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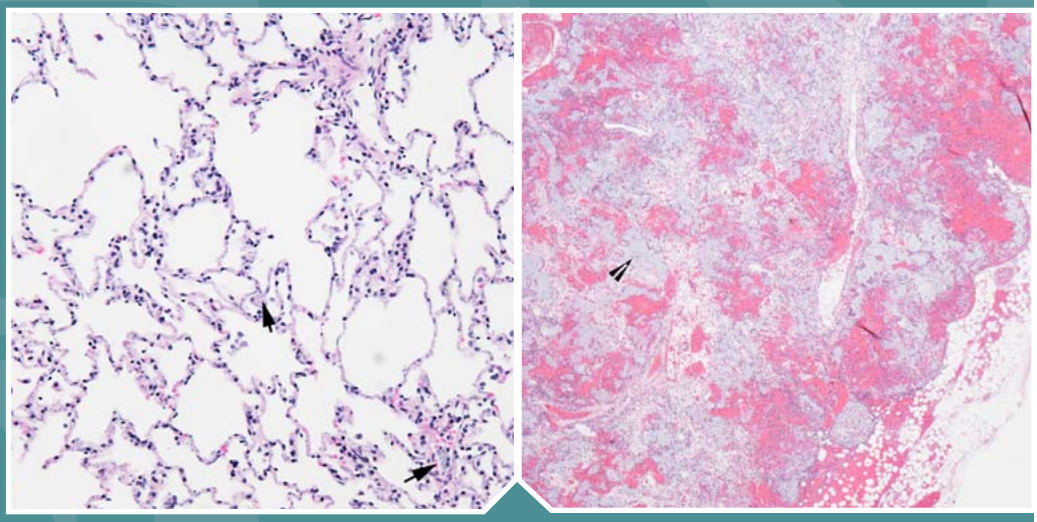
Acute Low Dose *Bacillus anthracis* Ames Inhalation Exposures in the Rabbit



Office of Research and Development
National Homeland Security Research Center

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Disclaimer

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This report has been reviewed by the Agency but does not necessarily reflect the Agency's views. No official endorsement should be inferred. EPA does not endorse the purchase or sale of any commercial products or services.

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Foreword

Following the events of September 11, 2001, the EPA's mission was expanded to address critical needs related to homeland security. Presidential Directives identify EPA as the primary federal agency responsible for the country's water supplies and for decontamination following a chemical, biological, and/or radiological attack.

As part of this expanded mission, the National Homeland Security Research Center (NHSRC) was established to conduct research and deliver products that improve the capability of EPA in carrying out its homeland security responsibilities. One focus area of this research is the compilation, development, and evaluation of information on the human health effects of pathogens that might be used by terrorists. Such information is critical to understanding the risks associated with biological contamination and supporting the development of site-specific cleanup goals, treatment technologies, and detection limits.

NHSRC has made this publication available to assist the response community to prepare for and recover from disasters involving microbial contamination. This information is intended to move EPA one step closer to achieving its homeland security goals and its overall mission of protecting human health and the environment while providing sustainable solutions to our environmental problems.

Jonathan Herrmann, Director
National Homeland Security Research Center

Executive Summary

Credible dose-response relationships are needed to more accurately assess the risk posed by exposure to low level *Bacillus anthracis* contamination during or following a release. The objective of this study was to evaluate physiological responses following an acute exposure to low doses of *B. anthracis* Ames spores.

Groups of five New Zealand White (NZW) rabbits were implanted with D70-PCT telemetery transmitters and subsequently aerosol challenged with average inhaled doses of 286 to 2.75×10^5 colony forming units (CFU). Control rabbits were challenged with irradiated (non-viable) spores as negative controls or with an average inhaled dose of 8.27×10^6 CFU as positive controls. The rabbits were then monitored for changes in non-specific parameters: activity levels, body temperature, heart and respiration rates, hematology, and serum chemistry. *Bacillus anthracis*-specific parameters were also measured and included bacteremia and presence of protective antigen (PA, a polypeptide produced by *B. anthracis*) in the serum (toxemia). All rabbits underwent necropsy and the lungs and any gross lesions were examined microscopically to identify anthrax-specific lesions.

All of the five positive control animals died on study; the mean time to death was 3.47 days. Four of the five rabbits that received an average inhaled dose of 2.75×10^5 CFU succumbed to infection with the mean time to death of 4.6 days. Two of the five rabbits that were exposed to an average inhaled dose of 2.54×10^4 CFU died 4.1 and 10.9 days post-challenge. All rabbits that received an average inhaled dose of 2.06×10^3 CFU or lower survived to the end of the study. Animals that succumbed to disease had pathological changes consistent with inhalational anthrax in the rabbit model, including pleural effusion and inflammation and bacilli observed in the lungs.

All animals that died on study were bacteremic, and 73% were positive for PA in serum. Increases in respiration, heart rate, and body temperature were observed in rabbits that

succumbed to anthrax. Neutrophila and increased liver enzymes were also associated with disease. Animals that survived to the end of the study never became bacteremic or toxemic.

These data suggest in the rabbit that an inhaled dose of *B. anthracis* spores at or above 2.54×10^4 CFU results in death and elicits measureable physiological changes. These data also suggest that in the rabbit model of disease, inhaled doses of 2.06×10^3 CFU or lower do not cause death or adverse changes in the measured physiological responses.

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Acronyms and Abbreviations

ALT	alanine aminotransferase
APS	aerodynamic particle sizer
AST	asparate aminotransferase
BBRC	Battelle Biomedical Research Center
CFU	colony forming unit
cm	centimeter
CRP	C-reactive protein
C_T	cycle threshold
dL	deciliter
DNA	deoxyribonucleic acid
DQA	Data Quality Audit
ED_{50}	effective dose 50%
EDTA	ethylene diamine tetraacetic acid
EF	edema factor
ELISA	enzyme-linked immunosorbent assay
EPA	U.S. Environmental Protection Agency
EU	endotoxin unit
FD	found dead
FS	final phase sacrifice
g	gram
HCT	hematocrit
Hg	mercury
HGB	hemoglobin
HRP	horseradish peroxidase
IgG	immunoglobulin G
InD	inhaled dose
kg	kilogram
L	liter
LDH	lactate dehydrogenase
LD_{50}	median lethal dose
LF	lethal factor

LOD ..... limit of detection
LOQ ..... limit of quantitation
µg ..... microgram
µL ..... microliter
µm ..... micrometer
mg ..... milligram
min ..... minute
mL ..... milliliter
mm ..... millimeter
MMAD ..... median aerodynamic diameter
MTT ..... 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide
NF_{50} ..... neutralization factor 50%
ng ..... nanogram
NHSRC ..... National Homeland Security Research Center
nM ..... nanomolar
NTC ..... no template control
NZW ..... New Zealand White
OD ..... optical density
PA ..... protective antigen
PCR ..... polymerase chain reaction
PLT ..... platelet count
QAP ..... Quality Assurance Plan
qPCR ..... quantitative real-time polymerase chain reaction
RDW ..... red cell distribution width
RNA ..... ribonucleic acid
rPA ..... recombinant protective antigen
rpoB ..... DNA-directed ribonucleic acid polymerase subunit beta
SDH ..... sorbitol dehydrogenase
SF ..... spray factor
SST ..... serum separator tube
TATV ..... total accumulated tidal volume
TNA ..... toxin neutralization assay
TNTC ..... too numerous to count

TSA ..... tryptic soy agar
TSAT ..... Technical System Audit
U ..... units
VAP ..... vascular access port
WBC ..... white blood cell
4PL ..... four parameter logistic log

1 Introduction

The U.S. Environmental Protection Agency's (EPA) is responsible for assessing the health risks associated with the intentional release of hazardous and toxic chemical, biological, and radiological threat agents. EPA is currently developing tools, technologies, and methodologies to advance microbial risk assessment to support site-specific cleanup, treatment, and detection for risk-based decision making. One of the greatest challenges faced by EPA is the assessment of the risk posed by low levels of biothreat agent contamination, either immediately following a release or after decontamination when low levels of residual agent may remain. *Bacillus anthracis*, the causative agent of anthrax, is one current focus of EPA risk assessment activities.

Bacillus anthracis is a gram-positive, rod-shaped, aerobic and/or facultative anaerobic, spore-forming bacterium. Each potential exposure route--gastrointestinal, cutaneous, and inhalation--manifests itself in different clinical symptoms, with inhalational anthrax being the most lethal. The incubation period usually varies from one to five days depending upon the dose and route of exposure. The onset of disease can be longer following inhalation exposure; some reports suggest a delayed onset of several weeks in low dose exposure or following removal of therapeutic intervention. The initial clinical signs and symptoms of inhalational anthrax are nonspecific and may include malaise, headache, fever, nausea, and vomiting. These are followed by a sudden onset of respiratory distress with dyspnea, stridor, cyanosis, and/or chest pain. The onset of respiratory distress is followed by shock and eventually death with close to 100% mortality rate (reviewed in Dixon et al., 1999).

Bacillus anthracis is considered to be one of the most significant biothreat agents due to the high lethality rates from inhalation exposure and the stability and persistence of the spore in the environment (Inglesby et al., 1999). The virulence of *B. anthracis* is predicated upon the production of an anti-phagocytic capsule and the anthrax toxins

(Drysdale et al., 2005; Acenzi et al., 2002). Three polypeptides, protective antigen (PA), lethal factor (LF), and edema factor (EF), interact to form two interlinked toxins of anthrax lethal toxin and edema toxin. The anthrax lethal toxin is produced when PA and LF combine; the edema toxin is produced when PA and EF combine. PA binds to a host cell receptor and is cleaved by a furin-like protease (Klimpel et al., 1992). The activated PA then forms a heptameric complex that competitively binds three molecules of LF and/or EF (Milne et al., 1994). The holotoxin is then taken up by the cell via receptor-mediated endocytosis. A decrease in endosomal pH produces a conformational change in the PA molecule, resulting in a pore structure for LF and EF translocation into the cytoplasm (Acenzi et al., 2002). LF is a zinc metalloprotease that inhibits mitogen-activated protein kinase signaling (Duesbery et al., 1998; Pellizzari et al., 1999; Vitale et al., 2000). EF, a calcium-dependent adenylate cyclase, increases cyclic adenosine monophosphate levels in susceptible cells, which results in altered water homeostasis and the inhibition of phagocytosis (Leppla 1982; O'Brien et al, 1985). Thus, both toxins inhibit the signaling cascades required for the activation of immune cells to combat disease.

An outbreak of inhalational anthrax in Sverdlovsk, Russia in 1979 provided the largest set of clinical specimens to study the pathology of human anthrax (Grinberg et al., 2001). Autopsies of outbreak victims consistently showed pathologic characteristics of inhalational anthrax, such as necrotic hemorrhage of the thoracic lymph nodes, hemorrhagic mediastinitis, and pleural effusion. Fifty percent of the cases involved hemorrhagic meningitis, and 92% showed signs of gastrointestinal tract involvement (i.e., submucosal hemorrhagic lesions). Quantitative microscopic findings showed that most of the severe pathologic lesions occurred in the mediastinum and mediastinal lymph nodes, the sites of initial replication of the bacterium. The investigators also observed peripheral transudate surrounding fibrin-rich edema, necrosis of veins and arteries, and apoptotic lymphocytes (Grinberg et al., 2001). The 2001 anthrax letter attacks resulted in five fatal cases of inhalational anthrax in the United States. Prior to hospital admission, common nonspecific symptoms included fever, malaise, and cough. Chest radiographs of these patients revealed pleural effusion

and lung infiltrates, with *B. anthracis* infection ultimately confirmed by culture (Jernigan et al., 2001).

Although almost 10 years have passed since the anthrax letter attacks of 2001, the lack of an acceptable dose-response relationship for *B. anthracis* continues to challenge the development of effective risk-based approaches for these events. *Bacillus anthracis* is the most highly studied of the currently known biothreat agents (Wilkening, 2006), yet there are significant data gaps in the dose-response assessment of low dose exposures (Gutting et al., 2008), the selection of appropriate animal models for low dose exposures (Leffel and Pitt, 2006), and necessary data for the extrapolation from animal to human dose-response relationships (Coleman et al., 2008). Collectively, these data gaps limit progress in the development of a dose-response relationship suitable for application for risk-based decision making.

With some exceptions (e.g., Druett et al., 1953), historical *B. anthracis* exposure studies with published dose-response data often relied on high doses that were associated with median or higher levels of lethality. Earlier studies were conducted with a variety of test species (e.g., guinea pigs, rabbits, various nonhuman primate species) and exposure products (e.g., differing *B. anthracis* strains, particle sizes). There are very few animal exposure studies that published raw dose-response data with adequate dose ranges for low dose extrapolation and sufficiently described exposure products to allow dosimetric adjustments.

There has been a significant increase in *B. anthracis* challenge studies conducted over the past 20 years. However, these studies were typically designed to test vaccine effectiveness (Fellows et al., 2001), assess the pathology of infection (Zaucha et al., 1998), or evaluate physiologic response to infection (Lawrence et al., 2009). Animals were challenged with doses that were multiples of the median lethal doses (LD_{50}) (e.g., doses that are 200 times or more of the identified median lethality value (e.g., Fellows et al., 2001)). While dose-response data may be obtained from control group animals that did not receive the vaccine, these groups typically show levels of mortality at or near 100%. As a result, these data are of limited value to assess dose-response relationships.

Conducted just over a century after the first identification of the rabbit as a potential

animal model for anthrax and published in 1886, the Zaucha et al. (1998) study has been identified as the definitive work characterizing the anthrax disease pathogenesis of rabbits. Zaucha et al. (1998) described widespread similarities in end-stage pathology between rabbits and humans, as well as concordance with nonhuman primates. Despite differences in pathology such as a more rapid disease progression for rabbits than humans including clinical evidence for regional lymph node involvement, Zaucha et al. (1998) concluded that rabbits were a suitable pathogenesis model for human disease. While there are a number of studies within the past 30 years that cite a LD_{50} for the New Zealand White (NZW) rabbit (*Oryctolagus cuniculus*), the published values are clustered near the Zaucha et al. (1998) value of 1.05×10^5 inhaled colony forming units (CFU)/animal and ranged from 10^5 to 1.1×10^5 CFU/animal. It should be noted that none of the studies present original dose-response data, and the actual values are cited as laboratory-derived values from an unpublished data set, as secondary data, or as personal communications.

In view of the lack of low dose exposure studies and the critical need for credible science to support risk-based cleanup decisions, this study was conducted to determine physiological responses following an acute exposure to low inhaled doses of *B. anthracis* Ames strain spores (hereafter referred to as *B. anthracis*) in the rabbit model of disease.

2 Materials and Methods

2.1 Test System

The protocol for the study, along with the methods referred to herein, is provided in Appendix A. All study deviations are documented in Appendix B. Thirty-five male pathogen-free NZW rabbits (*Oryctolagus cuniculus*) weighing approximately 3.5 kilograms (kg) were purchased from Covance (Denver, PA). Thirty rabbits were placed on study and the remaining five served as replacements. The study was performed at the Battelle Biomedical Research Center (BBRC) located in West Jefferson, OH. The rabbits were fitted with vascular access ports (VAP) at Covance before being shipped to the study facility. A veterinarian implanted a Data Sciences International (St. Paul, MN) model D70-PCT telemetric device in all of the rabbits prior the start of the study. Nasal swabs were taken and sent to Charles River Research Animal Diagnostic Services (Wilmington, MA) for *Bordetella bronchiseptica* testing to determine if there were any potential correlation with active *B. bronchiseptica* infection and responses in this study. The results of the testing are presented in Appendix C. *Bordetella bronchiseptica* status was not a criterion for rabbit placement on the study.

2.2 Randomization of Animals

Prior to challenge, the rabbits were randomized by weight into six groups of five rabbits per group (Table 1). The rabbits within each group were randomized for challenge order (based on ear tag numbers provided by the supplier). The SAS® software PLAN procedure (SAS Institute, Inc., Cary, NC) was used to randomize the animals. The rabbits were challenged according to randomization order and challenge dose group. For example, the rabbits in Group 1 were challenged first and the rabbits in Group 6 were challenged last. Prior to challenge, any animals with a malfunctioning vascular access port (VAP) or telemetric device were replaced with one of the five extra animals. Due to patency issues, all replacement animals with functional ports were used. Also, animals in the control groups (Groups 1 and 6) with working ports were exchanged with animals

from the experimental groups (Groups 2 through 5) with malfunctioning ports. The group switching occurred as follows: L23215 (formerly Group 1) was switched with L23223 (formerly Group 2); L23227 (formerly Group 6) was switched with L24213 (formerly Group 4); L23232 (formerly Group 4) was switched with L23205 (formerly Group 6).

Table 1. Study Design and Challenge Doses

Group	Targeted Inhaled Dose (CFU)	No. of Spore Challenges	No. of Rabbits
1 (Negative control*)	100 x LD_{50}†	1	5
2	100	1	5
3	1000	1	5
4	10,000	1	5
5	100,000	1	5
6 (High dose positive control)	100 x LD_{50}†	1	5

*Negative controls were challenged with irradiated spores
†LD_{50} = 1.05 x 10^5 CFU per Zaucha et al. (1998)

2.3 *Bacillus anthracis* Ames Strain Spores

Bacillus anthracis Ames strain spores (spore lot Ames B35) were used on this study. The spores were characterized and qualified prior to release for use (Table 2).

Table 2. Characterization of *Bacillus anthracis* Spores

Characterization	Acceptance Criteria	Results
Colony purity: Colony morphology on blood agar	Pure culture	Pure culture
% Vegetative cells	≤ 5%	0%
% Debris	≤ 5%	0.5%
% Spore refractility	≤ 5% Non-refractile spore	0.4% Non-refractile spore
Viable spore count	≥ 1 x10^9 CFU/mL	8.56 x 10^9 CFU/mL
Guinea pig LD_{50}	< 10 Spores/dose (intradermal)	3.67 Spores/dose (intradermal)
Endotoxin content	< 1.0 EU/mL	< 0.102 EU/mL
Phenol content	0.8 - 1.2%	0.99%

EU = Endotoxin unit
mL = milliliter

The spores were stored at 4ºC to 8ºC in 1.0% phenol, washed with endotoxin-free water four times, and stored at 4ºC to 8ºC until diluted for aerosolization. Prior to use, the spores were diluted to the appropriate concentration in endotoxin-free sterile water and 0.01% Tween 20. The spores were then stored in single use aliquots until time of use.

2.4 Aerosol Challenge Generation and Monitoring

Prior to the start of the study, spray factor (SF) testing was performed on the lower target inhaled doses of *B. anthracis* spores to ensure the challenge system was capable of achieving the low dose aerosol. It was confirmed that the targeted doses were achievable. Appendix D contains the methods and results of the preliminary SF study.

The SF was a numeric correlation between nebulizer concentration and the resulting generated aerosol concentration. The SF was calculated by dividing the aerosol concentration by the starting nebulizer suspension concentration and was used to predict aerosol concentration for a given starting suspension concentration. During SF testing the overall mean SF was determined. The formula for determining the SF is presented in Equation 1.

(1)

The aerosol system performance characteristics were evaluated by aerosolizing four target nebulizer concentrations of *B. anthracis* spores multiple times over a three day period. The target nebulizer concentrations tested over the three days were 1.0×10^4, 1.0×10^5, 1.0×10^6, and 1.0×10^7 CFU/mL. Specifically, each concentration was tested a total of nine times (three times per day over three days). Each test consisted of a 10-minute (min) period of aerosol generation and sample collection. The SF values determined in testing were used to plan the exposure durations needed in the actual single exposure study, the details of which are discussed in the paragraphs that follow.

On Study Day 0 (defined as Challenge Day), the rabbits were placed into a plethysmography chamber, passed into a Class III biosafety cabinet system, and aerosol challenged with targeted inhaled doses of 1.0×10^2, 1.0×10^3, 1.0×10^4, or 1.0×10^5 CFU of *B. anthracis* spores (Table 1). A high dose control group was challenged with 100 x LD_{50} value and the negative control group was exposed to an equivalent of 100 x LD_{50} value of gamma irradiated spores as described below.

A modified Microbiological Research Establishment type three-jet Collison nebulizer (BGI, Waltham, MA) with a precious fluid jar was used to generate a controlled delivery of aerosolized *B. anthracis* spores from a liquid suspension. These nebulizers were designed to generate aerosols having an approximate aerodynamic mean diameter of 1 to 2 micrometers (μm). Each nebulizer was characterized for a pressure that results in approximately 7.5 L/min flow, which normally is approximately 28.0 pounds per square inch, Collison nebulizer dependant.

Aerosol concentration and aerosol particle size distribution were determined by analysis of atmospheric samples drawn from the exposure chamber. The aerosolized spores were drawn into a plexiglass exposure chamber with internal dimensions of approximately 20.5 centimeters (cm) x 20.5 cm x 40 cm (length x width x height). Atmospheric samples were collected in an impinger (Model 7541; Ace Glass Inc.,

Vinland, NJ) filled with approximately 20 mL of sterile water that sampled at approximately 6.0 ± 0.3 L/min. The sampling rate was achieved by maintaining a vacuum of ≥18 inches Hg across the exhaust connection of the impinger to maintain the flow from the impinger critical orifice. The liquid in the impinger was diluted and enumerated by the spread plate technique to quantify culturable spore counts per mL; concentrations were reported in terms of CFU/mL. Enumeration results, along with the volume of liquid in the impinger, sampling rate, and sampling duration, were used in the calculation of the aerosol concentration expressed as CFU/L of air.

The aerosol particle size was determined during each exposure using an Aerodynamic Particle Sizer (APS Model 3321; TSI Inc., St. Paul, MN), which drew an atmospheric sample from the exposure chamber at 0.25 L/min with a diluter (1.0 L/min total with 0.75 L/min from the diluter and 0.25 L/min from the exposure chamber).

Whole body plethysmography was performed in real time on each animal during challenge to measure important respiratory parameters. These parameters (tidal volume, total accumulated tidal volume [TATV], and min volume) were calculated from the measured volumetric displacement of air caused by the movement of the thoracic cavity of an animal while it was in a sealed plethysmographic chamber. The data generated for each animal were used to determine the TATV, which along with the aerosol concentration, was used to calculate the inhaled dose. The rabbits were physically restrained within a plethysmography restraint device with the head protruding out of a port that was sealed with rubber dental dam material and held in place with two plexiglass guillotines. The plethysmograph was connected to a pneumotach (Hans Rudolph, Inc., Kansas City, MO) that was attached to a differential pressure transducer (Model DP-45; Validyne Engineering Corp., North Ridge, CA). Pressure differential measurements from inhalations and exhalations were transmitted to Biosystems XA Version 1.5.7 software (Biosystems XA, Buxco Electronics, Sharon, CT), which then calculated and recorded respiratory function. Prior to animal exposures, the Buxco software program was calibrated to establish unit (baseline) and air volume displacements from 5 to 40 mL to simulate animal respiration. This calibration was performed to encompass the respiration volume range

of the animal model to ensure accurate TATV measurements.

The inhalation exposure system data for each exposure were documented on appropriate forms to ensure proper system operation and to provide the needed information to quantify animal challenge conditions. Impinger sampling conditions and enumerated concentration results provided culturable bioaerosol challenge concentration, while plethysmography measurements documented the total inhaled volume. Total inhaled dose, as measured in CFU, was calculated from aerosol concentration and total inhaled volume. The number of LD_{50} was calculated by dividing the total inhaled dose by the reported inhalation LD_{50} for the rabbit. The reported LD_{50} value for rabbits is 105,000 inhaled CFU/animal (Zaucha et al., 1998).

Impinger samples were enumerated by the spread plate method (10^1 to 10^3 on tryptic soy agar [TSA] plates in triplicate), following serial dilutions to determine viable spore concentration. Diluted samples were mixed in a capped vial prior to subsequent dilutions. At different target dilutions, 0.1 mL was spread onto each of five TSA plates, which were placed in a secondary container and incubated. Impinger samples from the 1.0×10^2 and 1.0×10^3 CFU targeted inhaled doses were enumerated by spread plating and by growth on a filter. Briefly, 1.0 mL of the sample was passed through a sterile 0.45 µm filter (Nalgene) Analytical Test Filter Funnel (Catalog Number 145-0045; Fisher Scientific, Pittsburgh, PA), the filter was placed on top of a TSA plate, incubated for 24 to 72 hours at 37°C ± 2°C, and then enumerated. The impinger samples from the irradiated spores were plated neat to ensure sterility of the samples. After the incubation period, the plates were enumerated to determine the number of colonies on each plate. Impinger sample concentration was determined using Equation 2.

(2)

where C = CFU/mL
A = Average CFU per plate
D = Dilution factor.

The total inhaled dose (InD) was calculated from the impinger sample concentration, sampling parameters, and exposure duration (Equation 3). This equation assumes 100%

impinger sampling efficiency. The total number of viable spores captured during each exposure was the product of the impinger concentration (C) and the impinger sampler volume (V). The total number of viable spores was divided by the amount of air (S) that was sampled through the impinger during the exposure time (T). The aerosol concentration was $(C \times V)(S \times T)^{-1}$. The InD was calculated by the product of the aerosol concentration and the TATV.

(3)

where InD = Inhaled dose (CFU)
C = Impinger concentration (CFU/mL)
V = Impinger sampler volume (mL)
S = Sampling rate (6 L/min)
T = Exposure time (min)
$TATV$ = Total accumulated tidal volume (L).

A detailed schematic of the exposure system is located in Appendix E.

2.5 Stability of Aerosolized Spores

To determine the effect of aerosolization on spore stability, the nebulizer and impinger samples from the high dose group (Group 6) were plated after the challenges with and without heat treatment (30 min at 65°C ± 2°C). This process was repeated twice on the Group 6 impinger samples because the first heat treatment was done incorrectly and the nebulizer samples were not included. Briefly, the entire volume of impinger sample (~19 to 20 mL) contained in a 50 mL conical tube was placed in a 60°C water bath. However, it was unlikely that the samples reached the desired temperature for the necessary length of time because of the volume of sample and type of tube used. Therefore the process was repeated with modifications: 1.0 mL of impinger and nebulizer sample from Group 6 was transferred to a 2.0 mL vial and placed into a heating block. One mL of tap water was also placed in a similar vial containing a thermometer. These samples were incubated for 30 min at 65°C and enumerated by serial plating on TSA. The data collected from the second heat treatment were used to evaluate the difference between nebulizer and impinger samples. A two-tailed t-test (Microsoft Office Excel) was used to determine if any identified differences were statistically significant (Appendices F, H, I, J, and O).

2.6 Telemetric Monitoring

The rabbits were surgically implanted with telemetry units (D70-PCT transmitters, Data Sciences International) prior to being placed on study. Each D70-PCT transmitter contained one pressure lead and one biopotential lead. Body temperature, electrocardiogram activity, and cardiovascular function (heart rate and respiratory pressure) were monitored for 30 seconds every 15 min for seven days pre-challenge (baseline) and for 21 days post-challenge.

Each animal's cage was equipped with a Data Sciences International telemetry receiver. The transmitters, receivers, consolidation matrixes, cabling, and computers using the Dataquest A.R.T.™ data acquisition and analysis software are all components of the PhysioTel® Telemetry System. The Dataquest A.R.T.™ telemetry software collected the telemetry parameters mentioned above. The statistical methods used to analyze the telemetry data are presented in Appendix F. Telemetry surgery and histopathology were performed at Battelle Memorial Institute in Columbus, OH.

2.7 Clinical Observations and Body Weights

Following challenge, the rabbits were observed twice daily for survivability and clinical signs of illness that could be attributable to *B. anthracis* infection (e.g., moribund, respiratory distress, appetite, activity, and seizures) except on Study Day 12 when they were only observed in the morning. Animals were weighed on Study Days 0 (Challenge Day), 1, 2, 3, 7, 14 and 21.

2.8 Blood Collection

On Study Days -3, 1, 2, 3, 7, and 14 blood was collected into ethylene diamine tetraacetic acid (EDTA; ~1.5 mL) and serum separator tubes (SST; ~3.5 to 4.5 mL) (Table 3). Blood was drawn at 24, 48, and 72 hours (± 2 hours) from the median challenge time of each group. Blood samples also were taken from animals found dead or prior to euthanasia. At 21 days post-challenge, all surviving rabbits were terminally bled via cardiac puncture according to Table 3.

Serum was collected from blood samples in SSTs by centrifugation and was stored at ≤ -70°C until analyzed. Blood in EDTA tubes was stored at room temperature if used within four hours of collection; blood was stored at 2°C through 8°C if not analyzed within four hours. Blood was collected from

vascular access ports on Study Days -3, 1, 2, and 3 for both the EDTA tubes and SSTs. After Day 3, blood collected in the EDTA tubes was obtained from the medial auricular artery or the marginal ear vein. If a port failed, the medial auricular artery or the marginal ear vein was utilized for blood collection regardless of the sample time point. If a blood sample was not collected from either the port or other appropriate vessel, it was documented in the study file. Appendix G contains the exact blood collection times.

Table 3. Blood Collection Schedule and Volume

Tube Type	Study Day						
	-3	1	2	3	7	14	21
EDTA (~mL)	1.5	1.5	1.5	1.5	1.5	1.5	1.5*
SST (~mL)	4.5	3.5	3.5	3.5	3.5	3.5	15*
Total/day (~mL)	6	5	5	5	5	5	16.5

*Terminal blood sample, minimum draw volume

2.9 Protective Antigen ELISA

Serum samples were collected and stored in a freezer set to maintain ≤ -70ºC until evaluation of quantitative circulating PA levels by enzyme-linked immunosorbent assay (ELISA). Double affinity purified polyclonal, monospecific rabbit anti-PA immunoglobulin G (IgG) “capture antibody” was produced by Battelle (Columbus, OH). It was purified from recombinant PA (rPA)-vaccinated rabbit serum over first a Protein A column to bind all IgG antibodies, and then a PA column to specifically isolate anti-PA IgG antibodies. The “capture antibody” was used to coat the wells of a 96-well plate at a concentration of 2 micrograms per mL (µg/mL). The plates were blocked with skim milk and then incubated with rabbit serum samples containing native PA (Catalogue No. NR-164, Lot No. 5051797; BEI Resources, Manassas, VA), or a reference standard and quality control samples consisting of rPA spiked differentially into naive rabbit serum. The PA was detected by first incubating with diluted goat PA anti-serum, followed by incubation with a bovine anti-goat horseradish peroxidase (HRP)-conjugated secondary antibody (Santa Cruz Biologicals, Santa Cruz, CA), then a 2,2'-azinobis [3-

ethylbenzothiazoline-6-sulfonic acid]-diammonium salt substrate and a stop solution (both from Kirkegaard and Perry Laboratories, Gaithersburg, MD). The plates were read and the data were analyzed using a four parameter logistic-log (4PL) model to fit the eight point calibration curve. The concentrations of the PA in unknown samples were determined by computer interpolation from the plot of the reference standard curve data (Softmax Pro; Molecular Devices, Downington, PA). The assay was qualified using PA spiked into rabbit serum resulting in a qualified linear range, slope, and putative limit of detection (LOD) used for the assays. The statistical methods used to analyze the PA ELISA data are given in Appendix H.

2.10 Bacteremia

A portion of each blood sample from the EDTA collection tubes was tested for bacteremia by quantitative spread plate technique and quantitative real-time polymerase chain reaction (qPCR). The Day -3 samples were assayed for bacteremia via streaking an inoculating loop of collected blood on blood agar.

For qualitative bacteremia culture analysis, blood samples were streaked over blood agar plates and read for growth and morphology consistent with *B. anthracis* after a minimum incubation of 48 hours at 37°C ± 2°C. Quantitative counts were achieved by 10-fold serial dilutions of the blood samples in Dulbecco's Phosphate Buffered Saline (11.0 grams [g] NaCl, 5.7 g NaH_2PO_4, 1.3 g Na_2HPO_4 dissolved in 1.0 L of distilled water, pH adjusted to 6.2 and filter sterilized) from 1×10^1 to 1×10^9 and spread plating 100 microliters (µL) of each dilution on to TSA in triplicate. The plates were enumerated after 24 hour incubation at 37°C ± 2°C. In instances when a blood sample could not be obtained in an EDTA collection tube for quantitative bacteremia culture, the pellet from the SST sample was streaked on an agar plate to obtain qualitative results. Colonies with the morphology constant with *B. anthracis* ("ground glass"-like appearance) were enumerated to determine the viable bacterial load in the blood.

To perform qPCR, total nucleic acid was isolated from 100 µL rabbit peripheral whole blood using the fully automated bioMérieux NucliSENS® EasyMAG™ kit (bioMérieux, Durham, NC).
Based on published sequence data available in GenBank (Accession Number AE016879), oligonucleotides were designed

that would amplify a small deoxyribonucleic acid (DNA) fragment within the coding region of the *B. anthracis* DNA-directed ribonucleic acid (RNA) polymerase subunit beta (*rpoB*) gene (Table 4). The *rpoB* gene was selected because it is a highly conserved housekeeping gene. Due to its essential role in cellular metabolism, at least one copy is expected to be present in all bacteria. The qPCR assay was designed to be quantitative and not diagnostic. Therefore, the primers and probe used may detect other *Bacillus* species and the specificity of the assay was not determined.

Table 4. TaqMan® Gene Expression Assay for the *B. anthracis* rpoB_571 Gene

Name	Primer/Probe	Oligonucleotide Sequence (5'-3')	Accession No.*
rpoB_571	Forward	ATTCAAAACAGCGAAACCAA	AE016879
	Reverse	TCTATTAAGATTTATGCTCCTGAGTCAGA	
	Probe	6FAM-TGGAGTGGTAGAAGGTGA-NFQ	

*GenBank accession numbers are available online at http://www.ncbi.nlm.nih.gov/

qPCR reactions consisted of 1X TaqMan® Universal PCR Master Mix (AmpliTaq Gold® DNA Polymerase, AmpErase® UNG, dNTPs with dUTP, Passive Reference, and optimized buffer components [Applied Biosystems Inc., Foster City, CA]), 1X Gene Expression Assay mixture 900 nanomolar (nM) forward primer, 900 nM reverse primer, and 250 nM probe (dual-labeled with FAM™ at the 5′ end and a non-fluorescent quencher at the 3′ end; Table 4), nuclease-free distilled water, and either 5 µL of qualified reference standard plasmid or 5 µL of isolated nucleic acid in a total volume of 50 µL. qPCR was performed using an ABI PRISM® 7900HT Fast Sequence Detection System (Applied Biosystems Inc.) with the following conditions: 2 min at 50ºC, 10 min at 95ºC, followed by 40 cycles of 95ºC for 15 seconds and 60ºC for 1 min. All reactions were performed in triplicate, and each run contained a Nucleic Acid Isolation Negative Control (genomic DNA isolation procedure using nuclease-free distilled water), a Nucleic Acid Isolation Positive Control (genomic DNA isolated from a *B. anthracis* vegetative culture), and a master mix only control (no template control, NTC). Following acquisition, data were analyzed using the Sequence Detection System software (Applied Biosystems Inc.). Final reportable values were extrapolated

from the reference standard curve as long as a minimum of two test sample cycle threshold (C_T) values were within 0.50 of one another. The statistical methods used to analyze the bacteremia culture and qPCR data are located in Appendix H.

2.11 TNA/ELISA

To determine if the rabbits elicited an immune response following challenge, serum samples were analyzed by ELISA and high throughput toxin neutralization assay (TNA) as described below.

The ELISA was designed to quantify IgG antibodies against *B. anthracis* anthrax toxin PA using purified rPA as the solid-phase immobilized antigen, and an enzyme-conjugated anti-gamma chain secondary antibody was used as the reporter or signal system. The assay endpoint was reported as the serum mean concentration of anti-PA specific IgG (µg/mL).

Microtiter plates were coated with purified rPA. Unknown test samples, anti-PA IgG reference standard serum, and positive control sera are added to the microtiter plate. The PA-specific antibodies present in the samples/standards were allowed to bind to the rPA coated on the plate. After washing, the bound anti-PA antibodies were then detected by a species-specific anti-gamma chain IgG–HRP conjugate followed by addition of a peroxidase substrate. The optical density (OD) values for each plate were then read on a microplate reader (ELx800; BioTeK, Winooski, VT) at a wavelength of 405 nanometers using a 490 nanometer reference wavelength. The ELISA has both primary (plate-level) and secondary (test sample-level) acceptance criteria. The anti-PA IgG concentration of each passing test sample on passing plates was determined by taking the average of the acceptable concentrations from the eight-point dilution of the test sample back calculated from the standard curve. Results were reported in µg/mL of anti-PA IgG for each unknown test sample.

The TNA was designed to measure and qualify the functional ability of serum to neutralize *B. anthracis* lethal toxin (PA+LF) activity using an *in vitro* cytotoxicity assay. Specifically, cell viability was determined colorimetrically using a tetrazolium salt, 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) as the reporter or signal system. The serum-mediated neutralization of anthrax lethal toxin manifested as a suppression of

cytotoxicity, and hence preservation of cell viability.

Microtiter cell plates were seeded with J774A.1 cells and allowed to adhere. In separate microplates (prep plate), a serial dilution of the test samples and controls were prepared. Lethal toxin (LF+PA) was added to the prep plate and incubated to allow for lethal toxin neutralization by neutralizing antibodies. The contents of the prep plate were then transferred to the cell plate and incubated to allow intoxication to proceed. MTT was then added to the cell plates to allow viable cells to reduce the MTT dye. The OD values for each plate were read on a BioTek microplate reader (ELx800, BioTeK) at a wavelength of 570 nanometers using a 690 nanometer reference wavelength. The TNA Statistical Analysis System program then fit the seven-point serial dilutions of the reference serum standard and test sample serum OD values to a 4PL function, which was in turn used to calculate the reportable values (effective dose 50% [ED_{50}] and neutralization factor 50% [NF_{50}]).

2.12 Hematology and Clinical Chemistry

Complete hematological analysis was performed on blood samples collected in EDTA tubes using the Advia 120 Hematology Analyzer (Siemens Heathcare Diagnostics, Deerfield, IL) according to manufacturer's recommendations. Hematology analysis included the following parameters:

- White blood cell (WBC) count
- Neutrophil/lymphocyte ratio
- Differential leukocyte (absolute) count
- Hemoglobin (HGB)
- Hematocrit (HCT)
- Red blood cell count
- Mean corpuscular volume
- Mean corpuscular hemoglobin
- Mean corpuscular hemoglobin concentration
- Red cell distribution width (RDW)
- Platelet count (PLT)
- Mean platelet volume.

The values for the normal ranges of these parameters were identified by the manufacturer and were derived from mean values published by Schalm et al. (1975). The statistical methods used to evaluate the hematology data are presented in Appendix I.

Clinical chemistry was performed on all serum samples using Advia 1200 Chemistry analyzer (Siemens Heathcare Diagnostics) according to manufacturer's recommendations. Evaluation included the following parameters:

- Alanine aminotransferase (ALT)
- Asparate aminotransferase (AST)
- Total bilirubin
- Total protein
- Blood urea nitrogen
- Creatinine
- Calcium
- Sodium
- Potassium
- Chloride
- Phosphorus
- Lactate dehydrogenase (LDH)
- Sorbitol dehydrogenase (SDH)
- C-reactive protein (CRP).

The normal ranges of these parameters were determined by scientists based on mean values appearing in Thrall et al. (2004), Queensberry and Carpenter (1997), and University of Nebraska Institutional Animal Care and Use Committee (2009). The statistical methods used to analyze the clinical chemistry data are described in Appendix J.

2.13 Necropsy and Histopathology

Animals that succumbed to challenge or were found moribund and euthanized underwent gross necropsy. Surviving animals were euthanized and necropsied on Study Day 21. The lungs and gross lesions from each animal were collected. The meninges, ependyma, ventricles, frontal cortex, hippocampus, thalamus, cerebellum, and brain stem were included when gross lesions were present in the brain. The brain and standard sections of all other collected tissues were placed in 10% neutral buffered formalin, processed to approximately 5 µm slides, stained with hematoxylin and eosin, and examined histologically by a board-certified pathologist. All microscopic findings were graded semi-quantitatively according to the following scale, with the associated numerical score used to calculate average severity grades for each lesion by group:

- Minimal (Grade 1): the least detectible lesion
- Mild (Grade 2): an easily discernible lesion
- Moderate (Grade 3): a change affecting a large area of the represented tissue
- Marked (Grade 4): a lesion that approached maximal.

Gross and microscopic diagnoses were entered into the PATH/TOX SYSTEM (Xybion Medical Systems Corporation, Cedar Knolls, NJ) for data tabulation and analysis.

3 Results

3.1 Aerosol Challenges

To determine the physiological responses of low dose aerosol exposure to *B. anthracis* spores, four groups of five rabbits were exposed to targeted inhaled doses of 1 x 10^2 to 1 x 10^5 CFU. The individual challenge doses as well as the group means are listed in Table 5. Plate counts of the impinger samples revealed that mean actual inhaled doses were 2.86 x 10^2 (± 4.32 x 10^1) CFU to 2.75 x 10^5 (± 7.41 x 10^4) CFU. Group 6 animals were exposed to a targeted inhaled dose of 79 x LD_{50} equivalents and served as a positive control. The mass median aerodynamic diameter (MMAD) for challenge material for each group as determined by an aerodynamic particle sizer (APS) is presented in Table 5 and Appendix E, Figure 2. The MMAD values increased with the challenge dose because the APS instrument sizes all particles sampled and counted by the instrument. The APS does not distinguish between water droplets, debris particles, spores, etc. As a result, a greater proportion of small water droplet and debris were counted relative to spores at lower spore concentrations. As the spore concentration increased in the nebulizer, more spores were aerosolized and analyzed, thereby accounting for a larger percent of the total mass. The result was a shift to a larger measured particle size or MMAD. This occurred until a spore concentration was reached in the aerosol where the majority of particles counted and sized were only spores and the MMAD reflected predominately individual spores. As a result, the particle size did not shift any further since the spores were predominately the largest particle.

As a negative control, Group 1 rabbits were exposed to 100 x LD_{50} equivalents of gamma irradiated spores. The impinger plates from animal L23216 (Group 1) were positive for *B. anthracis* growth. The sample was re-plated and was still positive for *B. anthracis*; therefore the original results of 3 CFU/100 µL were reported. The rest of the impinger samples from Group 1 were sterile and the irradiated material was confirmed sterile by the U.S. Centers for Disease Control and Prevention prior to shipment to

the study facility. This suggested that growth most likely occurred because of contamination during the plating procedure of the individual sample.

Table 5. Individual and Group Mean Challenge Doses

Group	Animal ID	Inhaled Dose (CFU/ Animal)	Mean Inhaled Dose (SD) (CFU/Animal)	Challenge Dose (LD_{50})†	Mean Challenge Dose (LD_{50})	Mass Median Aerodynamic Diameter, (µm) (Geometric Standard Deviation)	Time to Death (day)
1	L23220	0.00	2.00 (4.58×10^{-1})	0.00	0.00 (0.00)	0.96 (1.33)	Survived
	L23216*	1.00×10^{1}		0.00			Survived
	L23218	0.00		0.00			Survived
	L23223	0.00		0.00			Survived
	L23222	0.00		0.00			Survived
2	L23215	3.22×10^{2}	2.86×10^{2} (4.32×10^{1})	3.00×10^{-3}	3.00×10^{-3} (4.10×10^{-4})	0.82 (1.48)	Survived
	L23206	2.98×10^{2}		3.00×10^{-3}			Survived
	L23210	2.18×10^{2}		2.00×10^{-3}			Survived
	L23219	3.21×10^{2}		3.00×10^{-3}			Survived
	L23211	2.73×10^{2}		3.00×10^{-3}			Survived
3	L23217	1.48×10^{3}	2.06×10^{3} (3.42×10^{2})	1.40×10^{-2}	1.9×10^{-2} (3.25×10^{-3})	0.92 (1.57)	Survived
	L23230	2.02×10^{3}		1.90×10^{-2}			Survived
	L23228	2.23×10^{3}		2.10×10^{-2}			Survived
	L23227	2.32×10^{3}		2.20×10^{-2}			Survived
	L23229	2.24×10^{3}		2.10×10^{-2}			Survived
4	L23235	1.76×10^{4}	2.54×10^{4} (5.21×10^{3})	1.68×10^{-1}	2.42×10^{-1} (4.96×10^{-2})	0.87 (1.59)	11
	L23205	2.73×10^{4}		2.60×10^{-1}			Survived
	L23225	2.59×10^{4}		2.47×10^{-1}			4
	L23231	2.41×10^{4}		2.29×10^{-1}			Survived
	L23207	3.19×10^{4}		3.04×10^{-1}			Survived
5	L23201	1.78×10^{5}	2.75×10^{5} (7.41×10^{4})	1.69	2.62 (7.06×10^{-1})	1.12 (1.33)	4
	L23234	2.96×10^{5}		2.82			6
	L23212	3.29×10^{5}		3.13			Survived
	L23200	2.19×10^{5}		2.09			3
	L23214	3.54×10^{5}		3.37			6
6	L23204	5.95×10^{6}	8.27×10^{6} (1.69×10^{6})	5.67×10^{1}	7.88×10^{1} (1.61×10^{1})	1.12 (1.31)	4
	L23203	8.86×10^{6}		8.44×10^{1}			5
	L23213	7.29×10^{6}		6.94×10^{1}			3
	L23221	8.88×10^{6}		8.46×10^{1}			2
	L23232	1.04×10^{7}		9.88×10^{1}			4

*Enumeration plates for this sample were positive for *B. anthracis*

†$LD_{50} = 1.05 \times 10^{5}$ CFU (Source: Zaucha et al., 1998)

Details of the aerosol challenge data were contained in Appendix E. A report characterizing the spray factors of the low dose aerosols was provided in Appendix D.

3.2 Stability of Aerosolized Spores

To determine the stability of spores after the aerosolization process, both nebulizer and impinger samples from the Group 6 exposure runs were subjected to heat shock (65°C for 30 min). Table 6 shows the heat shock results from individual samples. The mean pre-and post-heat shock nebulizer counts were 1.25×10^9 ($\pm 2.02 \times 10^8$) CFU and 1.21×10^9 ($\pm 2.05 \times 10^8$) CFU, respectively. There was no significant difference between the pre- and post-heat shock counts for the nebulizer samples (P = 0.55). There was a significant difference between the pre- and post-heat shock counts for the impinger samples (P = 0.02). The mean impinger pre-heat shock counts were 1.49×10^6 ($\pm 4.30 \times 10^5$) CFU/mL but dropped to 1.18×10^6 ($\pm 3.30 \times 10^5$) CFU/mL after the heat shock. This reduction in viable spores may have been caused by the spore coat being damaged during the aerosolization process. Also the impinger samples were processed twice for heat treatment which, while unlikely, may have had an impact on viability. The sample was processed twice because the first heat-shock procedure was done incorrectly as described in the Materials and Methods Section. The results from the failed heat treatment are presented in Appendix K.

Table 6. Heat Shock Enumerations

Sample Type	Sample ID	Enumeration Pre-Heat Shock (CFU/mL)	Enumeration Post-Heat Shock (CFU/mL)
Nebulizer	L23204	9.30×10^8	8.80×10^8
Nebulizer	L23203	1.48×10^9	1.26×10^9
Nebulizer	L23213	1.22×10^9	1.18×10^9
Nebulizer	L23221	1.33×10^9	1.27×10^9
Nebulizer	L23232	1.27×10^9	1.44×10^9
Impinger	L23204	7.28×10^5	6.16×10^5
Impinger	L23203	1.72×10^6	1.16×10^6
Impinger	L23213	1.68×10^6	1.34×10^6
Impinger	L23221	1.60×10^6	1.43×10^6
Impinger	L23232	1.73×10^6	1.35×10^6

3.3 Clinical Observations, Body Weights, and Mortality

Animals that succumbed to disease showed clinical signs consistent with inhalational anthrax in the rabbit model. Anorexia and lethargy were the most common observations prior to the animal's death. A complete list of individual clinical observations is presented in Appendix L. The body weights of the study rabbits remained consistent throughout the course of the study. Individual body weights are provided in Appendix M.

All of the rabbits in Groups 1 through 3 survived until the end of the study. Two of the five Group 4 rabbits died 4.14 days (L23225) and 10.85 days (L23235) post-challenge. Rabbits L23225 and L23235 were dosed with 2.6×10^4 CFU ($0.247 \times LD_{50}$) and 1.8×10^4 ($0.168 \times LD_{50}$), respectively. Four of the five Group 5 rabbits died, with a mean time to death of 4.64 days post-challenge. These animals received a mean inhaled dose of 2.8×10^5 ($\pm 7.41 \times 10^4$) CFU or $2.62 \times LD_{50} \pm 7.06 \times 10^{-1}$ equivalents of *B. anthracis* spores. All five of the high-dose control group (Group 6) animals succumbed to infection, with a mean time to death of 3.47 ± 1.18 days post-challenge. Appendix N contains the individual mortality data for each animal.

An overall Fisher's exact test on the mortality data was significant ($P < 0.0001$), indicating that the proportion of surviving animals in at least one of the groups was significantly different than those in the other groups. Based on the unadjusted Fisher's

exact tests, the proportions of surviving animals in the negative control group (Group 1) and in the two lowest dose groups (Groups 2 and 3) were significantly greater than those in Group 5 and in the high-dose control group (Group 6). However, after adjusting for the multiple comparisons, there were no significant pairwise differences between the groups since the multiple comparisons have a more stringent requirement for significance. The overall log-rank test was significant ($P < 0.0001$), indicating that the survival distribution in at least one of the groups was significantly different than those in the other groups. Based on the unadjusted log-rank tests, the times to death in the negative control group (Group 1) and Groups 2 and 3 were significantly greater than those in Groups 5 and the high dose control group (Group 6). Furthermore, Group 4 also experienced significantly greater times to death than the high dose control group (Group 6). However, after adjusting for the multiple pairwise comparisons, only the times to death in the negative control group (Group 1) and in the two lowest dose groups (Groups 2 and 3) were significantly greater than that in the high dose control group (Group 6). Figure 1 displays the Kaplan-Meier curves for each of the six dose groups. A dose-response relationship was observed, with increased target inhaled doses generally being associated with decreased times to death and greater mortality.

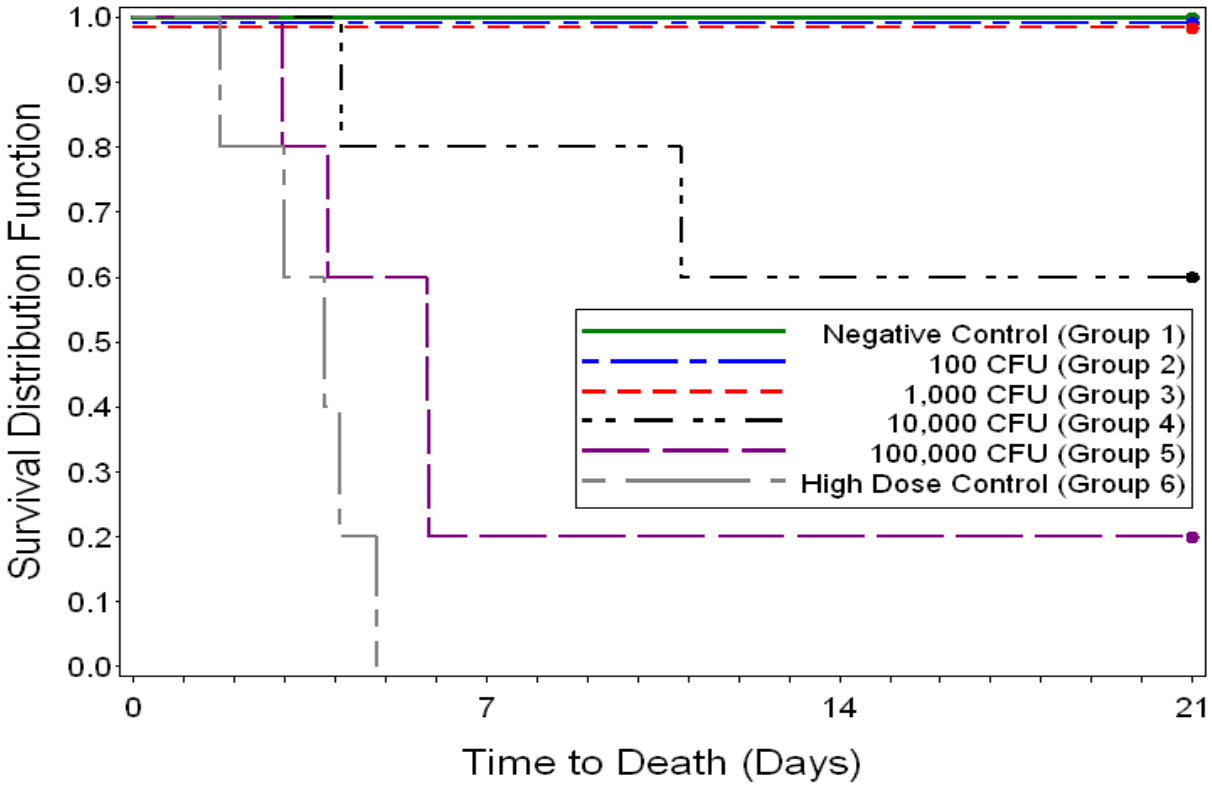

Figure 1. Kaplan-Meier curves representing time to death and survival data for each group.

The logistic regression model fitted to the survival data indicated a significant dose-response relationship with increased inhaled doses being associated with decreased probabilities of survival, as evidenced by the significant P-value associated with the estimated slope coefficient of -2.21 (P = 0.0147). The estimated LD_{50} was 5.18 x 10^4 CFU with a 95% Fieller confidence interval ranging from 6.14 x 10^3 CFU to 7.27 x 10^5 CFU. This study's estimated LD_{50} of 5.18 x 10^4 CFU was about half that of the commonly cited Zaucha et al. (1998) LD_{50} of 1.05 x 10^5 CFU. A list of the individual mortality data is located in Appendix N and complete statistical analysis can be found in Appendix O.

3.4 Telemetric Monitoring

To determine physiological responses to the various low spore doses in the NZW rabbits, telemetric devices were implanted in the animals and body temperature, electrocardiogram activity, and cardiovascular function (heart rate and respiratory pressure) were monitored for 30 seconds every 15 min. Each observation was then baseline adjusted according to the

associated clock time, and six-hour averages were computed for the baseline adjusted values using the following intervals: midnight - 06:00 (inclusive), 06:00 - 12:00 (inclusive), 12:00 - 18:00 (inclusive), and 18:00 - midnight (inclusive).

Estimates and exact binomial 95% confidence intervals for the proportion of abnormal animals were calculated within each group, and an overall two-sided Fisher's exact test was performed to determine if there was a significant difference between the proportions of abnormal animals in each group (at the 0.05 significance level). Table 7 contains the proportion of animals that were abnormal at any point during the study by group for each parameter, as well as the mean duration of abnormality for those groups having abnormal animals. Note that some animals died prior to becoming abnormal. In addition, Table 7 contains the results of Fisher's exact tests, comparing the proportion of animals that were abnormal in each group by parameter. There were no significant differences between the groups for any parameter.

Table 7. Abnormality Summaries by Parameter and Group Along with Fisher's Exact Tests Comparing the Proportion Abnormal in Each Group by Parameter

Parameter	Group	No. Abnormal/ Total	Proportion Abnormal (95% Confidence Interval)	Mean Duration of Abnormal (Days)[#]	Fisher's Group Effect (P-value)
Activity	1	0/5	0.00 (0.00, 0.52)	NA	0.0558
	2	1/5	0.20 (0.01, 0.72)	0	
	3	0/5	0.00 (0.00, 0.52)	NA	
	4	0/5	0.00 (0.00, 0.52)	NA	
	5	3/5	0.60 (0.15, 0.95)	0	
	6	0/5	0.00 (0.00, 0.52)	NA	
Heart rate	1	4/5	0.80 (0.28, 0.99)	8.69	1
	2	4/5	0.80 (0.28, 0.99)	11.63	
	3	3/5	0.60 (0.15, 0.95)	0.42	
	4	4/5	0.80 (0.28, 0.99)	8.81	
	5	4/5	0.80 (0.28, 0.99)	1.31	
	6	4/5	0.80 (0.28, 0.99)	1.31	
Respiratory rate	1	2/5	0.40 (0.05, 0.85)	0	0.2832
	2	4/5	0.80 (0.28, 0.99)	1.06	
	3	2/5	0.40 (0.05, 0.85)	5.38	
	4	2/5	0.40 (0.05, 0.85)	2.25	
	5	3/5	0.60 (0.15, 0.95)	2.08	
	6	5/5	1.00 (0.48, 1.00)	1.5	
Temperature	1	4/5	0.80 (0.28, 0.99)	6.19	0.8752
	2	3/5	0.60 (0.15, 0.95)	4.17	
	3	3/5	0.60 (0.15, 0.95)	1.67	
	4	2/5	0.40 (0.05, 0.85)	3.63	
	5	4/5	0.80 (0.28, 0.99)	2	
	6	4/5	0.80 (0.28, 0.99)	2.06	

[#]Means exclude those animals that were never abnormal
NA = There were no abnormal animals for this group

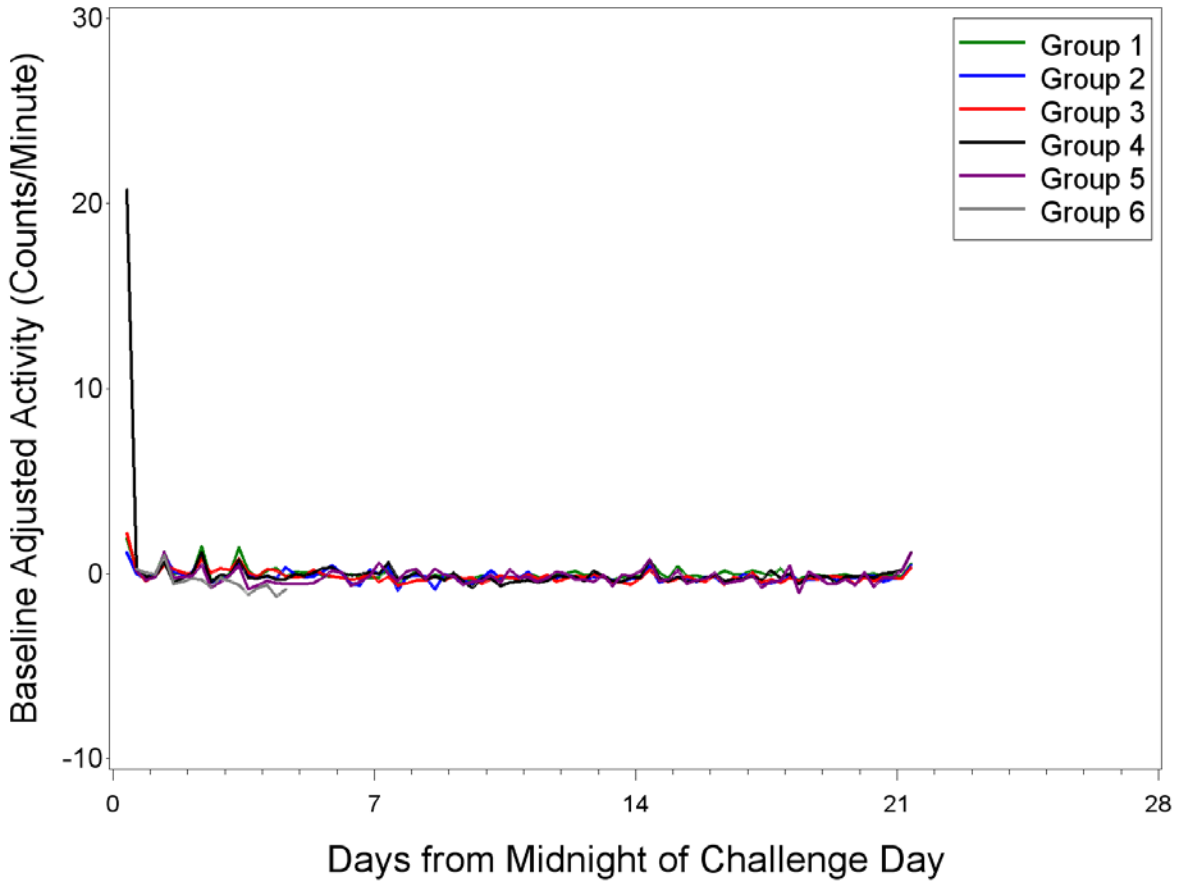

Figure 2. Plot of mean baseline adjusted activity values for each group.

A reading was categorized as abnormal if it fell outside of the criterion of three times the standard deviation above or three times the standard deviation below the mean baseline. Figure 2 illustrates the mean activity levels of the groups after challenge. Figure 3 shows the activity levels for each animal on study and highlights the variability within each group. Differences were observed in the activity levels of each group. For four consecutive six-hour intervals beginning on Study Day 3 at 12:00 - 18:00, there were significant decreases from baseline in Group 6. On Study Day 3 at 12:00 - 18:00 and Study Day 4 at 06:00 - 12:00, the mean decrease from baseline in Group 6 was significantly different from the mean increases from baseline in Groups 1 and 3.

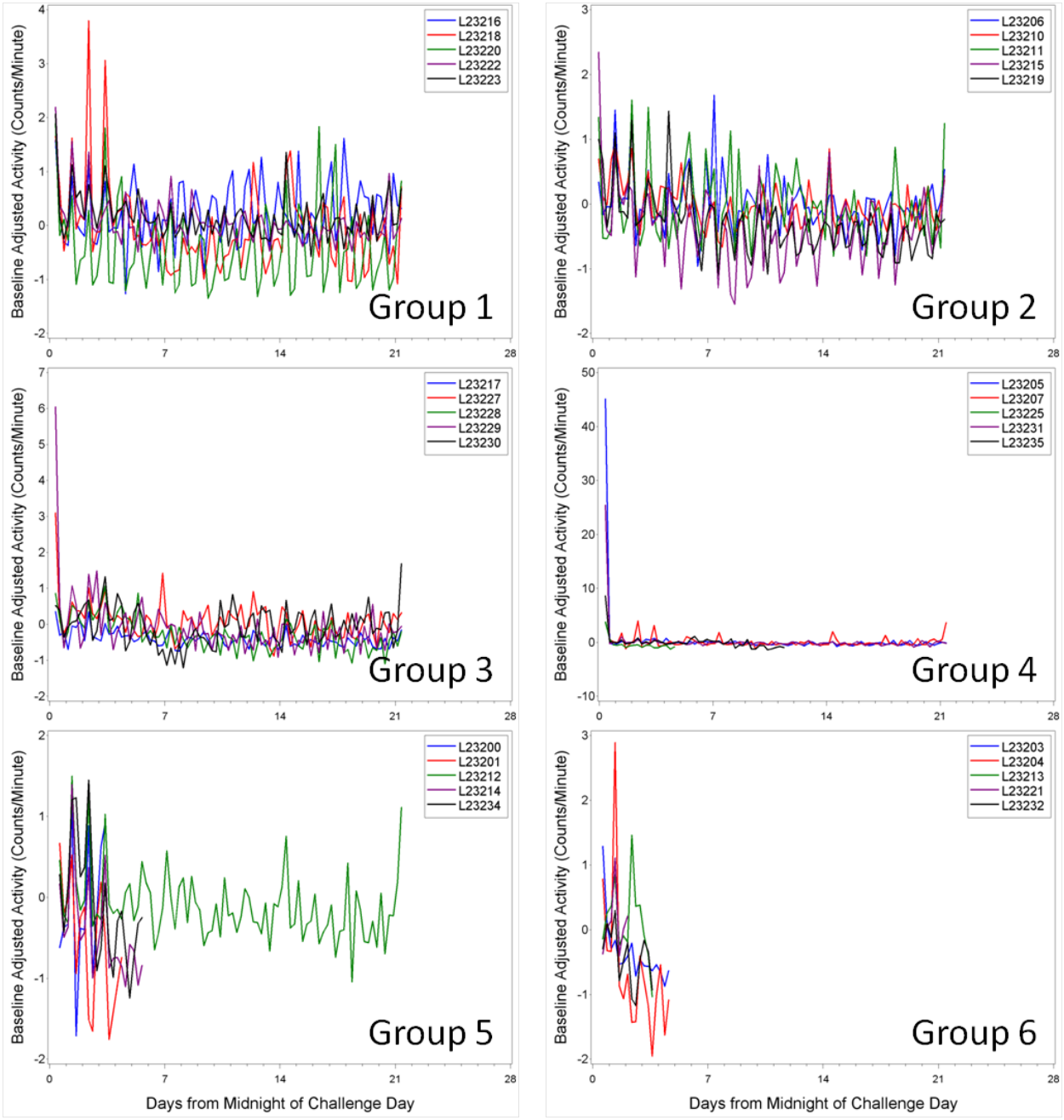

Figure 3. Plot of baseline adjusted activity values for each rabbit.

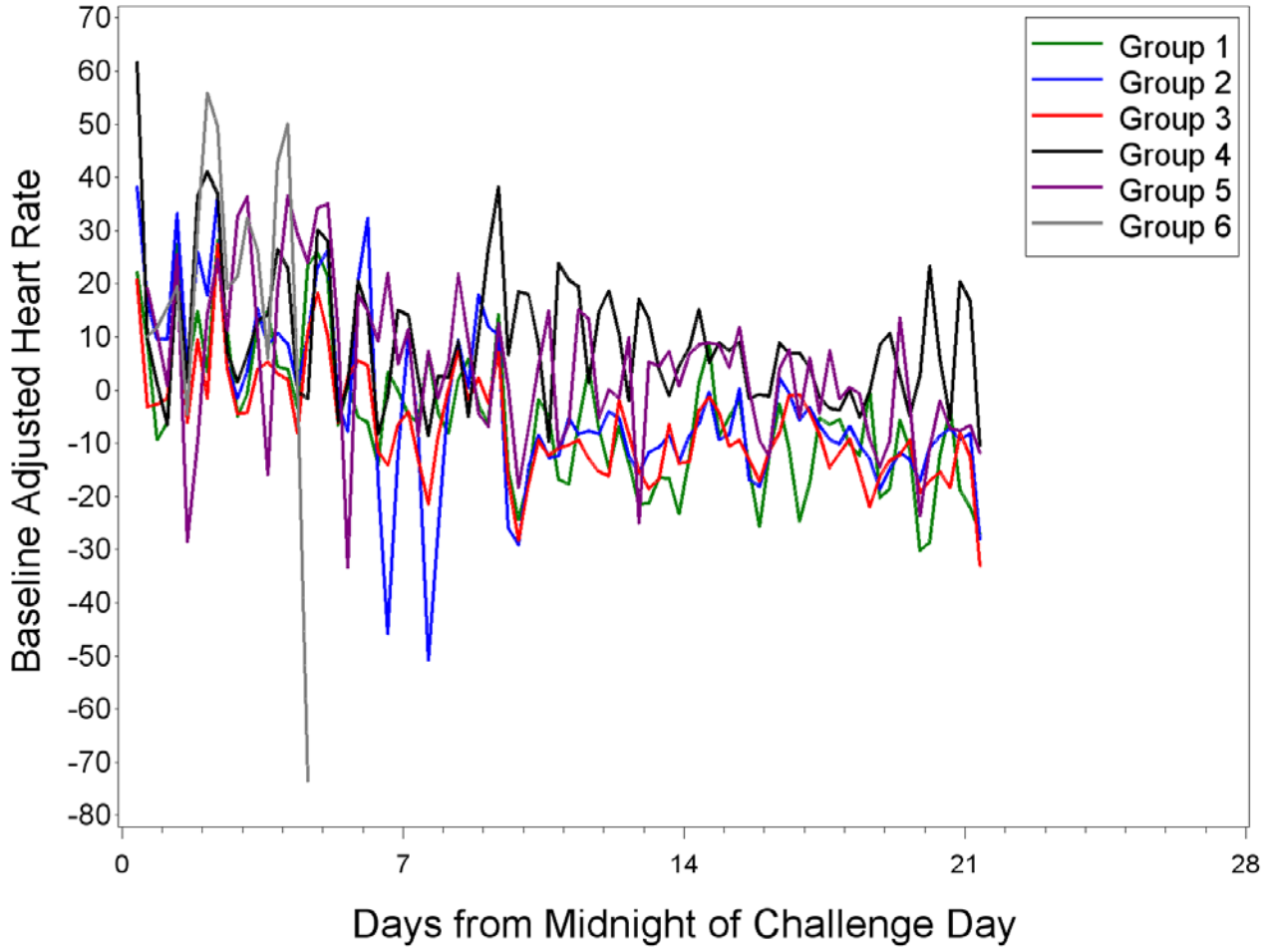

Figure 4. Plot of mean baseline adjusted heart rate values for each group.

Figure 4 illustrates the changes in heart rate by group mean over the course of the study. Figure 5 shows the heart rate of each rabbit over the course of the study. By Study Day 1 at 06:00 - 12:00, all groups had experienced significant increases from baseline, likely due to study-related activity in the animal room. The significant increases from baseline in Group 6 continued for most study times until Study Day 4. Groups 1 and 3 did not experience any significant increases from baseline after Study Day 4, whereas Group 4 continued to experience significant increases from baseline until Study Day 11. There was no definitive explanation for the differences in the length of time the animals experienced significant increases in heart rate. They were housed in the same room and exposed to the same environmental stimuli.

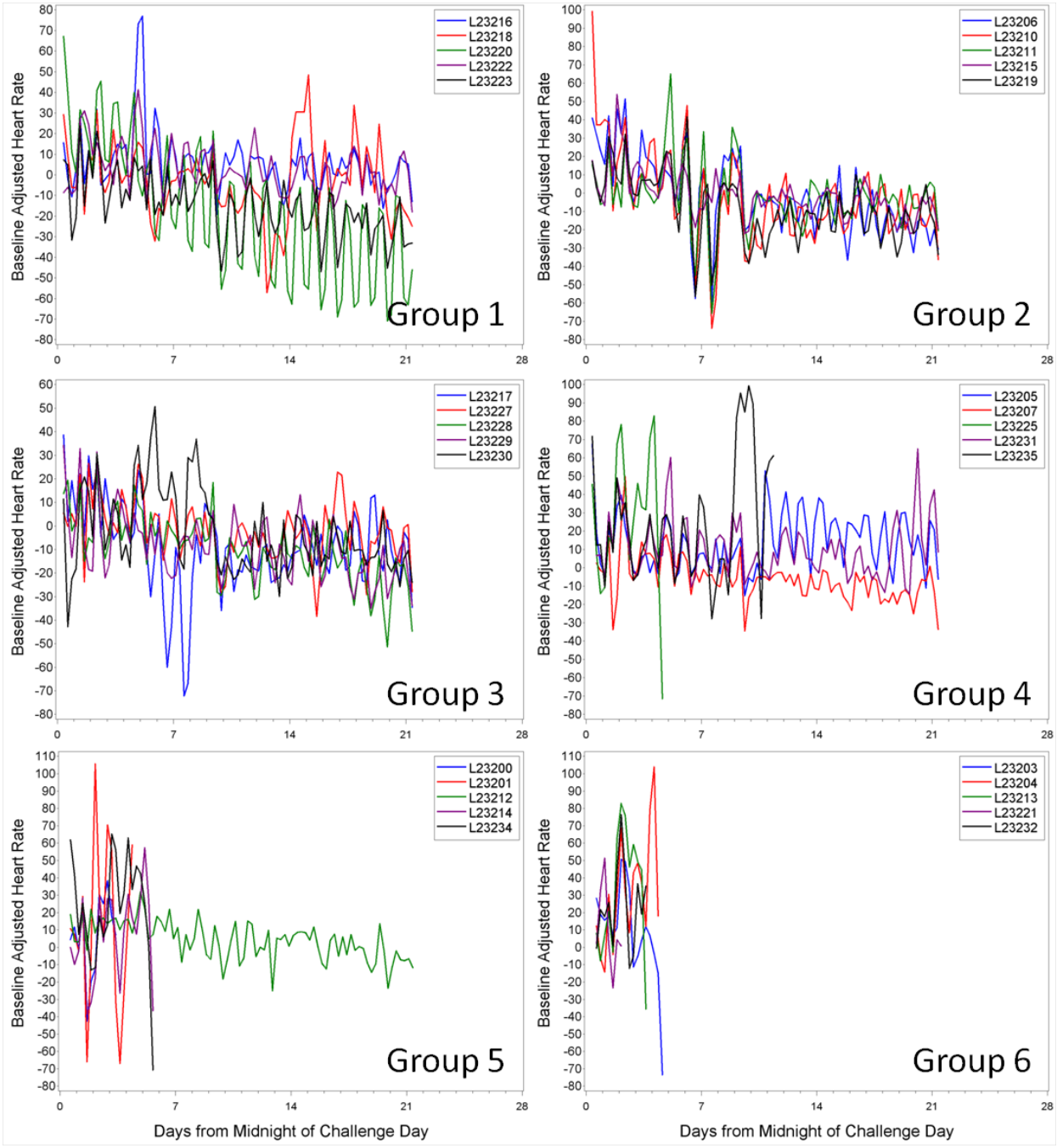

Figure 5. Plot of baseline adjusted heart rate values for each rabbit.

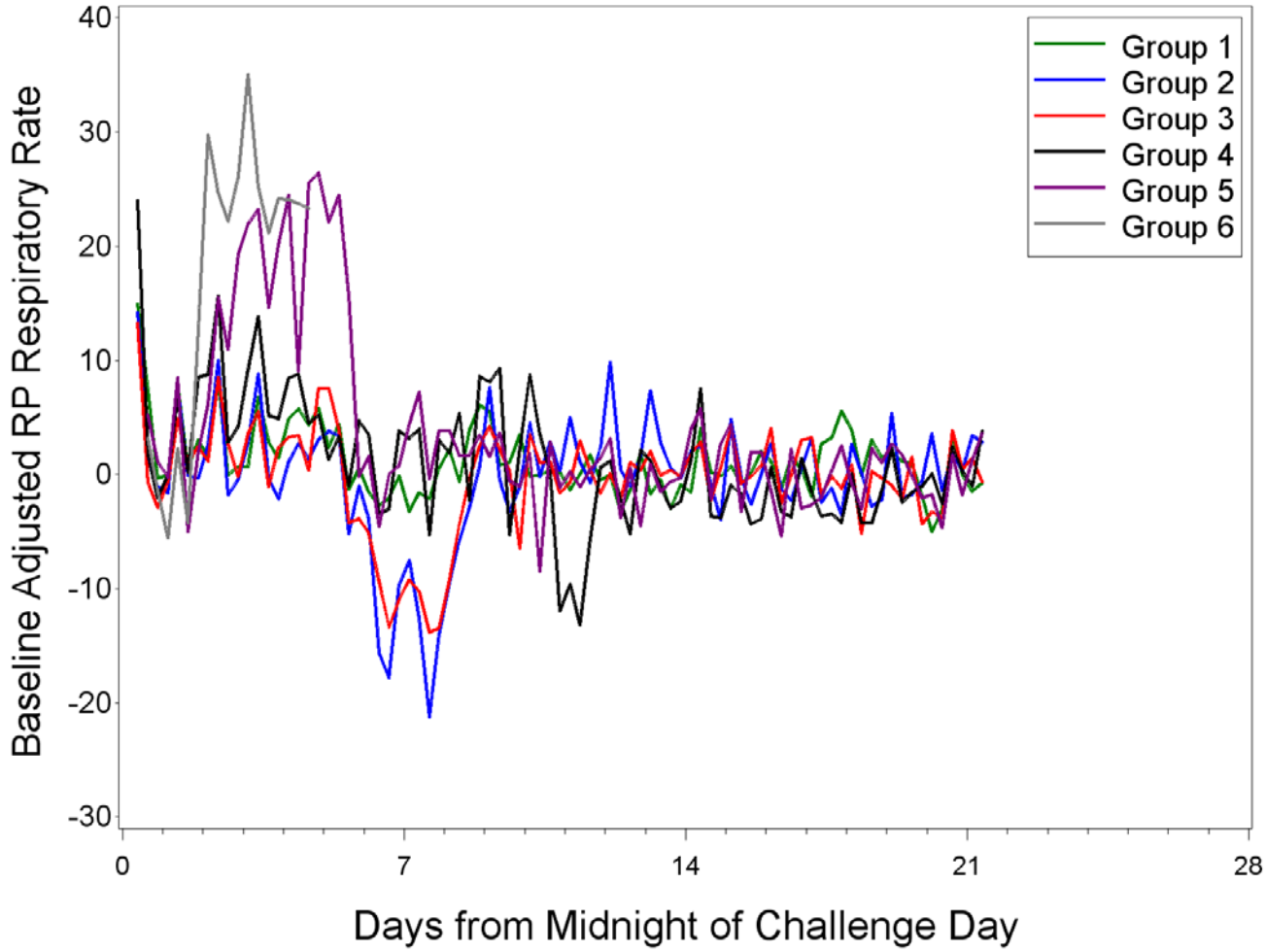

Figure 6. Plot of mean baseline adjusted respiratory rate values for each group.

Figure 6 illustrates the mean respiratory rates of the groups after challenge. Figure 7 shows the respiratory rates for each rabbit over the course of the study. Each rabbit that succumbed to disease showed increased respiration rates. The animals are distinguished by the truncated data lines in Figure 7 indicating that the rabbit died. In comparing group mean respiration rates, it was determined that there were significant increases from baseline in Group 5 from Study Day 2 at 06:00 - 12:00 through Study Day 5 at 12:00 - 18:00 and in Group 6 from Study Day 2 at midnight - 06:00 through Study Day 4 at 12:00 - 18:00, with the mean increase from baseline in Group 5 or 6 being significantly different than the mean change from baseline in at least one of the other groups during these study times.

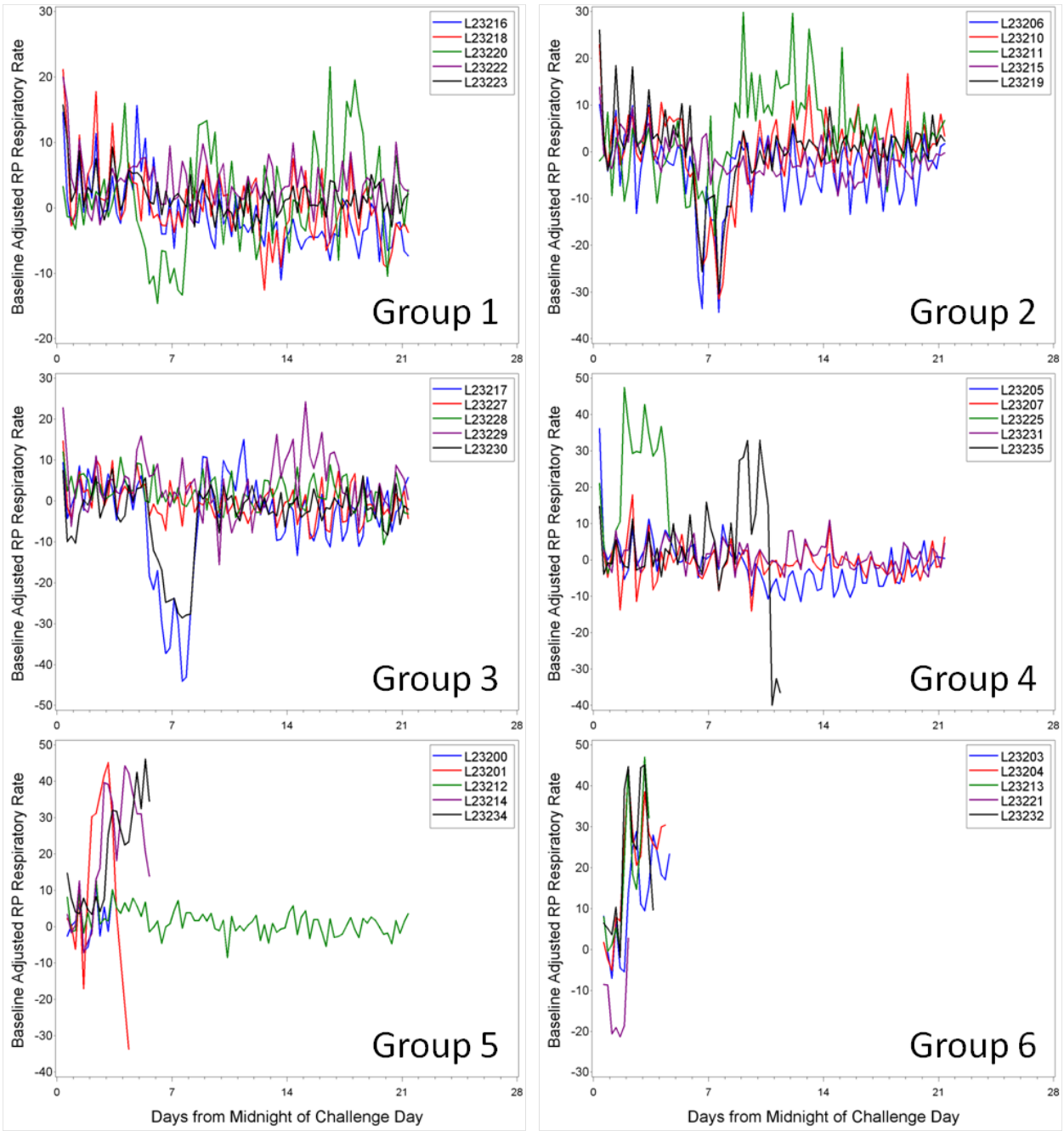

Figure 7. Plot of mean baseline adjusted respiratory rate values for each rabbit.

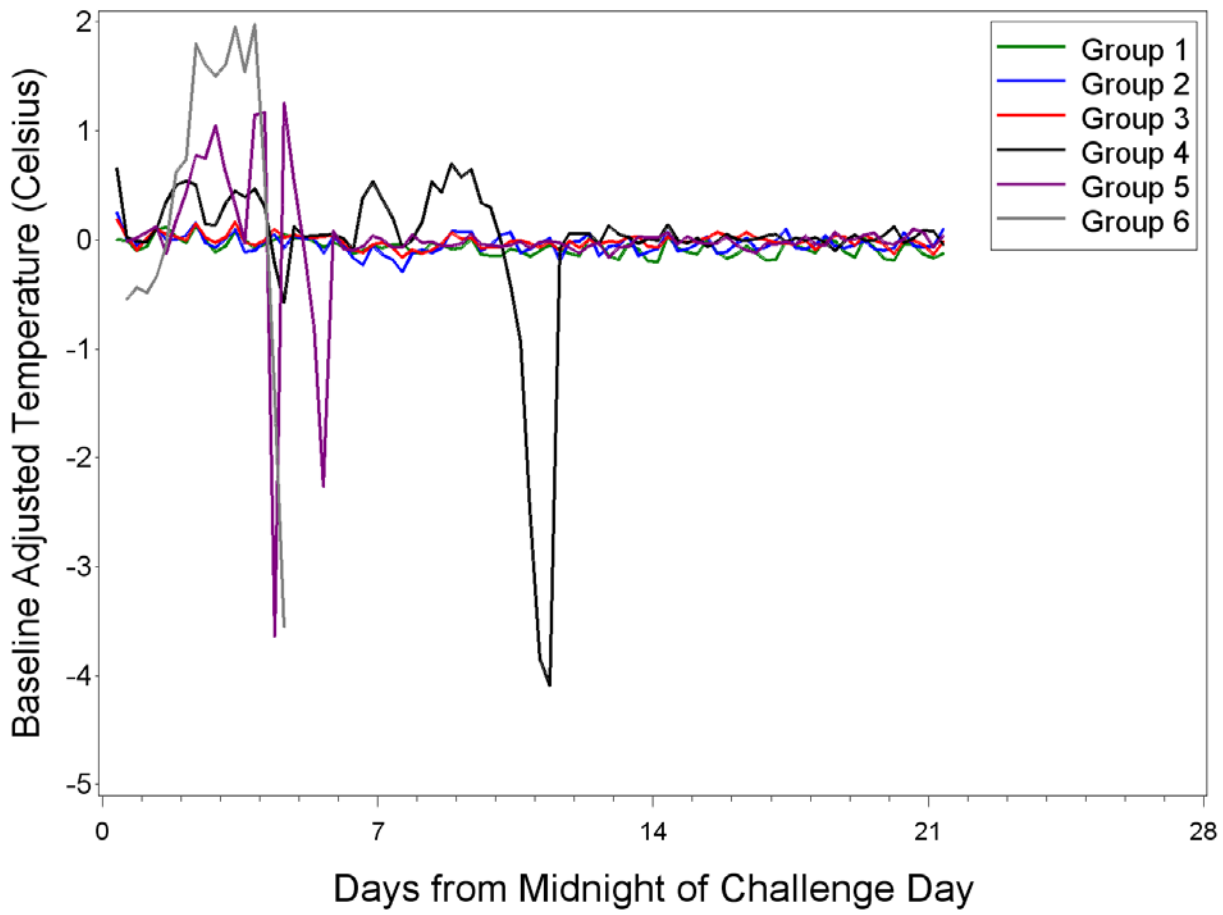

Figure 8. Plot of mean baseline adjusted temperature values for each group.

Figure 8 plots the mean body temperatures of the groups after challenge. Figure 9 shows the body temperatures for each rabbit over the course of the study. Like the respiration rate data, the body temperature of each rabbit that succumbed to disease increased just prior to death. Rabbit L23221 (Group 6) showed a decrease in body temperature starting at midnight of the challenge day when compared to baseline. The cause of this drop in body temperature is not known. The animal may have had an elevated temperature prior to challenge or there may have been a problem with the transponder. However, this animal still followed the group trend and showed a spike in body temperature prior to death.

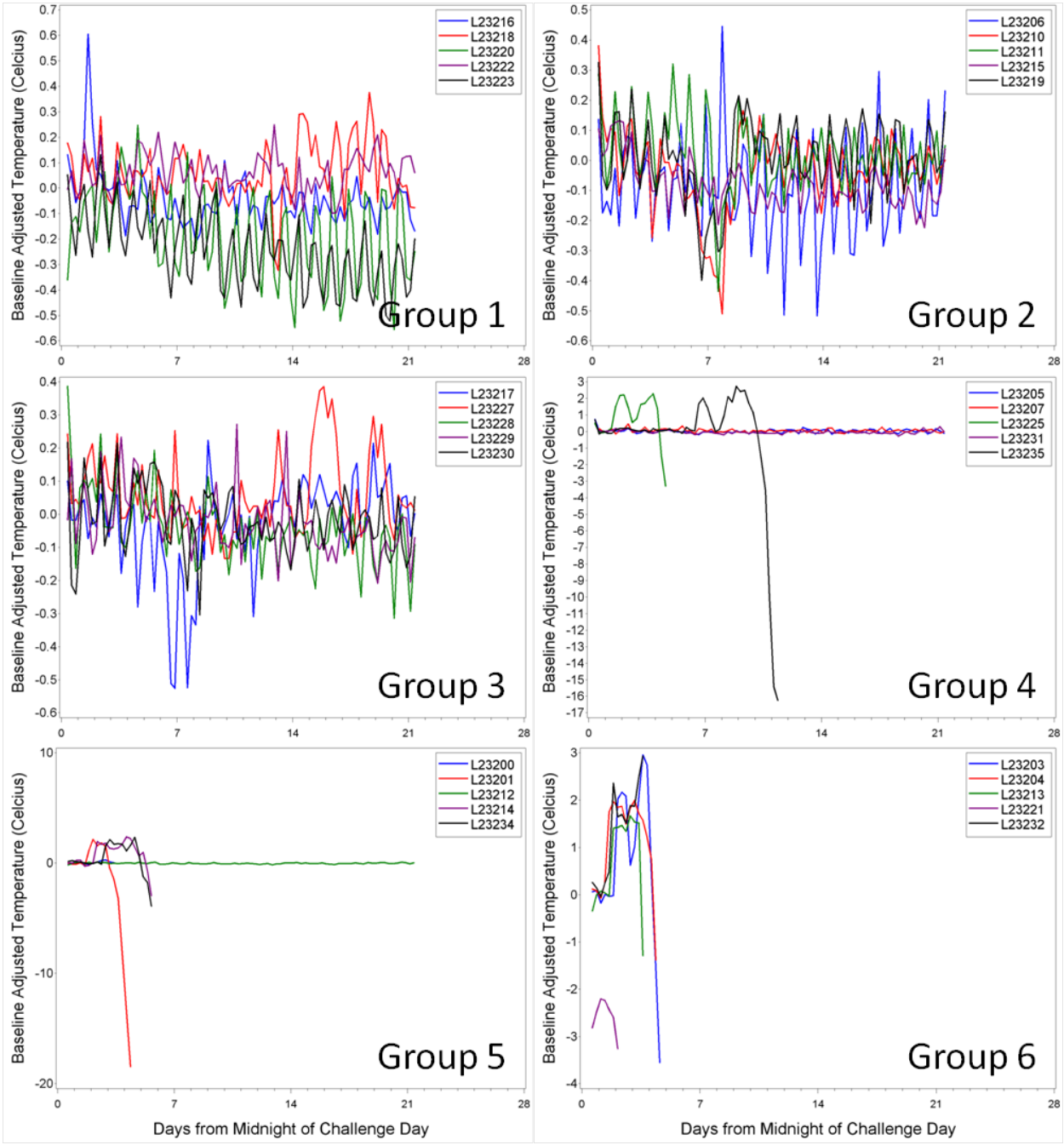

Figure 9. Baseline adjusted temperature values for each rabbit.

A comparison of group means showed that for seven consecutive six-hour intervals beginning on Study Day 2 at 06:00 - 12:00, there were significant increases in body temperature from baseline in Group 6. Excluding Study Day 3 at 12:00 - 18:00, the mean increases from baseline at these study times were significantly different from the mean changes from baseline in the lower dose groups (Groups 1 through 5). On Study Day 5 at 06:00 -12:00 and 12:00 - 18:00, there were significant decreases from baseline in Group 5 that were significantly different than the mean changes from baseline in Groups 1 through 4. The decrease in the Group 5 body temperature coincided with the time that the animals succumbed to disease. The increase in mean body temperature of Group 4 is attributable to the two animals that died in this group. Rabbit L23225 showed an increase in body temperature from approximately Study Day 2 to Study Day 4. This animal died on Study Day 4. Rabbit L23235 had an elevated body temperature ~8 through 11 days post-challenge and died on Study Day 11 (Figure 9).

Measurements on inspiratory time, expiratory time, respiration integral, and peak amplitude were also conducted (see Appendix F for figures and complete statistical analysis).

3.5 Circulating Levels of Protective Antigen

Toxemia was assessed over the course of the study via a PA ELISA which measured circulating levels of PA. All animals in Groups 1, 2, and 3 had observations less than the LOD (2.0 nanogram/mL [ng/mL]) at all time points assayed. One Group 4, three Group 5, and four Group 6 animals had detectable levels of circulating PA at some point during the study.

Rabbit L23225 (Group 4) had 51.199 ng/mL of circulating PA on Study Day 3 and this increased to 32,964.272 ng/mL on Study Day 4 when the terminal sample was taken. Rabbit L23235 (Group 4) never became positive for circulating PA but died on Study Day 11. This animal may have become positive after Study Day 7. However, the blood collection schedule did not collect a sample between Days 7 and 11, and a terminal blood sample was not obtained from this animal.

Rabbit L23201 (Group 5) had PA levels of 64.857 and 471.046 ng/mL on Study Days 2 and 3, respectively (no terminal sample was obtained). Rabbits L23214 and L23234

(Group 5) had 28.579 (Study 2) and 20.082 ng/mL (Study Day 3) of circulating PA, respectively. Terminal samples were not collected for either of these rabbits. Interestingly, rabbit L23200 (Group 5) never had detectable levels of PA but died on Study Day 3. The terminal sample for this animal was also below the LOD.

Rabbit L23203 (Group 6) had 2.833 and 1.631 ng/mL on Study Days 2 and 3, respectively. Rabbit L23232 had 2.107 ng/mL of circulating PA on Study Day 3. Rabbit L23213 (Group 6) had 125.095 ng/mL of circulating PA on Study Day 2 and a terminal level of 383.515 ng/mL. Rabbit L23221 (Group 6) was below the LOD on Study Day 1 and died on Study Day 2 prior to the blood draw, so no terminal sample was obtained. The complete statistical report is presented in Appendix H and the PA ELISA results for individual animals are contained in Appendix P.

3.6 Bacteremia

All animals in Groups 1, 2, and 3 were negative for *B. anthracis* bacteremia by culture on all study days. Two out of five rabbits in Group 4 were bacteremic at some point during the study, and both succumbed to disease. Rabbit L23225 (Group 4) had a bacterial burden of 4.53×10^3 CFU/mL of blood on Study Day 2 and 8.03×10^5 CFU/mL at the time of death. This animal was also positive on Study Day 3, but the bacterial load was below the limit of quantitation (LOQ) (2.5×10^3 CFU/mL). Rabbit L23235 (Group 4) had 1.70×10^6 CFU/mL at the time of death; all other samples for this animal were negative.

Four of the five Group 5 animals became bacteremic at some time point during the study. Three of the four animals that died in this group were bacteremic at the time of death. Rabbit L23214 (Group 5) was negative for bacteremia culture at the time of death (5.8 days post-challenge), but had 8.23×10^3 CFU/mL of blood on Study Day 2.

All of the Group 6 rabbits (5/5) had positive bacteremia cultures during the study. Only one animal (L23232) did not have positive bacteremia culture at the time of death (Study Day 4), but had 2.77×10^3 CFU/mL of blood on Study Day 3. A list of individual bacteremia culture results per animal is provided in Appendix Q.

Quantitative bacteremia was also assessed by qPCR targeting the *rpoB* gene. The two Group 4 animals that were positive by bacteremia culture (L23225 and L23235)

were also positive for qPCR. Rabbit L23225 was positive on Study Days 1 (21 copies/µL), 2 (18 copies/µL) and 3 (109 copies/µL). The animal died on Study Day 4 and no qPCR sample could be taken. The Day -3 sample for L23225 was also positive with a value of 234 copies/µL, suggesting the sample was contaminated with *B. anthracis* DNA. The sample was repeated, but still positive with a value of 15 copies/µL. It is possible that the qPCR assay detected another *Bacillus* species as this is a quantitative method and not used for identification.

The pPCR data mimicked the culture results for rabbit L23235 (Group 4); only the terminal sample was positive (7,394 copies/µL).

Four out of five Group 5 rabbits were positive for qPCR at some point during the study, and all four that died were positive at the time of death with a mean value of 7.2 x 10^4 (± 1.35 x 10^5) copies/µL. Four out of five rabbits in Group 6 were also positive for qPCR at some point during the study. Rabbit L23221 died on Study Day 2 prior to sample collection, and a qPCR sample could not be taken on Study Day 1. This animal was negative for qPCR at the time of death, but positive by culture (2.58 x 10^6 CFU/mL). A complete list of bacteremia and qPCR results is given in Appendix R. The complete statistical analysis of the bacteremia data can be found in Appendix H.

3.7 TNA/IgG ELISA

Individual TNA and IgG ELISA results are presented in Appendices S and T, respectively. All samples were below the LOQ for both assays. All ELISA samples had 0 µg/mL of anti-PA IgG except for one animal. Rabbit L23229 (Group 3) had levels of 0.386, 0.502, and 0.404 µg/mL on Study Days 7, 14, and 21. While these samples did produce results, they were well under the LOQ of 5.0 µg/mL and even the LOD of 1.0 µg/mL. The samples were repeated, but the ELISA plate did not pass acceptance criteria, therefore only the original data were reported.

3.8 Hematology and Clinical Chemistry

To further assess any physiological effects of low dose exposure to *B. anthracis*, whole blood and sera were assayed for a variety of hematological and clinical chemistry parameters (refer to Section 2.12 for a complete list of parameters). A list of individual animal hematology and clinical chemistry results for all hematology and clinical chemistry parameters is provided in

Appendices U and V, respectively. Values for the normal range of hematology and clinical parameters are also provided in Appendices U and V, respectively.

Appendices I and J contain the results of extensive statistical analyses of the hematological and clinical chemistry parameters, respectively. The statistical results provided in Appendices I and J were, as stated in the Appendices, utilized to perform a group-level analysis of hematological and clinical chemistry parameters. However, the interpretation of the group-level statistics in this study was hampered by the low number of starting animals per group and the further decrease in the group number from animal deaths due to the treatment effects. Additionally, the group means could be biased due to the presence of animals in a group that were a mixture of non-infected and infected individuals. In recognition of these concerns, an analysis was also conducted at the level of the individual animal comparing individual results to published normal ranges of parameter values described in the literature (see Appendices U and V for an identification of normal values). In the discussion below, the results of both the group and individual-level analyses were used to formulate findings based on these data.

The mammalian host responds to extracellular bacterial infection by increased hematopoiesis and neutrophilia. To determine if the rabbits responded to the various doses of *B. anthracis*, complete WBC counts and differentials were performed. For hematology parameters, the threshold for an abnormal parameter value was defined as each individual animal's baseline (Study Day -3) parameter value plus or minus two standard deviations. Since each animal had only one baseline value for each parameter, the standard deviation associated with each parameter was calculated using the baseline values of all animals. Animals were determined to have an abnormal parameter value when their observed value was above the upper threshold or below the lower threshold for that respective parameter.

There was a positive relationship between the challenge dose and circulating neutrophils, with an increase in the challenge dose resulting in an increase in the concentration of circulating neturophils. Table 8 shows the mean neutrophil levels by group over the study, and Figure 10 shows the neutrophil counts by individual rabbit. A

decrease in the time to neutrophilia was also observed with an increase in the challenge dose. The highest dose group (Group 6) had the greatest and earliest increase in neutrophils from baseline levels followed by Group 5 and then Group 4. However, only a few rabbits had levels above the normal range. Groups 1 through 3 did not exhibit a neutrophilic response following infection, but two Group 1 rabbits had higher than normal levels on Day -3.

Group 6 had a significant increase in neutrophils from baseline to Study Day 2, with a mean of 4.37 (± 1.53) x 10^3/µL compared to 1.68 (± 0.27) x 10^3/µL at baseline. This shift from baseline for Group 6 was statistically different from all other groups on this day ($P < 0.05$; Tukey Test). While this shift from baseline was significant, only two animals (L23204 and L23203) had levels outside of the normal range of 0.80 to 2.90 x 10^3/µL (Schalm et al., 1975).

Table 8. Mean Neutrophil Levels

		Neutrophil Counts ($10^3/\mu L$)						
		Day -3	**Day 1**	**Day 2**	**Day 3**	**Day 7**	**Day 14**	**Day 21**
Group 1	N	5	5	5	5	5	5	5
	Mean	2.5	1.34	1.3	1.3	1.5	1.17	0.91
	Std Dev	1.36	0.78	0.51	0.67	0.71	0.76	0.19
Group 2	N	5	5	5	4[a]	4[a]	5	4[a]
	Average	1.73	1.48	1.4	1.08	1.09	1.34	0.6
	Std Dev	0.34	0.21	0.33	0.2	0.18	0.48	0.14
Group 3	N	5	5	5	5	5	5	5
	Mean	1.64	1.52	1.46	1.4	1.44	1.45	0.75
	Std Dev	0.36	0.37	0.37	0.38	0.36	0.61	0.17
Group 4	N	5	5	5	5	4	3	3
	Mean	1.8	1.87	1.99	1.22	2.32	1.4	0.57
	Std Dev	0.35	0.66	1.07	0.72	1.25	0.12	0.18
Group 5	N	5	5	5	3	1	1	1
	Mean	2.59	1.94	2.17	3.48	1.32	0.82	0.56
	Std Dev	1.04	1.23	1.56	2.11	NA	NA	NA
Group 6	N	3[b]	4	3	3	--	--	--
	Mean	1.68	2.07	4.37	1.28	--	--	--
	Std Dev	0.27	0.25	1.53	0.69	--	--	--

N = Number of samples analyzed
NA = Not applicable (only one sample)
Std Dev = Standard deviation
-- = No rabbits alive at time point
[a] Only four out of the five rabbits alive at the time point were assayed
[b] Only three out of the five rabbits alive at the time point were assayed

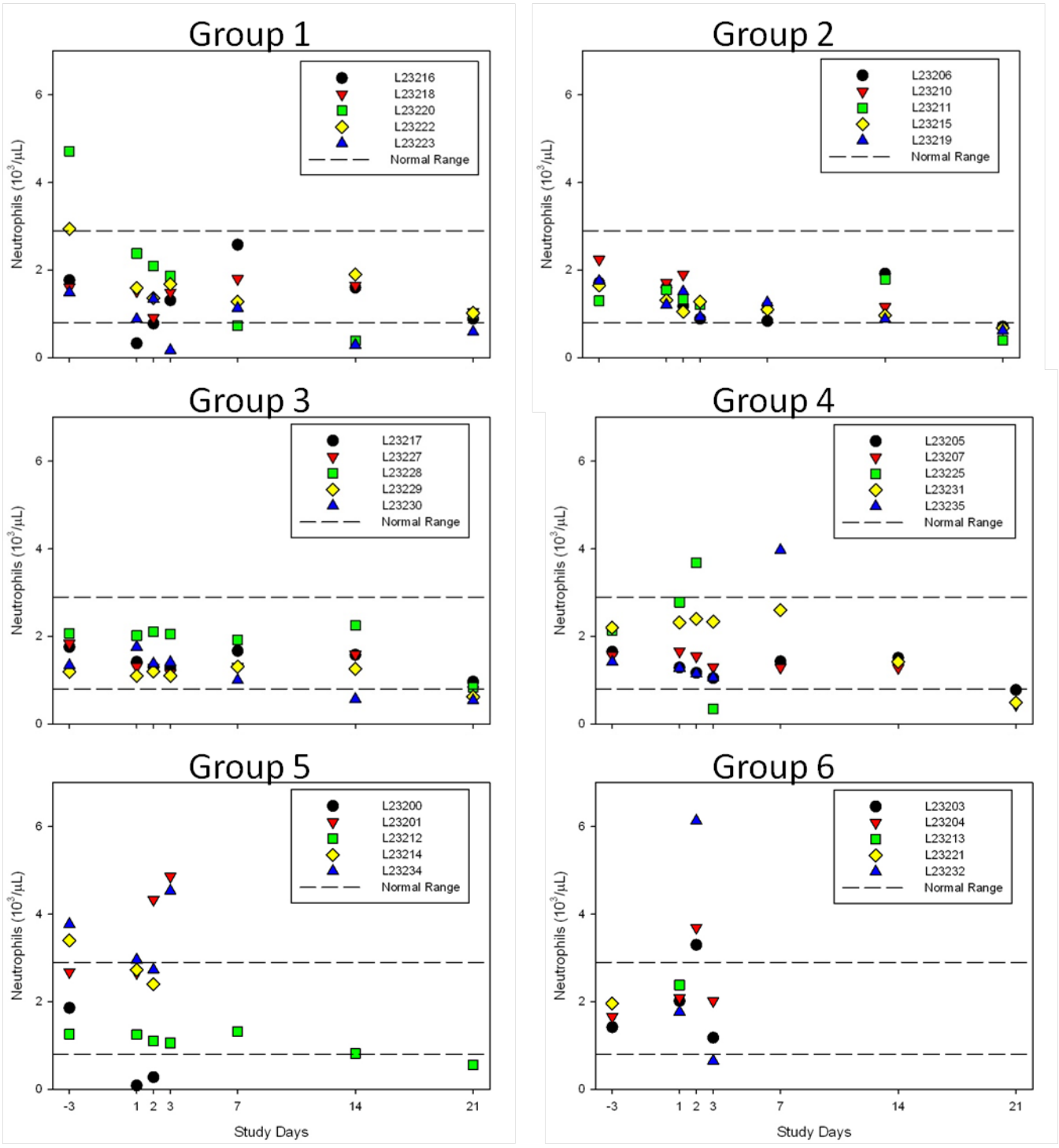

Figure 10. Neutrophil levels.

On Study Day 3, the neutrophil level peaked in Group 5. Two of the three rabbits alive at this time point in Group 5 had neutrophil levels of 4.86 and 4.53 x 10^3/µL (Figure 10). These two animals died prior to the next blood draw. Two Group 4 rabbits showed abnormally high neutrophil levels (L23225 [3.68 x 10^3/µL, Day 2] and L23235 [3.97 x 10^3/µL, Day 7]); both succumbed to infection. The other animals in this group were not neutrophilic at any time during the study.

While the level of circulating neutrophils was the only parameter that exhibited an obvious dose response, significant changes from baseline were observed in PLT, WBC counts, and the numbers of circulating neutrophils, lymphocytes, and monocytes. However, these slight variations in the measured parameters did not appear to be an effect of the *B. anthracis* challenge doses as there was no direct relationship between challenge dose and change in any of these parameters. Figures and complete statistical analysis of all hematological parameters are located in Appendix I.

CRP is an indicator of stress and non-specific inflammation. CRP can also be used as a marker for liver damage. Table 9 shows the levels of CRP during the course of the study, and Figure 11 shows the levels of CRP by individual animal. The negative control group (Group 1) had a mean CRP level of 1.80 mg/deciliter (dL) on Day -3. This abnormally high level of CRP was from two animals (L23220 and L23223) that had levels of 2.98 and 5.26 mg/dL, respectively (Figure 11). All groups had at least one animal with detectable levels of CRP through Study Day 3 (Figure 11).

There were significant increases to the CRP level in relation to the baseline level in Groups 2, 3, 5, and 6 at Study Day 1 ($P < 0.05$), with the increases in Groups 2 and 5 both significantly different than the decrease in Group 1. As a proportion of baseline, the levels for Groups 5 and 6 significantly increased at Study Day 2 ($P < 0.05$), with the increase in Group 6 significantly greater than those in Groups 1, 2, 3, and 4. There were also significant increases as a proportion of baseline in the levels for Groups 5 and 6 at Study Day 3 ($P < 0.05$). The increase in the CRP level of Group 6 was significantly different than the CRP level changes in Groups 1, 2, 3, and 4, while the increase in Group 5 was significantly different than the decrease in Group 1. Levels of CRP peaked on Study Day 3 in

Groups 4, 5, and 6; Group 6 demonstrated the highest level.

Table 9. Mean Levels of C-Reactive Protein over the Course of the Study

		C-Reactive Protein Levels (mg/dL)						
		Day -3	**Day 1**	**Day 2**	**Day 3**	**Day 7**	**Day 14**	**Day 21**
Group 1	N	5	5	5	5	5	5	5
	Mean	1.8	0.9	1.4	0.65	0.79	0.72	0.25
	Std Dev	2.27	0.97	1.46	0.66	1.2	1.06	0
Group 2	N	5	5	5	5	4[a]	3[b]	5
	Average	0.25	1.38	0.52	0.41	0.36	0.25	0.25
	Std Dev	0	0.61	0.31	0.35	0.22	0	0
Group 3	N	5	5	5	5	5	5	5
	Mean	0.36	0.93	0.4	0.31	0.25	0.37	0.31
	Std Dev	0.25	0.75	0.34	0.13	0	0.27	0.13
Group 4	N	5	5	5	5	4	3	3
	Mean	0.49	1.06	1.91	2.12	2.06	0.25	0.25
	Std Dev	0.55	1	3.39	4.18	3.62	0	0
Group 5	N	5	5	5	4	1	1	1
	Mean	0.4	1.78	1.44	2.9	0.25	0.25	0.25
	Std Dev	0.34	0.66	1.23	1.77	NA	NA	NA
Group 6	N	4[a]	4	3	3	--	--	--
	Mean	0.33	1.83	6.45	11.22	--	--	--
	Std Dev	0.14	0.97	2.03	1.63	--	--	--

N = Number of samples analyzed
NA = Not applicable (only one sample)
Std Dev = Standard deviation
-- = No rabbits alive at time point
[a] Only four out of the five rabbits alive at the time point were assayed
[b] Only three out of the five rabbits alive at the time point were assayed

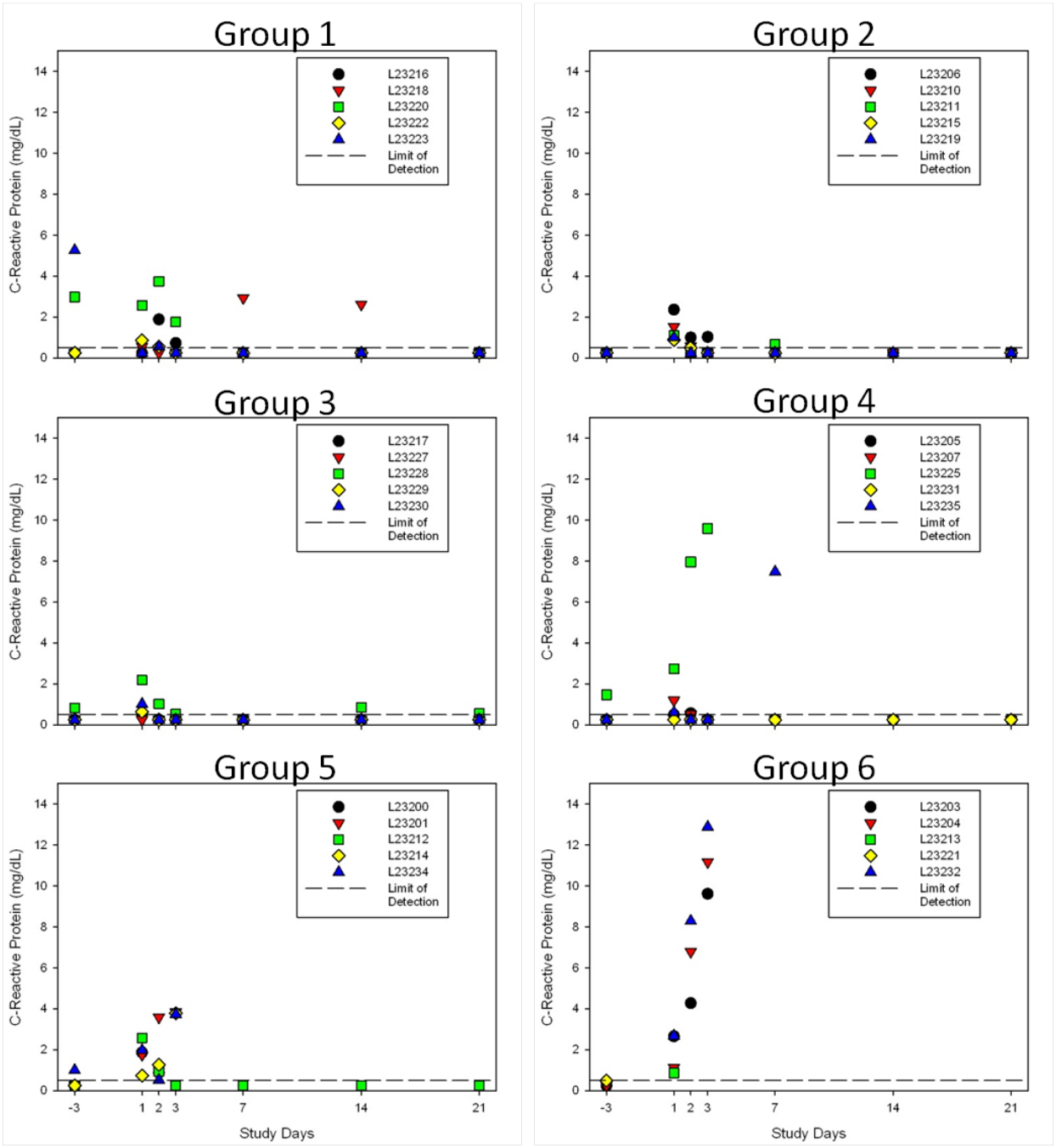

Figure 11. C-reactive protein levels.

The three animals from Group 6 that were alive on Study Day 3 had a mean CRP level of 11.22 mg/dL compared to 0.33 mg/dL at baseline. Groups 4 and 5 had mean levels of 2.12 (± 4.18) and 2.90 (± 1.77) mg/dL on Study Day 3. The increase in Group 4 stems from the one animal (L23225) that died on Study Day 4. The rest of the animals in this group had levels of < 0.5 mg/dL.

AST, ALT, LDH, and SDH were also used as markers for liver damage. Table 10 shows AST levels over the course of the study, and Figure 12 shows the AST levels by individual rabbit. The normal range for AST in rabbits is 0.0 to 120.0 units (U)/L (Thrall et al., 2004; Queensberry and Carpenter, 1997; and University of Nebraska Institutional Animal Care and Use Committee, 2009). Only the Group 5 mean levels on Days 2 and 3 were above the normal range (Table 10). However, there was a significant increase in AST as a proportion of baseline in Groups 5 and 6 on Study Day 2, and in Group 5 at Study Day 3. Group 5 showed the greatest increase in AST levels with a mean level of 565.0 U/L on Study Day 3. The increase in Group 5 at Study Day 3 was significantly different ($P < 0.0001$) than the changes in Groups 1, 2, 3, 4, and 6. There were significant increases as a proportion of baseline in Groups 2, 3, 4, and 5 at Study Day 3, and the increase in Group 4 was significantly different than the decrease in Group 1.

Among the other liver function parameters, there were similar results in terms of significance at Study Days 2, 3, and 7. At Study Day 2, the increase as a proportion of baseline in the high dose control group (Group 6) was significantly different than the change as a proportion of baseline in the negative control group (Group 1) for CRP and SDH. At Study Day 3, the increase as a proportion of baseline in the Group 5 was significantly different from the changes as a proportion of baseline in the lower dose groups (Groups 1 through 4) for ALT, LDH, and SDH.

Table 10. Mean Aspartate Aminotransferase Levels over the Course of the Study

		Aspartate Aminotransferase Levels (U/L)						
		Day -3	**Day 1**	**Day 2**	**Day 3**	**Day 7**	**Day 14**	**Day 21**
Group 1	N	5	5	5	5	5	5	5
	Mean	25.6	19.4	20.3	20.2	22.1	30.5	25
	Std Dev	10.6	3.7	4.5	3.4	4.7	13.5	6
Group 2	N	5	5	5	5	4[a]	3[b]	5
	Average	13.3	15.8	14.9	17.6	18.8	18.1	17.8
	Std Dev	2.5	2.6	1.8	2.6	5.1	1.7	2.6
Group 3	N	5	5	5	5	5	5	5
	Mean	12.5	14.6	15.9	13.6	16.5	48.9	17.1
	Std Dev	2	2.9	3.6	3.9	3.6	56.8	4.8
Group 4	N	5	5	5	5	4	3	3
	Mean	14.2	16.2	25.8	20.5	23.3	19.2	20.2
	Std Dev	2.9	4.8	26.5	3.4	5.1	4	5.3
Group 5	N	5	5	5	4	1	1	1
	Mean	17.2	20	154.3	565	23.7	20.5	21.8
	Std Dev	6.8	6.7	306.1	533.6	NA	NA	NA
Group 6	N	4[a]	4	3	3	--	--	--
	Mean	11.7	15.2	69.5	22.4	--	--	--
	Std Dev	1.9	1.9	22.2	4.8	--	--	--

N = Number of samples analyzed
NA = Not applicable (only one sample)
Std Dev = Standard deviation
-- = No rabbits alive at time point
[a] Only four out of the five rabbits alive at the time point were assayed
[b] Only three out of the five rabbits alive at the time point were assayed

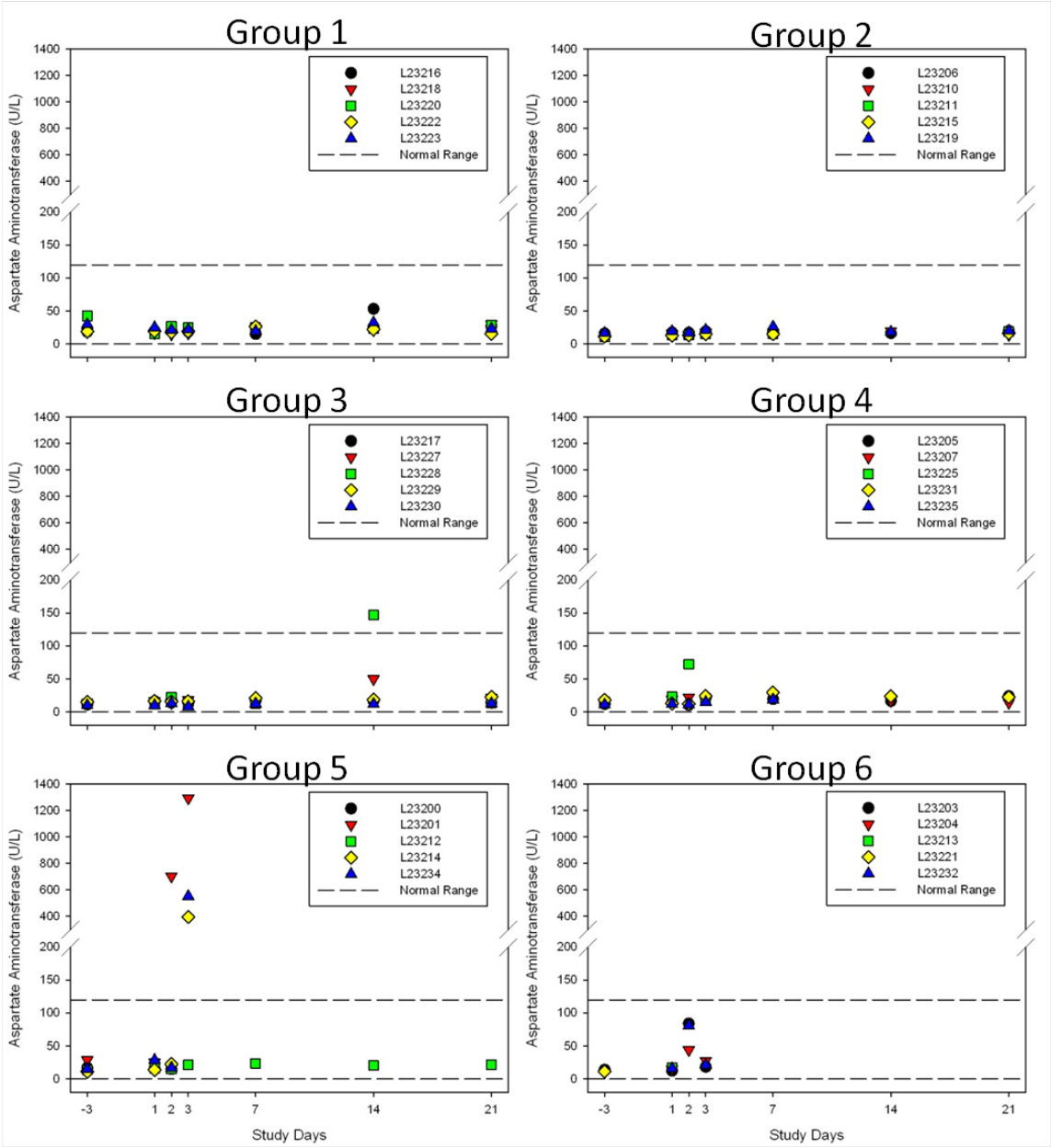

Figure 12. Aspartate aminotransferase levels.

3.10 Pathology

All rabbits were necropsied when they succumbed to infection or at the end of the study. Gross lesions in unscheduled death rabbits (rabbits that succumbed to challenge with *B. anthracis*) included discoloration of the brain (meninges), crusting of the skin, abdominal and thoracic cavity fluid, bronchial and mediastinal lymph node enlargement, small intestinal fluid, and thymic fluid. These gross lesions were typical of anthrax in rabbits (Zaucha et al., 1998) and correlated histologically with hemorrhage, necrosis, edema, and suppurative inflammation. Individual rabbit findings are presented in Table 11 and Appendix W. Lungs and gross lesions were examined microscopically for evidence of anthrax. Microscopic lesions typical of anthrax (Zaucha et al., 1998) were present in all rabbits that died on study and most surviving rabbits challenged with live spores, and included minimal to moderate suppurative inflammation [predominately degenerate and viable heterophils (polymorphonuclear cells)], necrosis, hemorrhage, fibrin and/or large rod-shaped bacteria in the lungs, bronchial and mediastinal lymph nodes, skin, small intestine, brain (meninges), and thymus. Lung lesions consisted of suppurative inflammation and bacteria generally found in the pulmonary interstitium, and associated with alveolar capillaries or larger pulmonary blood vessels (Figure 13).

Other anthrax-related lesions were found in lymph nodes, meninges, skin, thymus, and small intestine. Lymph node findings included hemorrhage, lymphoid necrosis, fibrin, and bacteria. Figure 14 and Figure 15 show representative lesions. There were minimal to mild intravascular bacteria, suppurative inflammation, and hemorrhage (with vascular necrosis) primarily in the meninges of the brain of one rabbit (L23232, Group 6). The skin of rabbit L23234 (Group 5) had areas of suppurative inflammation/edema, hemorrhage, necrosis, and intra- and extravascular bacteria. The thymuses of rabbits L23234 and L23232 had mild to moderate atrophy of lymphoid tissue and evidence of edema. L23234 also had intravascular bacteria in this tissue (Figure 16). Additionally, there was suppurative inflammation and intra- and extravascular bacteria in the small intestine (jejunum) of one rabbit (L23221, Group 6). Where suppurative inflammation occurred in tissues (other than the lung), it was generally associated with bacteria (bacilli).

Multinucleated giant cells “tending to organize” into microscopic granulomas in one animal (L23217,Group 3) were present in the lungs of some rabbits in all groups, including control (irradiated spore challenged) rabbits. These multinucleated giant cells were randomly distributed throughout the lung in ruptured or intact airways or less frequently in or near pulmonary blood vessels or lymphatics. The multinucleated giant cells often surrounded or contained birefringent foreign debris (Figure 17). These cells and debris were also seen in the lungs of two control animals; but occurred with greater frequency and severity in live spore challenged rabbits. Cell aggregates of this type were not described in a previous acute anthrax rabbit study (Zaucha et al., 1998); hence, it is uncertain if anthrax is contributory to the development of this lesion. While many pulmonary macrophages in challenged rabbits contained foamy or granular cytoplasm and cellular debris, bacilli were not identifiable in most cases.

Table 11. Pathology of the Rabbit Tissues

Group Number	Animal Number/ Death Status	Gross Findings	Microscopic Findings
1 100 LD_{50} (Irradiated)	101 (L23220)/FS		Lung: Unremarkable
	102 (L23216)/FS		Lung: Unremarkable
	103 (L23218)/FS		Lung: Multi-nucleated giant cells, minimal
	104 (L23223)/FS		Lung: Unremarkable
	105 (L23222)/FS		Lung: Multi-nucleated giant cells, minimal
2 100 CFU	201 (L23215)/FS		Lung: Inflammation, suppurative, minimal Lung: Multi-nucleated giant cells, minimal
	202 (L23206)/FS		Lung: Inflammation, suppurative, minimal
	203 (L23210)/FS		Lung: Unremarkable
	204 (L23219)/FS		Lung: Inflammation, suppurative, minimal Lung: Multi-nucleated giant cells, minimal
	205 (L23211)/FS		Lung: Unremarkable
3 1000 CFU	301 (L23217)/FS		Lung: Inflammation, suppurative, moderate Lung: Multi-nucleated giant cells, moderate Lung: Perivascular eosinophils, moderate
	302 (L23230)/FS		Lung: Inflammation, suppurative, minimal
	303 (L23228)/FS		Lung: Inflammation, suppurative, minimal Lung: Multi-nucleated giant cells, minimal
	304 (L23227)/FS		Lung: Bacteria (bacilli), minimal
	305 (L23229)/FS		Lung: Inflammation, suppurative, minimal
4 10,000 LD_{50}	401(L23235)/FD		Lung: Inflammation, suppurative, minimal
	402 (L23205)/FS		Lung: Inflammation, nonsuppurative, minimal Lung: Perivascular eosinophils, minimal
	403 (L23225)/FD	Cavity, abdominal: Fluid, red, ~30 mL Cavity, thoracic: Fluid, red, ~15 mL	Lung: Inflammation, suppurative, mild Lung: Bacteria (bacilli), moderate
	404 (L23231)/FS	Skin: Crust(s), hindlimb, red, left hind limb, 20 mm x 20 mm	Lung: Inflammation, nonsuppurative, minimal Skin: Necrosis, moderate
	405 (L23207)/FS		Lung: Multi-nucleated giant cells, mild Lung: Bacteria (bacilli), minimal Lung: Inflammation, nonsuppurative, minimal
5 100,000 LD_{50}	501 (L23201)/FD	Cavity, thoracic: Fluid, red, ~15 mL	Lung: Inflammation, suppurative, mild Lung: Multi-nucleated giant cells, mild Lung: Bacteria (bacilli), mild
	502 (L23234)/FD	Cavity, thoracic: Fluid, red, ~10 mL Skin: Crust(s), hindlimb, dark, left hindlimb, 30 mm 5 mm Skin: Crust(s), dark, back, 5 mm 5 mm Skin: Fluid, abdominal, clear, ventral abdomen, ~8 mL Thymus: Fluid, clear, ~8 mL	Lung: Inflammation, suppurative, moderate Lung: Multi-nucleated giant cells, mild Lung: Bacteria (bacilli), moderate Skin: Necrosis, mild Skin: Bacteria (bacilli), moderate Skin: Inflammation, suppurative, mild Skin: Hemorrhage, mild Thymus: Atrophy, lymphoid, moderate Thymus: Edema, mild Thymus: Bacteria, mild
	503 (L23212)/FS		Lung: Inflammation, suppurative, minimal

Table 11. (continued)

Group Number	Animal Number/ Death Status	Gross Findings	Microscopic Findings
5 100,000 LD_{50} (cont'd)	504 (L23200)/FD	Cavity, thoracic: Fluid, red, ~20 mL	Lung: Inflammation, suppurative, minimal Lung: Multi-nucleated giant cells, minimal
	505 (L23214)/FD		Lung: Inflammation, suppurative, mild Lung: Multi-nucleated giant cells, mild Lung: Bacteria (bacilli), mild
6 100 LD_{50}	601 (L23204)/FD	Cavity, thoracic: Fluid, red, ~15 mL	Lung: Inflammation, suppurative, mild Lung: Multi-nucleated giant cells, mild Lung: Bacteria (bacilli), mild
	602 (L23203)/FD		Lung: Inflammation, suppurative, mild Lung: Multi-nucleated giant cells, mild Lung: Bacteria (bacilli), mild
	603 (L23232)/FD	Brain: Discoloration(s), meninges, diffuse, red, affects all lobes Lymph node, bronchial: Enlarged, dark, 3x Lymph node, mediastinal: Enlarged, dark, 3x	Brain: Inflammation, suppurative, moderate Brain: Hemorrhage, mild Brain: Bacteria, minimal Lung: Inflammation, suppurative, moderate Lung: Bacteria (bacilli), minimal Lymph node, bronchial: Fibrin, minimal Hemorrhage, mild Necrosis, lymphoid, mild Bacteria (bacilli), minimal Lymph node, mediastinal: Fibrin, minimal Histiocytosis, mild Necrosis, lymphoid, mild Necrosis, vascular, mild Bacteria (bacilli), minimal Thymus: Atrophy, lymphoid, mild Thymus: Edema, mild
	604 (L23221)/FD	Cavity, abdominal: Fluid, red, ~60 mL Intestine, small: Fluid, jejunum, green, ~50 mL Abdomen was distended	Intestine, small: Inflammation, suppurative, mild Bacteria (bacilli), mild Lung: Inflammation, suppurative, mild Lung: Bacteria (bacilli), moderate
	605 (L23213)/FD	Lymph node, mediastinal: Enlarged, dark, 3x	Lung: Inflammation, suppurative, mild Lung: Bacteria (bacilli), minimal Lymph node, mediastinal: Fibrin, moderate Hemorrhage, moderate Necrosis, lymphoid, marked Bacteria (bacilli), marked

FD = Found dead

FS = Final phase sacrifice

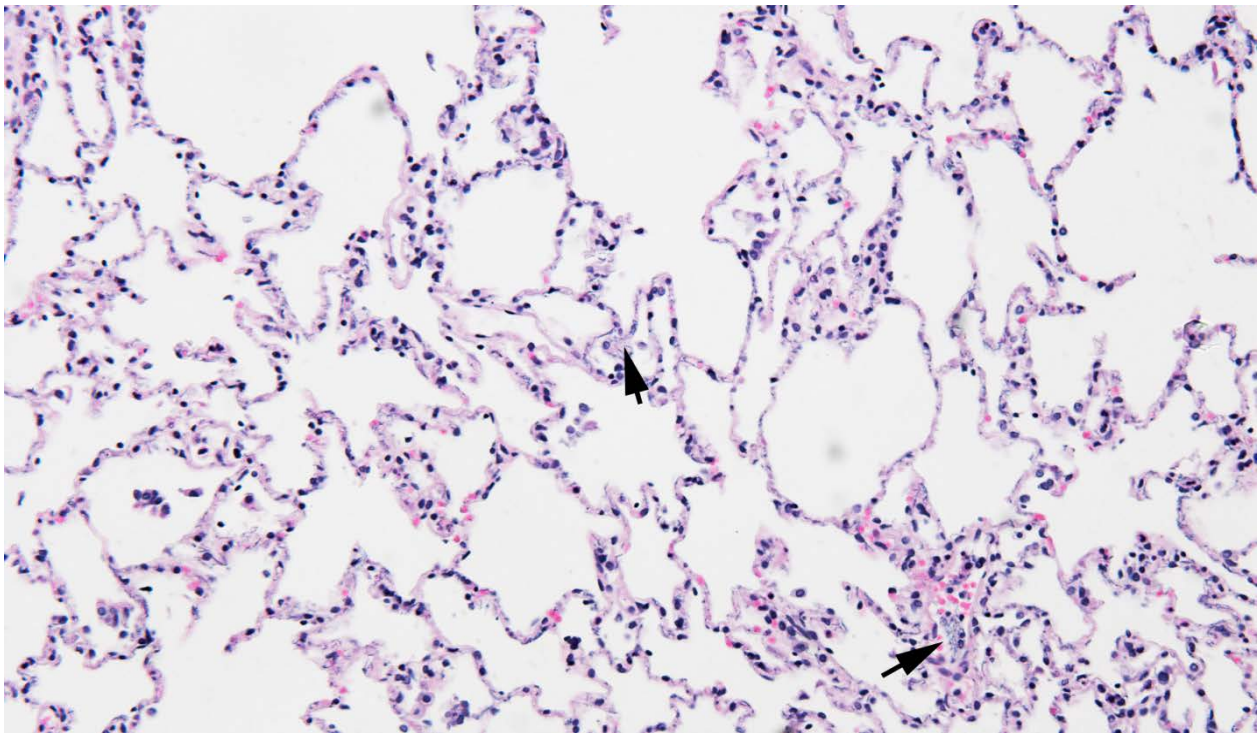

Figure 13. Hematoxylin and eosin stain of lung alveoli showing interstitial inflammation, and intravascular and interstitial *B. anthracis* bacilli (arrow) for rabbit L23225 (Group 4), 20X.

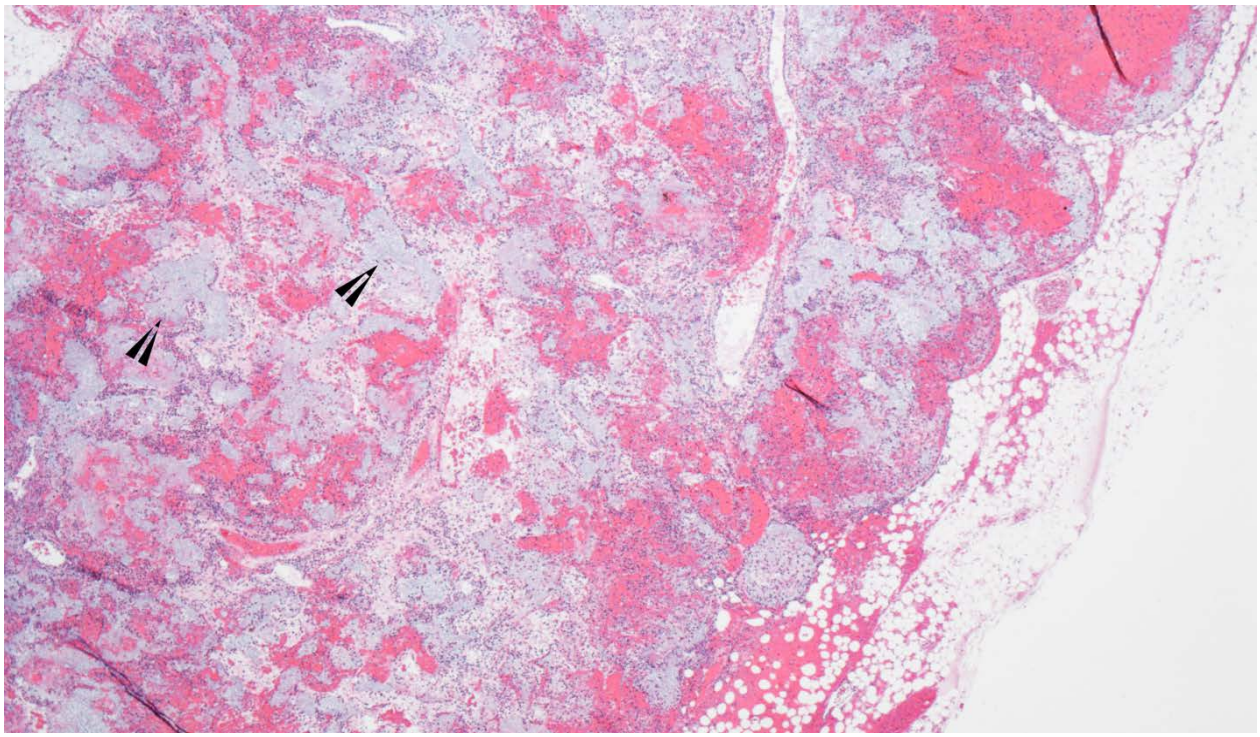

Figure 14. Hematoxylin and eosin stain of lymph node showing *B. anthracis* in the sinuses (arrow head) for rabbit L23213 (Group 6), 4X.

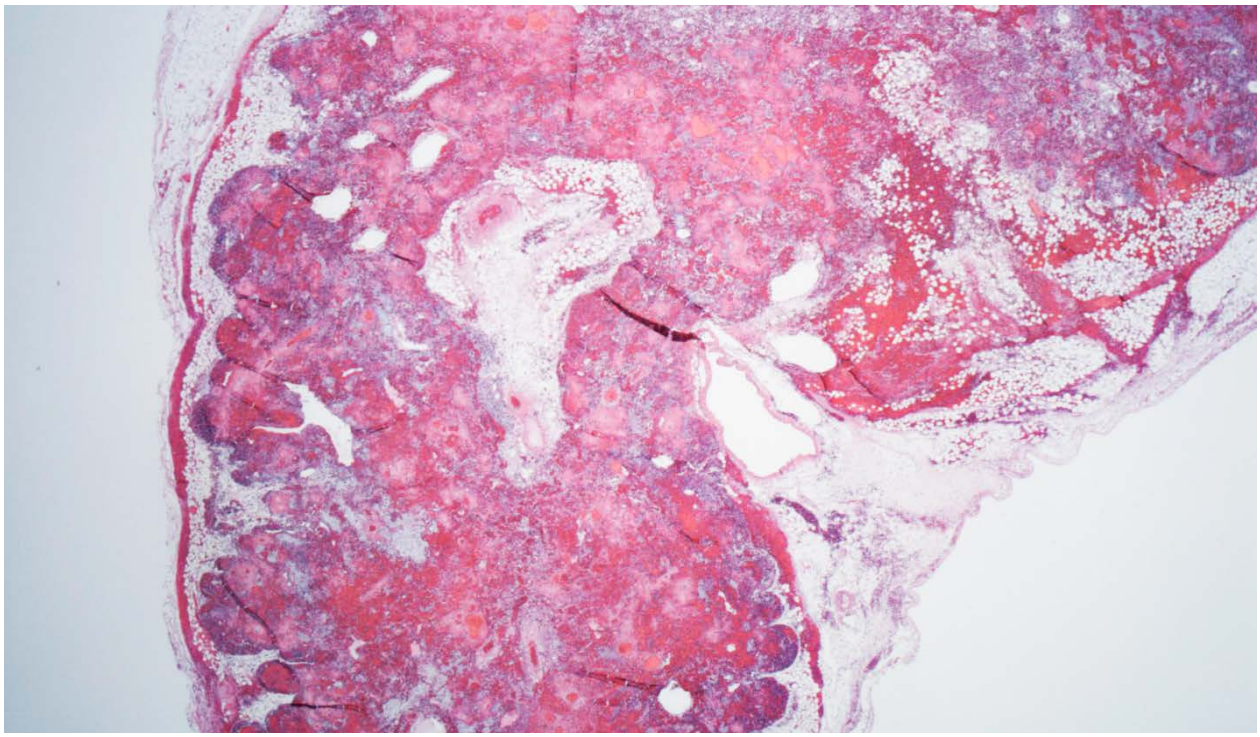

Figure 15. Hematoxylin and eosin stain of lymph node showing hemorrhage and lymphocyte depletion for rabbit L23213 (Group 6), 4X.

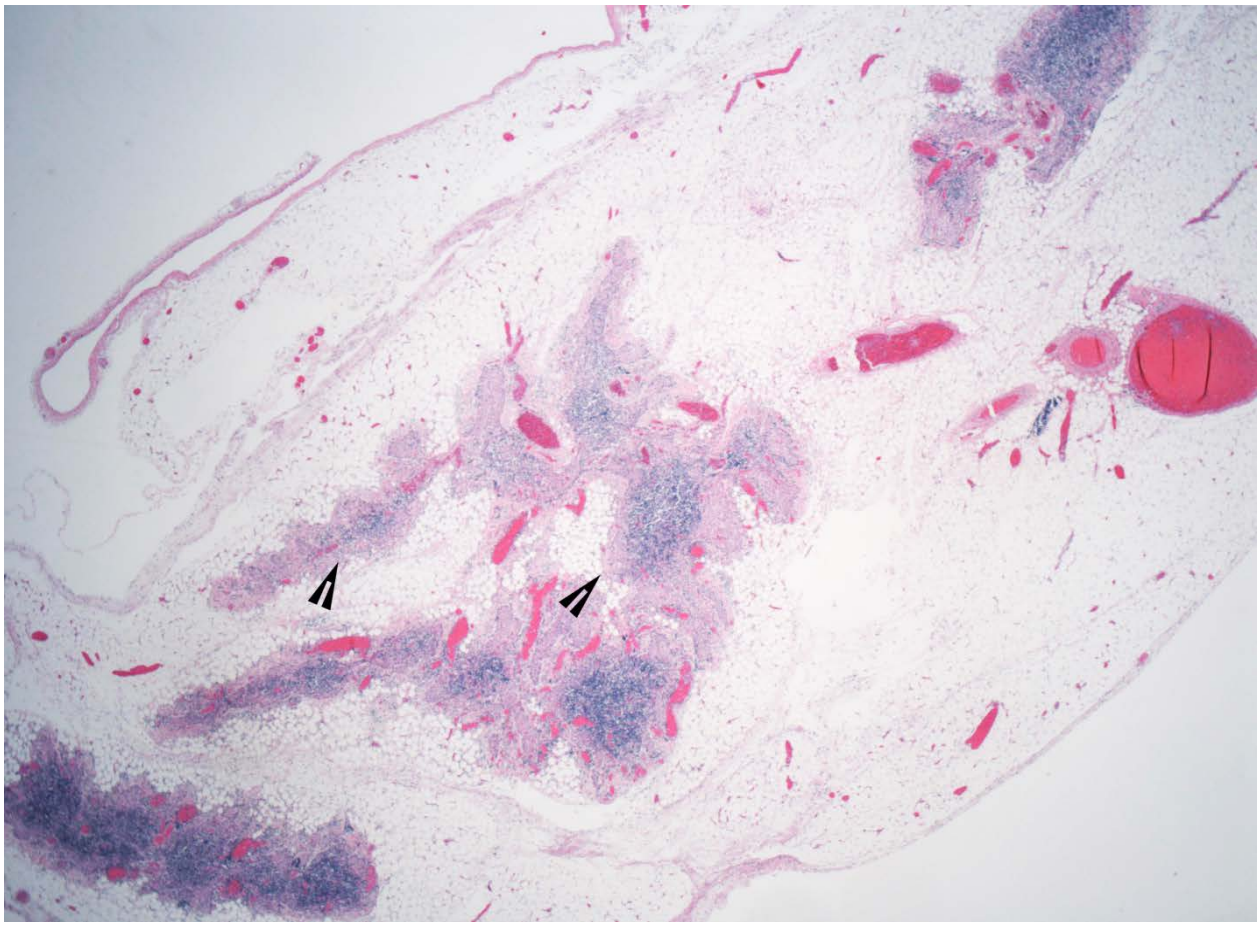

Figure 16. Hematoxylin and eosin stain of thymus from rabbit L23234 (Group 5) showing lymphocyte depletion (arrow heads), 4X.

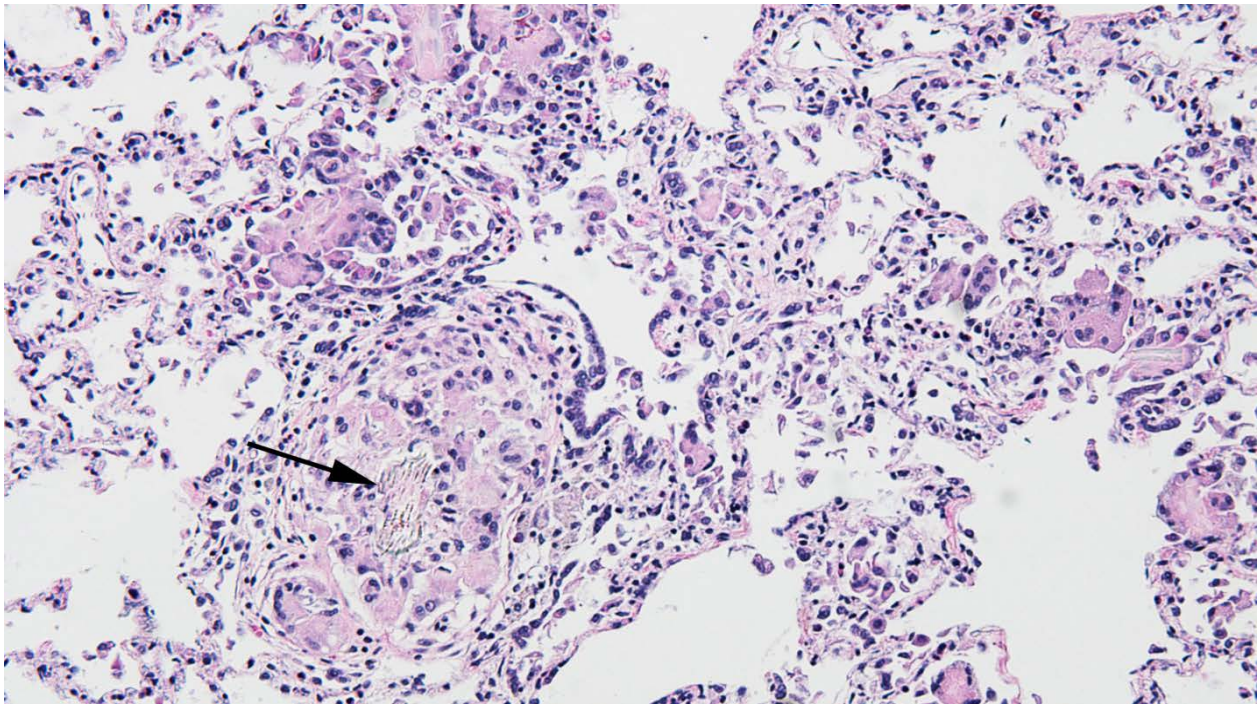

Figure 17. Hematoxylin and eosin stain of a lung section from rabbit L23204 (Group 6) showing multinucleated giant cell foreign body (arrow), 20X.

3.11 Quality Assurance

The procedures set forth in the EPA approved Quality Assurance Plan (QAP) prepared for this project were adhered to except in those instances that were documented as deviations (see Appendix B). In all there were seventeen study deviations; no facility deviations occurred during the study. Each deviation is listed in Table 12, along with the impact on the quality of the data and results reported herein. Technical System Audits (TSAT) and Data Quality Audits (DQA) performed for this study, along with dates they were performed, reported, addressed by the Study Director and project management, and closed, are listed in Table 13.

3.12 Archives

Records pertaining to the conduct of the study were documented in Battelle laboratory record books that were specific to this study. These records and the final report will be archived at Battelle.

Table 12. Deviations and Impacts on Data Quality and Results

Deviation Number	Deviation Type	Impact on Data Quality and Results
7891	Protocol	None. The dye is not necessary to visualize the colonies.
7893	SOP	None. The spores are not viable and do not need to be diluted or plated for enumeration.
8169	SOP	XI-009 deviation (numerous documentation deviations).
8170	Protocol	a) The protocol directed that the nebulizer and impinger samples from the high dose group (Group 6) be plated after the challenges with and without heat treatment (30 min at 65 ± 2°C). Only the impinger samples were heat shocked and plated on 9/18/09. The heat shock temperature was set at 60°C. b) Bacteremia testing was performed qualitatively from the blood pellet obtained in the SST following centrifugation for sample L23214 (Day 3). c) Bacteremia testing was performed qualitatively from the EDTA tube for sample L23232 (terminal).
8176	SOP	Study activities initiated before protocol was signed. As per the SOP (XI-107), "Process control is accomplished by following established procedures, methods, and study protocols." The preparation of the spores for challenge was initiated before the protocol was signed by the study director.
8177	SOP	The challenge material with dilution IDs "1020-3e4", "1020-3e5", and "1020-3e6" was not prepared and enumerated at least one day prior to the challenge.
8178	SOP	Per SOP X-202, the acceptable colony range is 25 to 250. Samples with results falling outside this range were not repeated. Results below this range will be reported as "+." For sample L23221 (terminal), the results will be calculated from the obtained results. For dilution ID "E4", counts of 255, 260, and Too Numerous To Count (TNTC) were obtained. The plates with the quantifiable values will be used to calculate a reportable result of 2.58×10^6.
8476	Study	Minimal. The technicians performed the necessary assignments to carry out the study activities properly but failed to record and/or verify all required information on the forms for accuracy and completeness at the end of each task.
8477	Study	Minimal. The animals that had missed afternoon observation on 9/30/09 were all observed as normal the morning of 9/30/09 and the following morning on 10/1/09. The animals that had a missed AM observation on 10/9/09 were scheduled for euthanasia that morning and each animal was handled individually during that procedure so any adverse conditions would have been noted. The study was not negatively impacted in either instance.
8581	Study	None. The challenge material described above was prepared on the day of the challenge from the "1020SSF 1e7" material prepared on 8/27/09, which had been enumerated prior to the day of challenge. The 3e4, 3e5 and 3e6 challenge material was then enumerated by two analysts the day of challenge. The enumerated values were accepted by the study director and aerosol director and spray factor calculations were determined based on these enumerated values.

Deviation Number	Deviation Type	Impact on Data Quality and Results
8582	Study	Minimal. The pre-run sample is enumerated as a verification of the aerosol system and the enumeration values obtained are not utilized in any calculation. The enumeration values obtained from runs 4, 5, and 6 will be reported and utilized in the spray factor calculations. For the starting material from 9/3/09, the challenge enumeration from the previous day (7.86×10^6 CFU/mL) will be utilized in the spray factor calculations.
8584	Study	**a)** Minimal. The filters used for the procedure cannot be verified. The impinge samples were filter and reportable results were obtained. b) Minimal. The equipment and reagents are accurately documented on the form. All other dates were accurately documented on the form. c) Minimal. The sample was plated appropriately and a reportable result was obtained. 8.62×10^5 CFU/mL will be used in the spray factor calculation.
8591	Study	Minimal. The system is still operating at a negative pressure as is necessary for correct operation. Due to variations in animal respiration, it is difficult to avoid some readings over -0.2″ H_2O.
8612	Study	None. The reason for taking blood samples from the ear after Day 2 was to avoid false positive bacteremia results because of a colonized port. All animals with positive bacteremias on Day 3 succumbed to disease suggesting the positive results were from systemic infection and not a colonized port.
8713	SOP	Minor documentation errors.
9383	SOP	TNA plates (111809-376 to -378), 111809-379 to -380, 111909-381) failed primary acceptance criteria but will be passed and the data reported per the study director.
9410	SOP	1) BBRC SOP VI-077 section V.D. “Raw data must be saved on a daily basis to a diskette.” Raw data was unable to be saved to electronic media due to instrument malfunction. 2) BBRC SOP VI-077 section V.F. “Parameters for the analytical methods will then be repeated after a study sample has been completed.” Quality control material was unable to be analyzed at end of sample analysis due to instrument malfunction.

Table 13. Technical System Audit and Data Quality Audit Dates

Audit Name	Audit Type	Inspection Start Date	Date Reported	Response Date	Closure Date
Aerosol challenge; Blood collections; Weights; Hematology, Bacteremia plating	TSAT	9/18/2009	10/12/2009	2/1/2010	2/26/2010
IgG ELISA, PA ELISA, QPCR, TNA	TSAT	10/22/2009	12/7/2009	2/1/2010	5/25/2010
Quarantine telemetry implant and housing	TSAT	11/9/2009	11/23/2009	1/21/2010	1/22/2010
Clinical Chemistry/Hematology	DQA	11/20/2009	12/8/2009	12/9/2009	2/25/2010
In vivo Binder	DQA	11/20/2009	12/8/2009	12/9/2009	2/9/2010
Micro and Aerosol Telemetry Binders B0004642 and 4547	DQA	12/1/2009	12/14/2009	1/7/2010	1/25/2010
DNA Isolation and QPCR	DQA	12/2/2009	12/8/2009	12/11/2009	1/25/2010
Telemetry Binder	DQA	12/2/2009	12/8/2009	1/9/2010	1/25/2010
PA ELISA Binder	DQA	12/11/2009	12/21/2009	12/21/2009	2/25/2010
Spray Factor – Aerosol portion binder	DQA	1/25/2010	1/27/2010	1/27/2010	2/9/2010
Spray Factor – Micro binder	DQA	1/25/2010	1/27/2010	1/27/2010	2/9/2010
ELISA Study Binder	DQA	2/10/2010	2/12/2010	4/6/2010	5/25/2010
TNA Binder B0005163	DQA	2/10/2010	2/12/2010	4/6/2010	5/25/2010
DNA Isolation and Real-time PCR, Binder 2	DQA	3/19/2010	3/22/2010	3/22/2010	5/25/2010

4 Discussion and Conclusions

The anthrax letter attacks of 2001 resulted in 12 cases of cutaneous and 11 cases of inhalational anthrax. Five of the 11 individuals with the inhalational form of the disease died (Jernigan et al., 2001). The processing of these spore-containing letters through the U.S. mail resulted in the contamination of numerous government and private facilities (Canter, 2005). As part of the remedial process, a standard of nondetection of culturable spores was used to confirm that the decontamination of buildings was successful. Almost ten years later, the criterion for remediation remains nondetection (Coleman et al., 2008). One reason for the continuing lack of a risk-based standard is the unavailability of credible dose-response data for *B. anthracis* that could be used to estimate the risk posed by low dose inhalation exposures. This study was proposed to develop dose-response data that could be used to begin to fill the data gap present in the low dose range.

The objective of this work was to determine physiological responses following an acute inhalation exposure of *B. anthracis* in the NZW rabbit at various low dose concentrations. NZW rabbits were exposed to targeted inhaled doses of 1.0×10^2, 1.0×10^3, 1.0×10^4, and 1.0×10^5 CFU of *B. anthracis*. Preliminary SF studies were conducted to ensure that the lower doses could be successfully delivered. The actual doses were approximately 2.06 to 2.86 times the targeted inhaled dose and remained relatively consistent from animal to animal within a group. Based on the actual doses administered, the LD_{50} was determined to be 5.18×10^4 CFU, which is about 50% of the published LD_{50} of 1.05×10^5 from Zaucha et al. (1998). The Zaucha et al. (1998) LD_{50} value falls within the confidence intervals established for this study of 6.14×10^3 CFU to 7.27×10^5 CFU. As no confidence intervals were identified in Zaucha et al. (1998), it is not possible to determine how the LD_{50} presented here compares to the variability in the Zaucha data. It is also possible that selection of dose inputs (i.e., targeted versus actual dose) that were used in Zaucha et al. (1998) dose-response

analysis may also have affected the value of their calculated LD_{50}, if similar variability between the targeted versus actual doses were present in their study.

As described in this report, Group 6 served as a positive control group and these rabbits were challenged with a targeted inhaled dose of 1.00×10^7 CFU (actual equaled 8.27×10^6 CFU). Each of these five positive control animals succumbed to infection with a mean time to death of 3.47 days post-challenge. All of the animals in this group were bacteremic prior to death and all animals except one were shown to be toxemic at some time in the study. In addition, the positive control animals showed increases in respiration rate, heart rate, body temperature, and circulating neutrophils prior to death, and had pathology findings consistent with inhalational anthrax. Enzymes indicative of liver damage (AST) were also elevated in this group; however, there was no gross evidence of liver damage. These observations correspond to the findings of Lawrence et al. (2009), who evaluated the physiological response of Dutch Belted Rabbits exposed to 1.00×10^7 *B. anthracis* spores via nasal instillation. The Dutch Belted Rabbits also presented with tachycardia, fever, and neutrophilia prior to death, which corresponded to the observations in the positive control animals (Lawrence et al., 2009). This strain of rabbit also showed significant increase in ALT levels. Interestingly, no significant increase in AST was observed in the Dutch Belted Rabbits, whereas there was a significant increase in the NZW rabbits from Groups 5 and 6 in the current study. This may be the result of differences in the two strains of rabbit used in the studies.

In Group 5, four out of five of the rabbits that received $2.75 \text{ X } 10^5$ CFU showed results consistent with those observed in the positive control group and died on study. The pathology in this group was not as extensive as that seen in the positive control group, but was generally consistent with pathology previously reported (Zaucha et al., 1998) for inhalational anthrax in the rabbit model. The only animal survivor in Group 5 (L23212) received an actual inhaled dose of 3.29×10^5 CFU. This animal did not become bacteremic or toxemic and did not demonstrate increases in heart rate, respiration, or body temperature. End of study pathology examination showed minimal acute inflammation in the lung,

which may or may not have been induced by exposure to the *B. anthracis.*

Group 4 animals received an average inhaled dose of 2.54 x 10^4 CFU and only two out of five rabbits succumbed to infection. However, the two animals that died (L23235 and L23225) were bacteremic at the time of death. Rabbit L23235 never become toxemic (i.e., circulating levels of PA were not found to be elevated) suggesting that detectable levels of circulating PA may not be an appropriate marker for disease. However, both of the animals that succumbed showed increases in heart and body temperature prior to death.

All animals that received inhaled doses less than 2.06 x 10^3 CFU (i.e., Groups 2 and 3) survived to the end of the study and were not observed to be bacteremic or toxemic at any time. Rabbit L23227 (Group 3) was noted as having bacteria (Bacilli) in the lung upon microscopic examination; however, it cannot be confirmed that these were *B. anthracis*. The entire lung was fixed in 10% buffered formalin, so the tissue could not be cultured. As the animal was never bacteremic but bacilli were observed in the lungs, it was possible, though unlikely, that a few of the spores germinated in the lungs of L23227 but never established a systemic infection. None of the surviving rabbits seroconverted, suggesting that a *B. anthracis* infection was not established.

Nine of the rabbits on study were positive for *Bordetella bronchiseptica* prior to challenge with *B. anthracis.* All challenge groups (except Group 6) contained at least one positive animal. The *B. bronchiseptica* infection was not found to affect survival or any physiological parameters measured. (Please note two samples were labeled L23223, both samples were negative and one of the samples was most probably from animal L23233.)

During histopathologic examination, it was also noted that many of the rabbits had multi-nucleated giant cells in the lungs. This finding did not correspond with challenge dose or *B.bronchiseptica* infection. However, it is possible that these lesions were caused by an indirect effect of prolonged anthrax septicemia on macrophage function resulting in altered foreign particle clearance. Macrophage dysfunction has been described as occurring late in sepsis (Pahuja et al., 2008). Inhaled debris or emboli from indwelling vascular access ports may have also contributed to the development of multinucleated giant cells/granulomas. Pulmonary multinucleated

giant cells/granulomas, thromboemboli, and perivascular eosinophils have been described in animals fitted with indwelling vascular access ports (Taketoh et al., 2009). Further research is required to determine if this pathological finding was caused by exposure to the *B. anthracis* spores.

Table 14 showed the physiological changes that occurred after challenge with various doses of *B. anthracis* spores. These data presented a profile of the physiological responses of the rabbit model when challenged with various doses of *B. anthracis* spores. The table illustrated that as the challenge dose increases, so do the number of parameters affected.

In the present study there was a trend toward increases in these heart and respiration parameters in the higher challenge dose groups relative to the pre-challenge baseline. Increased heart and respiration rates were commonly associated with death, but a few animals showed increases in these parameters and lived. Temperature was the most consistent indicator of disease outcome. All rabbits that had a febrile response died on study. Increased neutrophils and increased liver enzymes have been associated with inhalational anthrax in the rabbit model (Lawrence et al., 2009). However, if one were to use literature-based values to determine departures from normality, only seven out of the 11 animals that died would have been found to exhibit neutrophilia and only three of the 11 that died would have been found to exhibit elevated levels of AST. The majority of rabbits had detectable levels of CRP regardless of levels of the challenge dose, indicating that this parameter was not a good indicator of disease outcome.

While the above parameters show a physiological response to exposure over a certain threshold, they are not specific and may be caused by a myriad of bacteria. The diagnostic indicators assayed here were bacteremia and toxemia. These specific indicators were only detected in animals that succumbed to disease, suggesting that if the bacteria were able to escape the lungs and spread systemically then the result is death. Overall, the data indicate that challenge doses of *B. anthracis* below the level sufficient to establish systemic infection do not produce observable physiological responses; however, doses that trigger a response result in death.

Table 14. Summary Table of Responses Measured by Rabbit

Animal ID	Dose Group	Inhaled Dose (CFU/ Animal)	Heart Rate[a]	Resp. Rate[a]	Temp[a]	Neut. Levels[b]	CRP[b]	AST[b]	Tox	Bact	Time to Death (days)
L23220	1	0	↔	↑↓	↔	↑↓	↑	↔	-	-	NA
L23216	1	1.00×10^1	↑	↑	↔	↔	↑	↔	-	-	NA
L23218	1	0	↑	↔	↔	↔	↑	↔	-	-	NA
L23223	1	0	↔	↔	↔	↔	↔	↔	-	-	NA
L23222	1	0	↔	↔	↔	↑	↑	↔	-	-	NA
L23215	2	3.22×10^2	↔	↔	↔	↓	↑	↔	-	-	NA
L23206	2	2.98×10^2	↔	↓	↔	↓	↑	↔	-	-	NA
L23210	2	2.18×10^2	↔	↓	↔	↓	↑	↔	-	-	NA
L23219	2	3.21×10^2	↔	↓	↔	↓	↑	↔	-	-	NA
L23211	2	2.73×10^2	↑	↑	↔	↓	↑	↔	-	-	NA
L23217	3	1.48×10^3	↓	↓	↔	↔	↔	↔	-	-	NA
L23230	3	2.02×10^3	↑	↓	↔	↓	↑	↔	-	-	NA
L23228	3	2.23×10^3	↔	↔	↔	↓	↑	↑	-	-	NA
L23227	3	2.32×10^3	↔	↔	↔	↓	↔	↔	-	-	NA
L23229	3	2.24×10^3	↔	↑	↔	↓	↑	↔	-	-	NA
L23235	4	1.76×10^4	↑	↑	↑	↑	↑	↔	-	+	11
L23205	4	2.73×10^4	↔	↑	↔	↔	↔	↔	-	-	NA
L23225	4	2.59×10^4	↑	↔	↑	↑↓	↑	↔	+	+	4
L23231	4	2.41×10^4	↑	↔	↔	↓	↔	↔	-	-	NA
L23207	4	3.19×10^4	↔	↔	↔	↓	↑	↔	-	-	NA
L23201	5	1.78×10^5	↑↓	↑	↑	↑	↑	↑	+	+	4
L23234	5	2.96×10^5	↑	↑	↑	↑	↑	↑	+	+	6
L23212	5	3.29×10^5	↔	↔	↔	↓	↑	↔	-	-	NA
L23200	5	2.19×10^5	↓	↑	↑	↓	↔	↔	-	+	3
L23214	5	3.54×10^5	↑	↑	↑	↑	↑	↑	+	+	6
L23204	6	5.95×10^6	↑	↑	↑	↑	↑	↔	+	+	4
L23203	6	8.86×10^6	↑	↑	↑	↑	↑	↔	+	+	5
L23213	6	7.29×10^6	↑	↑	↑	↔	↑	↔	+	+	3
L23221	6	8.88×10^6	↑	↑	↑	↔	↑	↔	-	+	2
L23232	6	1.04×10^7	↑	↑	↑	↓	↑	↔	+	+	4

↑ = Increases in a parameter
↓ = Decreases in a parameter
↔ = No change in the parameter
\+ = Positive or bacteremia culture or toxemia
\- = Negative for bacteremia culture or toxemia
[a] = Changes based on baseline
[b] = Changes base on normal ranges

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APPENDIX A
STUDY PROTOCOL

Rabbit Single Dose Anthrax Telemetry Study

Study No. 1020-CG920503

BBRC Director:	James A. Blank, Ph.D., D.A.B.T.
BBRC Associate Director:	Jason M. Mott, D.V.M., Ph.D.
Study Director:	Jason E. Comer, Ph.D.
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Approval Signatures

Jason E. Comer, Ph.D. — 9-15-09
Study Director — Date

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Gregory V. Stark, Ph.D. — Date
Statistician

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Katherine A.B. Knostman, D.V.M., Ph.D., D.A.C.V.P — Date
Pathologist

Gloria Sivko, D.V.M., Ph.D. — 9.15.09
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Jason M. Mott, D.V.M., Ph.D. — 9-15-09
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Stephen M. Miller, D.V.M.. — 9-15-09
Study Veterinarian — Date

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Sarah C. Taft, Ph.D. — Date
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Charles D. Lawrie — 9/16/2009
Quality Process Coordinator — Date

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1.0 INTRODUCTION

Bacillus anthracis, the etiologic agent of anthrax, is a gram-positive, rod-shaped, aerobic and/or facultative anaerobic, spore-forming bacterium. Each route of human infection, including gastrointestinal, cutaneous, and inhalation, manifests in different clinical symptoms, with inhalational anthrax being the most lethal. The incubation period usually varies from 12 hours to five days depending upon the dose and route of entry. The onset of disease can be longer following inhalation exposure and some reports suggest a delayed onset of several weeks in low-dose exposure or following removal of therapeutic intervention. The initial clinical signs and symptoms of inhalation anthrax are nonspecific and may include malaise, headache, fever, nausea, and vomiting. These are followed by a sudden onset of respiratory distress with dyspnea, stridor, cyanosis, and/or chest pain. The onset of respiratory distress is followed by shock and eventually death with close to 100% mortality.

Anthrax is considered a serious biological terrorist and military threat due to the high lethality rates of inhalation exposure and the stability of the *B. anthracis* spore. The virulence of *B. anthracis* spores is predicated upon the production of an anti-phagocytic capsule and two proteinaceous toxins. Three polypeptides, protective antigen (PA), lethal factor (LF), and edema factor (EF), interact to form two interlinked toxins. PA and LF combine to produce anthrax lethal toxin (LT), and PA and EF combine to produce edema toxin (ET). PA binds to a host cell receptor and is cleaved by furin-like protease. The activated PA then forms a heptameric complex which competitively binds three molecules of LF and/or EF. The holotoxin is then taken up by the cell via receptor-mediated endocytosis. A decrease in endosomal pH results in a conformational change in the PA molecule resulting in a pore structure for LF and EF translocation into the cytoplasm. LF is a zinc metalloprotease that inhibits mitogen activated protein kinase signaling. EF, a calcium-dependent adenylate cyclase, increases cyclic adenosine monophosphate levels in susceptible cells and results in altered water hemostasis and the inhibition of phagocytosis. Thus both toxins inhibit the signaling cascades required for the activation of immune cells.

An unfortunate outbreak of inhalational anthrax in Sverdlovsk, Russia provided the largest set of clinical specimens to study the pathology of human anthrax. Necropsies of victims of the outbreak consistently showed pathologic characteristics of inhalational anthrax including, necrotic hemorrhage of the thoracic lymph nodes, hemorrhagic mediastinitis, and pleural effusion. Fifty percent of the cases involved hemorrhagic meningitis, and 92 % showed signs of gastrointestinal tract involvement (i.e., submucosal hemorrhagic lesions).

Quantitative microscopic findings showed that most of the severe pathologic lesions occurred in the mediastinum and mediastinal lymph nodes, the sites of initial replication of the bacterium. The investigators also observed peripheral transudate surrounding fibrin-rich edema, necrosis of veins and arteries, and apoptotic lymphocytes.

The 2001 anthrax letter attacks resulted in five fatal cases of inhalational anthrax in the United States. Prior to hospital admission, common nonspecific symptoms included fever, malaise, and cough. Chest radiographs of these patients reveal pleural effusion and lung infiltrates and anthrax infection was confirmed by culture.

The objective of this study is to determine physiological markers of disease following a single exposure of *B. anthracis* Ames strain spores.

2.0 LOCATION OF TESTING FACILITIES

This study will be performed by Battelle Memorial Institute, Biomedical Research Center (BBRC) located at State Route 142, West Jefferson, OH 43162. Telemetry surgery and histopathology and will be performed at Battelle Memorial Institute, 505 King Ave., Columbus, OH 43201.

3.0 STUDY OBJECTIVES

The objective of this study is to determine physiological markers of disease following a single exposure of *B. anthracis* Ames strain spores.

4.0 TEST SYSTEM

Animals: Thirty-five (35) male pathogen free New Zealand White (NZW) rabbits (*Oryctolagus cuniculus*) weighing at least 3.5 kg will be ordered for this study. Thirty rabbits will be placed on study with the remaining five serving as replacements. Rabbit age is not used as a criterion for placement on study. Rabbits will be purchased from Covance. Prior to receipt, rabbits will be fitted with vascular access ports at Covance. A Battelle veterinarian will implant a Data Sciences International model D70-PCTP telemetric devices into the rabbits prior the start of the study. The rabbits shall be in good health, free of malformations, and exhibit no signs of clinical disease. The identity of each rabbit will be confirmed before and after each procedure (challenge, monitoring, and bleeds) by ear tags and verified against cage cards. Five extra animals will be ordered and implanted with telemetric devices in case of vascular access port or telemetric device failure.

5.0 STUDY DESIGN

5.1 Randomization of Animals: Prior to challenge, rabbits will be randomized by weight into six groups of five. The rabbits within each group will also be randomized for challenge order (based on ear tag numbers provided by supplier). The rabbits will be challenged according to randomization order and challenge dose group. For example, the rabbits in Group 1 will be challenged first and the rabbits in Group 6 will be challenged last. Prior to challenge, any animals with a malfunctioning vascular access ports or telemetric devices will be replaced with one of the five extra animals.

5.2 Aerosol Challenge Generation and Monitoring: On Study Day 0 (Study Day 0 defined as Challenge Day), rabbits will be placed into a plethysmography chamber, passed into a Class III cabinet system, and aerosol challenged with targeted doses of 100,000, 10,000, 1,000, or 100 CFUs of *B. anthracis* (Ames strain) spores. A high dose control group will be challenged with 100 LD50s and the negative control group will exposed to an equivalent of 100LD50s of gamma irradiated spores. The Ames LD_{50} value (105,000 cfu) published from USAMRIID (Zaucha, 1998) will be used.

Aqueous suspension of *B. anthracis* (Ames strain) will be aerosolized as per SOP BBRC. XIII-001. Serial dilutions of impinger samples will be plated onto TSA plates and enumerated as per SOP BBRC. X-054.

Impinger samples that are expected to have concentrations lower than can be enumerated by spread plating will be both plated and filtered. Briefly, 1 mL of the sample will passed through a sterile 0.45 micron filter (Nalgene) Analytical Test Filter Funnel, (Fisher catalog number 145-0045), the filter will placed on top of a TSA plate and incubated for 24-72 hours at 37°C ± 2°C. The filter will then be stained with an appropriate dye to visualize the bacteria on the white filter. Another aliquot of the sample will be enumerated by spread plating undiluted sample and a 1:10 dilution of the sample.

The aerosol challenge duration will be based upon an estimated aerosol challenge concentration and a cumulative minute volume gathered "real" time throughout the exposure.

To determine the affect of aerosolization on the spore coat the nebulizer and impinger samples from the high dose group (Group 6) will be plated after the challenges with and without heat treatment (30 minutes at 65 ± 2 °C).

Following aerosol challenge, rabbits will be held in individual stainless steel cages and monitored for clinical signs of illness, body temperature, heart rate, respiration rate and survivability for 21 days post-challenge.

Table 1. Study Design and Challenge Doses.

Group	Spore dose (CFU)	Number of Spore Challenges	# of Rabbits
1 (neg) control*	100X LD_{50}	1	5
2	100	1	5
3	1000	1	5
4	10,000	1	5
5	100,000	1	5
6 (high dose control)	100X LD_{50}	1	5

* Negative controls will be challenge with irradiated spores.

5.3 Animal Weights: Animals will be on weighed on Study Days 0 (challenge day), 1, 2, 3, 7, 14 and 21.

5.4 Blood Collection Schedule: On Study Days -3, 1, 2, 3, 7 and 14 blood will be collected into EDTA (~1.5 mL) and SST tubes (~3.5 – 4.5 mL) (Table 2). Blood taken on Study Days 1, 2, and 3 will be drawn 24, 48, and 72 hours (± 2hours), respectively, based on median challenge time of each group. If possible a blood sample will be taken from animals found dead or prior to euthanasia. On Study Day 21 all surviving rabbits with be terminally bleed via cardiac puncture according to Table 2.

Blood samples collected into SST tubes will be processed to serum in accordance with SOP BBRC. V-033. Blood in EDTA tubes will be stored at room temperature if utilized within 4 hours of collection; blood will be stored at 2-8 °C if not analyzed within 4 hours. Sera will be stored at ≤ -70 °C until needed.

Blood samples will be collected from vascular access ports on Study Days -3, 1 and 2 for both the EDTA and SST collection tubes. After Day 2 blood collected in the EDTA tubes will be obtained from the medial auricular artery or the marginal ear vein. Blood collected in SST tubes will continued to be obtained from the vascular access port. If a port fails, the medial auricular artery, the marginal ear vein, or other appropriate vasculature may be utilized for blood collection if attainable. If a blood sample cannot be collected from either the port or other appropriate vasculature based on study director discretion, it will be documented in the study file.

Table 2. Blood collection schedule

	Study Day						
Tube Type	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21
EDTA (~ml)	1.5	1.5	1.5	1.5	1.5	1.5	1.5 *
SST (~ml)	4.5	3.5	3.5	3.5	3.5	3.5	15 *
Total per day~(ml)	6	5	5	5	5	5	16.5

*Terminal Blood sample, minimum draw volume.

5.5 Priority usage of whole blood and sera:
Whole Blood: Bacteremia via quantitative plating >>> Hematology >>> Bacteremia via Quantitative PCR
Sera: Circulating PA ELISA >>> Retention sample (maximum of 750 µl if possible) >>> Clinical Chemistry >>> anti-PA IgG ELISA and TNA

5.6 Toxemia Assessment: A portion of all the serum collected will be analyzed for circulating PA via the quantitative PA ELISA according to SOP BBRC. X-180.

5.7 TNA/ELISA: To determine if the rabbits elicit an immune response following challenge, serum samples will be analyzed by ELISA and htp-TNA according to SOPs BBRC. X-101 and X-143.

5.8 Bacteremia: A portion of each blood sample from the EDTA collection tubes will be tested for bacteremia by quantitative spread plate technique (SOP BBRC. X-202), and quantitative real-time PCR (SOP BBRC. X -146). The Day -3 samples will be assayed for bacterema via the qualitative plating technique bacteremia (SOP BBRC. X-096). Please note, if the blood samples cannot be plated in real-time, ~400 µl of blood from the EDTA tube will be transferred to another appropriate tube and stored on ice until plated or DNA isolation.

5.9 Hematology and Clinical Chemistry : Hematology will be performed on blood samples collected in EDTA tubes using the Advia Hematology Analyzer according to SOP BBRC. VI-066. Hematology evaluation will include but not be limited to the following parameters:

- White blood cell count (WBC)
- N/L ratio (neutrophil/lymphocyte ratio)
- Differential leukocyte (absolute) count

- Hemoglobin (HGB)
- Hematocrit (HCT)
- Red blood cell count (RBC)
- Mean corpuscular volume (MCV)
- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular hemoglobin concentration (MCHC)
- Red cell distribution width (RDW)
- Platelet count (PLT)
- Mean platelet volume (MPV)

Clinical Chemistry will be performed on all serum samples using Advia 1200 Chemistry analyzer. According to SOP BBRC. VI-077. Evaluation will include, but not be limited to, the following parameters:

- Alanine aminotransferase (ALT)
- Asparate aminotransferase (AST)
- Total bilirubin
- Total protein
- Blood urea nitrogen (BUN)
- Creatinine
- Calcium
- Sodium
- Potassium
- Chloride
- Phosphorus
- Lactate Dehydrogenase (LDH)
- Sorbitol Dehydrogenase (SDH)
- C-Reactive Protein (CRP)

5.10 Clinical Observations: Following challenge rabbits with be observed twice daily for clinical signs of illness and survivability due to anthrax infection (e.g., moribund, respiratory distress, appetite, activity, and seizures).

5.11 Telemetric Monitoring: Rabbits will be surgically implanted with telemetry units (D70-PCTP transmitters, Data Sciences International) prior to being placed on study (allowing sufficient time to allow recovery from the implantation procedure). Each D70-PCT transmitter contains one pressure lead and one biopotential lead. Body temperature, ECG activity, and cardiovascular function (heart rate and respiratory pressure) will be monitored at least 30 seconds every 15 minutes for 7 days pre-

challenge (baseline) and for 21 days post-challenge according to SOP MREF.VI-087. Event markers will not be logged. If the telemetry implants fail post-challenge, the affected parameters will not be recorded; however the animal will stay on study to collect other study data (i.e. clinical observations, biological samples, clinical pathology, etc.).

Each animal's cage will be equipped with a Data Sciences International telemetry receiver. The transmitters, receivers, consolidation matrixes, cabling, and computers utilizing the Dataquest A.R.T.™ data acquisition and analysis software are all components of the PhysioTel® Telemetry System. The Dataquest A.R.T.™ telemetry software will collect the telemetry parameters above.

5.12 Necropsy and Histopathology: Animals that succumb to challenge, or are found moribund and are euthanized, will undergo a gross necropsy. Surviving animals will be euthanized and necropsied on Study Day 21. The lungs from each rabbit will be collected and examined histopathologically.

6.0 ANIMAL CARE AND HUSBANDRY

6.1 Quarantine

Rabbits will be quarantined for 7 days prior to study initiation and will be visually inspected and released by the study veterinarian prior to study. Animals will be observed a minimum of twice per day during the quarantine period.

6.2 Veterinary Care

Discomfort and distress will be limited to that which is unavoidable in the conductance of scientifically valuable research. Animals that develop non-study related illness or injury will be evaluated by a Battelle veterinarian for determination of treatment or disposition. In such cases, and if in the opinion of the Study Director and a Battelle veterinarian an animal is in a moribund state, that animal will be euthanized. No treatment will be given for study related signs with the exception that rabbits meeting the Criteria for Euthanasia will be euthanized.

6.3 Criteria for Euthanasia

The sequelae leading to death in the subcutaneous and inhalation rabbit model have been published by Zaucha *et al.* and confirmed in our laboratory. Although there is a trend for decreased survival time with increasing dose, it is minimal. Fulminating disease appears to be an all-or-none response, and no protracted illness has ever been

observed, regardless of the dose. Abnormal clinical observations are not generally apparent until approximately 24-hours before death, at which time rabbits become progressively lethargic and weak. Several rabbits have exhibited brief periods of excitation and hyperactivity within hours or minutes before death. These rabbits had brain or meningeal lesions at necropsy.

The following criteria have been pre-established for euthanasia: presence of any seizure (denoting meningitis or encephalitis), respiratory distress, dyspnea, or forced abdominal respirations, unresponsive to touch or external stimuli, and moribundity.

Rabbits that are judged to be moribund by a highly trained life sciences technician, Battelle veterinarian, or by the Study Director will be euthanized after an individual blind to treatment concurs with the decision to euthanize. Concurrence will not be required in instances when the animal is observed seizing.

Rabbits that are euthanized will be sedated with Acepromazine or other approved anesthetic and then administered an overdose of a euthanasia agent containing pentobarbital or other American Veterinary Medical Association (AVMA) approved method of euthanasia.

6.4 Husbandry

Housing: Rabbits will be housed individually in stainless steel cages on racks equipped with automatic watering systems.

Lighting: The light/dark cycle will be approximately 12 hours each per day, using fluorescent lighting.

Temperature: Animal room temperatures will be maintained according to Battelle SOPs.

Humidity: The relative humidity of animal rooms will be maintained according to Battelle SOPs.

Diet: PMI, Inc. Certified Rabbit Chow® will be fed per Battelle SOP No. BBRC. VII-013. No contaminants that would affect the results of the study are known to be present in the feed.

Water: Water is supplied from the Battelle water system and will be available *ad libitum* during the entire study. Water is analyzed at a minimum once per year. Analysis is carried out following Battelle SOPs. No contaminants that would affect the results of the study are known to be present in the water.

Enrichment: To promote and enhance the psychological well being of rabbits, enrichment will be as described in Battelle SOP No. BBRC. VII-040.

7.0 ANALYTICAL AND STATISTICAL PLAN

7.1 Sample Size:

A sample size of 5 animals per group is sufficient to detect group effects in a one-way ANOVA model with greater than 80% power, when a two standard deviation difference in group means is present.

7.2 Statistical Methods:

Survival data from the groups will be compared using a Fisher's exact test. To explore difference in time to death a log-rank test will be conduced or Cox proportional hazards model will be fitted.

For, circulating PA levels, TNA, ELISA, each hematology parameter, each clinical chemistry parameter, quantitative bacteremia and PCR data descriptive statistics will be produced for each animal at each sample collection time. As no negative control animals are expected to survive, baseline values for animals will be used in the assessment of these endpoints, with each animal serving as its own control. Mean changes in each parameter will be compared to baseline, to evaluate any change in health status. An analysis of variance (ANOVA) model will be fitted to determine if parameters changed significantly from baseline and whether there were significant differences between groups. In addition, baseline data will be used to estimate a threshold for each parameter to use in determining whether individual animals are abnormal. Summary statistics for the proportion of animals that are abnormal in each group will be produced for each parameter. The proportion abnormal in each group will be compared using Fisher's exact tests for each parameter..

Telemetry endpoints may be smoothed by adjusting the post-challenge values to baseline averages calculated for each individual animal or other appropriate method. Statistical evaluation of dose-response curves may be made at specified time intervals during the post-challenge period. Alternatively, time to onset of altered telemetric parameters may be evaluated using Cox proportional hazard models with dose as an explanatory variable.

7.3 Missing Value Handling:

All animals used in this study will be individually identified and accounted for at the conclusion of the study. Mortality will be recorded as it occurs to the nearest hour and/or day. If animals are removed from the study for appropriate reasons, mortality will be reported as a percentage of the total animals remaining. Similarly, if individual sample results or other measurements are not obtained for appropriate reasons, all available results will be included in the analysis.

8.0 RECORDS TO BE MAINTAINED

8.1 Animals

Animals surviving the challenge(s) will be euthanized on Study Day 21.

8.2 Specimens

Specimens generated during this study (tissue, histology slides, sera, etc.) will be shipped to the sponsor, if requested, or disposed of in accordance with SOP BBRC. VII-011.

8.3 Study Records and Materials

All records applicable to this study will be maintained in compliance with BBRC procedures.

8.4 Study Reports

Reports generated for this study will be written to provide all appropriate information to the sponsor. The final report will contain all general information on the study.

8.5 Sponsor Study Audits

The documentation specific to this study will only be made available to representatives of the sponsor, independent auditors contracted through the Sponsor, or other designees of the Sponsor. This model development study is intended to serve as the model for subsequent government and non-government therapeutic efficacy studies.

9.0 BIOSAFETY CONSIDERATIONS

Containment Level: The aerosol exposure system will be contained in a class III biosafety cabinet within the BL-3 laboratory. Rabbits will be housed in the BL-3 for up to 28 days post-challenge, after which all surviving rabbits will be anesthetized, have a terminal bleed taken, and be euthanized. If rabbits are removed from the study prematurely, mortality will be reported as a percentage of the total animals remaining. All animals that die or are euthanized will be double bagged, autoclaved, and incinerated.

Biohazard Safety: Personnel handling anthrax challenged rabbits will wear appropriate personal protective equipment (PPE) as described in Battelle SOPs. Additionally, all personnel working with anthrax or anthrax-exposed animals have received appropriate vaccination. Only antibiotic sensitive strains of anthrax will be used on this study.

Agents Used in this Protocol – *Bacillus anthracis,* Ames strain

Other toxic chemicals to be used include sodium hypochlorite and vaporized hydrogen peroxide for decontamination requirements.

A Battelle Environment, Safety and Health Officer has been provided the opportunity to review the procedures required to execute this study.

10.0 REFERENCES

Battelle SOP Number BBRC. IV-002, "Standard Operating Procedure (SOP) for Monitoring Room Lighting in Animal Rooms."

Battelle SOP Number BBRC. IV-008, "Standard Operating Procedure (SOP) for Monitoring Temperature and Humidity Conditions Using Automated HVAC Control and Monitoring Systems."

Battelle SOP Number BBRC.V-033, "Standard Operating Procedure (SOP) for the Processing of Blood, Fecal or Urine Specimens Prior to Analysis."

Battelle SOP Number BBRC.V-061, "Standard Operating Procedure (SOP) for Performing the Rapid Protective Antigen Screening Assay using Serum."

Battelle SOP Number BBRC.VI-066, "Standard Operating Procedure (SOP) for the Operation and Maintenance of the Siemens (formerly Bayer) Advia®120 Hematology analyzer."

Battelle SOP Number BBRC.VI-077, "Standard Operating Procedure (SOP) for the Operation and Maintenance of the Siemens (formerly Bayer) Advia® 1200 Chemistry analyzer."

Battelle SOP Number BBRC.VI-086, "Standard Operating Procedure (SOP) for Use and Maintenance of the Data Sciences International (DSI) Telemetry System."

Battelle SOP Number BBRC. VII-002, "Standard Operating Procedure (SOP) for Feed Source, Storage, Handling, and Analysis."

Battelle SOP Number BBRC. VII-006, "Standard Operating Procedure (SOP) for Animal Euthanasia at the Battelle Biomedical Research Center (BBRC)."

Battelle SOP Number BBRC. VII-020, "Standard Operating Procedure (SOP) for the Collection of Blood Samples from Animals."

Battelle SOP Number BBRC. VII-010, "Standard Operating Procedure (SOP) for Clinical Observations of Animals at the Battelle Biomedical Research Center (BBRC)."

Battelle SOP Number BBRC. VII-011, "Standard Operating Procedure (SOP) for Receipt, Handling, Shipping, and Disposal of Test Materials, Analytical Samples and Controlled Substances."

Battelle SOP Number BBRC. VII-013, "Standard Operating Procedure (SOP) for Care of Rabbits."

Battelle SOP Number BBRC. VII-026, "Standard Operating Procedure for Receipt, Quarantine, Monitoring, and Release of Experimental Animals."

Battelle SOP Number BBRC. VII-040, "Standard Operating Procedure for Environmental Enhancement/Enrichment Plan to Promote the Physiological Well-Being of species other than Non-Human Primates."

Battelle SOP Number BBRC. VIII-003, "Standard Operating Procedure (SOP) for Supplying Water and Monitoring Water Quality of the Manual and Automatic Watering Systems."

Battelle SOP Number BBRC. X-096, "Standard Operating Procedure (SOP) for the Qualitative Analysis of Bacteria in Blood and Tissue."

Battelle SOP Number BBRC. X-054, "Standard Operating Procedure (SOP) for Enumeration of BL-2 and BL-3 Bacterial Samples via the Spread Plate Technique."

Battelle SOP Number BBRC. X-146 "Standard Operating Procedure (SOP) for Performing Quantitative Real-Time Poymerase Chain Reaction (qPCR) Using Reference Standard Materials."

Battelle SOP Number BBRC. X-165, "Standard Operating Procedure (SOP) for Performing Qualitative Real-Time Polymerase Chain Reaction (PCR)."

Battelle SOP Number BBRC. X-180, "Standard Operating Procedure (SOP) for Enzyme Linked Immunosorbent Assay (ELISA) Detection of *Bacillus anthracis* Circulating Protective Antigen in Sera."

Battelle SOP Number BBRC. X-202, "Standard Operating Procedure (SOP) the Enumeration of Bacteria via the Spread Plate Technique."

Battelle SOP Number BBRC. XIII-001, "Standard Operating Procedure (SOP) for the Aerosol Exposure System to Challenge Non-Human Primates and Rabbits to Aerosolized Agent."

Battelle SOP Number BBRC. X-146, "Standard Operating Procedure (SOP) for Performing Quantitative Real-Time Polymerase Chain Reaction (QPCR) Using Reference Standard Materials."

Battelle SOP Number BBRC. VI-044, "Standard Operating Procedure (SOP) for the Operation and Maintenance of the Biomedic Data System DAS-6007 Handheld Probe and the DAS-5002 Notebook Unit."

Battelle SOP Number BBRC. VII-040, "Standard Operating Procedure (SOP) for Environmental Enhancement/Enrichment Plan to Promote the Psychological Well-Being of Species Other Than Non-human Primates."

Battelle SOP Number BBRC. X-101, "Standard Operating Procedure (SOP) for Enzyme Linked Immunosorbent Assay (ELISA) Detection of *Bacillus anthracis* PA-Specific IgG in Sera."

Battelle SOP Number BBRC. X-143, "Standard Operating Procedure (SOP) for the High Throughput Toxin Neutralization Assay (htp-TNA) Proper."

Inglesby, T.V., Henderson, D.A., Bartlett, J.G., Ascher, M.S., Eitzen, E., Friedlander, A.M., Hauer, J., McDade, J., Osterholm, M.T., O'Toole, T., Parke, G., Perl, T.M., Russell, P.K., and K. Tonat. 1999. Anthrax as a biological weapon: medical and public health management. Working Group on Civilian Biodefense. JAMA. 281:1735-1745.

Zaucha, GM; Pitt, LM; Estep, J; Ivins, BE; and Friedlander, AM (1998). The pathology of experimental anthrax in rabbits exposed by inhalation and subcutaneous inoculation. Arch. Pathol. Lab. Med. 122: 982-992.

Rabbit Single Dose Anthrax Telemetry Study

BBRC Protocol Amendment No. #1

Change No. # 1

Used to read:

5.5 Priority usage of whole blood and sera:
Whole Blood: Bacteremia via quantitative plating >>> Hematology >>> Bacteremia via Quantitative PCR
Sera: Circulating PA ELISA >>> Retention sample (maximum of 750 µl if possible) >>> Clinical Chemistry >>> anti-PA IgG ELISA and TNA.

Now reads (changes in bold):

5.5 Priority usage of whole blood and sera:
Whole Blood: Bacteremia via quantitative plating >>> Hematology >>> Bacteremia via Quantitative PCR
Sera: Circulating PA ELISA >>> **Clinical Chemistry >>> Retention sample (maximum of 750 µl if possible)** >>> anti-PA IgG ELISA and TNA.

Reason for Change:
The client and study director decided that clinical chemistry was more important than the retention sample.

Impact on Study:
There is a positive impact on the study. Determining the clinical chemistry status of each animal will help identify biomarkers of disease.

Effective Date: 10/21/09

Approved By:

Jason E. Comer, Ph.D.
Study Director

10.23.09
Date

See attachment for signature

Sarah C. Taft, Ph.D.
Sponsor Representative

Date

Quality Assurance Review and Registration:

Charles D. Lawrie
Quality Process Coordinator

10/26/09
Date

Approved By:

Jason E. Comer, Ph.D.
Study Director

Date

Sarah C. Taft

Sarah C. Taft, Ph.D.
Sponsor Representative

10/22/2009
Date

Quality Assurance Review and Registration:

Charles D. Lawrie
Quality Process Coordinator

Date

APPENDIX B

STUDY DEVIATIONS

BATTELLE BIOMEDICAL RESEARCH CENTER
DEVIATION FORM

Deviation No. (Assigned by QAU): 7891	**CAQ No. (Assigned by QAU):** N/A

Standard or Procedure Deviated:

☒ **Protocol (Number):** 1020
☐ **SOP (Number):**
☐ **Method (Number):**
☐ **GLP (Section):**
☐ **Other:**

Type of Deviation (check one):

☐ **Facility**

☒ **Study (fill out study info)** **Study Number:** 1020-CG920503

Study Title: Rabbit Single Dose Anthrax Telemetry Study

Record Affected (describe Title, Binder name, location, Form no. etc.): Microbiology Binder; Form No Microbio-454 and Microbio-455

Date of Deviation(s): 9/19/09

Description of Deviation:
The filtered impinger samples will not be stained with dye.

Cause of Deviation:
Colonies are visible on the filters without the use of dye.

Corrective Action:
The staff members will count the colonies without the use of dye.

Impact of Deviation: None. The dye is not necessary to visualize the colonies.

If deviation is planned, effective date: 9/18/09

Deviation form Prepared by/Date: TRM 9/18/09

Deviation Reviewed and Corrective Action Accepted by/Date: JEC 9.23.09

Deviation Reviewed and Registered by QAU/Date: [signature] 9/25/09

BATTELLE BIOMEDICAL RESEARCH CENTER
DEVIATION FORM

Deviation No. (Assigned by QAU): ~~7890~~ 7893 WN MR 9/25/09 **CAQ No. (Assigned by QAU):** N/A

Standard or Procedure Deviated:

☐ **Protocol (Number):**
☒ **SOP (Number):** X-054
☐ **Method (Number):**
☐ **GLP (Section):**
☐ **Other:**

Type of Deviation (check one):

☐ **Facility**

☒ **Study (fill out study info)** **Study Number:** 1020-CG920503

Study Title: Rabbit Single Dose Anthrax Telemetry Study

Record Affected (describe Title, Binder name, location, Form no. etc.): Microbiology Binder; Form No Microbio-454 and Microbio-455

Date of Deviation(s): 9/18/09

Description of Deviation:
The irradiated spores (from the challenge suspension, pre-run, nebulizer samples, and impinger samples) will not be enumerated as per SOP X-054.

Cause of Deviation:
These spores are not viable as confirmed by testing conducted at the CDC. Enumeration of these samples should not result in colonies.

Corrective Action:
Each sample will be directly plated onto TSA. For the challenge suspension, 0.1 mL of the neat sample will be plated in quintuplicate. For the pre-run sample, the nebulizer sample (from each group 1 animal), and the impinger sample (from each group 1 animal), 0.1mL of each neat sample will be plated onto an individual plate.

Impact of Deviation: None. The spores are not viable and do not need to be diluted or plated for enumeration.

If deviation is planned, effective date: 9/18/09

Deviation form Prepared by/Date: TRM 9/18/09

Deviation Reviewed and Corrective Action Accepted by/Date: JEC 9.23.09

Deviation Reviewed and Registered by QAU/Date: [signature] 9/25/09

BATTELLE BIOMEDICAL RESEARCH CENTER
DEVIATION FORM

Deviation No. (Assigned by QAU): 8476 | **CAQ No. (Assigned by QAU):** 8100

Standard or Procedure Deviated:

☐ **Protocol (Number):**
☒ **SOP (Number): XI-009-02**
☐ **Method (Number):**
☐ **GLP (Section):**
☐ **Other:**

Type of Deviation (check one):

☐ **Facility**

☒ **Study (fill out study info)** **Study Number: 1020-CG920503**

Study Title: Rabbit Single Dose Anthrax Telemetry Study

Record Affected (describe Title, Binder name, location, Form no. etc.):
I. Rabbit Daily Room Activity Schedule; Form No. General-078
II. Weight and Clipping Record; Form No. Weightsht-013
III. Small Animal Daily Observation Sheet; Form No. MREF Animal Care-004
IV. Rabbit Clinical Observations (BID); Form No. obsRabbit-003
V. Collection, Transfer, and Storage of Whole Blood; Form No. Microbio-372
Vivo Binder

Date of Deviation(s):
I. 9/30/09
II. 9/18/09, 9/19/09, 9/23/09, 9/25/09, 10/9/09
III. 9/21/09
IV. 9/18/09,9/25/09, 9/27/09, 9/30/09
V. 9/19/09, 9/20/09, 9/21/09, 9/22/09, 9/23/09, 10/9/09

Description of Deviation:
SOP states, "For all records it must be absolutely clear what was done, when it was done, by whom it was done, who entered the documentation, and when it was entered."

Cause of Deviation:
I. The technician responsible for recording PM activity failed to record time, date, initials and check the appropriate boxes for activities performed.
II. On 9/18/09, the technician recording a correction for animal L23230 failed to use a proper footnote for a comment. On 9/19/09, the technician responsible for study activity failed to record date and initials verifying animals ID's, weighed by, checked sex, clipped by, and balance information on the form at the end of the procedures. On 9/23/09, the technician recording weight identification for calibration failed to properly finish a correction with a correction code, date, and initials. On 9/25/09 and 10/09/09, the technician responsible for study activity failed to record date and initials for weighing and checking the sex/ID of the animals at the time of the activity.
III. The technician responsible for AM observations for animals L23202 and L23209 failed to properly record the time on the sheet but it can be verified as 0730 from the daily activity schedule.

BATTELLE BIOMEDICAL RESEARCH CENTER
DEVIATION FORM

Deviation No. (Assigned by QAU): 8476	**CAQ No. (Assigned by QAU):** 8100

IV. On 9/18/09, the technician responsible for PM observations failed to record the date at the end of study activity for Groups 4, 5, and 6. On 9/25/09, the technician responsible for PM observations for Group 4 animals failed to record date and initials at the end of study activity. On 9/27/09, the technician responsible for AM observations failed to record their initials at the end of study activity.
V. On 9/16/09, the technicians responsible for the blood draw on animals L23217 and L23221 failed to finish a correction by recording date and initials for each instance. On 9/20/09, the technician responsible for the blood draw for animal L23213 failed to record a time the blood draw actually occurred. Also on 9/20/09, the technicians responsible for the terminal blood draw on animal L23221 failed to record date and initials for transferring and receiving the sample, verifying the label information and a time the sample was actually obtained. On 9/21/09, the technician responsible for the terminal blood draw on animal L23200 failed to record date and initials for transferring the sample. On 9/22/09, the technician responsible for the terminal blood draw for animals L23201 and L23232 failed to record the animals ID on the form. A time was recorded for each animal, and it is being assumed based on the previous order of the animals on blood sheets that L23201should have been recorded first at the 0838 time and then L23232 recorded second at the 0900 time. This is how the animals and times are being reported on the blood draw tables. On 9/23/09, the technician responsible for the terminal blood draw on animal L23203 failed to record a time the sample was actually obtained. On 10/9/09, the technician responsible for the blood draw on animal L23212 failed to record date and initials for the study activity.

Corrective Action:
Technicians were asked to review SOP XI-009 for documentation and proper procedures and reminded of the importance of recording and reviewing all study forms at the completion of study activity for accuracy and completeness.

Impact of Deviation:
Minimal. The technicians performed the necessary assignments to carry out the study activities properly but failed to record and/or verify all required information on the forms for accuracy and completeness at the end of each task.

If deviation is planned, effective date:

Deviation form Prepared by/Date: JJm 1/6/10

Deviation Reviewed and Corrective Action Accepted by/Date: JEC 1/11/10

Deviation Reviewed and Registered by QAU/Date: [signature] 1/11/10

BATTELLE BIOMEDICAL RESEARCH CENTER
DEVIATION FORM

Deviation No. (Assigned by QAU): 8477 | **CAQ No. (Assigned by QAU):** 8100

Standard or Procedure Deviated:

☒ **Protocol (Number): 1020**
☐ **SOP (Number):**
☐ **Method (Number):**
☐ **GLP (Section):**
☐ **Other:**

Type of Deviation (check one):

☐ **Facility**

☒ **Study (fill out study info)** **Study Number:1020-CG920503**

Study Title: Rabbit Single Dose Anthrax Telemetry Study

Record Affected (describe Title, Binder name, location, Form no. etc.):
Small Animal Daily Observation Sheet; Form No. MREF Animal Care-004,
Rabbit Clinical Observations (BID); Form No. obsRabbit-003,
Vivo Binder

Date of Deviation(s): 9/30/09, 10/9/09

Description of Deviation:
Protocol states, "Following challenge rabbits with be observed twice daily for clinical signs of illness and survivability due to anthrax infection (e.g., moribund, respiratory distress, appetite, activity, and seizures)."

Cause of Deviation:
On 9/30/09, the technician responsible for performing the PM observations failed to record the observations on the proper forms for the challenged animals as well as the remaining extra animals.
On 10/9/09, the technician responsible for performing the AM observations prior to euthanasia failed to record the observations on the proper forms.

Corrective Action:
Technicians were asked to review the protocol and documentation procedures. They were also reminded of the importance of recording and reviewing all study forms at the completion of study activity for accuracy and completeness.

Impact of Deviation:
Minimal. The animals that had a missed afternoon observation on 9/30/09 were all observed as normal the morning of 9/30/09 and the following morning on 10/1/09. The animals that had a missed AM observation on 10/9/09 were scheduled for euthanasia that morning and each animal was handled individually during that procedure so any adverse conditions would have been noted. The study was not negatively impacted in either instance.

If deviation is planned, effective date:

BATTELLE BIOMEDICAL RESEARCH CENTER DEVIATION FORM	
Deviation No. (Assigned by QAU): 8477	**CAQ No. (Assigned by QAU):** 8100
Deviation form Prepared by/Date: LRB 10-13-09	
Deviation Reviewed and Corrective Action Accepted by/Date: JTEC 11/11/10	
Deviation Reviewed and Registered by QAU/Date: [signature] 11/11/10	

BATTELLE BIOMEDICAL RESEARCH CENTER
DEVIATION FORM

Deviation No. (Assigned by QAU): 8581 | **CAQ No. (Assigned by QAU):** N/A

Standard or Procedure Deviated:

☐ **Protocol** (Number and Amendment No. if applicable):
☒ **SOP** (Number and Revision Number): X-072-09
☐ **Method** (Number and Revision Number):
☐ **GLP (Section):**
☐ **Other:**

Type of Deviation (check one):

☐ **Facility**

☒ **Study (fill out study info)** **Study Number:** 1020-CG920503

Study Title: Rabbit Single Dose Anthrax Telemetry Study

Record Affected (describe Title, Binder name, location, Form no. etc.): Microbiology Binder; Form No. Microbio-185, Microbio-454, and Microbio-455

Date of Deviation(s): 9/2/09, 9/3/09, and 9/4/09

Description of Deviation:
Per SOP X-072 (section V.E.), "Challenge spore suspensions may be prepared and aliquoted on the day of challenge (for non-aerosol challenges only)" and (section V.E.5) "The challenge suspension must be enumerated by 2 analysts at least one day prior to the day of the challenge for all aerosol challenges." The spray factor material with dilution IDs"1020SF-1e6", "1020SF-1e5" and "1020SF-1e4" was not prepared and enumerated at least 1 day prior to challenge.

Root Cause of Deviation:
It is preferred that samples at a concentration of $1x10^6$ cfu/mL or less are not stored overnight. The study director decided that it would be best to prepare these dilutions of the challenge material on the day of challenge.

Corrective Action:
The staff members prepared the spray factor material on the day of challenge and enumerated it twice (once by two different staff members).

Impact of Deviation: None. The challenge material described above was prepared on the day of challenge from the "1020SF 1e7" material prepared on 8/27/09, which had been enumerated prior to the day of challenge. The 3e4, 3e5 and 3e6 challenge material was then enumerated by two analysts the day of challenge. The enumerated values were accepted by the study director and aerosol director and spray factor calculations were determined based on these enumerated values.

If deviation is planned, effective date: n/a

Deviation Form Prepared by/Date: TRM 12.29.09

BATTELLE BIOMEDICAL RESEARCH CENTER **DEVIATION FORM**	
Deviation No. (Assigned by QAU): 8581	**CAQ No. (Assigned by QAU):** N/A
Deviation Reviewed and Corrective Action Accepted by/Date (Study Director or Responsible Individual): JEC 1.26.10	
Deviation Reviewed and Corrective Action Accepted by/Date (Supervisor, Supervisor Representative, or Group Manager): Circle One: Vivo, Micro, Mol Tox, Aerosol, Chemistry, BDS, MCB, Facility, QA, Study Management, Other KSS 1/26/10	
Deviation Reviewed and Registered by QAU/Date: Wharrie 1/27/2010 ~~Wharrie 1/26/2010~~ ①	☑ Category I ☐ Category II (See SOP XI-023 for details)

① Inadvertently signed before SD/Supervisor sign off; not registered @ this time. [illegible] 1/26/2010

BATTELLE BIOMEDICAL RESEARCH CENTER
DEVIATION FORM

Deviation No. (Assigned by QAU): 8582 | **CAQ No. (Assigned by QAU):** N/A

Standard or Procedure Deviated:

☐ **Protocol** (Number and Amendment No. if applicable):
☒ **SOP** (Number and Revision Number): X-054-06
☐ **Method** (Number and Revision Number):
☐ **GLP (Section):**
☐ **Other:**

Type of Deviation (check one):

☐ **Facility**

☒ **Study (fill out study info)** **Study Number:** 1020-CG920503

Study Title: Rabbit Single Dose Anthrax Telemetry Study

Record Affected (describe Title, Binder name, location, Form no. etc.): Microbiology Spray Factor Binder; Form No. Microbio-455

Date of Deviation(s): 9/2/09, 9/3/09, and 9/4/09

Description of Deviation:
Per SOP X-054 (section V.B.19), "if no dilutions result in a majority of plates containing between 25-250 colonies or none of the dilutions yield mean counts within the 25-250 range, the sample will be repeated". The following samples did not meet these criteria, but were not re-plated.

Spray Factor Date	Sample ID	Result (cfu/mL)
9/2/09	Pre-run	$1.00x10^1$
9/2/09	Day 1 Run 4-1e5	$1.00x10^2$
9/2/09	Day 1 Run 5-1e5	$1.22x10^2$
9/2/09	Day 1 Run 6-1e5	$1.88x10^2$
9/3/09	Pre-run	$1.80x10^1$
9/3/09	Day 2 Run 4-1e5	$1.80x10^1$
9/3/09	Day 2 Run 5-1e5	$3.80x10^1$
9/3/09	1020SF-1e7 (starting material)	0
9/4/09	Pre-run	$2.80x10^1$
9/4/09	Day 3 Run 4-1e5	$1.30x10^2$
9/4/09	Day 3 Run 5-1e5	$1.90x10^2$
9/4/09	Day 3 Run 6-1e5	$2.14x10^2$

Root Cause of Deviation:
Each sample except for "1020SF-1e7" is of such low concentration that additional enumeration would not yield counts within the acceptable range. The study director and aerosol director decided not to have these samples re-enumerated or plated using a filter method. It is unknown why the enumeration of sample "1020SF-1e7" did not yield any colonies. It appears that the sample was not appropriately diluted or plated.

BATTELLE BIOMEDICAL RESEARCH CENTER
DEVIATION FORM

Deviation No. (Assigned by QAU): 8582	**CAQ No. (Assigned by QAU):** N/A

Corrective Action:
A suggestion was made that samples anticipated to be very low in concentration be enumerated via X-054 and X-199 (at the study director's discretion) in attempts to obtain the best possible values which meet the acceptance criteria.

Impact of Deviation: Minimal. The pre-run sample is enumerated as a verification of the aerosol system and the enumeration values obtained are not utilized in any calculation. The enumeration values obtained from runs 4, 5, and 6 will be reported and utilized in the spray factor calculations. For the starting material from 9/3/09, the challenge enumeration from the previous day (7.86×10^6 cfu/mL) will be utilized in the spray factor calculations.

If deviation is planned, effective date: n/a

Deviation Form Prepared by/Date: HM 12.29.09

Deviation Reviewed and Corrective Action Accepted by/Date (Study Director or Responsible Individual): JEC 1.26.10

Deviation Reviewed and Corrective Action Accepted by/Date (Supervisor, Supervisor Representative, or Group Manager):
Circle One: Vivo, Micro, Mol Tox, Aerosol, Chemistry, BDS, MCB, Facility, QA, Study Management, Other KSE 1/26/10

Deviation Reviewed and Registered by QAU/Date: [signature] 1/27/2010

☑ Category I
☐ Category II
(See SOP XI-023 for details)

BATTELLE BIOMEDICAL RESEARCH CENTER
DEVIATION FORM

Deviation No. (Assigned by QAU): 8584 | **CAQ No. (Assigned by QAU):** N/A

Standard or Procedure Deviated:

☐ **Protocol** (Number and Amendment No. if applicable):
☒ **SOP** (Number and Revision Number): XI-009-02
☐ **Method** (Number and Revision Number):
☐ **GLP (Section):**
☐ **Other:**

Type of Deviation (check one):

☐ **Facility**

☒ **Study (fill out study info)** **Study Number:** 1020-CG920503

Study Title: Rabbit Single Dose Anthrax Telemetry Study

Record Affected (describe Title, Binder name, location, Form no. etc.): Microbiology Spray Factor Binder;

a. Form No. Microbio-380 and Microbio-455
b. Form No. Microbio-169
c. Form No. Microbio-454

Date of Deviation(s):

a. 9/2/09, 9/3/09, and 9/4/09
b. 8/31/09
c. 9/4/09

Description of Deviation:
Per SOP XI-009 (section V.A.5), study data, including materials used, must be recorded clearly, accurately, legibly, completely and promptly.

a. The materials used to filter the impinger samples (Runs 4, 5, and 6 (1e5)) were not documented. KEW 1.26.10
b. The date documented for the equipment/reagent verification is incorrect. KS 1/26/10 ①
c. The dilution scheme detailed for sample "1020SF – 1e6 (Day 3)" is not the dilution scheme actually created and plated. This form indicates that final dilutions plated were 10^3 through 10^5, when actually 10^4 through 10^6 were plated per Form No. Microbio-455. KS 1/26/10 ①

Root Cause of Deviation:

a. The technicians did not document this information.
b. The technician inadvertently documented 3/31/09 instead of 8/31/09.
c. The technician did not verify that the dilution scheme on Microbio-454 matched the "final dilutions plated" column on Microbio-455.

Corrective Action:

a. The technicians have been reminded to document all relevant materials used during a procedure and asked to review the SOP detailing documentation.
b. The technician was reminded to accurately document all dates and asked to review the SOP detailing documentation.

① Staff member is out of the office deviation acknowledged by supervisor

BATTELLE BIOMEDICAL RESEARCH CENTER
DEVIATION FORM

Deviation No. (Assigned by QAU): 8584	**CAQ No. (Assigned by QAU):** N/A

c. The technician was reminded to accurately document all relevant materials and to carefully verify all pre-typed information. The technician was also asked to review the SOP detailing documentation.

Impact of Deviation:

a. Minimal. The filters used for the procedure cannot be verified. The impinger samples were filter and reportable results were obtained.
b. Minimal. The equipment and reagents are accurately documented on the form. All other dates were accurately documented on the form.
c. Minimal. The sample was plated appropriately and a reportable result was obtained. 8.62×10^5 cfu/mL will be used in the spray factor calculations.

If deviation is planned, effective date: n/a

Deviation Form Prepared by/Date: TRM 1.21.10

Deviation Reviewed and Corrective Action Accepted by/Date (Study Director or Responsible Individual)**:** JEC 1.26.10

Deviation Reviewed and Corrective Action Accepted by/Date (Supervisor, Supervisor Representative, or Group Manager)**:**
Circle One: Vivo, Micro, Mol Tox, Aerosol, Chemistry, BDS, MCB, Facility, QA, Study Management, Other
JEC 1/26/10

Deviation Reviewed and Registered by QAU/Date: 1/27/2010

☒ Category I
☐ Category II
(See SOP XI-023 for details)

BATTELLE BIOMEDICAL RESEARCH CENTER
DEVIATION FORM

Deviation No. (Assigned by QAU): 8591	**CAQ No. (Assigned by QAU):** N/A

Standard or Procedure Deviated:

☐ **Protocol** (Number and Amendment No. if applicable):
☒ **SOP** (Number and Revision Number): **BBRC SOP XIII-001** At Revision # 002 1/27/2010
☐ **Method** (Number and Revision Number):
☐ **GLP** (Section):
☐ **Other:**

Type of Deviation (check one):

☐ **Facility**

☒ **Study (fill out study info)** **Study Number:** 1020-CG920503

Study Title: Rabbit Single Dose Anthrax Telemetry Study

Record Affected (describe Title, Binder name, location, Form no. etc.): Form No. Aerosol-010

Date of Deviation(s): 9/18/09

Description of Deviation: SOP XIII-001 requires that, "Readjust the exhaust pump valve (if needed) so the magnehelic gauge reads in the range of 0.0 to -0.2 inches of H2O" The reading was recorded as ≥ -.25"H_2O at various points.

Root Cause of Deviation: Magnehelic gauge actually reads from approximately 0.25 to -0.25"H_2O; when an animal's muzzle is in the chamber breathing, the point in time the reading was taken, the gauge was at the -0.25" mark. At approximately 5 minutes, this could have been due to the fact that the valve to the APS, for taking a sample, was also open at the time. When the valve is open, the vacuum in the chamber increases, and in turn caused the gauge to read more negative for the duration of the 10 second APS sample. Also, rabbits can cause the readings to be in this range simply by placing their head further into the box or by moving and creating a better seal.

Corrective Action: SOP will be reviewed/clarified to specify how to estimate the pressure when an animal is breathing; when an animal breathes, the needle moves back and forth over a certain range of pressure. This may occur at various points on the gauge itself and a median reading is taken.

Impact of Deviation: Minimal. The system is still operating at a negative pressure as is necessary for correct operation. Due to variations in animal respiration, it is difficult to avoid some readings over -0.20"H_2O.

If deviation is planned, effective date: N/A

Deviation Form Prepared by/Date: DAF 122909

Deviation Reviewed and Corrective Action Accepted by/Date (Study Director or Responsible Individual): JEC 1.27.10

Deviation Reviewed and Corrective Action Accepted by/Date (Supervisor, Supervisor Representative, or Group Manager):
Circle One: Vivo, Micro, Mol Tox, Aerosol, Chemistry, BDS, MCB, Facility, QA, Study Management, Other
Roy Barnewall 1/27/10

Deviation Reviewed and Registered by QAU/Date: M Laurie 1/27/2010	☑ Category I ☐ Category II (See SOP XI-023 for details)

BATTELLE BIOMEDICAL RESEARCH CENTER
DEVIATION FORM

Deviation No. (Assigned by QAU): 8612	**CAQ No. (Assigned by QAU):** 7886

Standard or Procedure Deviated:

☒ **Protocol** (Number and Amendment No. if applicable):
☐ **SOP** (Number and Revision Number):
☐ **Method** (Number and Revision Number):
☐ **GLP** (Section):
☐ **Other:**

Type of Deviation (check one):

☐ **Facility**

☒ **Study (fill out study info)** **Study Number: 1020-CG920503**

Study Title: Rabbit Single Dose Anthrax Telemetry Study

Record Affected (describe Title, Binder name, location, Form no. etc.):
Collection, Transfer, and Storage of Whole Blood; Form No. Microbio-372

Date of Deviation(s): 9/21/09

Description of Deviation: Protocol states The critical phase auditor observed technicians taking blood samples from the vascular access ports when they should have collected blood from the ear vein. "Blood samples will be collected from vascular access ports on Study Days -3, 1 and 2 for both the EDTA and SST collection tubes. After Day 2 blood collected in the EDTA tubes will be obtained from the medial auricular artery or the marginal ear vein." During a critical phase audit Z. Willenberg noted that the Day 3 bloods were collected from the vascular access ports. This cannot be verified in the study records as the blood collection sites are not captured on the form.

Root Cause of Deviation: The technicians did not review the protocol prior to the start of the Day 3 blood draws.

Corrective Action: The technicians were reminded to review the protocol prior to study related activities. Also the blood collection form will be reviewed to determine if the blood collection site should be captured on the paper work. DAP 1/29/10 mDE 1/29/10 DRD 1/29/10

Impact of Deviation: None. The reason for taking blood samples from the ear after Day 2 was to avoid false positive bacteremia results because of a colonized port. All animals with positive bacteremias on Day 3 succumbed to disease suggesting the positive results were from systemic infection and not a colonized port.

If deviation is planned, effective date: N/A

Deviation Form Prepared by/Date: JEC 2.1.10

Deviation Reviewed and Corrective Action Accepted by/Date (Study Director or Responsible Individual): JEC 2.1.10

Deviation Reviewed and Corrective Action Accepted by/Date (Supervisor, Supervisor Representative, or Group Manager):
Circle One: Vivo, Micro, Mol Tox, Aerosol, Chemistry, BDS, MCB, Facility, QA, Study Management, Other
CW 2/1/10

BATTELLE BIOMEDICAL RESEARCH CENTER DEVIATION FORM	
Deviation No. (Assigned by QAU): 8612	**CAQ No. (Assigned by QAU):** 7886
Deviation Reviewed and Registered by QAU/Date: Charles Laurie 2/1/2010	☑ Category I ☐ Category II (See SOP XI-023 for details)

APPENDIX C
BORDETELLA RESULTS

Charles River Research Animal Diagnostic Services
251 Ballardvale Street, Wilmington, MA 01887 USA
Tel: 800-338-9680 Fax: 978-658-7698

Sponsor: Battelle Memorial Institute **Accession #: 2009-040733**

Diagnostic Summary Report

1425 Plain City-Georgesville Rd.
State Rt. 142
West Jefferson, OH 43162 USA

Attn: Jason Comer

Tel: 614-424-5825

Received: 15 Sep 2009
Approved: 18 Sep 2009, 11:14

Bill Method: PO# V114984001710
Test Specimen: Respiratory

Sample Set	Service (# Tested)	Profile	Assay	Tested	+	+/-	?
#1	Bacteriology (34)	Bordetella Screen - Respiratory	***B. bronchiseptica***	34	**9**	0	0

+ = Positive, +/- = Equivocal, ? = Indeterminate

Summary Remarks

BACTERIOLOGY
Bordetella bronchiseptica has not been demonstrated to be pathogenic in rabbits, and is considered to be of no clinical consequence in immunocompetent animals.

Service Approvals

Service	Approved By*	Date
Bacteriology	Richard D. Fister	18 Sep 2009, 11:14

To assure the SPF status of your research animal colonies, it is essential that you understand the sources, pathobiology, diagnosis and control of pathogens and other adventitious infectious agents that may cause research interference. We have summarized this important information in infectious agent **Technical Sheets**, which you can view by visiting http://www.criver.com/info/disease_sheets.

**This report has been electronically signed by laboratory personnel. The name of the individual who approved these results appears in the header of this service report. All services are performed in accordance with and subject to General Terms and Conditions of Sale found in the Charles River Laboratories-Research Models and Services catalogue and on the back of invoices.*

Charles River Research Animal Diagnostic Services
251 Ballardvale Street, Wilmington, MA 01887 USA
Tel: 800-338-9680 Fax: 978-658-7698

Sponsor: Battelle Memorial Institute **Accession #: 2009-040733**

Product: Not Indicated **Test Specimen:** Respiratory **Received:** 15 Sep 2009

Bacteriology Results Report

Department Review: Approved by Richard D. Fister, 18 Sep 2009, 11:14*

Bordetella Screen - Respiratory

Sample #: / Code :	1 L23226	2 L23216	3 L23218	4 L23215	5 L23222	6 L23223	7 L23206	8 L23210	9 L23219	10 L23211
B. bronchiseptica	-	2	1	-	-	-	1	-	-	-
Other	-	-	-	-	-	-	-	-	-	-

Sample #: / Code :	11 L23217	12 L23230	13 L23228	14 L23232	15 L23229	16 L23235	17 L23213	18 L23225	19 L23231	20 L23207
B. bronchiseptica	-	-	2	-	-	-	-	1	1	2
Other	-	-	-	-	-	-	-	-	-	-

Sample #: / Code :	21 L23201	22 L23234	23 L23212	24 L23200	25 L23214	26 L23204	27 L23203	28 L23205	29 L23221	30 L23227
B. bronchiseptica	1	-	-	-	1	-	-	-	-	-
Other	-	-	-	-	-	-	-	-	-	-

Sample #: / Code :	31 L23220	32 L23223	33 L23202	34 L23209
B. bronchiseptica	-	-	-	-
Other	-	-	-	-

Remarks:
- = Negative/No Growth; 1 = Rare/Few Colonies; 2 = Several Colonies; 3 = Moderate Growth; 4 = Heavy Growth;NI = Not Interpreted: culture could not be interpreted due to overgrowth of Proteus; NT = Not Tested.

**This report has been electronically signed by laboratory personnel. The name of the individual who approved these results appears in the header of this service report.*

APPENDIX D
SPRAY FACTOR REPORT

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List of Acronyms

APS ..... aerodynamic particle sizer
BBRC ..... Battelle Biomedical Research Center
BCS III ..... Class III biological safety cabinet
CFU ..... colony forming unit
cm ..... centimeter
d ..... diameter
GSD ..... geometric standard deviation
HEPA ..... high efficiency particulate air
InD ..... inhaled dose
LD_{50} ..... median lethal dose
L ..... liter
MFC ..... mass flow controller
min ..... minute
mL ..... milliliter
mm ..... millimeter
MMAD ..... mass median aerodynamic diameter
SOP ..... standard operating procedure
SF ..... spray factor
TSA ..... tryptic soy agar
µm ..... micrometer

1. Introduction

The large animal exposure system at the Battelle Biomedical Research Center (BBRC) has been developed by Battelle and used extensively in aerosol studies to deliver high doses of *Bacillus anthracis* spores to animals ($\geq 1.1 \times 10^7$ colony forming units [CFU] inhaled). The purpose of this Spray Factor characterization study was to determine whether aerosol concentrations could be achieved in the system that would allow for low doses of spores (10^2-10^4 CFU inhaled) to be delivered to rabbits.

2. Methods

The aerosol system performance characteristics were evaluated by aerosolizing four target nebulizer concentrations of *B. anthracis* spores multiple times over a three day period. The target nebulizer concentrations tested over the three days were 1.0 $x10^4$, 1.0 $x10^5$, 1.0 $x10^6$, and 1.0 $x10^7$CFU/milliliter (mL). Specifically, each concentration was tested a total of a total of nine times each (three times per day over three days). Each test consisted of a 10-minute (min) period of aerosol generation and sample collection. The overall mean SF was determined. A schematic of the exposure system is shown in Figure 1.

The Spray Factor (SF) is a numeric correlation between nebulizer concentration and the resulting generated aerosol concentration. The SF is calculated by dividing the aerosol concentration by the starting nebulizer suspension concentration and is used to predict aerosol concentration for a given starting suspension concentration. The formula for determining the Spray Factor is presented below (Equation1):

$$\text{Spray Factor} = \frac{\dfrac{\text{Impinger Conc. (CFU/mL) x Impinger Volume (mL)}}{\text{Impinger Sample Rate (L/min) x Test Duration (min)}}}{\text{Nebulizer Conc (CFU/mL) x 1000 mL/L}} = \frac{\text{Aerosol Conc (CFU/L)}}{\text{Nebulizer Conc (CFU/L)}} \quad (1)$$

Once the SF is determined, the value can be used for future studies to predict how many liters of aerosol an animal must inhale to achieve a particular intended dose. In this case, the value was used in study protocol 1020 which targeted inhaled doses of 1 $x10^2$, 1 $x10^3$, 1 $x10^4$, and 1 $x10^5$ CFU.

The practical application of the SF is that during an aerosol exposure when the nebulizer concentration is known the aerosol concentration that will be produced can be calculated and the amount an animal must inhale to reach the desired target dosage can be be determined (breathing rate unknown). For example:

- A target total inhaled dosage of 100 CFU is chosen
- An exposure period is unknown due to individual animal breathing rate and volumes
- The starting nebulizer concentration is 1.66 x 10^4 CFU /mL
- Given that the aerosol concentration equation is:
  $Conc_{(aerosol)}$ CFU/L = SF x $Conc_{(nebulizer)}$ CFU/L

- Given that the SF in this study was determined experimentally to be 4.58×10^{-7}
  $Conc_{aerosol} = 1.66 \times 10^{4}$ CFU/L x 1000 mL/L x (4.58×10^{-7})
  $Conc_{aerosol}$ = 7.6 CFU/L
- The target inhaled volume = 100 CFU/7.6 CFU/L = 13 L to inhale.

3. Experimental Setup and Test Conditions

The Battelle large animal exposure system can be divided into five subsystems. Below is a description of each subsystem.

3.1 Aerosol Generation Subsystem

Air was supplied to the system by an in-house air system filtered through two high efficiency particulate air (HEPA) capsule filters and a carbon filter. The air was split into dilution airflow of approximately 8.6 L/min and nebulizer bypass airflow of approximately 7.5 L/min, maintained by mass flow controllers (MFC). The dilution air was humidified via a bubbler as needed to maintain humidity within a range of 52% - 97%. A modified microbiological research establishment type three-jet Collison nebulizer (BGI, Waltham, MA) with a precious fluid jar was used to generate a controlled delivery of aerosolized *B. anthracis* spores (spore lot Ames B35) from a liquid suspension. These nebulizers are designed to generate aerosols having an approximate mean diameter of 1-2 micrometers (μm). Each nebulizer was characterized for a pressure that results in an approximately 7.5 L/min flow, which is approximately 25-36 pounds per square inch, Collison nebulizer dependant.

3.2 Delivery Subsystem

After the agent aerosol was generated by the Collison nebulizer, it exited the Collison and traveled down a 3.75 centimeter (cm) diameter, 40 cm long cylinder (mixing tube) that mixed and dried the aerosol with dilution air. The aerosol then entered the top of the exposure chamber through another cylinder with a tapered 14 cm long slit on each side. The total airflow entering the exposure chamber was approximately 16 L/min. The aerosol entered the chamber through these slits to fill the exposure chamber, washed over the exposure target (muzzle or head), and was then exhausted out of the exposure chamber through another cylinder at the bottom that contained slots on two sides, each 19.5 cm in length. The aerosol was pulled through the chamber using a vacuum pump that maintained a slight negative pressure (from -0.18 to -0.01 inches of water) within the exposure chamber, as measured using a differential pressure gauge (magnehelic). The exhaust aerosol was filtered by two HEPA cartridge filters before exiting the system.

3.3 Exposure Chamber

The exposure chamber was a plexiglass box with internal dimensions of approximately 20.5 x 20.5 x 40 cm (length x width x height). A port approximately 15 cm in diameter was located on one side of the chamber where an animal's head or muzzle entered into the exposure chamber. Rubber dental dam material was stretched across the opening and held in place with an o-ring gasket. The animal's head or muzzle was pushed through a small hole in the dental dam, producing a seal to decrease leakage around the opening. Four additional ports are located in the chamber: two ports for collection of aerosol samples (one for enumeration and one for aerosol particle sizing), one port to measure temperature and humidity, and one port to measure the differential pressure within the exposure chamber in relation to the surrounding atmosphere within the Class III biological safety cabinet (BSC III). Thus, the sampling from the impinger and aerodynamic particle sizer (APS) spectrometer and exposure of the animal all occur from the same chamber. The aerosol system was operated within a self-contained BSC III.

3.4 Sampling/Monitoring

Aerosol concentration and aerosol particle size distribution were determined by analysis of atmospheric samples drawn from the exposure chamber. The atmospheric samples were collected in an impinger (Model 7541, Ace Glass Inc.) filled with approximately 20 mL of sterile water that sampled at approximately 6.0 ±0.3 L/min. The sampling rate was achieved by maintaining a vacuum of ≥ 18 inches Hg across the exhaust connection of the impinger to maintain the flow from the impinger critical orifice. The liquid in the impinger was diluted and enumerated by the spread plate technique to quantify viable spore counts per milliliter. Concentrations are reported in terms of CFU/mL. Enumeration results, along with the volume of liquid in the impinger, sampling rate, and sampling duration, are used in the calculation of the aerosol concentration expressed as CFU/L of air.

The aerosol particle size was determined during each test using an APS spectrometer, which draws an atmospheric sample from the exposure chamber at 0.25 L/min with a diluter (1.0 L/min total with 0.75 L/min from the diluter and 0.25 L/min from the exposure chamber). An APS was used because of its advantages over other methods. These advantages include near real-time data

measurements, aerodynamic diameter measurements, ease of instrument operation, and the generation of electronic data that is easy to process and export to a report.

4. Inhalation Results

The inhalation exposure system data for each exposure were documented on appropriate forms to ensure proper system operation and to provide the needed information to quantify animal challenge conditions. Impinger sampling conditions and enumerated concentration results provided viable bioaerosol challenge concentration while plethysmography measurements documented the total inhaled volume. Total inhaled dose (CFU) was calculated from aerosol concentration and total inhaled volume. Tables 1 through 3 show the daily aerosol testing results. The mean SF value from all testing was 4.58×10^{-7}. The number of median lethal dose equivalents (LD_{50} value) was calculated by dividing the total inhaled dose by the reported inhalation LD_{50} for each particular species of animal. The reported LD_{50} value for rabbits is 105,000 CFU (Zaucha et al., 1998) and for Rhesus macaques is 55,000 CFU (Ivins et al., 1998).

4.1 Impinger Sample Analysis

Impinger samples were enumerated by the spread plate method following serial dilutions to determine viable spore concentration. Diluted samples were mixed in a capped vial prior to subsequent dilutions. At different target dilutions, 0.1 mL was spread onto each of five tryptic soy agar (TSA) plates, which were placed in a secondary container and incubated at the appropriate temperature for the appropriate time. After the incubation period, the plates were enumerated to determine the number of colonies on each plate. Impinger sample concentration (C) was determined using the equation below (Equation 2):

$$C = (A \cdot D) / 0.1\ mL \qquad (2)$$

C = CFU/mL (impinger sample concentration)
A = average CFU per plate
D = dilution factor (serial dilution with countable colonies)

4.2 Inhaled Dose Calculation

The total inhaled dose (InD) was calculated from the impinger sample concentration, sampling parameters, and exposure duration according to the equation below. This equation assumes 100 % impinger sampling efficiency, which is based on evidence from previous studies in other laboratories. The total number of viable spores captured during each exposure was the product of

the impinger concentration and the impinger volume (C x V). The total number of viable organisms was divided by the amount that was sampled through the impinger during the exposure time (S x T). The aerosol concentration was (C x V) x $(S \times T)^{-1}$. The inhaled dose was the product of the aerosol concentration multiplied by the total accumulated tidal volume (see Equation 3 below):

$$InD = (C \times V)\,(S \times T)^{-1}(TATV) \qquad (3)$$

InD = inhaled dose (CFU)
C = Impinger concentration (CFU/mL)
V = Impinger sampler volume (mL)
S = sampling rate (~6 L/min)
T = Exposure time (min)
TATV = Total accumulated tidal volume (L)

5. Particle Size Results

The aerodynamic size of aerosol particles primarily dictates aerosol transport characteristics, and in the case of inhalation studies, the sites of lung deposition. The aerodynamic equivalent diameter is the diameter of a sphere, with density = 1 g/cm^3, that has the same terminal settling velocity as the aerosol being evaluated. For inhalation exposures, the mass median aerodynamic diameter (MMAD) of the aerosol is typically reported along with the geometric standard deviation. Aerosol size distribution plays a critical role in inhalation studies. The biological effects of inhaled aerosols can be dependent upon the sites and degree of deposition within the respiratory tract. Further, the size and shape of inhaled aerosols is a critical factor in determining deposition mechanisms and the extent of penetration into the lung and alveolar regions. As a general rule, aerosols with aerodynamic particle sizes ≤5 μm are desired for inhalation studies. Above this size, a larger portion of the aerosol is deposited in the upper respiratory tract (Hinds, 1999). It is important to know the aerosol particle size since large particles containing bacterial organisms deposited in the upper respiratory tract may not cause disease, or may require a higher quantity (dosage) to cause disease or may cause only an upper respiratory disease. Therefore, if the objective is to maximize deep lung deposition, then an aerosol with a size on the order of 1 to 5 μm (or lower, as opposed to larger aerosols) is desired.

Figures 2 through 5 show log probability plots representing the average of all APS particle size distributions obtained for each concentration tested over the three days of testing. The MMAD and geometric standard deviation (GSD) are also shown.

The MMAD for the log probability plot (Figure 2) was determined from averaging the cumulative median size (50% mass) from the aerosol size distributions obtained from the APS for all aerosol exposures (Equation 4). The GSD was determined from taking the cumulative average of the GSD calculated by the APS for each exposure test. The GSD represents one standard deviation for a normal distribution, and is determined by the following equation:

$$GSD = d84\%/d50\% \quad (4)$$

Where d84% is the particle size diameter in μm (d) at a cumulative % mass of 84% and d50% is the particle size diameter (d) at a cumulative mass of 50% (Hinds, 1999).

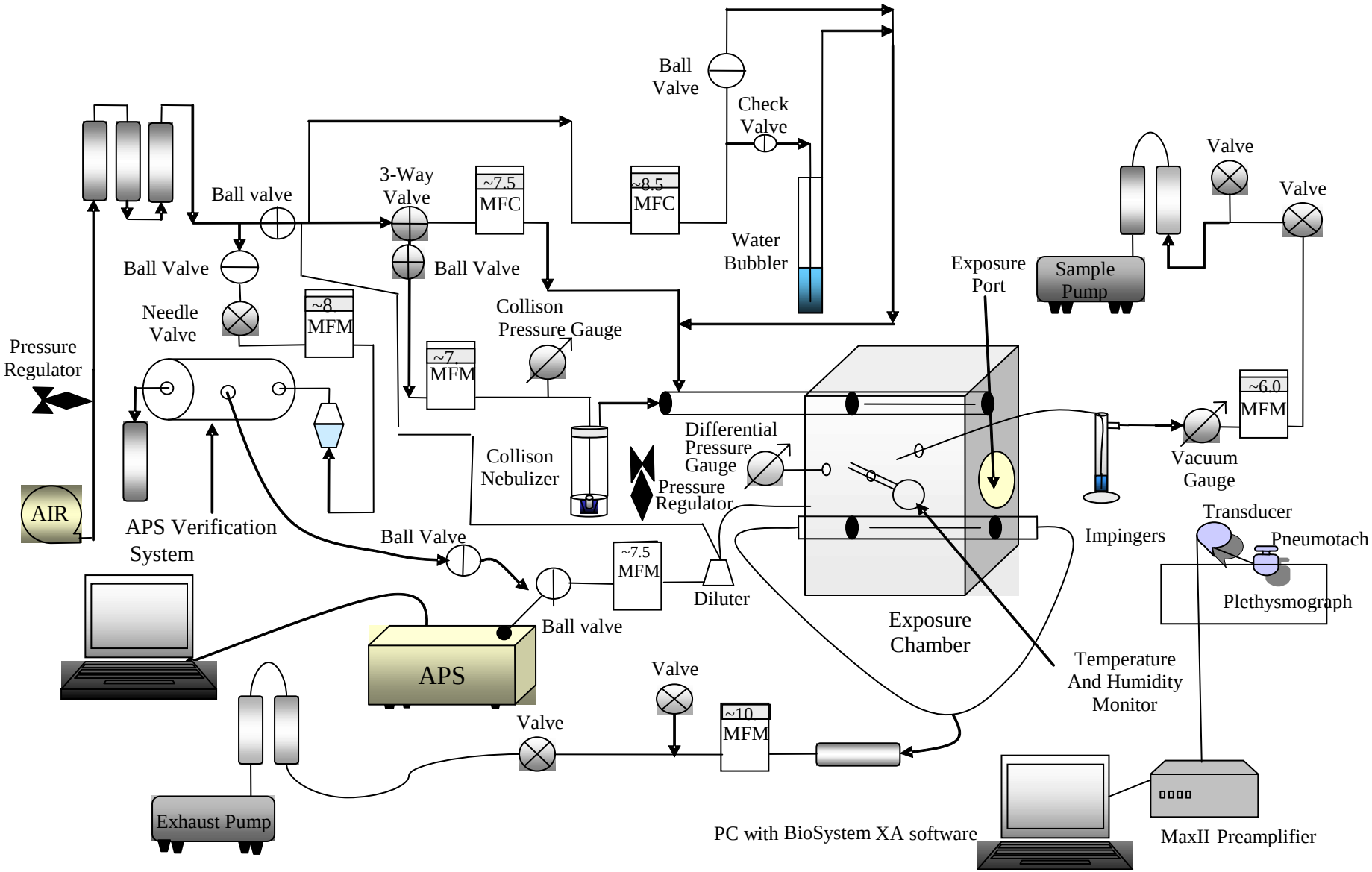

Figure 1. Exposure system diagram. APS, aerodynamic particle sizer; MFC, mass flow controller; MFM, mass flow meter

Table 1. Low Dose Spray Factor, (09-02-09)

Neb (CFU/mL)	Impinger (CFU/mL)	Impinger Volume (mL)	Impinger (Total CFU)	Sample Rate (L/min)	Sample Time (min)	Aerosol (CFU/L)	Total Volume inhaled (L)	Mortality	Spray Factor	Sample ID Run
8.55E+03	8.40E+00	19.00	1.60E+02	6.00	10.00	3	NA	NA	3.11E-07	1
8.55E+03	1.36E+01	19.00	2.58E+02	6.00	10.00	4	NA	NA	5.04E-07	2
8.55E+03	1.58E+01	18.50	2.92E+02	6.00	10.00	5	NA	NA	5.70E-07	3
8.17E+04	1.00E+02	19.20	1.92E+03	6.00	10.00	32	NA	NA	3.92E-07	4
8.17E+04	1.22E+02	19.20	2.34E+03	6.00	10.00	39	NA	NA	4.78E-07	5
8.17E+04	1.88E+02	19.20	3.61E+03	6.00	10.00	60	NA	NA	7.36E-07	6
7.03E+05	5.98E+02	19.00	1.14E+04	6.00	10.00	189	NA	NA	2.69E-07	7
7.03E+05	1.11E+03	18.00	2.00E+04	6.00	10.00	333	NA	NA	4.74E-07	8
7.03E+05	1.16E+03	19.00	2.20E+04	6.00	10.00	367	NA	NA	5.23E-07	9
7.86E+06	6.90E+03	19.00	1.31E+05	6.00	10.00	2185	NA	NA	2.78E-07	10
7.86E+06	7.64E+03	18.80	1.44E+05	6.00	10.00	2394	NA	NA	3.05E-07	11
7.86E+06	8.92E+03	19.00	1.69E+05	6.00	10.00	2825	NA	NA	3.59E-07	12

NA Not applicable

Table 2. Low Dose Spray Factor, (09-03-09)

Neb (CFU/mL)	Impinger (CFU/mL)	Impinger Volume (mL)	Impinger (Total CFU)	Sample Rate (L/min)	Sample Time (min)	Aerosol (CFU/L)	Total Volume inhaled (L)	Mortality	Spray Factor	Sample ID Run
8.55E+03	8.40E+00	19.00	1.60E+02	6.00	10.00	3	NA	NA	3.11E-07	1
8.55E+03	1.36E+01	19.00	2.58E+02	6.00	10.00	4	NA	NA	5.04E-07	2
8.55E+03	1.58E+01	18.50	2.92E+02	6.00	10.00	5	NA	NA	5.70E-07	3
8.17E+04	1.00E+02	19.20	1.92E+03	6.00	10.00	32	NA	NA	3.92E-07	4
8.17E+04	1.22E+02	19.20	2.34E+03	6.00	10.00	39	NA	NA	4.78E-07	5
8.17E+04	1.88E+02	19.20	3.61E+03	6.00	10.00	60	NA	NA	7.36E-07	6
7.03E+05	5.98E+02	19.00	1.14E+04	6.00	10.00	189	NA	NA	2.69E-07	7
7.03E+05	1.11E+03	18.00	2.00E+04	6.00	10.00	333	NA	NA	4.74E-07	8
7.03E+05	1.16E+03	19.00	2.20E+04	6.00	10.00	367	NA	NA	5.23E-07	9
7.86E+06	6.90E+03	19.00	1.31E+05	6.00	10.00	2185	NA	NA	2.78E-07	10
7.86E+06	7.64E+03	18.80	1.44E+05	6.00	10.00	2394	NA	NA	3.05E-07	11
7.86E+06	8.92E+03	19.00	1.69E+05	6.00	10.00	2825	NA	NA	3.59E-07	12

NA Not applicable

Table 3. Low Dose Spray Factor, (09-04-09)

Neb (CFU/mL)	Impinger (CFU/mL)	Impinger Volume (mL)	Impinger (Total CFU)	Sample Rate (L/min)	Sample Time (min)	Aerosol (CFU/L)	Total Volume inhaled (L)	Mortality	Spray Factor	Sample ID Run
8.55E+03	8.40E+00	19.00	1.60E+02	6.00	10.00	3	NA	NA	3.11E-07	1
8.55E+03	1.36E+01	19.00	2.58E+02	6.00	10.00	4	NA	NA	5.04E-07	2
8.55E+03	1.58E+01	18.50	2.92E+02	6.00	10.00	5	NA	NA	5.70E-07	3
8.17E+04	1.00E+02	19.20	1.92E+03	6.00	10.00	32	NA	NA	3.92E-07	4
8.17E+04	1.22E+02	19.20	2.34E+03	6.00	10.00	39	NA	NA	4.78E-07	5
8.17E+04	1.88E+02	19.20	3.61E+03	6.00	10.00	60	NA	NA	7.36E-07	6
7.03E+05	5.98E+02	19.00	1.14E+04	6.00	10.00	189	NA	NA	2.69E-07	7
7.03E+05	1.11E+03	18.00	2.00E+04	6.00	10.00	333	NA	NA	4.74E-07	8
7.03E+05	1.16E+03	19.00	2.20E+04	6.00	10.00	367	NA	NA	5.23E-07	9
7.86E+06	6.90E+03	19.00	1.31E+05	6.00	10.00	2185	NA	NA	2.78E-07	10
7.86E+06	7.64E+03	18.80	1.44E+05	6.00	10.00	2394	NA	NA	3.05E-07	11
7.86E+06	8.92E+03	19.00	1.69E+05	6.00	10.00	2825	NA	NA	3.59E-07	12

NA Not applicable

1x10^{4}cfu/mL Spray Factor log probability size distribution plot.

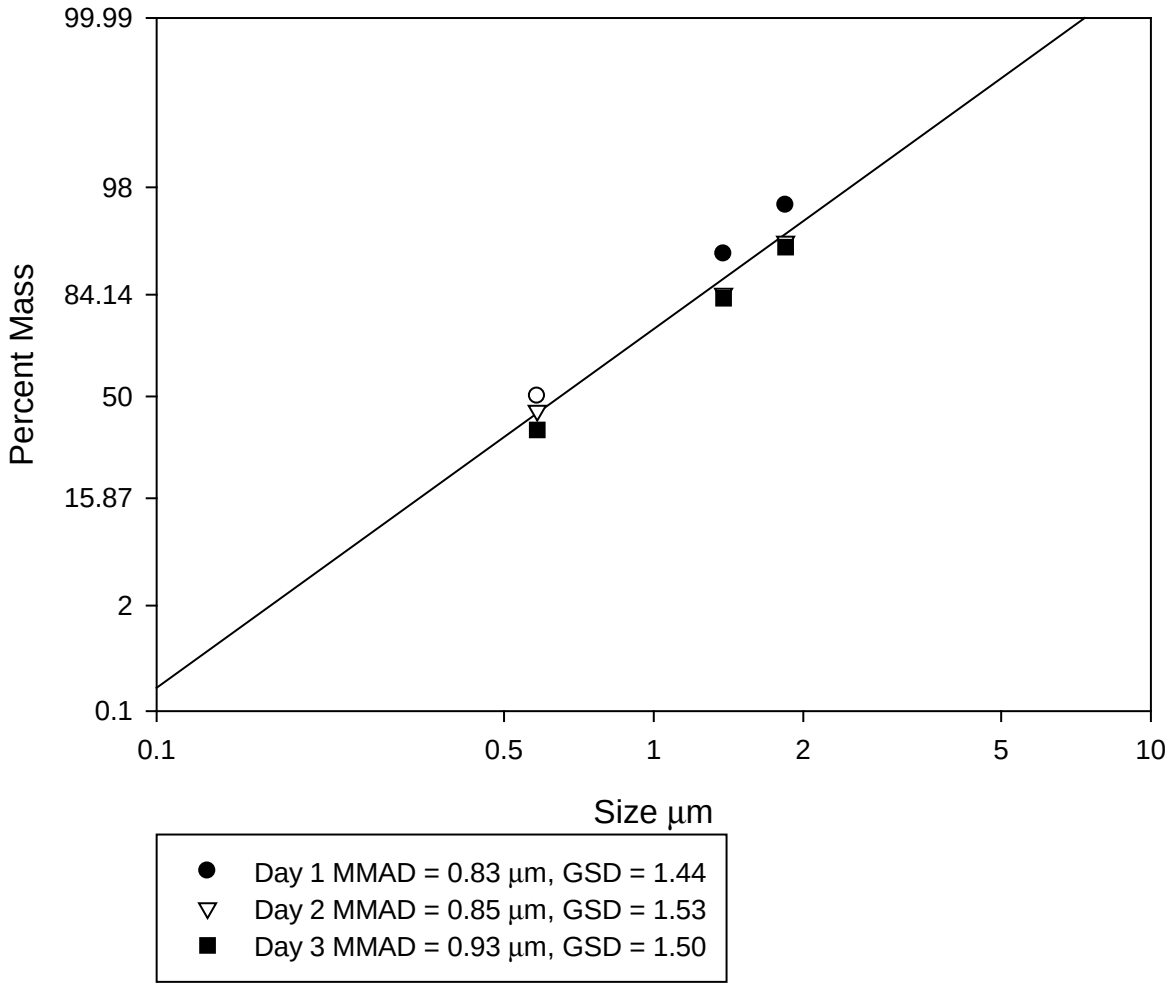

Figure 2. 1x10^{4}CFU/mL Spray factor log probability size distribution plot.

MMAD, mass median aerodynamic diameter; GSD, geometric standard deviation

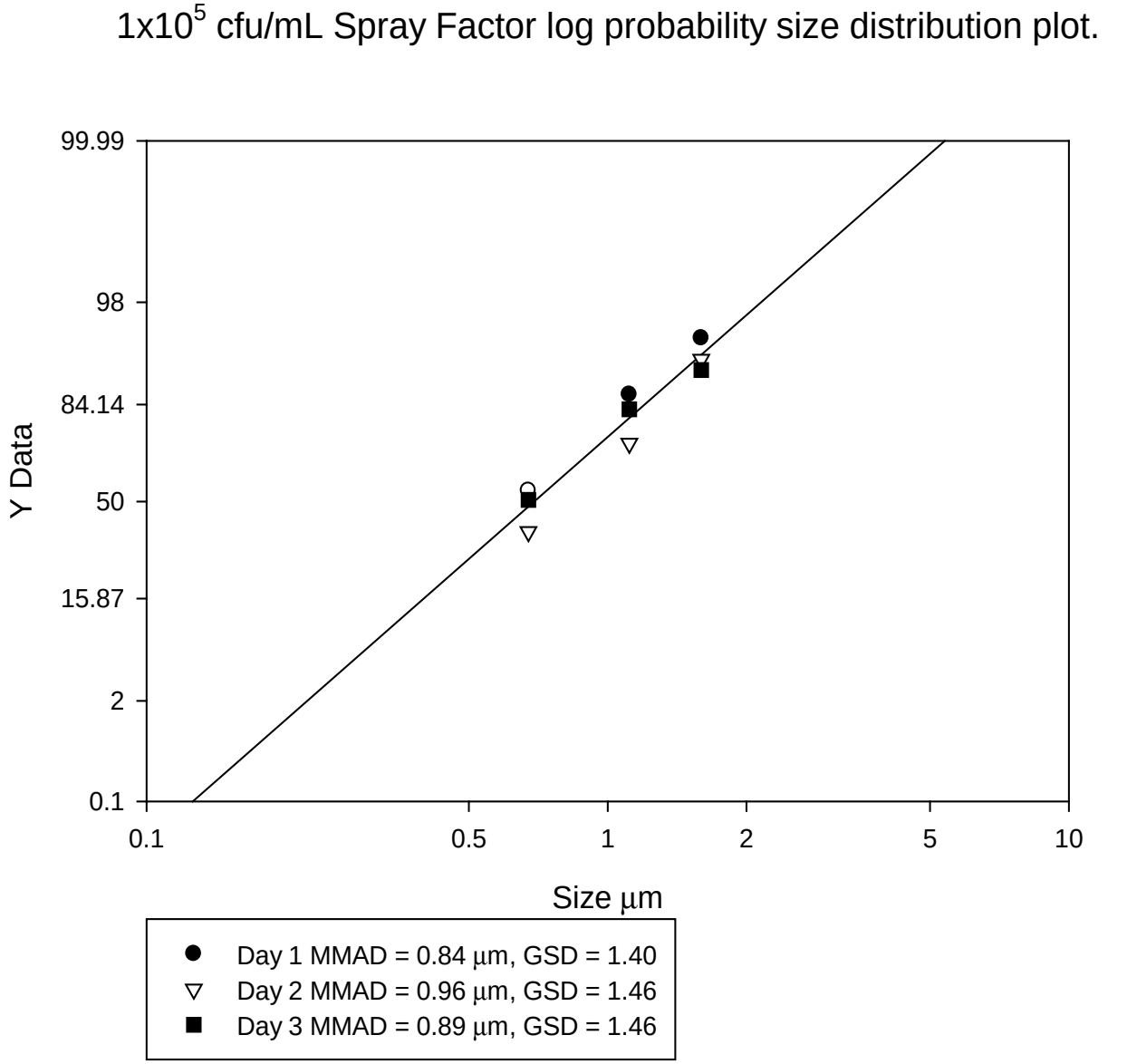

Figure 3. 1x10^{5}CFU/mL Spray factor log probability size distribution plot.

MMAD, mass median aerodynamic diameter; GSD, geometric standard deviation

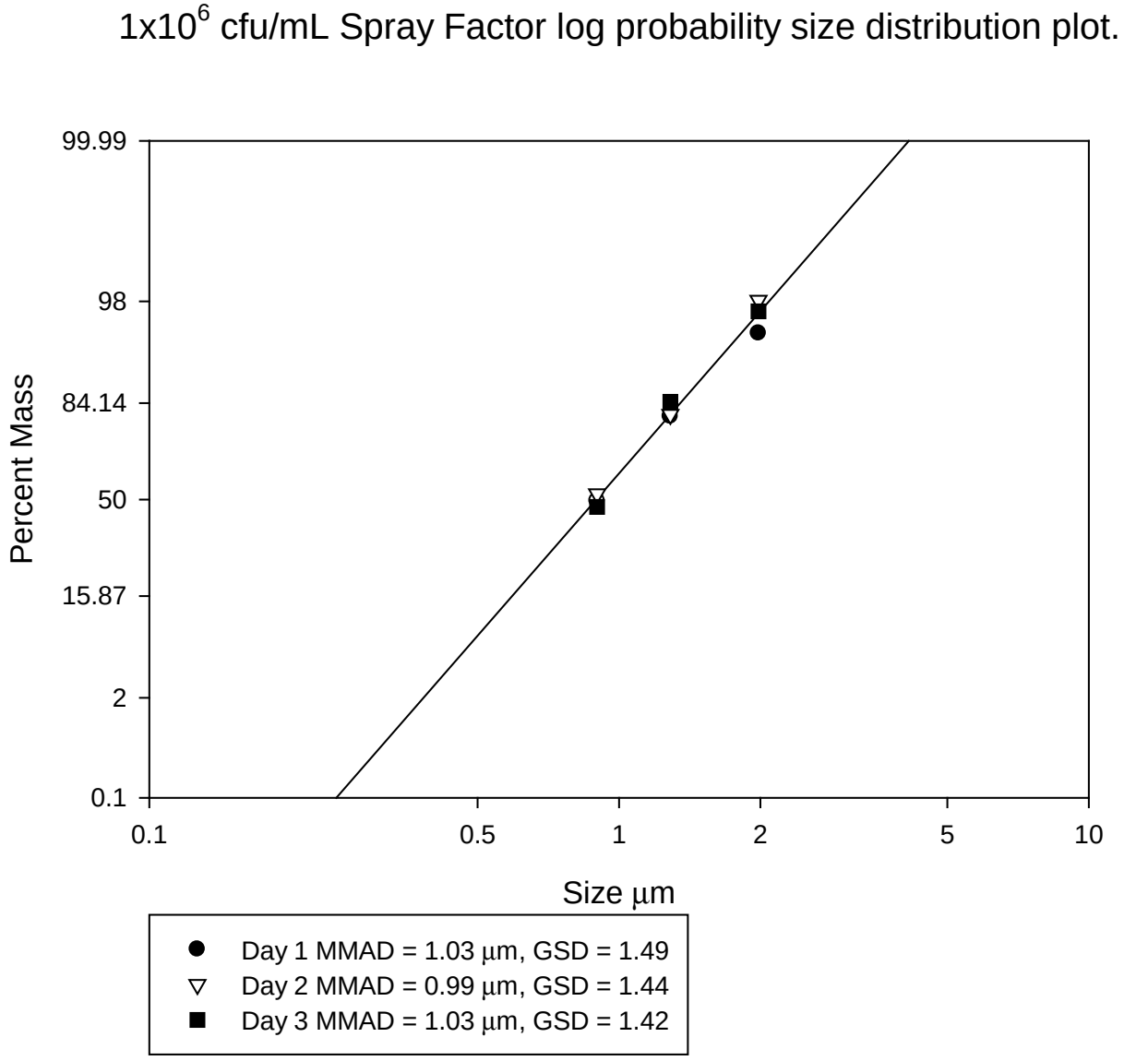

Figure 4. 1x10^{6}CFU/mL Spray factor log probability size distribution plot.

MMAD, mass median aerodynamic diameter; GSD, geometric standard deviation

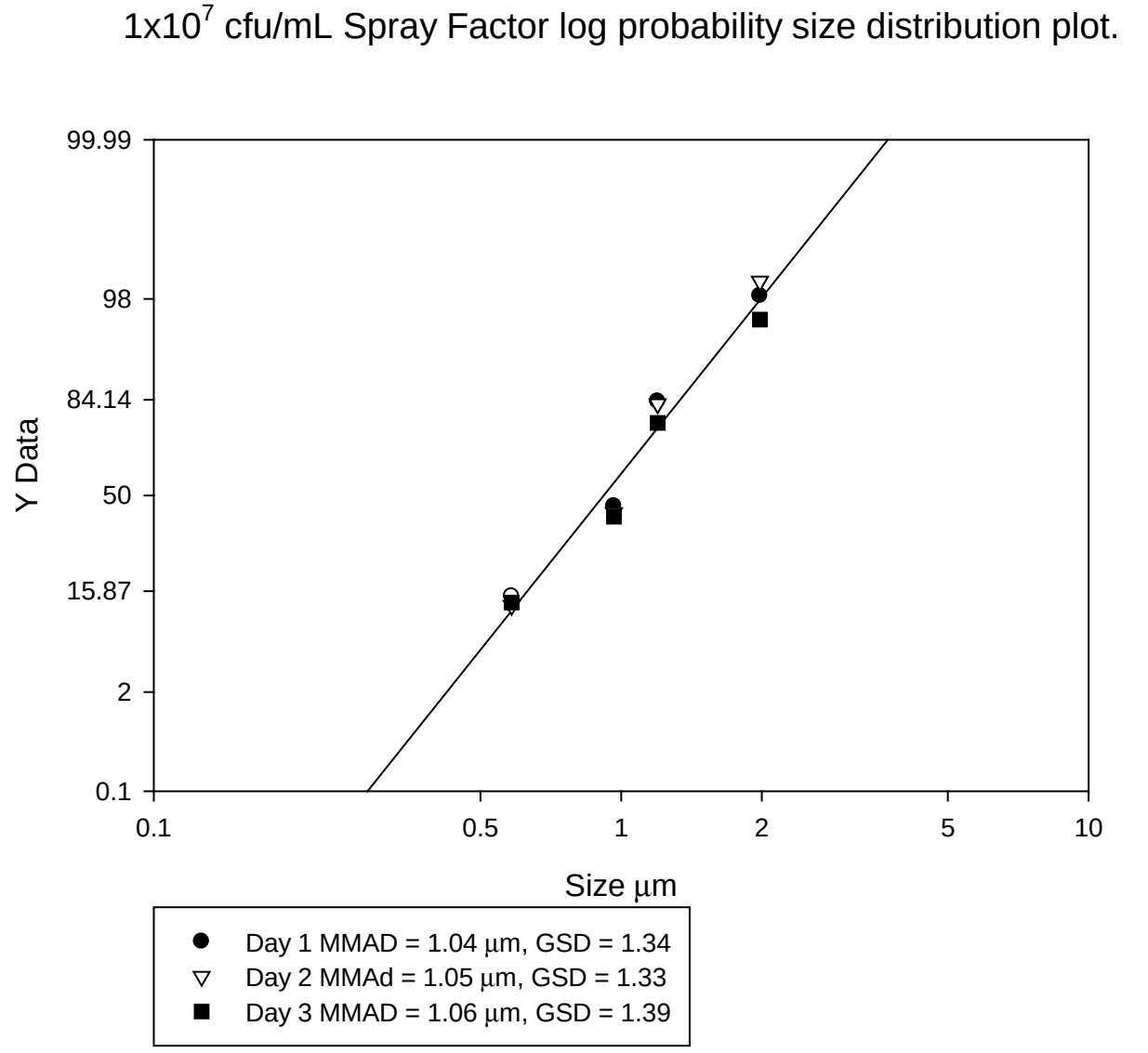

Figure 5. 1x10^{7}CFU/mL Spray factor log probability size distribution plot.

MMAD, mass median aerodynamic diameter; GSD, geometric standard deviation

6. Conclusions

This testing determined that the BBRC's large animal aerosol system is capable of generating aerosol concentrations that will allow for low doses of spores (10^2-10^4 CFU inhaled) to be delivered to rabbits.

7. References

1. Hinds, William C. 1999. Aerosol Technology: Properties, Behavior, and Measurement of Airborne Particles. Second Edition. Wiley-Interscience, John Wiley & Sons Inc., New York, NY: Pages 94-97.

2. Zaucha, G.M., Pitt, L.M., Estep, J., Ivins, B.E., and Friedlander, A.M., 1998. The pathology of experimental rabbits exposed by inhalation and subcutaneous inoculation. Arch. Pathol. Lab. Med. 122(11):982-992.

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APPENDIX E
AEROSOL REPORT

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List of Acronyms

APS ..... aerodynamic particle sizer
BBRC ..... Battelle Biomedical Research Center
BSC III ..... Class III biological safety cabinet
C ..... impinger concentration
cm ..... centimeter
CFU ..... colony forming units
D ..... dilution factor
d ..... diameter
GSD ..... geometric standard deviation
HEPA ..... high efficiency particulate air
InD ..... total inhaled dose
L ..... liter
LD_{50} Value ..... median lethal dose equivalent
MFC ..... mass flow controller
MFM ..... mass flow meter
min ..... minute
mL ..... milliliter
MMAD ..... mass median aerodynamic diameter
S ..... sampling rate
SOP ..... standard operating procedure
T ..... exposure time
TATV ..... total accumulated tidal volume
TSA ..... tryptic soy agar
µm ..... micrometer
V ..... impinger sampler volume
WBP ..... whole body plethysmography

1. Methods

Standard operating procedures (SOPs) developed by Battelle and utilized for other similar studies were followed during animal aerosol exposure challenges.

2. Experimental Setup and Conditions

The Battelle large animal exposure system (Figure 1) can be divided into five subsystems: Aerosol Generation Subsystem, Delivery System, Exposure Chamber, Sampling/Monitoring, and Plethysmography. Below is a description of each subsystem.

2.1 Aerosol Generation Subsystem

Air was supplied to the system by an in-house air system filtered through two high efficiency particulate air (HEPA) capsule filters and a carbon filter. The air was split into dilution airflow of approximately 8.6 liters per minute (L/min) and a nebulizer/bypass airflow of approximately 7.5 L/min, maintained by mass flow controllers (MFC). The dilution air was humidified via a bubbler as needed to maintain humidity within a desired range of 74% to 83%. A modified microbiological research establishment type three-jet Collison nebulizer (BGI, Waltham, MA) with a precious fluid jar was used to generate a controlled delivery of aerosolized *B. anthracis* spores (spore lot Ames B35) from a liquid suspension. These nebulizers are designed to generate aerosols having an approximate mean diameter of 1 to 2 micrometer (μm). Each nebulizer was characterized for a pressure that results in an approximately 7.5 L/min flow, which normally is approximately 28.0 pounds per square inch, Collison nebulizer dependant.

2.2 Delivery Subsystem

After the agent aerosol was generated by the Collison nebulizer, it exited the Collison and traveled down a 3.75 centimeter (cm) diameter, 40 cm long cylinder (mixing tube) that mixed the aerosol with dilution air. The aerosol then entered the top of the exposure chamber through another cylinder with a tapered 14 cm long slit on each side. The total airflow entering the exposure chamber was approximately 16 L/min. The aerosol entered the chamber through these slits to fill the exposure chamber, washed over the exposure target (muzzle or head), and was then exhausted out of the exposure chamber through another cylinder at the bottom that

contained slots on two sides, each 19.5 cm in length. The aerosol was pulled through the chamber using a vacuum pump that maintained a slight negative pressure (from -0.25 to -0.01 inches of water) within the exposure chamber, as measured using a differential pressure gauge (magnehelic). The exhaust aerosol was filtered by two HEPA cartridge filters before exiting the system.

2.3 Exposure Chamber

The exposure chamber was a plexiglass box with internal dimensions of approximately 20.5 x 20.5 x 40 cm (length x width x height). A port approximately 15 cm in diameter was located on one side of the chamber where an animal's head or muzzle entered into the exposure chamber. Rubber dental dam material was stretched across the opening and held in place with an o-ring gasket. The animal's head or muzzle was pushed through a small hole in the dental dam, producing a seal to decrease leakage around the opening. Four additional ports are located in the chamber: two ports for collection of aerosol samples (one for enumeration and one for aerosol particle sizing), one port to measure temperature and humidity, and one port to measure the differential pressure within the exposure chamber in relation to the surrounding atmosphere within the Class III biological safety cabinet (BSC III). Thus, the sampling from the impinger and Aerodynamic Particle Sizer (APS) spectrometer and exposure of the animal all occur from the same chamber. The aerosol system was operated within a self-contained BSC III.

2.4 Sampling/Monitoring

Aerosol concentration and aerosol particle size distribution were determined by analysis of atmospheric samples drawn from the exposure chamber. The atmospheric samples were collected in an impinger (Model 7541, Ace Glass Inc.) filled with approximately 20 milliliter (mL) of sterile water. Chamber air was drawn into and through the impinger at approximately 6.0 ±0.3 L/min. The sampling rate was achieved by maintaining a vacuum of ≥18 inches Hg across the exhaust connection of the impinger to maintain the flow from the impinger critical orifice. The liquid in the impinger was diluted and enumerated by the spread plate technique to quantify viable spore counts per mL. Concentrations are reported in terms of CFUs per mL (CFU/mL) of impinger liquid. Enumeration results, along with the volume of liquid in the

impinger, sampling rate, and sampling duration, are used in the calculation of the aerosol concentration expressed as CFU/L of air.

The aerosol particle size was determined during each exposure using an APS spectrometer, which draws an atmospheric sample from the exposure chamber at 0.25 L/min with a diluter (1.0 L/min total with 0.75 L/min from the diluter and 0.25 L/min from the exposure chamber). An APS was used because of its advantages over other methods. These advantages include near real-time data measurements, aerodynamic diameter measurements, ease of instrument operation, and the generation of electronic data that is easy to process and export to a report.

2.5 Plethysmography

Whole body plethysmography (WBP) was performed real-time on each animal during agent challenge to measure important respiratory parameters. These parameters (tidal volume, total accumulated tidal volume [TATV], and minute volume) were calculated from the measured volumetric displacement of air caused by the movement of the thoracic cavity of an animal while it was in the sealed chamber (the plethysmograph). The data generated for each animal were used to determine the TATV, which along with the aerosol concentration were used in calculating the inhaled dose. Rabbits were physically restrained within a plethysmograph restraint device with the head protruding out of a port that was sealed with rubber dental dam material and held into place with two plexiglass guillotines. The plethysmograph was connected to a pneumotach (Hans Rudolph, Inc., Kansas City, MO) that was attached to a differential pressure transducer (Model DP-45; Validyne Engineering Corp., North Ridge, CA). Pressure differential measurements from inhalations and exhalations were transmitted to Biosystems XA Version 1.5.7 software (Biosystems XA, Buxco Electronics, Sharon, CT) which then calculated and recorded respiratory function. Prior to animal exposures, the Buxco software program was calibrated to establish unit (baseline) and air volume displacements from 5 to 40 mL to simulate animal respiration within the plethysmograph. This calibration was performed to encompass the respiration volume range of the animal model for accurate TATV measurements.

3. Inhalation Results

The inhalation exposure system data for each exposure were documented on appropriate forms to ensure proper system operation and to provide the needed information to quantify animal challenge conditions. Impinger sampling conditions and enumerated concentration results were used to calculate the viable bioaerosol challenge concentration while plethysmography measurements documented the total inhaled volume. Total inhaled dose (CFU) was calculated from aerosol concentration and total inhaled volume. The number of median lethal dose equivalents (LD_{50} value) was calculated by dividing the total inhaled dose by the reported inhalation LD_{50} for the rabbit. The reported LD_{50} value for rabbits is 105,000 CFU (Zaucha et al., 1998). Table 1 shows the inhalation results for this study.

3.1. Impinger Sample Analysis

Impinger samples were enumerated by the spread plate method following serial dilutions to determine viable spore concentration. Diluted samples were mixed in a capped vial prior to subsequent dilutions. At different target dilutions, 0.1 mL was spread onto each of five tryptic soy agar (TSA) plates, which were placed in a secondary container and incubated at the appropriate temperature for the appropriate time. After the incubation period, the plates were enumerated to determine the number of colonies on each plate. Impinger sample concentration (C) was determined using Equation 1:

$$C = (A \cdot D) / 0.1\ mL \qquad (1)$$

C = CFU/mL
A = average CFU per plate
D = dilution factor

3.2 Inhaled Dose Calculation

The total inhaled dose (InD) was calculated from the impinger sample concentration, sampling parameters, and exposure duration according to the equation below. The total number of viable spores captured during each exposure was the product of the impinger concentration (C) and the impinger volume (V). The total number of viable organisms was divided by the amount that was sampled through the impinger during the exposure time (T). The aerosol concentration was (C x

V) $(S \times T)^{-1}$. The inhaled dose was the product of the aerosol concentration multiplied by the total accumulated tidal volume (Equation 2).

$$InD = (C \times V)\,(S \times T)^{-1}(TATV) \qquad (2)$$

InD = Total inhaled dose (CFU)

C = Impinger concentration (CFU/mL)

V = Impinger sampler volume (mL)

S = Sampling rate (6 L/min)

T = Exposure time (min)

TATV = Total accumulated tidal volume (L)

4. Particle Size Results

Figure 2 shows a log probability plot representing the average of all APS particle size distributions obtained from exposure testing. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) are also shown.

The MMAD for the log probability plot (Figure 2) was determined from averaging the cumulative median size (50% mass) from the aerosol size distributions obtained from the APS for all aerosol exposures (Equation 3). The GSD was determined from taking the cumulative average of the GSD calculated by the APS for each exposure test. The GSD represents one standard deviation for a normal distribution, and is determined by the following equation:

$$GSD = d84\%/d50\% \quad (3)$$

Where d84% is the particle size diameter (d) at a cumulative % mass of 84% and d50% is the particle size diameter (d) at a cumulative mass of 50% (Hinds, 1999).

Table 1. Inhalation Exposure Report for Rabbits, (09-18-09)

Rabbit ID	Nebulizer Conc. (CFU/mL)	Impinger Conc. (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol Conc. (CFU/L)	Total Accum. Tidal Volume Inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD_{50} Equivalents	Spray Factor
L23220	0.00E+00	0.00E+00	19.00	0.00E+00	6	7.00	0.00E+00	10.2	0	0.000	NA
L23216*	0.00E+00	3.00E+01	18.75	5.63E+01	6	9.15	1.02E+00	10.0	10	0.000	NA
L23218	0.00E+00	0.00E+00	18.75	0.00E+00	6	8.88	0.00E+00	10.0	0	0.000	NA
L23223	0.00E+00	0.00E+00	19.25	0.00E+00	6	7.77	0.00E+00	10.0	0	0.000	NA
L23222	0.00E+00	0.00E+00	19.00	0.00E+00	6	7.08	0.00E+00	10.0	0	0.000	NA
L23215	1.66E+04	5.12E+01	19.00	9.73E+02	6	7.00	2.32E+01	13.9	322	0.003	1.40E-06
L23206	1.66E+04	4.78E+01	19.40	9.27E+02	6	7.00	2.21E+01	13.5	298	0.003	1.33E-06
L23210	1.66E+04	5.34E+01	19.20	1.03E+03	6	7.85	2.18E+01	10.0	218	0.002	1.31E-06
L23219	1.66E+04	3.74E+01	19.20	7.18E+02	6	5.60	2.14E+01	15.0	321	0.003	1.29E-06
L23211	1.66E+04	3.46E+01	19.00	6.57E+02	6	6.03	1.82E+01	15.0	273	0.003	1.09E-06
L23217	1.37E+05	2.55E+02	19.40	4.95E+03	6	7.00	1.18E+02	12.6	1484	0.014	8.60E-07
L23230	1.37E+05	3.98E+02	19.40	7.72E+03	6	7.00	1.84E+02	11.0	2022	0.019	1.34E-06
L23228	1.37E+05	4.82E+02	19.40	9.35E+03	6	7.05	2.21E+02	10.1	2233	0.021	1.61E-06
L23227	1.37E+05	6.00E+02	19.20	1.15E+04	6	8.25	2.33E+02	10.0	2327	0.022	1.70E-06
L23229	1.37E+05	6.00E+02	19.00	1.14E+04	6	8.48	2.24E+02	10.0	2241	0.021	1.64E-06
L23235	1.65E+06	4.12E+03	19.20	7.91E+04	6	7.47	1.76E+03	10.0	17649	0.168	1.07E-06
L23205	1.65E+06	4.04E+03	19.20	7.76E+04	6	7.00	1.85E+03	14.8	27333	0.260	1.12E-06
L23225	1.65E+06	5.10E+03	19.60	1.00E+05	6	7.00	2.38E+03	10.9	25942	0.247	1.44E-06
L23231	1.65E+06	5.12E+03	19.00	9.73E+04	6	7.00	2.32E+03	10.4	24088	0.229	1.40E-06
L23207	1.65E+06	5.40E+03	19.40	1.05E+05	6	7.00	2.49E+03	12.8	31927	0.304	1.51E-06
L23201	1.96E+07	2.78E+04	19.20	5.34E+05	6	7.00	1.27E+04	14.0	177920	1.694	6.48E-07
L23234	1.96E+07	4.94E+04	19.20	9.48E+05	6	7.00	2.26E+04	13.1	295835	2.817	1.15E-06
L23212	1.96E+07	5.56E+04	19.40	1.08E+06	6	7.00	2.57E+04	12.8	328728	3.131	1.31E-06
L23200	1.96E+07	2.17E+04	19.60	4.25E+05	6	4.78	1.48E+04	14.8	219482	2.090	7.57E-07
L23214	1.96E+07	4.14E+04	19.40	8.03E+05	6	5.68	2.36E+04	15.0	353504	3.367	1.20E-06
L23204	1.05E+09	7.52E+05	19.40	1.46E+07	6	6.13	3.97E+05	15.0	5949755	56.664	3.78E-07
L23203	1.05E+09	1.83E+06	19.00	3.48E+07	6	7.00	8.28E+05	10.7	8858071	84.363	7.88E-07
L23213	1.05E+09	1.59E+06	19.20	3.05E+07	6	6.98	7.29E+05	10.0	7289398	69.423	6.94E-07
L23221	1.05E+09	1.85E+06	19.20	3.55E+07	6	7.00	8.46E+05	10.5	8880000	84.571	8.05E-07
L23232	1.05E+09	1.83E+06	19.20	3.51E+07	6	7.00	8.37E+05	12.4	10373486	98.795	7.97E-07

First five animal exposures were with irradiated spores, CFU/mL = 0
* Sample was likely contaminant as the aerosol system was decontaminated and showed no growth. Also all other irradiated samples were no growth
NA = not applicable. The spray factor cannot be calculated for these samples as the starting concentration enumeration is zero as there were not viable from irradiation

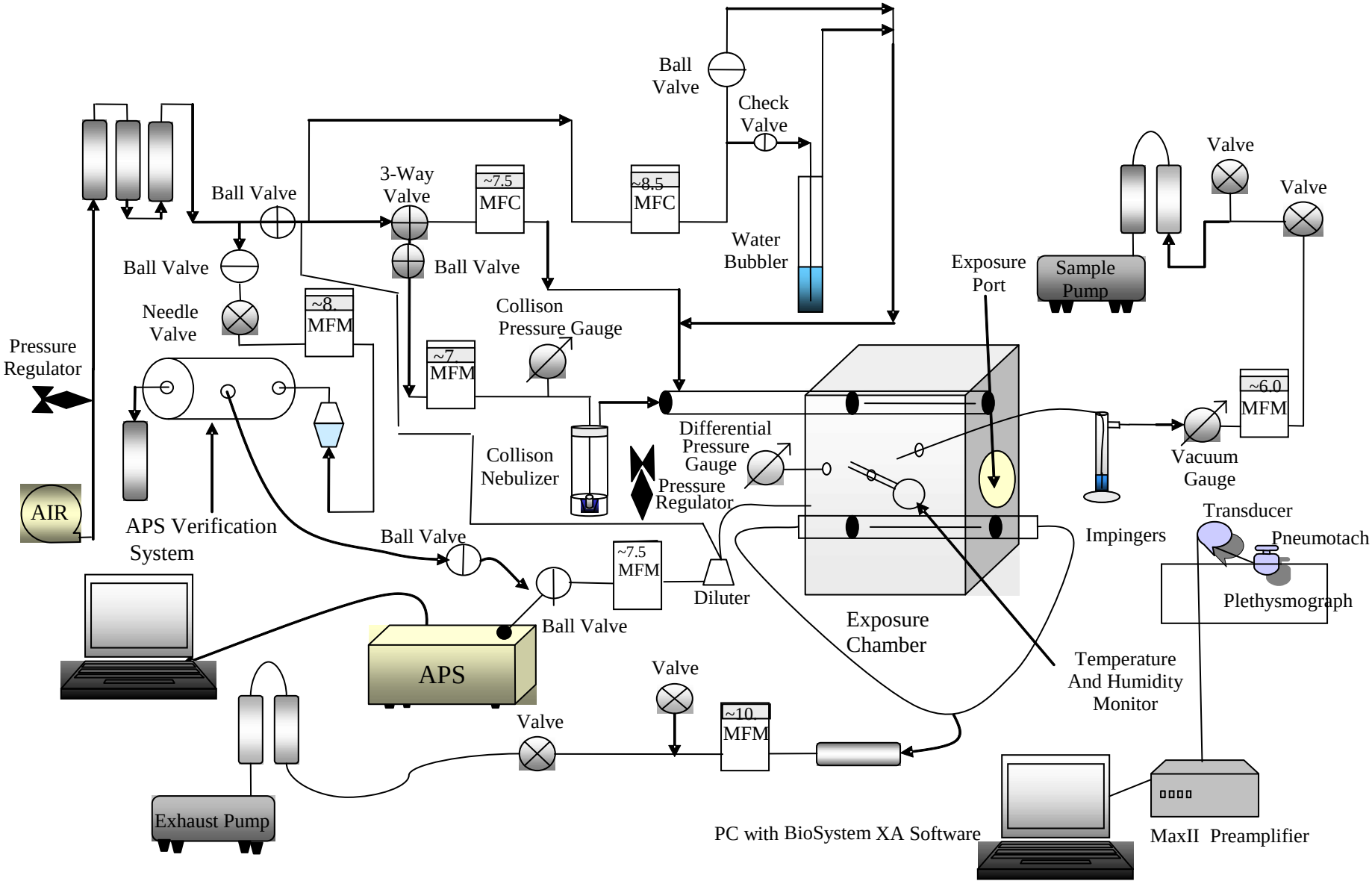

Figure 1. Exposure system diagram.

APS = Aerodynamic Particle Sizer, MFC = mass flow controller, MFM = mass flow meter

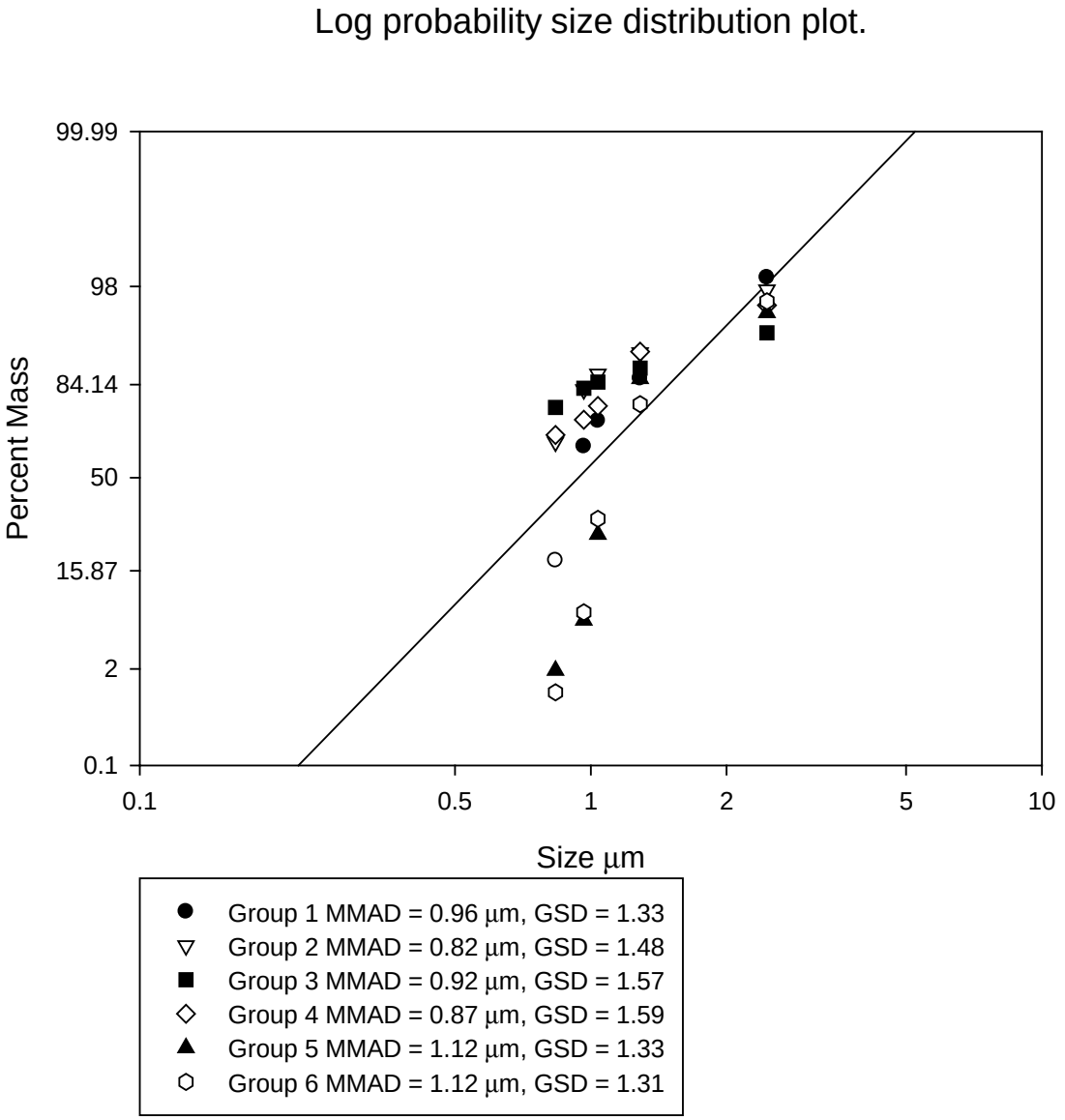

Figure 2. Log probability size distribution plot.

MMAD, mass median aerodynamic diameter; GSD, geometric standard deviation

5. References

1. Hinds, William C. Aerosol Technology: Properties, Behavior, and Measurement of Airborne Particles. Second Edition 1999. Wiley-Interscience, John Wiley & Sons Inc., New York, NY: Pages 94-97.

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STATISTICAL ANALYSIS OF TELEMETRY DATA

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List of Acronyms

ANOVA ..... Analysis of variance
BBRC ..... Battelle Biomedical Research Center
BPM ..... Beats per minute
CFU ..... Colony forming unit
LD_{50} ..... Median lethal dose
LDH ..... Lactate Dehydrogenase
mm ..... millimeter
N ..... Number of animals
RCPM ..... Respiratory cycles per minute
RP ..... Respiratory period

1. Introduction

This report summarizes the statistical analysis of telemetry data collected under Battelle Biomedical Research Center (BBRC) Study No. 1020-CG920503. Thirty (30) male pathogen-free New Zealand White rabbits *(Oryctolagus cuniculus)* were randomized into six Groups, with each Group having five animals. Animals were aerosol-challenged on Study Day zero with *Bacillus anthracis* (Ames strain) spores as indicated in Table 1.

Table 1. Study Design

Group	Number of Animals per Group	Target Spore Dose (CFU)
1[a]	5	100 x LD_{50}
2	5	100
3	5	1,000
4	5	10,000
5	5	100,000
6[b]	5	100 x LD_{50}

CFU Colony forming units.
a Spores are gamma-irradiated (negative control).
b High dose control.
LD_{50} Median lethal dose.

Telemetry data were collected for activity, heart rate, respiratory period (RP) expiratory time, RP inspiratory time, RP integral, RP peak amplitude, RP respiratory rate, and body temperature. The telemetry data were collected for at least 30 seconds every 15 minutes throughout the study. Approximately seven days of baseline data were collected prior to challenge for each animal, while the post-challenge data were collected for surviving animals up to 21 days following challenge. All telemetry data collected after an animal's time of death were excluded from the statistical analysis, as were all records that had each respiratory parameter recorded as missing and an activity recorded as either missing or zero. Furthermore, extreme observations were identified during baseline for Animal L23234 on September 14, 2009, at 16:45; therefore, these observations were also excluded prior to the statistical analysis.

2. Statistical Methods

The analysis described below was performed separately for each animal and for each of the animal's telemetry parameters. The mean telemetry value was computed for every 15 minute clock time (00:00, 00:15, …, 23:45) at baseline. Each observation was then baseline adjusted according to the associated clock time. Six-hour averages were computed for the baseline adjusted values using the following intervals: midnight-6AM (inclusive), 6AM-Noon (inclusive), Noon-6PM (inclusive), and 6PM-midnight (inclusive). The standard deviation of each six-hour average at baseline was calculated and used to form the upper and lower limits for indications of abnormality. The upper limit was defined to be three standard deviations above zero, while the lower limit was defined to be three standard deviations below zero. An animal was found to be abnormal if two consecutive baseline adjusted six-hour averages were outside the upper or lower limits following challenge. The time of onset for abnormality was defined as the time associated with the second abnormal value during the first occurrence of two consecutive abnormal values following challenge. The end of abnormality was defined as the time associated with the second abnormal value during the last occurrence of two consecutive abnormal values following challenge. Therefore, the duration of abnormality was defined as the difference between the time associated with the end of abnormality and the time associated with the onset of abnormality.

In order to determine if the baseline adjusted telemetry results were significantly different between the Groups, the following analysis of variance (ANOVA) model was fitted separately at each study time:

$$Y_{dij} = \mu + \text{Group}_i + \varepsilon_{ij} \qquad (1)$$

where Y_{dij} is the baseline adjusted six-hour average telemetry value for the *j*th animal in Group *i* (*i*=1 to 6) at study time *d*, μ is an overall constant, and ε_{ij} is the random error left unexplained by the model. Least square mean estimates from the ANOVA models were calculated and approximate t-tests were performed to determine if, for each Group, there was a significant shift in the telemetry values between baseline and each study time, after adjusting for the clock time. This t-test was performed to determine if the mean baseline adjusted telemetry value is significantly different from zero. Additionally, Tukey's multiple comparisons procedure

was performed to determine which pairs of groups had mean baseline adjusted telemetry values that were significantly different from each other. Under the Tukey procedure, the set of all comparisons within each parameter and Study Day combination are made at a joint 95% confidence level.

Estimates and exact binomial 95% confidence intervals for the proportion of abnormal animals were calculated within each Group and an overall two-sided Fisher's exact test was performed to determine if there was a significant difference between the proportions of abnormal animals in each Group. For those groups with abnormal animals, the mean duration of abnormality was also calculated.

For each telemetry parameter, an overall log-rank test was performed to determine if there was a significant difference between the times to abnormality within each Group. Similarly for each parameter, an overall log-rank test was performed to determine if there was a significant difference between the duration of abnormality within each Group. If the overall log-rank test for a parameter was significant, then pairwise log-rank tests were performed to evaluate all pairwise Group comparisons. The Bonferroni-Holm adjustment was used to maintain an overall 0.05 level of significance among the multiple pairwise comparisons made within each telemetry parameter. For those parameters associated with a significant overall log-rank test, Kaplan-Meier estimates were plotted for each Group.

All statistical analyses were conducted using SAS® (SAS Institute Inc.; Cary, NC; version 9.1) and all results were reported at the 0.05 level of significance. Estimates and exact binomial confidence intervals for the proportion of abnormal animals were calculated using the MEANS procedure. All ANOVA models were fitted using the MIXED or GLM procedure. All Fisher's exact tests were performed using the FREQ procedure, and all log-rank tests were performed using the LIFETEST procedure. The MULTTEST procedure was used to maintain an overall 0.05 level of significance among the multiple pairwise comparisons made within each parameter.

3. Results

Since the animals were challenged at various times on Study Day 0 and the clock time is of interest when analyzing telemetry data, all figures are presented in terms of "days from midnight of challenge day" instead of "hours post-challenge". Figures 1a through 8f display the baseline adjusted six-hour averages for the animals within each group for each of the telemetry parameters. Figures 9 through 16 display the mean baseline adjusted six-hour averages within each group for each of the telemetry parameters, respectively.

Tables 2 through 9 contain test results within each group at each study time, testing whether the mean baseline adjusted value was significantly different from zero (at the 0.05 level) for each telemetry parameter. In each cell, the estimate of the mean baseline adjusted value is shown for that parameter, Group, and study time. Following the estimate, an up arrow (↑) indicates that the mean baseline adjusted value was significantly greater than zero, while a down arrow (↓) indicates that it was significantly less than zero. These tables also contain group effect p-values for each study time, as well as test results from the Tukey's pairwise comparisons procedure that were used to identify pairs of groups with significantly different mean baseline adjusted values. Under the Tukey procedure, the set of comparisons within each parameter and study time is made at a joint 95% confidence level. Each significant difference is shown as the estimated difference between the mean baseline adjusted values for the pair of groups under consideration, the direction of the comparison (i.e., which group experienced a larger mean baseline adjusted value), and the corresponding Tukey-adjusted p-value. P-values less than 0.05 provide evidence of a significant difference.

The results on some Study Days were based on smaller sample sizes due to missing data or due to animal deaths prior to the end of the study. All animals in Group 6 died prior to the end of Study Day 4 and all but one animal in Group 5 died prior to the end of Study Day 5. A summary of the results listed in Tables 2 through 9 is presented below for each parameter.

- **Activity (Table 2, Figures 1a-f)**: For four consecutive six-hour intervals beginning on Study Day 3 at Noon-6PM, there were significant decreases from baseline in Group 6. On Study Day 3 at Noon-6PM and Study Day 4 at 6AM-Noon, the mean

decrease from baseline in Group 6 was significantly different than the mean increases from baseline in Groups 1 and 3. There were no significant decreases from baseline in Group 1 following Study Day 13.

- **Heart Rate (Table 3, Figures 2a-f)**: By Study Day 1 at 6AM-Noon, all groups had experienced significant increases from baseline. The significant increases from baseline in Group 6 continued for most study times until Study Day 4. Groups 1 and 3 did not experience any significant increases from baseline after Study Day 4, while Group 4 continued to experience some significant increases from baseline until Study Day 11.

- **RP Expiratory Time (Table 4, Figures 3a-f)**: Group 1 rarely experienced significant shifts from baseline, while Group 5 had more significant decreases from baseline than any other Group. For eight consecutive six-hour intervals beginning on Study Day 6 at 6AM-Noon, there were significant increases from baseline in Group 3. For four consecutive six-hour intervals beginning on Study Day 4 at Noon-6PM, the mean decrease from baseline in Group 5 was significantly different than the mean change from baseline in at least one of the other lower dose groups (Groups 1 through 4).

- **RP Inspiratory Time (Table 5, Figures 4a-f)**: On Study Days 2 through 4, there were often significant decreases from baseline in Groups 5 and 6. Groups 2 and 3 never experienced significant decreases from baseline, but did experience some significant increases from baseline on Study Days 6 through 8. From Study Day 2 through 5, the mean decreases from baseline in Group 5 were often significantly different than the mean changes from baseline in Group 2.

- **RP Integral (Table 6, Figures 5a-f)**: For ten consecutive six-hour intervals beginning on Study Day 3 at 6AM-Noon, there were significant increases from baseline in Group 5. On Study Day 4 at Noon-6PM and 6PM-Midnight, the mean increase from baseline in Group 5 was significantly different than the mean change

from baseline in Group 1. Groups 3 and 4 did not experience any significant shifts from baseline during the study.

- **Peak Amplitude (Table 7, Figures 6a-f)**: There were no significant shifts from baseline in Groups 3 or 4, and only one significant decrease from baseline in Group 6 during the study. Groups 1, 2, and 5 experienced some significant increases from baseline, but no significant decreases from baseline.

- **RP Respiratory Rate (Table 8, Figures 7a-f)**: There were typically significant increases from baseline in Group 5 from Study Day 2 at 6AM-Noon through Study Day 5 at Noon-6PM and in Group 6 from Study Day 2 at Midnight-6AM through Study Day 4 at Noon-6PM. The mean increase from baseline in Group 5 or 6 was often significantly different than the mean change from baseline in at least one of the other groups during these study times. Group 1 experienced only one significant increase from baseline after Study Day 2.

- **Temperature (Table 9, Figures 8a-f)**: For seven consecutive six-hour intervals beginning on Study Day 2 at 6AM-Noon, there were significant increases from baseline in Group 6. Excluding Study Day 3 at Noon-6PM, the mean increases from baseline at these study times were significantly different than the mean changes from baseline in at least three of the lower dose groups (Groups 1 through 5). On Study Day 5 at 6AM-Noon and Noon-6PM, there were significant decreases from baseline in Group 5 that were significantly different than the mean changes from baseline in Groups 1 through 4.

Table 10 contains the proportion of animals that were abnormal at any point during the study by group for each parameter as well as the mean duration of abnormality for those groups with abnormal animals. Note that some animals died prior to becoming abnormal. In addition, Table 10 contains the results of Fisher's exact tests comparing the proportion of animals that were abnormal in each group by parameter. There were no significant differences between the groups for any parameter.

Table 11 contains the results of the overall log-rank tests for each parameter comparing the times to abnormality within each Group. Activity, RP expiratory time, RP inspiratory time, RP integral, RP peak amplitude, RP respiratory rate, and temperature all had significant differences between the groups at the 0.05 level; therefore, pairwise comparisons for these parameters are presented in Table 12. The pairwise comparisons are not dependent upon the ordering of the groups being compared (e.g., comparing Group 1 to Group 2 is equivalent to comparing Group 2 to Group 1); therefore, the cells in the lower left portion of the table are shaded out for each parameter. Prior to the adjustment for multiple pairwise comparisons, the times to abnormal activity in Groups 1, 3, and 4 were significantly greater than that in Group 5. After adjusting for the multiple pairwise comparisons, the times to abnormal activity were not significantly different for any pair of Groups. Prior to the adjustment for multiple pairwise comparisons, the times to abnormal RP expiratory time in Groups 1, 2, and 4 were significantly greater than that in Group 6. After adjusting for the multiple pairwise comparisons, the time to an abnormal RP expiratory time in Group 4 was no longer significantly different from that in Group 6. Prior to the adjustment for multiple pairwise comparisons, the times to abnormal RP inspiratory time in Groups 1 through 5 were significantly greater than that in Group 6. After adjusting for the multiple pairwise comparisons, the times to abnormal RP inspiratory time in Groups 3 and 5 were no longer significantly different from that in Group 6. Prior to the adjustment for multiple pairwise comparisons, the times to abnormal RP integral in Groups 1 through 5 were significantly greater than that in Group 6. After adjusting for the multiple pairwise comparisons, the times to abnormal RP integral in Groups 2, 4, and 5 were no longer significantly different from that in Group 6. Prior to the adjustment for multiple pairwise comparisons, the times to abnormal RP peak amplitude in Groups 2 through 4 were significantly greater than that in Group 6. After adjusting for the multiple pairwise comparisons, the times to abnormal RP peak amplitude in Groups 3 and 4 were no longer significantly different from that in Group 6. Prior to the adjustment for multiple pairwise comparisons, the times to abnormal RP respiratory rate in Groups 2 through 5 were significantly greater than that in Group 6. After adjusting for the multiple pairwise comparisons, the times to abnormal RP respiratory rate in Groups 3 through 5 were no longer significantly different from that in Group 6. Prior to the adjustment for multiple pairwise comparisons, the time to abnormal temperature in Group 2 was significantly greater than that in Group 6. After adjusting for the multiple pairwise comparisons, the times to

abnormal temperature were not significantly different for any pair of groups. Figures 17 through 23 display the Kaplan-Meier curves associated with time to abnormality for activity, RP expiratory time, RP inspiratory time, RP integral, RP peak amplitude, RP respiratory rate, and temperature, respectively. The dots displayed throughout the Kaplan-Meier curves indicate that the time to abnormality for an animal could not be observed beyond the indicated study time. For example, if an animal were to die prior to experiencing abnormality then the time to abnormality for that animal would be unobserved and censored at the animal's time of death.

Table 13 contains the results of the overall log-rank tests for each parameter comparing the duration of abnormality within each group. Heart rate, RP expiratory time, RP inspiratory time, and RP respiratory rate all had significant differences between the groups at the 0.05 level; therefore, pairwise comparisons for these parameters are presented in Table 14. Prior to the adjustment for multiple pairwise comparisons, the duration of abnormal heart rate in Group 2 was significantly greater than those in Groups 3 and 6, and the duration of abnormal heart rate in Group 4 was significantly greater than that in Group 3; however, these differences were no longer significant after the adjustment for multiple comparisons. Prior to the adjustment for multiple pairwise comparisons, the duration of abnormal RP expiratory time in Group 3 was significantly greater than those in Groups 2, 4, and 5, and the duration of abnormal RP expiratory time in Group 4 was significantly greater than that in Group 5; however, these differences were no longer significant after the adjustment for multiple comparisons. No significant pairwise differences were identified for duration of abnormal RP inspiratory time. Prior to the adjustment for multiple pairwise comparisons, the duration of abnormal RP respiratory rates in Groups 5 and 6 were significantly greater than that in Group 1; however, these differences were no longer significant after the adjustment for multiple comparisons. Figures 24 through 27 display the Kaplan-Meier curves associated with duration of abnormality for heart rate, RP expiratory time, RP inspiratory time, and RP respiratory rate, respectively. The dots displayed throughout the Kaplan-Meier curves indicate that the duration of abnormality for an animal could not be observed beyond the indicated study time. For example, if an animal were still abnormal at its time of death or at the end of the study, then the duration of abnormality for that animal would be unobserved. Note the since the animals in Groups 5 and 6 died early in the study, the durations of abnormality in the lower dose groups may appear greater than those in the higher dose Groups.

4. Conclusions

All animals in high dose control group (Group 6) died prior to the end of Study Day 4 and all but one animal in the 100,000 colony forming unit (CFU) dose group (Group 5) died prior to the end of Study Day 5. Prior to the death of these animals, there were typically more significant shifts from baseline in these two groups than there were in the lower dose groups (Groups 1 through 4). In terms of the proportion of animals that became abnormal during the study, there were no significant differences between the groups for any parameter. In terms of time to abnormality, there were significant differences between the groups for RP expiratory time, RP inspiratory time, RP integral, RP peak amplitude, RP respiratory rate, and temperature. For each of these telemetry parameters, the time to abnormality in at least one of the lower dose groups (Groups 1 through 5) was significantly greater than that in the high dose control group (Group 6) prior to the adjustments for multiple comparisons. The duration of abnormality was found to be significantly different between the groups for heart rate, RP expiratory time, RP inspiratory time, and RP respiratory rate; however, there were no significant pairwise differences between the groups for any parameter after the adjustment for multiple comparisons.

Table 2. Summary of the ANOVA Results for the Baseline Adjusted Six-Hour Averages for Activity (Counts/Minute)

Activity									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value[#]
		1	2	3	4	5	6		
0	6AM - Noon	1.88	1.14	2.18	20.73 ↑	NA	NA	0.0108 *	-18.85 (1<4) 0.0218 -19.59 (2<4) 0.0169 -18.55 (3<4) 0.0242
	Noon - 6PM	0.25	0.01	0.17	0.23	0.19	0.25	0.9680	
	6PM - Midnight	-0.13	-0.27 ↓	-0.39 ↓	-0.15	-0.34 ↓	0.03	0.0591	
1	Midnight - 6AM	-0.10	-0.04	-0.16	-0.19	-0.18	-0.05	0.9309	
	6AM - Noon	1.16 ↑	1.01 ↑	0.45	0.60	1.16 ↑	1.00 ↑	0.4351	
	Noon - 6PM	-0.03	0.13	0.27	-0.49	-0.23	-0.56 ↓	0.2111	
	6PM - Midnight	0.06	-0.07	0.04	-0.23	-0.21	-0.43 ↓	0.2812	
2	Midnight - 6AM	-0.08	0.07	-0.03	-0.01	-0.12	-0.26	0.7192	
	6AM - Noon	1.47 ↑	1.09 ↑	0.82	1.14 ↑	0.49	-0.31	0.3065	
	Noon - 6PM	-0.32	-0.46	0.05	-0.45	-0.75 ↓	-0.74 ↓	0.2913	
	6PM - Midnight	-0.32	-0.34	0.28	-0.09	-0.46 ↓	-0.24	0.1738	
3	Midnight - 6AM	-0.01	-0.10	0.20	-0.12	-0.09	-0.40 ↓	0.3672	
	6AM - Noon	1.42 ↑	0.61	0.79	0.76	0.43	-0.62	0.0549	-2.03 (6<1) 0.0216
	Noon - 6PM	0.14	-0.10	0.03	-0.22	-0.83 ↓	-1.14 ↓	0.0123 *	-1.27 (6<1) 0.0226 -1.17 (6<3) 0.0426
	6PM - Midnight	-0.24	-0.24	-0.15	-0.28	-0.64 ↓	-0.78 ↓	0.2053	
4	Midnight - 6AM	0.18	-0.16	0.24	-0.11	-0.40	-0.60 ↓	0.0584	
	6AM - Noon	0.29	-0.25	0.19	-0.30	-0.51 ↓	-1.25 ↓	0.0084 *	-1.54 (6<1) 0.0086 -1.43 (6<3) 0.0159
	Noon - 6PM	-0.16	0.34	-0.15	-0.31	-0.57	-0.86	0.3001	
	6PM - Midnight	0.07	-0.04	-0.17	-0.04	-0.55 ↓	NA	0.3588	
5	Midnight - 6AM	0.05	-0.21	-0.14	-0.12	-0.56 ↓	NA	0.3593	
	6AM - Noon	0.10	-0.15	0.20	0.06	-0.51	NA	0.5507	
	Noon - 6PM	-0.11	0.12	-0.02	0.31	-0.22	NA	0.6473	
	6PM - Midnight	-0.17	0.45	-0.16	0.32	0.18	NA	0.2767	

Table 2. (Continued)

Activity									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value[#]
		1	2	3	4	5	6		
6	Midnight - 6AM	-0.22	0.02	-0.21	0.03	0.06	NA	0.3050	
	6AM - Noon	-0.02	-0.55 ↓	-0.29 ↓	-0.09	-0.65 ↓	NA	0.0546	
	Noon - 6PM	-0.14	-0.61 ↓	-0.27	-0.01	-0.44	NA	0.3610	
	6PM - Midnight	-0.22	0.21	0.03	0.07	-0.13	NA	0.8504	
7	Midnight - 6AM	-0.23	-0.03	-0.45 ↓	-0.01	0.57	NA	0.0613	
	6AM - Noon	0.26	0.42	-0.18	0.61	-0.01	NA	0.4694	
	Noon - 6PM	-0.61 ↓	-0.90 ↓	-0.62 ↓	-0.26	-0.39	NA	0.1903	
	6PM - Midnight	-0.24	-0.14	-0.50 ↓	-0.19	0.13	NA	0.7041	
8	Midnight - 6AM	0.17	-0.12	-0.37	0.06	0.24	NA	0.3299	
	6AM - Noon	-0.28	-0.17	-0.37	-0.30	-0.31	NA	0.9866	
	Noon - 6PM	-0.05	-0.86 ↓	-0.15	-0.17	0.26	NA	0.0738	
	6PM - Midnight	-0.16	-0.03	-0.35	-0.35	-0.06	NA	0.7871	
9	Midnight - 6AM	-0.01	-0.20	-0.14	0.04	-0.15	NA	0.6375	
	6AM - Noon	-0.44	-0.40	-0.27	-0.40	-0.60	NA	0.9764	
	Noon - 6PM	-0.29	-0.56 ↓	-0.23	-0.77 ↓	-0.44	NA	0.6225	
	6PM - Midnight	-0.12	-0.25	-0.55 ↓	-0.29	-0.41	NA	0.5496	
10	Midnight - 6AM	-0.18	0.16	-0.29	-0.28	-0.08	NA	0.3360	
	6AM - Noon	-0.28	-0.24	-0.11	-0.66 ↓	-0.49	NA	0.5618	
	Noon - 6PM	-0.19	-0.20	-0.24	-0.47	0.24	NA	0.8145	
	6PM - Midnight	-0.23	-0.25	-0.27	-0.42	-0.23	NA	0.9734	
11	Midnight - 6AM	0.04	0.09	-0.02	-0.37	-0.19	NA	0.6544	
	6AM - Noon	-0.18	-0.41	-0.30	-0.48	-0.43	NA	0.8700	
	Noon - 6PM	-0.34	-0.36	-0.05	-0.41	-0.24	NA	0.8022	
	6PM - Midnight	-0.03	-0.23	-0.43 ↓	-0.11	0.11	NA	0.5817	

Table 2. (Continued)

Activity									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value[#]
		1	2	3	4	5	6		
12	Midnight - 6AM	0.02	-0.03	-0.26	-0.14	0.00	NA	0.7983	
	6AM - Noon	0.14	-0.19	-0.13	-0.17	-0.30	NA	0.9165	
	Noon - 6PM	-0.07	-0.26	-0.19	-0.39	-0.31	NA	0.9298	
	6PM - Midnight	-0.03	-0.19	-0.07	0.14	-0.45	NA	0.8764	
13	Midnight - 6AM	-0.10	-0.19	-0.15	-0.12	-0.07	NA	0.9977	
	6AM - Noon	-0.36 ↓	-0.35 ↓	-0.43 ↓	-0.38	-0.66	NA	0.9379	
	Noon - 6PM	-0.20	-0.45 ↓	-0.46 ↓	-0.43	-0.08	NA	0.8172	
	6PM - Midnight	-0.10	-0.29	-0.58 ↓	-0.21	-0.11	NA	0.3947	
14	Midnight - 6AM	-0.16	-0.29	-0.26	-0.22	0.13	NA	0.8195	
	6AM - Noon	0.71 ↑	0.42	0.22	0.73 ↑	0.75	NA	0.6456	
	Noon - 6PM	0.06	-0.47	-0.31	-0.23	-0.38	NA	0.7358	
	6PM - Midnight	-0.26	-0.37 ↓	-0.42 ↓	-0.39	-0.33	NA	0.9676	
15	Midnight - 6AM	0.38	-0.19	-0.26	-0.18	0.20	NA	0.1260	
	6AM - Noon	-0.14	-0.25	-0.17	-0.45 ↓	-0.54	NA	0.6691	
	Noon - 6PM	-0.12	-0.43 ↓	-0.35	-0.40	-0.33	NA	0.7044	
	6PM - Midnight	-0.25	-0.18	-0.42 ↓	-0.22	-0.29	NA	0.8763	
16	Midnight - 6AM	-0.09	-0.21	-0.06	-0.06	0.04	NA	0.8285	
	6AM - Noon	0.16	-0.57	-0.60 ↓	-0.39	-0.57	NA	0.3062	
	Noon - 6PM	-0.04	-0.42	-0.18	-0.33	-0.43	NA	0.7484	
	6PM - Midnight	-0.04	-0.05	-0.33	-0.27	-0.35	NA	0.6439	
17	Midnight - 6AM	0.16	-0.10	0.06	-0.14	-0.10	NA	0.8302	
	6AM - Noon	0.03	-0.50	-0.48	-0.33	-0.74	NA	0.5444	
	Noon - 6PM	-0.13	-0.51 ↓	-0.31	0.17	-0.42	NA	0.4936	
	6PM - Midnight	0.27	-0.40	-0.49	-0.25	-0.40	NA	0.2189	

Table 2. (Continued)

Activity									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value[#]
		1	2	3	4	5	6		
18	Midnight - 6AM	-0.18	-0.04	-0.24	0.00	0.42	NA	0.6554	
	6AM - Noon	-0.33	-0.41	-0.40	-0.57	-1.04	NA	0.8305	
	Noon - 6PM	-0.11	-0.32	-0.24	-0.11	0.08	NA	0.9095	
	6PM - Midnight	-0.06	-0.31	-0.32	-0.16	-0.15	NA	0.8823	
19	Midnight - 6AM	-0.15	-0.19	-0.14	-0.13	-0.55	NA	0.9254	
	6AM - Noon	-0.12	-0.37	-0.15	-0.53	-0.56	NA	0.7316	
	Noon - 6PM	-0.02	-0.21	-0.23	-0.13	-0.30	NA	0.9703	
	6PM - Midnight	-0.20	-0.24	-0.56 ↓	-0.15	-0.61	NA	0.3986	
20	Midnight - 6AM	-0.07	-0.11	-0.21	-0.12	0.05	NA	0.9503	
	6AM - Noon	0.00	-0.09	-0.38	-0.23	-0.70	NA	0.5638	
	Noon - 6PM	-0.13	-0.42	-0.20	0.00	-0.22	NA	0.9150	
	6PM - Midnight	-0.13	-0.26	-0.23	0.04	-0.23	NA	0.9008	
21	Midnight - 6AM	-0.14	-0.26	-0.26	0.19	0.19	NA	0.4512	
	6AM - Noon	0.53	0.47	0.32	1.12	1.11	NA	0.8033	

Cells contain all pairwise comparisons that are statistically significant at the 0.05 level. The format within each cell is (1) the difference of means, (2) the relationship between the corresponding pair of Group means shown in parentheses [For example, "(1<6)" indicates that the mean baseline adjusted value for Group 6 was significantly greater than that for Group 1], and (3) the Tukey-adjusted p-value.

↑, ↓ Indicate that the mean baseline adjusted value was significantly different from zero (at the 0.05 level). "↑" indicates that the mean at the study time was greater than that at baseline, while "↓" indicates that the mean at the study time was less than that at baseline.

NA Data not available for this Group at this study time.

* Group effect was significant at the 0.05 level.

Table 3. Summary of the ANOVA Results for the Baseline Adjusted Six-Hour Averages for Heart Rate (BPM)

Heart Rate									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value[#]
		1	2	3	4	5	6		
0	6AM - Noon	22.13	38.16 ↑	20.69	61.63 ↑	NA	NA	0.1750	
	Noon - 6PM	9.15	16.48	-3.19	9.69	19.16 ↑	10.06	0.4798	
	6PM - Midnight	-9.31	9.57	-2.72	1.24	10.93	11.83	0.2873	
1	Midnight - 6AM	-6.05	9.65	-1.52	-6.56	0.83	15.52 ↑	0.1053	
	6AM - Noon	27.36 ↑	33.18 ↑	22.08 ↑	22.06 ↑	25.39 ↑	19.43 ↑	0.0479 *	-13.75 (6<2) 0.0377
	Noon - 6PM	3.56	3.95	-6.01	1.58	-28.50 ↓	-4.56	0.1363	
	6PM - Midnight	14.82	26.04 ↑	9.49	36.46 ↑	-9.64	30.53 ↑	0.0369 *	-46.10 (5<4) 0.0332
2	Midnight - 6AM	3.52	17.84	-1.56	41.09 ↑	14.28	55.84 ↑	0.0269 *	-57.40 (3<6) 0.0391
	6AM - Noon	28.14 ↑	36.44 ↑	27.34 ↑	36.90 ↑	24.72 ↑	49.64 ↑	0.0208 *	-22.30 (3<6) 0.0458 -24.92 (5<6) 0.0203
	Noon - 6PM	11.01	4.56	4.04	7.13	11.25	19.14 ↑	0.6420	
	6PM - Midnight	-4.89	-1.46	-4.53	1.54	32.64 ↑	21.42 ↑	0.0242 *	
3	Midnight - 6AM	-0.62	3.60	-4.25	6.77	36.37 ↑	32.30 ↑	0.0036 *	-36.99 (1<5) 0.0271 -40.61 (3<5) 0.0127 -36.54 (3<6) 0.0450
	6AM - Noon	12.44	15.27	3.84	13.03	10.81	26.33 ↑	0.5870	
	Noon - 6PM	14.85	8.38	5.23	14.09	-15.95	5.97	0.2819	
	6PM - Midnight	4.37	10.68	3.13	26.41 ↑	18.81	42.87 ↑	0.1818	
4	Midnight - 6AM	3.89	8.72	1.96	23.02	36.57 ↑	50.02 ↑	0.1760	
	6AM - Noon	-3.43	0.59	-8.08	-0.23	29.32 ↑	1.58	0.0063 *	-32.74 (1<5) 0.0114 -28.72 (2<5) 0.0315 -37.40 (3<5) 0.0034 -29.55 (4<5) 0.0257
	Noon - 6PM	23.70 ↑	10.09	10.94	-1.61	24.05	-73.48 ↓	0.0148 *	-97.18 (6<1) 0.0078 -83.57 (6<2) 0.0258 -84.42 (6<3) 0.0240 -97.53 (6<5) 0.0118
	6PM - Midnight	25.82 ↑	23.19 ↑	18.23 ↑	30.08 ↑	34.23 ↑	NA	0.7694	

Table 3. (Continued)

Heart Rate									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4	5	6		
5	Midnight - 6AM	21.30	26.30 ↑	10.22	27.96 ↑	35.06 ↑	NA	0.6243	
	6AM - Noon	-6.57	-2.71	-6.06	-5.89	10.87	NA	0.2251	
	Noon - 6PM	1.10	-7.76	2.49	1.20	-33.34 ↓	NA	0.1507	
	6PM - Midnight	-5.01	20.59 ↑	5.58	20.45	17.81	NA	0.3331	
6	Midnight - 6AM	-6.17	32.34 ↑	4.61	14.83	14.83	NA	0.0108 *	-38.51 (1<2) 0.0059
	6AM - Noon	-14.08 ↓	-16.97 ↓	-11.64 ↓	-8.28	9.24	NA	0.2547	
	Noon - 6PM	3.29	-45.77 ↓	-14.09	-1.34	21.90	NA	0.0024 *	-49.06 (2<1) 0.0039 -44.42 (2<4) 0.0138 -67.67 (2<5) 0.0224
	6PM - Midnight	-0.09	-11.88	-6.49	15.08	4.97	NA	0.3944	
7	Midnight - 6AM	-4.65	10.34	-4.05	14.10	11.39	NA	0.2104	
	6AM - Noon	-6.14	-7.30 ↓	-12.88 ↓	2.48	-8.55	NA	0.0609	-15.36 (3<4) 0.0307
	Noon - 6PM	6.77	-50.80 ↓	-21.39 ↓	-8.60	7.30	NA	0.0081 *	-57.57 (2<1) 0.0054
	6PM - Midnight	-4.09	-26.79 ↓	-8.76	2.58	-1.46	NA	0.5341	
8	Midnight - 6AM	-8.15	-1.49	0.05	2.38	4.92	NA	0.8162	
	6AM - Noon	1.99	9.44	7.29	9.28	21.83	NA	0.5319	
	Noon - 6PM	5.81	0.53	-1.85	-5.00	9.41	NA	0.4976	
	6PM - Midnight	-2.83	17.94 ↑	2.25	10.61	-4.43	NA	0.2638	
9	Midnight - 6AM	-6.13	12.02	-2.44	26.43 ↑	-6.89	NA	0.1802	
	6AM - Noon	14.16	10.28	7.12	38.18 ↑	12.51	NA	0.1979	
	Noon - 6PM	-14.99	-26.09 ↓	-16.70	6.58	0.77	NA	0.4072	
	6PM - Midnight	-24.35	-29.06 ↓	-28.26 ↓	18.49	-18.28	NA	0.1295	
10	Midnight - 6AM	-16.81	-14.29	-17.86	17.98	-7.18	NA	0.2626	
	6AM - Noon	-1.77	-8.47	-9.50	8.67	4.56	NA	0.0986	
	Noon - 6PM	-4.83	-12.67 ↓	-12.32 ↓	-9.65	14.93	NA	0.3436	
	6PM - Midnight	-16.81	-12.38	-10.93	23.82 ↑	-10.72	NA	0.0850	

Table 3. (Continued)

Heart Rate									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value[#]
		1	2	3	4	5	6		
11	Midnight - 6AM	-17.74	-5.32	-10.29	20.73	-6.43	NA	0.1160	
	6AM - Noon	-6.17	-8.31	-9.44	19.39 ↑	15.13	NA	0.0331 *	-28.83 (3<4) 0.0484
	Noon - 6PM	4.01	-7.67 ↓	-12.90 ↓	1.38	13.35 ↑	NA	0.0006 *	-11.68 (2<1) 0.0317 -16.91 (3<1) 0.0019 -21.02 (2<5) 0.0248 -14.28 (3<4) 0.0219 -26.25 (3<5) 0.0049
	6PM - Midnight	-7.16	-8.26	-15.36	14.77	-5.33	NA	0.2565	
12	Midnight - 6AM	-14.76	-4.05	-16.15	18.67	0.06	NA	0.1212	
	6AM - Noon	-6.85	-5.22	-1.92	10.63	-1.49	NA	0.2468	
	Noon - 6PM	-13.58	-12.45	-8.20	-1.94	9.82	NA	0.5515	
	6PM - Midnight	-21.46 ↓	-15.53	-15.30	17.07	-24.99	NA	0.0791	
13	Midnight - 6AM	-21.35 ↓	-11.69	-18.55 ↓	13.29	5.28	NA	0.0997	
	6AM - Noon	-16.44 ↓	-10.73	-16.66 ↓	3.90	4.48	NA	0.1082	
	Noon - 6PM	-16.60 ↓	-8.58	-6.37	-1.10	7.27	NA	0.1870	
	6PM - Midnight	-23.27 ↓	-13.32	-13.81	4.51	0.71	NA	0.2204	
14	Midnight - 6AM	-12.76	-8.66	-13.46	7.87	6.72	NA	0.5930	
	6AM - Noon	1.41	-6.29	-3.77	15.14	8.56	NA	0.2483	
	Noon - 6PM	8.37	-0.42	-1.26	5.20	8.90	NA	0.6183	
	6PM - Midnight	-8.64	-9.24	-4.24	8.87	8.51	NA	0.7039	
15	Midnight - 6AM	-5.00	-8.34	-10.56	7.38	4.28	NA	0.8378	
	6AM - Noon	-1.98	0.19	-9.41	8.99	11.75	NA	0.2340	
	Noon - 6PM	-14.87 ↓	-16.83 ↓	-13.12 ↓	-1.59	0.01	NA	0.3325	
	6PM - Midnight	-25.70 ↓	-18.09	-17.12	-0.81	-9.34	NA	0.5227	

Table 3. (Continued)

Heart Rate									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value[#]
		1	2	3	4	5	6		
16	Midnight - 6AM	-12.28	-12.01	-11.47	-1.12	-12.45	NA	0.9359	
	6AM - Noon	-2.69	2.18	-8.02	8.87	3.98	NA	0.2531	
	Noon - 6PM	-11.13 ↓	-0.62	-0.96	6.92	7.58	NA	0.0915	
	6PM - Midnight	-24.61 ↓	-5.68	-0.85	7.00	-5.17	NA	0.3300	
17	Midnight - 6AM	-17.43	-3.23	-4.12	4.32	6.03	NA	0.5937	
	6AM - Noon	-5.23	-6.63	-8.33 ↓	-1.39	-4.26	NA	0.7868	
	Noon - 6PM	-6.51	-9.24	-14.64 ↓	-3.37	7.35	NA	0.3718	
	6PM - Midnight	-5.46	-10.13	-11.76	-3.76	-1.70	NA	0.9874	
18	Midnight - 6AM	-10.17	-6.82	-9.20	0.06	0.49	NA	0.9563	
	6AM - Noon	-12.37 ↓	-10.43 ↓	-15.17 ↓	-5.12	-0.64	NA	0.3569	
	Noon - 6PM	0.05	-12.90 ↓	-21.89 ↓	-0.21	-9.31	NA	0.0744	
	6PM - Midnight	-20.26	-18.68	-16.27	8.53	-14.45	NA	0.4851	
19	Midnight - 6AM	-18.57	-14.95	-13.30	10.71	-9.59	NA	0.3551	
	6AM - Noon	-5.63	-11.79	-12.21	2.38	13.55	NA	0.3871	
	Noon - 6PM	-11.08	-13.06	-9.37	-4.81	-2.15	NA	0.9026	
	6PM - Midnight	-30.22 ↓	-17.05	-19.29	2.58	-23.59	NA	0.4236	
20	Midnight - 6AM	-28.60 ↓	-10.81	-17.07	23.28	-10.93	NA	0.0661	-51.88 (1<4) 0.0363
	6AM - Noon	-12.21 ↓	-8.68 ↓	-15.41 ↓	5.73	-2.10	NA	0.0180 *	-17.94 (1<4) 0.0363 -21.15 (3<4) 0.0122
	Noon - 6PM	-3.55	-7.23	-18.30 ↓	-5.57	-7.17	NA	0.1732	
	6PM - Midnight	-18.76 ↓	-9.34	-7.99	20.38	-7.68	NA	0.1357	
21	Midnight - 6AM	-22.02 ↓	-8.19	-12.43	16.81	-6.63	NA	0.1787	
	6AM - Noon	-27.02 ↓	-28.01 ↓	-32.98 ↓	-10.38	-11.79	NA	0.1486	

Cells contain all pairwise comparisons that are statistically significant at the 0.05 level. The format within each cell is (1) the difference of means, (2) the relationship between the corresponding pair of Group means shown in parentheses [For example, "(1<6)" indicates that the mean baseline adjusted value for Group 6 was significantly greater than that for Group 1], and (3) the Tukey-adjusted p-value.

↑, ↓ Indicate that the mean baseline adjusted value was significantly different from zero (at the 0.05 level). "↑" indicates that the mean at the study time was greater than that at baseline, while "↓" indicates that the mean at the study time was less than that at baseline.

* Group effect was significant at the 0.05 level.

NA Data not available for this Group at this study time.

Table 4. Summary of the ANOVA Results for the Baseline Adjusted Six-Hour Averages for RP Expiratory Time (Seconds)

RP Expiratory Time									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value[#]
		1	2	3	4	5	6		
0	6AM - Noon	-0.12 ↓	-0.17 ↓	-0.11 ↓	-0.26 ↓	NA	NA	0.2401	
	Noon - 6PM	-0.05	-0.06	0.03	-0.03	-0.04	-0.02	0.4223	
	6PM - Midnight	0.01	0.02	0.07	0.06	-0.01	0.03	0.7305	
1	Midnight - 6AM	-0.01	-0.04	0.07	0.01	-0.02	0.02	0.4677	
	6AM - Noon	-0.06	-0.12 ↓	-0.04	-0.07 ↓	-0.10 ↓	-0.03	0.2536	
	Noon - 6PM	0.02	-0.03	-0.02	0.03	0.08	0.02	0.5652	
	6PM - Midnight	0.01	-0.04	-0.03	-0.07	0.01	-0.08	0.6755	
2	Midnight - 6AM	-0.01	-0.08	0.00	-0.13 ↓	-0.01	-0.22 ↓	0.0754	
	6AM - Noon	-0.08	-0.14 ↓	-0.11 ↓	-0.17 ↓	-0.10	-0.17 ↓	0.7225	
	Noon - 6PM	0.03	0.02	-0.02	0.00	-0.05	-0.12	0.4864	
	6PM - Midnight	0.01	0.00	-0.03	-0.01	-0.15 ↓	-0.17 ↓	0.1569	
3	Midnight - 6AM	0.00	-0.09	-0.04	-0.08	-0.20 ↓	-0.21 ↓	0.1083	
	6AM - Noon	-0.07	-0.15 ↓	-0.08	-0.12 ↓	-0.16 ↓	-0.17 ↓	0.5627	
	Noon - 6PM	-0.01	-0.03	0.00	-0.04	-0.03	-0.07	0.9725	
	6PM - Midnight	-0.01	-0.01	0.01	-0.08	-0.19 ↓	-0.12	0.4552	
4	Midnight - 6AM	-0.06	-0.11	-0.02	-0.11	-0.23 ↓	-0.18	0.4925	
	6AM - Noon	-0.07	-0.09	0.00	-0.10	-0.05	-0.14	0.8742	
	Noon - 6PM	-0.03	-0.05	-0.01	-0.01	-0.22 ↓	-0.12	0.0291 *	-0.19 (5<1) 0.0496 -0.21 (5<3) 0.0294 -0.21 (5<4) 0.0230
	6PM - Midnight	-0.08 ↓	-0.07	-0.08 ↓	-0.07	-0.27 ↓	NA	0.0215 *	-0.19 (5<1) 0.0312 -0.20 (5<2) 0.0233 -0.19 (5<3) 0.0355 -0.20 (5<4) 0.0354

Table 4. (Continued)

RP Expiratory Time									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value[#]
		1	2	3	4	5	6		
5	Midnight - 6AM	-0.04	-0.13 ↓	-0.07	0.00	-0.29 ↓	NA	0.0235 *	-0.25 (5<1) 0.0442 -0.30 (5<4) 0.0185
	6AM - Noon	-0.02	-0.11 ↓	-0.05	0.01	-0.18 ↓	NA	0.0375 *	-0.19 (5<4) 0.0422
	Noon - 6PM	-0.01	0.03	0.01	0.00	-0.10 ↓	NA	0.2616	
	6PM - Midnight	0.01	-0.04	0.05	-0.03	0.00	NA	0.7478	
6	Midnight - 6AM	-0.03	0.05	0.10	0.00	-0.03	NA	0.8364	
	6AM - Noon	0.05	0.26 ↑	0.22 ↑	0.07	0.09	NA	0.3494	
	Noon - 6PM	0.02	0.32 ↑	0.25 ↑	0.05	-0.05	NA	0.1491	
	6PM - Midnight	-0.02	0.18	0.27 ↑	-0.01	-0.04	NA	0.2888	
7	Midnight - 6AM	0.00	0.13	0.26 ↑	-0.03	-0.08	NA	0.3826	
	6AM - Noon	0.01	0.19	0.30 ↑	0.02	-0.07	NA	0.2783	
	Noon - 6PM	0.01	0.43 ↑	0.32 ↑	0.04	-0.02	NA	0.1932	
	6PM - Midnight	-0.06	0.35 ↑	0.35 ↑	-0.05	-0.06	NA	0.2118	
8	Midnight - 6AM	-0.08	0.22	0.36 ↑	-0.07	-0.09	NA	0.2848	
	6AM - Noon	-0.01	0.06	0.09	-0.02	-0.03	NA	0.5805	
	Noon - 6PM	-0.06	0.01	0.00	0.01	-0.02	NA	0.8485	
	6PM - Midnight	-0.09 ↓	-0.06	-0.01	-0.09 ↓	-0.08	NA	0.6223	
9	Midnight - 6AM	-0.09 ↓	-0.13 ↓	-0.05	-0.12 ↓	-0.04	NA	0.6489	
	6AM - Noon	-0.03	-0.05	-0.03	-0.06	-0.06	NA	0.9538	
	Noon - 6PM	-0.02	0.02	0.00	0.01	0.02	NA	0.9842	
	6PM - Midnight	-0.05	-0.05	0.08	-0.08	0.04	NA	0.1717	
10	Midnight - 6AM	-0.04	-0.08	-0.05	-0.10	-0.08	NA	0.9062	
	6AM - Noon	-0.01	-0.05	0.00	0.00	0.11	NA	0.2995	
	Noon - 6PM	-0.04	-0.06	0.03	0.02	-0.04	NA	0.5821	
	6PM - Midnight	-0.01	-0.01	0.02	0.15	0.00	NA	0.4683	

Table 4. (Continued)

RP Expiratory Time									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value[#]
		1	2	3	4	5	6		
11	Midnight - 6AM	-0.04	-0.10	0.02	0.09	0.05	NA	0.2024	
	6AM - Noon	-0.01	-0.04	-0.03	0.15 ↑	0.04	NA	0.0499 *	-0.19 (2<4) 0.0494
	Noon - 6PM	-0.04	-0.03	-0.02	0.06	0.00	NA	0.7158	
	6PM - Midnight	-0.02	-0.04	0.02	-0.04	-0.02	NA	0.8422	
12	Midnight - 6AM	-0.05	-0.21 ↓	-0.01	-0.04	-0.06	NA	0.0151 *	-0.16 (2<1) 0.0470 -0.19 (2<3) 0.0128
	6AM - Noon	0.06	-0.06	0.01	0.04	0.08	NA	0.2259	
	Noon - 6PM	0.03	-0.03	-0.01	0.07	0.00	NA	0.7445	
	6PM - Midnight	0.00	-0.02	0.01	-0.08	0.12	NA	0.6903	
13	Midnight - 6AM	0.04	-0.10	-0.05	-0.09	-0.01	NA	0.6185	
	6AM - Noon	0.04	-0.06	-0.02	0.05	0.05	NA	0.5210	
	Noon - 6PM	0.03	-0.03	-0.02	0.01	0.03	NA	0.8851	
	6PM - Midnight	-0.02	-0.01	-0.02	-0.04	0.05	NA	0.9753	
14	Midnight - 6AM	0.02	-0.08	-0.03	-0.08	-0.06	NA	0.6841	
	6AM - Noon	-0.04	-0.06	-0.04	-0.06	-0.05	NA	0.9880	
	Noon - 6PM	-0.04	-0.02	-0.01	0.00	0.07	NA	0.8709	
	6PM - Midnight	-0.02	0.05	-0.02	-0.01	-0.03	NA	0.8871	
15	Midnight - 6AM	-0.04	-0.09	-0.08	-0.03	-0.07	NA	0.9737	
	6AM - Noon	-0.02	-0.06	-0.03	0.00	0.08	NA	0.6182	
	Noon - 6PM	0.00	0.02	-0.02	0.01	0.01	NA	0.9869	
	6PM - Midnight	-0.03	-0.05	-0.04	-0.02	-0.01	NA	0.9935	
16	Midnight - 6AM	-0.05	-0.08	-0.06	-0.09	0.07	NA	0.8983	
	6AM - Noon	0.03	-0.08	-0.01	0.04	0.11	NA	0.3066	
	Noon - 6PM	0.01	-0.01	-0.02	0.00	-0.01	NA	0.9973	
	6PM - Midnight	-0.02	-0.04	-0.04	-0.06	0.09	NA	0.8114	

Table 4. (Continued)

RP Expiratory Time									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value[#]
		1	2	3	4	5	6		
17	Midnight - 6AM	0.00	-0.12 ↓	-0.05	-0.02	0.08	NA	0.4033	
	6AM - Noon	-0.04	0.00	0.02	0.04	0.07	NA	0.4028	
	Noon - 6PM	-0.04	-0.01	0.01	0.01	0.04	NA	0.9455	
	6PM - Midnight	-0.05	0.02	0.01	-0.01	0.00	NA	0.8740	
18	Midnight - 6AM	-0.04	-0.09	-0.01	-0.03	-0.01	NA	0.8591	
	6AM - Noon	-0.01	-0.05	0.06	0.05	0.06	NA	0.4214	
	Noon - 6PM	-0.05	0.01	-0.02	0.00	0.00	NA	0.8972	
	6PM - Midnight	-0.05	0.02	-0.02	-0.05	-0.01	NA	0.9006	
19	Midnight - 6AM	-0.06	-0.11	-0.01	-0.08	-0.09	NA	0.8350	
	6AM - Noon	-0.03	-0.02	0.01	0.03	-0.02	NA	0.8810	
	Noon - 6PM	0.00	-0.02	-0.02	-0.02	0.00	NA	0.9941	
	6PM - Midnight	0.03	-0.02	0.05	-0.05	0.01	NA	0.4663	
20	Midnight - 6AM	0.08	-0.11	0.04	-0.06	0.06	NA	0.1805	
	6AM - Noon	0.00	-0.06 ↓	0.02	0.01	0.07	NA	0.2019	
	Noon - 6PM	-0.03	-0.06	-0.04	-0.02	0.00	NA	0.9120	
	6PM - Midnight	-0.03	-0.05	-0.01	-0.06	0.04	NA	0.8750	
21	Midnight - 6AM	-0.04	-0.11	-0.03	-0.04	-0.02	NA	0.8630	
	6AM - Noon	-0.02	-0.09	0.00	-0.04	0.04	NA	0.7086	

Cells contain all pairwise comparisons that are statistically significant at the 0.05 level. The format within each cell is (1) the difference of means, (2) the relationship between the corresponding pair of Group means shown in parentheses [For example, "(1<6)" indicates that the mean baseline adjusted value for Group 6 was significantly greater than that for Group 1], and (3) the Tukey-adjusted p-value.

↑, ↓ Indicate that the mean baseline adjusted value was significantly different from zero (at the 0.05 level). "↑" indicates that the mean at the study time was greater than that at baseline**,** while "↓" indicates that the mean at the study time was less than that at baseline.

* Group effect was significant at the 0.05 level.

NA Data not available for this Group at this study time.

Table 5. Summary of the ANOVA Results for the Baseline Adjusted Six-Hour Averages for RP Inspiratory Time (Seconds)

RP Inspiratory Time									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value [#]
		1	2	3	4	5	6		
0	6AM - Noon	-0.12 ↓	-0.05	-0.05	-0.13	NA	NA	0.5769	
	Noon - 6PM	-0.08 ↓	0.02	0.01	-0.01	-0.04	-0.02	0.3054	
	6PM - Midnight	-0.01	0.01	0.02	0.00	-0.04	-0.01	0.8324	
1	Midnight - 6AM	-0.02	0.04	-0.03	-0.02	0.02	0.10	0.5210	
	6AM - Noon	-0.10 ↓	-0.03	-0.02	-0.07	-0.05	0.03	0.5017	
	Noon - 6PM	-0.03	0.02	0.00	0.00	0.06	0.08	0.5192	
	6PM - Midnight	-0.07	0.01	0.00	-0.02	-0.02	-0.03	0.9754	
2	Midnight - 6AM	-0.03	-0.02	-0.02	-0.03	-0.10	-0.15 ↓	0.6075	
	6AM - Noon	-0.09	-0.06	-0.03	-0.07	-0.14 ↓	-0.15 ↓	0.3835	
	Noon - 6PM	-0.04	0.00	-0.01	-0.02	-0.10 ↓	-0.13 ↓	0.2892	
	6PM - Midnight	-0.03	0.00	0.01	-0.07	-0.19 ↓	-0.14 ↓	0.0163 *	-0.19 (5<2) 0.0450 -0.20 (5<3) 0.0264
3	Midnight - 6AM	-0.02	-0.02	-0.03	-0.08	-0.21 ↓	-0.21 ↓	0.0471 *	
	6AM - Noon	-0.07 ↓	-0.04	-0.01	-0.09 ↓	-0.20 ↓	-0.14 ↓	0.0030 *	-0.16 (5<2) 0.0153 -0.19 (5<3) 0.0027
	Noon - 6PM	-0.05	0.02	0.02	-0.02	-0.01	-0.08	0.9027	
	6PM - Midnight	-0.03	0.02	-0.01	0.03	-0.17 ↓	-0.14 ↓	0.0238 *	-0.19 (5<2) 0.0417 -0.19 (5<4) 0.0403
4	Midnight - 6AM	-0.03	0.05	-0.04	-0.04	-0.21 ↓	-0.11	0.0158 *	-0.26 (5<2) 0.0059
	6AM - Noon	-0.05	0.03	-0.04	-0.04	0.42	-0.14	0.6463	
	Noon - 6PM	-0.06 ↓	0.03	0.01	-0.02	-0.11 ↓	-0.15 ↓	0.0083 *	-0.14 (5<2) 0.0222
	6PM - Midnight	-0.05	-0.02	-0.03	-0.02	-0.15 ↓	NA	0.2084	
5	Midnight - 6AM	-0.02	0.00	-0.07	-0.03	-0.15 ↓	NA	0.1974	
	6AM - Noon	-0.07	0.03	-0.01	-0.04	-0.20 ↓	NA	0.0661	-0.23 (5<2) 0.0400
	Noon - 6PM	0.01	0.05	0.06	0.01	-0.12 ↓	NA	0.0875	
	6PM - Midnight	-0.02	0.01	0.09	-0.04	0.00	NA	0.5178	

Table 5. (Continued)

RP Inspiratory Time									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
		1	2	3	4	5	6		
6	Midnight - 6AM	0.04	0.14 ↑	0.10	-0.09	-0.02	NA	0.1870	
	6AM - Noon	0.01	0.20 ↑	0.13	0.00	-0.03	NA	0.1424	
	Noon - 6PM	-0.01	0.24 ↑	0.13	0.00	0.05	NA	0.1743	
	6PM - Midnight	0.01	0.15	0.18 ↑	-0.04	0.00	NA	0.3579	
7	Midnight - 6AM	0.08	0.25 ↑	0.18	-0.04	-0.02	NA	0.4037	
	6AM - Noon	0.02	0.16	0.15	-0.06	-0.05	NA	0.3044	
	Noon - 6PM	0.02	0.35 ↑	0.23	0.07	0.04	NA	0.3027	
	6PM - Midnight	0.02	0.25	0.31 ↑	0.00	0.02	NA	0.3127	
8	Midnight - 6AM	0.01	0.25 ↑	0.21	0.04	0.01	NA	0.4975	
	6AM - Noon	0.01	0.12 ↑	0.12 ↑	-0.07	-0.01	NA	0.0755	
	Noon - 6PM	-0.01	0.08 ↑	0.01	0.03	0.00	NA	0.3558	
	6PM - Midnight	-0.03	0.04	-0.02	-0.02	-0.01	NA	0.9100	
9	Midnight - 6AM	-0.04	-0.04	-0.03	-0.01	0.02	NA	0.9855	
	6AM - Noon	0.00	0.04	0.00	-0.05	-0.02	NA	0.6900	
	Noon - 6PM	0.00	0.06	0.00	0.14 ↑	-0.01	NA	0.1542	
	6PM - Midnight	-0.02	0.07	0.06	0.07	0.00	NA	0.7119	
10	Midnight - 6AM	0.05	-0.02	-0.03	-0.02	0.04	NA	0.7881	
	6AM - Noon	-0.01	0.03	0.00	-0.02	0.06	NA	0.9130	
	Noon - 6PM	0.03	0.02	-0.02	0.06	0.01	NA	0.5486	
	6PM - Midnight	0.01	0.01	0.01	0.40 ↑	0.04	NA	0.3972	
11	Midnight - 6AM	0.08	-0.02	0.02	0.33 ↑	-0.05	NA	0.3772	
	6AM - Noon	0.00	0.00	-0.02	0.48 ↑	-0.04	NA	0.3051	
	Noon - 6PM	0.01	0.03	0.02	0.06	0.00	NA	0.9059	
	6PM - Midnight	0.03	-0.01	0.02	0.04	-0.03	NA	0.8926	
12	Midnight - 6AM	0.04	-0.01	0.00	0.03	-0.05	NA	0.9776	
	6AM - Noon	0.01	0.01	0.03	0.05	-0.02	NA	0.9734	
	Noon - 6PM	0.00	0.04	-0.01	0.05	0.00	NA	0.7948	
	6PM - Midnight	-0.01	-0.01	-0.01	0.05	-0.02	NA	0.8308	

Table 5. (Continued)

RP Inspiratory Time									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
		1	2	3	4	5	6		
13	Midnight - 6AM	0.01	-0.04	0.02	0.05	-0.01	NA	0.9004	
	6AM - Noon	-0.03	0.01	0.07	0.01	-0.02	NA	0.6330	
	Noon - 6PM	0.03	0.02	0.02	0.08	-0.03	NA	0.7101	
	6PM - Midnight	0.04	0.02	0.04	0.11	-0.03	NA	0.8018	
14	Midnight - 6AM	0.02	0.05	0.00	0.07	-0.03	NA	0.9191	
	6AM - Noon	-0.04	0.00	0.01	-0.03	-0.04	NA	0.8979	
	Noon - 6PM	0.04	0.04	0.05	0.10 ↑	-0.01	NA	0.7026	
	6PM - Midnight	0.02	0.06	0.02	0.11	-0.02	NA	0.6394	
15	Midnight - 6AM	0.01	-0.01	0.02	0.05	-0.02	NA	0.9720	
	6AM - Noon	0.02	0.04	0.06	0.07	-0.03	NA	0.9512	
	Noon - 6PM	0.00	0.04	0.04	0.09	-0.04	NA	0.6844	
	6PM - Midnight	0.01	0.05	0.03	0.11	-0.05	NA	0.6423	
16	Midnight - 6AM	0.01	0.01	0.01	0.08	-0.01	NA	0.9477	
	6AM - Noon	-0.01	0.05	0.07	0.06	-0.03	NA	0.6528	
	Noon - 6PM	-0.01	0.05	0.04	0.07	-0.02	NA	0.5922	
	6PM - Midnight	0.02	0.00	0.00	0.04	-0.03	NA	0.9458	
17	Midnight - 6AM	0.02	0.02	-0.02	0.05	-0.02	NA	0.9622	
	6AM - Noon	-0.02	0.03	0.04	0.06	-0.04	NA	0.6357	
	Noon - 6PM	-0.01	0.02	0.00	0.07	-0.04	NA	0.5859	
	6PM - Midnight	-0.05	0.04	0.01	0.13	-0.03	NA	0.3056	
18	Midnight - 6AM	-0.01	0.00	-0.01	0.03	0.00	NA	0.9878	
	6AM - Noon	0.02	0.01	0.01	0.06	-0.03	NA	0.8818	
	Noon - 6PM	-0.01	0.03	0.02	0.09	-0.04	NA	0.4446	
	6PM - Midnight	0.02	0.01	0.02	0.11	-0.02	NA	0.8310	

Table 5. (Continued)

RP Inspiratory Time									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
		1	2	3	4	5	6		
19	Midnight - 6AM	0.03	-0.04	0.05	0.05	0.04	NA	0.8401	
	6AM - Noon	-0.01	0.03	0.05	0.04	-0.02	NA	0.8834	
	Noon - 6PM	-0.01	0.05	-0.01	0.06	0.01	NA	0.4585	
	6PM - Midnight	0.02	0.01	0.03	0.11	0.03	NA	0.8963	
20	Midnight - 6AM	0.05	0.01	0.05	0.04	-0.02	NA	0.9567	
	6AM - Noon	0.05	0.06	0.05	0.05	-0.02	NA	0.9282	
	Noon - 6PM	-0.04	0.02	0.01	0.00	-0.02	NA	0.7146	
	6PM - Midnight	0.02	0.02	0.02	0.05	-0.02	NA	0.9783	
21	Midnight - 6AM	0.09	0.00	0.01	0.07	-0.02	NA	0.7896	
	6AM - Noon	0.05	0.05	0.04	0.03	-0.05	NA	0.9663	

Cells contain all pairwise comparisons that are statistically significant at the 0.05 level. The format within each cell is (1) the difference of means, (2) the relationship between the corresponding pair of Group means shown in parentheses [For example, "(1<6)" indicates that the mean baseline adjusted value for Group 6 was significantly greater than that for Group 1], and (3) the Tukey-adjusted p-value.

↑, ↓ Indicate that the mean baseline adjusted value was significantly different from zero (at the 0.05 level). "↑" indicates that the mean at the study time was greater than that at baseline**,** while "↓" indicates that the mean at the study time was less than that at baseline.

* Group effect was significant at the 0.05 level.

NA Data not available for this Group at this study time.

Table 6. Summary of the ANOVA Results for the Baseline Adjusted Six-Hour Averages for RP Integral (mmHg-seconds)

RP Integral									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
		1	2	3	4	5	6		
0	6AM - Noon	2.24	3.49 ↑	1.28	1.66	NA	NA	0.7033	
	Noon - 6PM	0.51	3.29 ↑	-0.37	0.00	0.44	-0.74	0.2196	
	6PM - Midnight	-1.54	1.37	0.01	-1.20	-0.74	-1.80	0.6757	
1	Midnight - 6AM	-0.44	1.99	0.50	0.11	0.18	-2.39 ↓	0.1565	
	6AM - Noon	1.76	2.94 ↑	-0.09	1.10	0.92	-0.73	0.2933	
	Noon - 6PM	-0.66	2.59	0.10	-0.96	0.17	-1.03	0.4688	
	6PM - Midnight	-0.94	1.58	-0.12	0.41	0.89	0.68	0.8248	
2	Midnight - 6AM	0.44	2.13	0.75	-0.15	0.00	2.70 ↑	0.4145	
	6AM - Noon	1.58	2.56 ↑	0.81	1.52	1.17	2.64 ↑	0.7702	
	Noon - 6PM	0.67	2.02	0.60	-0.25	1.17	2.55	0.6164	
	6PM - Midnight	-0.66	1.88	0.50	-0.93	1.63	2.98	0.5503	
3	Midnight - 6AM	-0.44	3.21	0.52	-0.63	2.11	3.64	0.5136	
	6AM - Noon	0.36	3.11	1.66	0.95	4.74 ↑	2.95	0.3794	
	Noon - 6PM	-1.59	2.89	-0.14	0.13	4.44 ↑	1.63	0.2926	
	6PM - Midnight	-1.66	1.49	-0.04	-0.99	8.74 ↑	1.57	0.0843	
4	Midnight - 6AM	1.86	2.97	0.64	-1.20	7.42 ↑	1.40	0.2447	
	6AM - Noon	2.38	3.52	0.84	1.46	5.41 ↑	1.34	0.6689	
	Noon - 6PM	-1.02	3.71	1.19	1.82	8.54 ↑	2.59	0.0905	-9.56 (1<5) 0.0468
	6PM - Midnight	0.22	2.52	0.98	1.13	9.26 ↑	NA	0.0443 *	-9.04 (1<5) 0.0347
5	Midnight - 6AM	1.42	3.09	1.13	-0.24	8.81 ↑	NA	0.0814	
	6AM - Noon	-0.02	3.27	1.10	-0.80	7.90 ↑	NA	0.0762	
	Noon - 6PM	0.97	2.36	0.87	-0.31	6.00 ↑	NA	0.1822	
	6PM - Midnight	-2.20	1.49	0.53	0.16	0.09	NA	0.8268	

Table 6. (Continued)

RP Integral									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
		1	2	3	4	5	6		
6	Midnight - 6AM	1.00	1.07	0.14	-0.99	0.67	NA	0.9048	
	6AM - Noon	-0.34	-0.30	-1.07	-1.45	-1.13	NA	0.9871	
	Noon - 6PM	-0.87	0.19	-2.65	0.04	0.53	NA	0.7906	
	6PM - Midnight	-1.52	-0.86	-1.99	0.21	0.98	NA	0.9254	
7	Midnight - 6AM	-0.93	-0.17	-1.17	-0.33	2.09	NA	0.9647	
	6AM - Noon	0.37	-0.24	-1.54	-0.49	0.31	NA	0.9631	
	Noon - 6PM	-0.49	-0.71	-2.10	-0.39	0.57	NA	0.9477	
	6PM - Midnight	-0.85	-1.90	-2.46	0.74	0.73	NA	0.8496	
8	Midnight - 6AM	3.59	-0.25	-1.93	1.64	2.07	NA	0.3266	
	6AM - Noon	2.72	0.01	0.25	1.00	0.71	NA	0.7167	
	Noon - 6PM	1.36	1.87	0.78	0.90	0.29	NA	0.9726	
	6PM - Midnight	1.53	1.66	0.17	1.26	1.67	NA	0.9604	
9	Midnight - 6AM	3.93 ↑	2.31	1.74	1.65	1.08	NA	0.8573	
	6AM - Noon	-0.66	2.40	0.88	1.18	1.20	NA	0.8766	
	Noon - 6PM	-0.16	1.57	0.62	0.47	-0.29	NA	0.9570	
	6PM - Midnight	2.10	1.44	1.01	1.22	0.24	NA	0.9660	
10	Midnight - 6AM	4.31 ↑	2.86	1.61	1.76	1.82	NA	0.8578	
	6AM - Noon	2.61	1.86	1.54	0.79	0.66	NA	0.9014	
	Noon - 6PM	1.50	2.84 ↑	1.12	0.99	0.94	NA	0.8673	
	6PM - Midnight	0.73	0.93	0.66	0.68	0.59	NA	0.9999	
11	Midnight - 6AM	3.25	2.64	0.85	1.11	0.13	NA	0.7956	
	6AM - Noon	0.76	1.82	0.96	0.53	-0.75	NA	0.9554	
	Noon - 6PM	0.40	2.77	1.41	0.49	-0.11	NA	0.8094	
	6PM - Midnight	0.23	1.90	0.78	1.00	-0.23	NA	0.9543	

Table 6. (Continued)

RP Integral									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value [#]
		1	2	3	4	5	6		
12	Midnight - 6AM	2.89	3.72 ↑	0.99	1.86	0.36	NA	0.7254	
	6AM - Noon	-0.84	1.95	1.19	0.27	-2.10	NA	0.8306	
	Noon - 6PM	-0.87	1.65	1.15	0.33	-0.18	NA	0.8695	
	6PM - Midnight	2.93 ↑	1.28	1.27	1.04	-1.59	NA	0.6757	
13	Midnight - 6AM	3.35	2.80	0.81	1.56	-0.49	NA	0.7574	
	6AM - Noon	1.17	1.94	0.83	0.37	-2.10	NA	0.7661	
	Noon - 6PM	0.49	1.46	1.05	0.70	-0.85	NA	0.9543	
	6PM - Midnight	1.40	1.38	0.69	0.50	-0.99	NA	0.9485	
14	Midnight - 6AM	0.62	2.08	0.28	1.45	0.60	NA	0.9190	
	6AM - Noon	1.18	2.27	0.94	1.55	-0.18	NA	0.9459	
	Noon - 6PM	0.92	1.45	0.16	0.53	-0.09	NA	0.9362	
	6PM - Midnight	2.14	0.18	0.24	0.31	-0.36	NA	0.8027	
15	Midnight - 6AM	4.25	1.82	0.21	1.57	0.45	NA	0.6991	
	6AM - Noon	3.38 ↑	1.09	-0.04	0.80	-1.05	NA	0.5057	
	Noon - 6PM	1.82	1.89	0.56	0.56	-1.05	NA	0.8633	
	6PM - Midnight	1.71	0.90	0.50	0.61	-0.61	NA	0.9418	
16	Midnight - 6AM	3.02	2.16	0.93	1.41	-0.39	NA	0.8231	
	6AM - Noon	1.32	2.18	0.57	0.15	-1.24	NA	0.8094	
	Noon - 6PM	-3.94	1.37	0.78	0.32	-0.23	NA	0.6457	
	6PM - Midnight	-2.14	0.39	0.61	0.36	-0.15	NA	0.8928	
17	Midnight - 6AM	2.14	2.00	0.60	0.82	0.19	NA	0.9301	
	6AM - Noon	2.20	0.76	0.55	0.23	-1.11	NA	0.7516	
	Noon - 6PM	1.20	1.79	0.80	0.30	-0.11	NA	0.9522	
	6PM - Midnight	2.28	0.10	0.60	0.52	-0.08	NA	0.8214	

Table 6. (Continued)

RP Integral									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
		1	2	3	4	5	6		
18	Midnight - 6AM	3.23	1.50	0.60	0.69	1.24	NA	0.8738	
	6AM - Noon	3.09	1.65	0.43	0.00	-1.09	NA	0.6480	
	Noon - 6PM	1.82	0.92	1.41	0.57	-0.24	NA	0.9351	
	6PM - Midnight	2.75	-1.56	0.64	1.09	0.02	NA	0.6083	
19	Midnight - 6AM	4.88	-1.16	0.76	1.41	0.90	NA	0.6568	
	6AM - Noon	1.79	1.16	0.88	0.74	0.73	NA	0.9853	
	Noon - 6PM	-2.78	2.25	0.94	0.69	0.76	NA	0.7263	
	6PM - Midnight	-1.11	1.82	0.31	1.50	0.86	NA	0.8321	
20	Midnight - 6AM	2.65	2.74	0.81	2.97	0.50	NA	0.8471	
	6AM - Noon	3.26	2.77	0.72	0.88	-0.24	NA	0.7397	
	Noon - 6PM	1.91	2.75 ↑	1.10	1.36	1.11	NA	0.8947	
	6PM - Midnight	2.55	1.68	-0.01	1.54	0.11	NA	0.7967	
21	Midnight - 6AM	4.23	2.54	0.44	3.16	0.19	NA	0.8189	
	6AM - Noon	2.95	2.93	0.34	1.46	-0.24	NA	0.7819	

Cells contain all pairwise comparisons that are statistically significant at the 0.05 level. The format within each cell is (1) the difference of means, (2) the relationship between the corresponding pair of Group means shown in parentheses [For example, "(1<6)" indicates that the mean baseline adjusted value for Group 6 was significantly greater than that for Group 1], and (3) the Tukey-adjusted p-value.

↑, ↓ Indicate that the mean baseline adjusted value was significantly different from zero (at the 0.05 level). "↑" indicates that the mean at the study time was greater than that at baseline, while "↓" indicates that the mean at the study time was less than that at baseline.

* Group effect was significant at the 0.05 level.

NA Data not available for this Group at this study time.

Table 7. Summary of the ANOVA Results for the Baseline Adjusted Six-Hour Averages for RP Peak Amplitude (mmHg)

RP Integral									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
		1	2	3	4	5	6		
0	6AM - Noon	1.67	3.74	1.04	2.10	NA	NA	0.8270	
	Noon - 6PM	-0.24	5.33 ↑	-0.80	-0.07	0.87	-2.05	0.1343	
	6PM - Midnight	-1.67	2.57	0.38	-1.40	-0.35	-2.90	0.5993	
1	Midnight - 6AM	-0.97	2.83	0.94	0.42	1.30	-4.24 ↓	0.1878	
	6AM - Noon	1.45	4.09 ↑	-0.17	1.19	2.01	-1.55	0.2983	
	Noon - 6PM	-2.12	5.11 ↑	0.44	-0.78	0.58	-1.59	0.3334	
	6PM - Midnight	-2.01	2.95	-0.57	0.56	1.17	0.22	0.7236	
2	Midnight - 6AM	-2.04	3.22	1.31	-0.45	0.85	2.69	0.4724	
	6AM - Noon	0.67	3.00	0.71	2.11	1.48	3.27	0.8323	
	Noon - 6PM	0.57	4.15 ↑	1.04	0.28	0.00	3.80	0.5169	
	6PM - Midnight	-3.48	3.62	0.74	-1.51	-1.65	4.53	0.5000	
3	Midnight - 6AM	-2.23	4.51	0.64	-1.26	-0.22	4.82	0.4630	
	6AM - Noon	-2.84	4.40	2.63	0.99	4.30	4.27	0.3589	
	Noon - 6PM	-6.29	5.23	-0.31	0.91	5.04	2.65	0.3355	
	6PM - Midnight	-5.12	3.06	-0.49	-1.34	10.09	1.82	0.3083	
4	Midnight - 6AM	-0.23	4.48	0.65	-2.82	8.29	1.71	0.4069	
	6AM - Noon	2.65	5.65	1.54	2.40	5.90	1.88	0.8614	
	Noon - 6PM	-6.63	6.52	2.04	3.37	10.48	5.22	0.2266	
	6PM - Midnight	-2.71	3.88	0.85	1.87	11.46 ↑	NA	0.1547	
5	Midnight - 6AM	-1.89	4.22	1.50	0.28	10.93 ↑	NA	0.1543	
	6AM - Noon	-4.31	5.35	1.90	-0.36	8.59	NA	0.2867	
	Noon - 6PM	0.21	5.01 ↑	1.93	0.26	8.09 ↑	NA	0.2393	
	6PM - Midnight	-6.51	3.12	1.37	0.54	0.09	NA	0.6691	
6	Midnight - 6AM	-0.43	3.56	1.46	-1.06	1.31	NA	0.6959	
	6AM - Noon	-2.56	4.12	-0.08	-1.39	-1.46	NA	0.6643	
	Noon - 6PM	-4.30	5.15	-2.84	0.83	0.93	NA	0.4539	
	6PM - Midnight	-7.48	1.89	-2.16	0.16	1.83	NA	0.7760	

Table 7. (Continued)

RP Peak Amplitude									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
		1	2	3	4	5	6		
7	Midnight - 6AM	-7.03	3.11	-0.27	-1.17	3.07	NA	0.7142	
	6AM - Noon	-0.98	3.50	-0.15	-0.81	-0.39	NA	0.8695	
	Noon - 6PM	-2.60	5.39	-1.25	0.50	1.38	NA	0.5821	
	6PM - Midnight	-3.33	0.88	-2.45	1.06	1.51	NA	0.9415	
8	Midnight - 6AM	5.06	2.86	-1.88	2.59	3.02	NA	0.5989	
	6AM - Noon	4.68	1.40	0.79	1.76	0.80	NA	0.8114	
	Noon - 6PM	2.26	4.44	1.16	2.34	0.77	NA	0.8554	
	6PM - Midnight	2.47	2.65	-0.82	1.63	2.50	NA	0.9027	
9	Midnight - 6AM	4.79	2.63	2.42	2.18	1.53	NA	0.9250	
	6AM - Noon	-0.13	4.08	0.84	1.48	1.26	NA	0.8598	
	Noon - 6PM	0.46	3.45	0.85	2.04	-0.86	NA	0.8841	
	6PM - Midnight	3.20	2.51	1.93	2.16	0.31	NA	0.9819	
10	Midnight - 6AM	5.94 ↑	3.53	2.24	2.48	2.64	NA	0.8881	
	6AM - Noon	4.23	3.17	2.69	1.77	1.30	NA	0.9395	
	Noon - 6PM	2.86	5.19 ↑	1.87	2.71	1.51	NA	0.8556	
	6PM - Midnight	1.97	1.77	1.15	2.20	0.86	NA	0.9978	
11	Midnight - 6AM	4.91	3.39	1.46	2.72	-0.07	NA	0.8516	
	6AM - Noon	2.15	3.20	1.70	2.13	-1.99	NA	0.9077	
	Noon - 6PM	0.99	5.35 ↑	2.52	1.75	-0.44	NA	0.7146	
	6PM - Midnight	1.01	3.46	1.22	1.33	-0.63	NA	0.9427	
12	Midnight - 6AM	4.07	5.18 ↑	1.36	2.28	0.15	NA	0.7681	
	6AM - Noon	0.01	3.49	2.14	0.92	-3.18	NA	0.8399	
	Noon - 6PM	-0.12	3.89	1.83	1.64	-0.56	NA	0.8342	
	6PM - Midnight	5.41 ↑	2.61	1.79	1.31	-2.45	NA	0.6653	

Table 7. (Continued)

RP Peak Amplitude									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
		1	2	3	4	5	6		
13	Midnight - 6AM	5.49	3.33	1.14	1.91	-1.22	NA	0.7669	
	6AM - Noon	3.62	3.49	1.48	0.75	-3.71	NA	0.6213	
	Noon - 6PM	2.37	3.27	1.57	1.90	-1.47	NA	0.9361	
	6PM - Midnight	2.82	2.80	0.57	0.60	-1.33	NA	0.8746	
14	Midnight - 6AM	1.03	3.04	-0.23	1.83	0.48	NA	0.8635	
	6AM - Noon	1.40	3.87	1.35	2.20	-1.02	NA	0.8799	
	Noon - 6PM	2.14	3.62	0.12	1.91	-0.19	NA	0.8278	
	6PM - Midnight	3.61	1.42	-0.21	0.90	-0.97	NA	0.7984	
15	Midnight - 6AM	5.79	2.54	0.16	2.50	-0.03	NA	0.7381	
	6AM - Noon	5.17	2.19	-0.46	1.71	-1.94	NA	0.5767	
	Noon - 6PM	3.48	4.46	0.22	1.67	-2.01	NA	0.7417	
	6PM-Midnight	3.40	1.87	0.37	1.41	-1.47	NA	0.8627	
16	Midnight - 6AM	4.47	2.86	1.26	1.83	-0.55	NA	0.8353	
	6AM - Noon	3.21	3.73	1.18	0.45	-2.16	NA	0.7434	
	Noon - 6PM	-6.28	3.53	0.78	1.11	-0.74	NA	0.5414	
	6PM - Midnight	-3.14	1.04	0.34	0.27	-0.35	NA	0.9131	
17	Midnight - 6AM	4.33	2.80	0.34	0.85	0.48	NA	0.8213	
	6AM - Noon	3.82	0.90	0.79	0.76	-2.15	NA	0.7241	
	Noon - 6PM	2.00	3.38	0.80	0.98	-0.50	NA	0.9208	
	6PM - Midnight	2.99	0.51	0.63	1.12	-0.20	NA	0.9219	
18	Midnight - 6AM	3.84	1.70	0.69	0.73	2.38	NA	0.9228	
	6AM - Noon	4.71	2.10	0.87	0.45	-2.28	NA	0.7303	
	Noon - 6PM	3.16	1.56	2.08	1.68	-1.00	NA	0.9537	
	6PM - Midnight	4.61	-4.09	0.82	1.93	0.13	NA	0.5928	

Table 7. (Continued)

RP Peak Amplitude									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
		1	2	3	4	5	6		
19	Midnight - 6AM	6.87	-4.85	1.03	1.83	0.94	NA	0.6274	
	6AM - Noon	2.45	1.96	1.55	1.36	1.07	NA	0.9974	
	Noon - 6PM	-5.26	5.13	1.09	1.85	1.35	NA	0.5935	
	6PM - Midnight	-2.05	3.15	0.82	2.61	1.51	NA	0.8228	
20	Midnight - 6AM	3.42	3.78	1.87	4.36	0.74	NA	0.9380	
	6AM - Noon	5.36	4.80	2.04	1.63	-0.50	NA	0.8250	
	Noon - 6PM	3.15	5.16 ↑	1.82	2.91	2.06	NA	0.8839	
	6PM - Midnight	4.42	3.13	0.18	2.58	0.34	NA	0.8237	
21	Midnight - 6AM	6.14	3.52	0.71	4.76	0.52	NA	0.8258	
	6AM - Noon	4.30	5.13	0.85	2.40	0.41	NA	0.8081	

Cells contain all pairwise comparisons that are statistically significant at the 0.05 level. The format within each cell is (1) the difference of means, (2) the relationship between the corresponding pair of Group means shown in parentheses [For example, "(1<6)" indicates that the mean baseline adjusted value for Group 6 was significantly greater than that for Group 1], and (3) the Tukey-adjusted p-value.

↑, ↓ Indicate that the mean baseline adjusted value was significantly different from zero (at the 0.05 level). "↑" indicates that the mean at the study time was greater than that at baseline**,** while "↓" indicates that the mean at the study time was less than that at baseline.

* Group effect was significant at the 0.05 level.

NA Data not available for this Group at this study time.

Table 8. Summary of the ANOVA Results for the Baseline Adjusted Six-Hour Averages for RP Respiratory Rate (RCPM)

RP Respiratory Rate									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
		1	2	3	4	5	6		
0	6AM - Noon	14.94 ↑	14.20 ↑	13.27 ↑	23.99 ↑	NA	NA	0.3889	
	Noon - 6PM	7.35 ↑	1.52	-0.74	1.71	5.19	3.14	0.3478	
	6PM - Midnight	-0.28	-1.08	-2.89	-2.03	1.11	-1.39	0.7682	
1	Midnight - 6AM	-0.08	-1.66	-0.67	-0.07	-0.23	-5.58 ↓	0.5608	
	6AM - Noon	7.97 ↑	7.74 ↑	4.89	6.77 ↑	8.47 ↑	2.23	0.6242	
	Noon - 6PM	0.50	-0.08	1.01	0.22	-5.01	-4.24	0.6622	
	6PM - Midnight	3.00	-0.33	2.43	8.52	1.98	12.70	0.6897	
2	Midnight - 6AM	1.64	2.59	1.18	8.74	6.16	29.74 ↑	0.0098 *	-28.10 (1<6) 0.0156 -27.16 (2<6) 0.0207 -28.56 (3<6) 0.0136
	6AM - Noon	8.22 ↑	9.99 ↑	8.51 ↑	15.64 ↑	15.51 ↑	24.62 ↑	0.0082 *	-16.40 (1<6) 0.0112 -14.64 (2<6) 0.0283 -16.12 (3<6) 0.0131
	Noon - 6PM	-0.03	-1.77	2.68	2.83	10.96 ↑	22.17 ↑	0.0188 *	-22.20 (1<6) 0.0353 -23.94 (2<6) 0.0199
	6PM - Midnight	0.67	-0.39	-0.17	4.21	19.32 ↑	26.12 ↑	0.0047 *	-25.45 (1<6) 0.0304 -26.51 (2<6) 0.0223 -26.29 (3<6) 0.0237
3	Midnight - 6AM	0.71	2.81	3.57	9.15	22.00 ↑	35.03 ↑	0.0056 *	-34.32 (1<6) 0.0109 -32.23 (2<6) 0.0186 -31.46 (3<6) 0.0225
	6AM - Noon	6.78	8.79 ↑	5.52	13.83 ↑	23.19 ↑	25.19 ↑	0.0024 *	-16.41 (1<5) 0.0366 -18.41 (1<6) 0.0242 -17.67 (3<5) 0.0211 -19.67 (3<6) 0.0142
	Noon - 6PM	2.85	-0.31	-1.09	5.15	14.63 ↑	21.16 ↑	0.0116 *	-21.46 (2<6) 0.0302 -22.25 (3<6) 0.0232
	6PM - Midnight	1.43	-2.13	2.36	4.88	20.13 ↑	24.15 ↑	0.0096 *	-22.26 (2<5) 0.0353 -26.28 (2<6) 0.0286

Table 8. (Continued)

RP Respiratory Rate									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value [#]
		1	2	3	4	5	6		
4	Midnight - 6AM	4.86	1.13	3.26	8.46	24.46 ↑	24.11 ↑	0.0379 *	
	6AM - Noon	5.77	2.72	3.44	8.78	9.05	23.74 ↑	0.5764	
	Noon - 6PM	4.34	1.34	0.38	4.48	25.55 ↑	23.32 ↑	<0.0001 *	-21.21 (1<5) 0.0008 -24.22 (2<5) 0.0002 -21.98 (2<6) 0.0236 -25.17 (3<5) 0.0001 -22.94 (3<6) 0.0172 -21.08 (4<5) 0.0009
	6PM - Midnight	5.80	3.06	7.54 ↑	5.19	26.45 ↑	NA	0.0094 *	-20.65 (1<5) 0.0179 -23.39 (2<5) 0.0068 -18.92 (3<5) 0.0327 -21.26 (4<5) 0.0200
5	Midnight - 6AM	2.39	3.83	7.53 ↑	1.29	22.15 ↑	NA	0.0079 *	-19.76 (1<5) 0.0094 -18.32 (2<5) 0.0168 -20.86 (4<5) 0.0087
	6AM - Noon	4.41	3.39	3.91	3.32	24.47 ↑	NA	0.0181 *	-20.06 (1<5) 0.0302 -21.08 (2<5) 0.0215 -20.56 (3<5) 0.0256 -21.14 (4<5) 0.0286
	Noon - 6PM	-1.30	-5.17	-4.19	-0.97	15.71 ↑	NA	0.0350 *	-20.87 (2<5) 0.0274 -19.90 (3<5) 0.0375
	6PM - Midnight	0.53	-1.02	-3.85	4.72	-0.17	NA	0.7259	
6	Midnight - 6AM	-1.56	-3.88	-5.14	3.48	1.60	NA	0.4260	
	6AM - Noon	-2.73	-15.63 ↓	-9.40 ↓	-3.44	-4.56	NA	0.1580	
	Noon - 6PM	-2.05	-17.84 ↓	-13.37 ↓	-3.05	0.00	NA	0.2067	
	6PM - Midnight	-0.13	-9.67	-10.92	3.85	0.80	NA	0.3063	

Table 8. (Continued)

RP Respiratory Rate									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value [#]
		1	2	3	4	5	6		
7	Midnight - 6AM	-3.26	-7.53	-9.24 ↓	3.15	4.46	NA	0.2431	
	6AM - Noon	-1.60	-12.62 ↓	-10.26 ↓	3.98	7.21	NA	0.0952	
	Noon - 6PM	-2.09	-21.23 ↓	-13.81 ↓	-5.28	-0.36	NA	0.2698	
	6PM - Midnight	0.60	-13.98 ↓	-13.45 ↓	2.99	3.84	NA	0.1553	
8	Midnight - 6AM	2.19	-9.46 ↓	-9.21	1.88	3.86	NA	0.1832	
	6AM - Noon	-0.65	-5.76 ↓	-4.29	5.41	1.66	NA	0.0812	
	Noon - 6PM	3.88	-2.90	-0.31	-2.25	1.67	NA	0.4326	
	6PM - Midnight	6.07	0.80	2.57	8.59 ↑	3.46	NA	0.5806	
9	Midnight - 6AM	5.53	7.64	4.17	8.09	1.57	NA	0.9467	
	6AM - Noon	0.83	-0.52	2.20	9.31 ↑	3.66	NA	0.4782	
	Noon - 6PM	1.05	-3.21	0.31	-5.30	-0.55	NA	0.7769	
	6PM - Midnight	3.51	-1.04	-6.44 ↓	2.34	-1.07	NA	0.0557	-9.96 (3<1) 0.0432
10	Midnight - 6AM	-0.19	4.51	3.53	8.76	1.82	NA	0.6950	
	6AM - Noon	-0.03	-0.17	1.02	3.51	-8.48	NA	0.6532	
	Noon - 6PM	0.32	2.87	1.11	0.18	2.85	NA	0.9668	
	6PM - Midnight	0.19	0.30	-1.61	-11.95 ↓	-1.13	NA	0.3108	
11	Midnight - 6AM	-1.33	4.97	-0.71	-9.62 ↓	0.24	NA	0.2549	
	6AM - Noon	0.06	1.10	2.93	-13.21 ↓	-1.08	NA	0.1188	
	Noon - 6PM	1.71	-0.73	0.41	-5.67	0.43	NA	0.6131	
	6PM - Midnight	-0.52	2.35	-1.66	0.62	1.40	NA	0.8203	
12	Midnight - 6AM	-0.01	9.82 ↑	0.07	1.16	3.14	NA	0.2730	
	6AM - Noon	-3.26	0.43	-2.02	-2.26	-3.78	NA	0.5419	
	Noon - 6PM	-1.31	-0.97	1.02	-5.20	0.57	NA	0.7670	
	6PM - Midnight	1.90	1.57	0.39	2.15	-4.47	NA	0.7925	

Table 8. (Continued)

RP Respiratory Rate									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
		1	2	3	4	5	6		
13	Midnight - 6AM	-1.70	7.36	2.05	1.23	0.93	NA	0.5224	
	6AM - Noon	-0.42	2.82	-0.09	-1.08	-1.52	NA	0.9446	
	Noon - 6PM	-2.99	0.56	0.36	-2.95	-0.63	NA	0.8384	
	6PM - Midnight	-0.90	-0.31	-0.16	-2.36	-0.28	NA	0.9885	
14	Midnight - 6AM	-1.54	2.00	1.79	1.21	3.94	NA	0.6784	
	6AM - Noon	4.06	2.59	2.88	7.51 ↑	5.76	NA	0.7714	
	Noon - 6PM	0.19	-0.86	-2.27	-3.67	-2.15	NA	0.8935	
	6PM - Midnight	-0.11	-3.93	0.55	-3.81	2.70	NA	0.5351	
15	Midnight - 6AM	0.75	4.84	4.15	-0.94	4.40	NA	0.8602	
	6AM - Noon	-0.80	-0.29	-0.79	-1.67	-3.25	NA	0.9898	
	Noon - 6PM	0.13	-2.60	-0.12	-4.33	1.96	NA	0.8740	
	6PM - Midnight	2.02	0.05	0.78	-3.90	1.95	NA	0.6715	
16	Midnight - 6AM	1.14	2.71	4.02	0.63	-1.41	NA	0.8788	
	6AM - Noon	-1.28	-1.24	-2.52	-3.24	-5.39	NA	0.9069	
	Noon - 6PM	1.46	-2.29	-0.10	-3.72	2.20	NA	0.9234	
	6PM - Midnight	0.53	0.52	2.98	1.39	-2.93	NA	0.6619	
17	Midnight - 6AM	-1.94	3.17	3.24	-1.51	-2.65	NA	0.1515	
	6AM - Noon	2.68	-2.44	-1.72	-3.66	-1.99	NA	0.2958	
	Noon - 6PM	3.21	-1.20	-0.17	-3.43	0.35	NA	0.6888	
	6PM - Midnight	5.54 ↑	-3.51	-1.20	-4.22	2.52	NA	0.0584	
18	Midnight - 6AM	3.92	2.68	0.80	0.04	-0.76	NA	0.8647	
	6AM - Noon	-0.08	0.16	-5.15	-4.22	-3.02	NA	0.5430	
	Noon - 6PM	3.01	-2.80	0.24	-4.22	2.27	NA	0.3684	
	6PM - Midnight	1.49	-2.18	-0.31	-1.31	1.01	NA	0.6707	

Table 8. (Continued)

RP Respiratory Rate									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
		1	2	3	4	5	6		
19	Midnight - 6AM	1.97	5.36 ↑	-0.94	2.32	2.68	NA	0.3552	
	6AM - Noon	1.17	-2.01	-2.11	-2.45	1.76	NA	0.1728	
	Noon - 6PM	1.00	-1.77	1.55	-1.56	-0.07	NA	0.8515	
	6PM - Midnight	-1.87	-0.58	-4.31 ↓	-1.02	-2.06	NA	0.7114	
20	Midnight - 6AM	-4.99 ↓	3.55	-3.26	0.09	-1.75	NA	0.0913	
	6AM - Noon	-3.01 ↓	-1.43	-3.82 ↓	-2.57	-4.66	NA	0.6915	
	Noon - 6PM	3.37	1.90	3.84	2.49	1.72	NA	0.9639	
	6PM - Midnight	0.00	0.33	0.48	0.32	-1.78	NA	0.9815	
21	Midnight - 6AM	-1.47	3.42 ↑	1.41	-0.97	1.32	NA	0.2172	
	6AM - Noon	-0.72	2.79	-0.67	3.81	3.61	NA	0.2952	

Cells contain all pairwise comparisons that are statistically significant at the 0.05 level. The format within each cell is (1) the difference of means, (2) the relationship between the corresponding pair of Group means shown in parentheses [For example, "(1<6)" indicates that the mean baseline adjusted value for Group 6 was significantly greater than that for Group 1], and (3) the Tukey-adjusted p-value.

↑, ↓ Indicate that the mean baseline adjusted value was significantly different from zero (at the 0.05 level). "↑" indicates that the mean at the study time was greater than that at baseline**,** while "↓" indicates that the mean at the study time was less than that at baseline.

* Group effect was significant at the 0.05 level.

NA Data not available for this Group at this study time.

Table 9. Summary of the ANOVA Results for the Baseline Adjusted Six-Hour Averages for Temperature (Celsius)

Temperature									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
		1	2	3	4	5	6		
0	6AM - Noon	0.00	0.25 ↑	0.19 ↑	0.66 ↑	NA	NA	0.0007 *	-0.66 (1<4) 0.0004 -0.41 (2<4) 0.0181 -0.47 (3<4) 0.0068
	Noon - 6PM	-0.01	0.01	0.02	0.02	-0.01	-0.54 ↓	0.5165	
	6PM - Midnight	-0.10	-0.05	-0.09	-0.01	0.02	-0.43	0.6981	
1	Midnight - 6AM	-0.06	0.00	0.01	-0.02	0.07	-0.48 ↓	0.3151	
	6AM - Noon	0.09	0.12	0.10	0.10	0.12	-0.34	0.5419	
	Noon - 6PM	0.12	0.00	0.05	0.35	-0.12	-0.05	0.9181	
	6PM - Midnight	0.03	0.00	0.03	0.50	0.18	0.63	0.8355	
2	Midnight - 6AM	-0.02	0.04	0.00	0.54	0.44	0.74	0.7995	
	6AM - Noon	0.13	0.16	0.15	0.51 ↑	0.77 ↑	1.80 ↑	0.0001 *	-1.67 (1<6) 0.0002 -1.64 (2<6) 0.0003 -1.65 (3<6) 0.0003 -1.29 (4<6) 0.0046 -1.03 (5<6) 0.0330
	Noon - 6PM	0.00	-0.03	0.02	0.14	0.75 ↑	1.61 ↑	<0.0001 *	-1.61 (1<6) 0.0001 -1.64 (2<6) 0.0001 -1.59 (3<6) 0.0002 -1.47 (4<6) 0.0004
	6PM - Midnight	-0.11	-0.07	-0.02	0.14	1.05 ↑	1.50 ↑	<0.0001 *	-1.16 (1<5) 0.0036 -1.61 (1<6) 0.0001 -1.12 (2<5) 0.0051 -1.57 (2<6) 0.0002 -1.07 (3<5) 0.0077 -1.52 (3<6) 0.0003 -0.91 (4<5) 0.0292 -1.37 (4<6) 0.0011

Table 9. (Continued)

Temperature									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
		1	2	3	4	5	6		
3	Midnight - 6AM	-0.06	0.01	0.02	0.33	0.62 ↑	1.60 ↑	0.0021 *	-1.66 (1<6) 0.0028 -1.60 (2<6) 0.0041 -1.58 (3<6) 0.0045 -1.27 (4<6) 0.0302
	6AM - Noon	0.07	0.09	0.16	0.45	0.31	1.95 ↑	0.0017 *	-1.88 (1<6) 0.0021 -1.86 (2<6) 0.0023 -1.79 (3<6) 0.0035 -1.51 (4<6) 0.0171 -1.64 (5<6) 0.0081
	Noon - 6PM	0.00	-0.11	-0.02	0.39	-0.03	1.54 ↑	0.3398	
	6PM - Midnight	-0.07	-0.09	-0.05	0.47	1.15 ↑	1.98 ↑	0.0042 *	-2.05 (1<6) 0.0107 -2.07 (2<6) 0.0097 -2.02 (3<6) 0.0119
4	Midnight - 6AM	-0.04	-0.01	0.00	0.30	1.17 ↑	0.58	0.0260 *	-1.21 (1<5) 0.0286 -1.19 (2<5) 0.0336 -1.18 (3<5) 0.0354
	6AM - Noon	0.00	0.05	0.10	-0.22	-3.63	-1.45	0.6945	
	Noon - 6PM	0.05	-0.07	0.02	-0.57	1.25 ↑	-3.55 ↓	0.0033 *	-3.60 (6<1) 0.0101 -3.48 (6<2) 0.0134 -3.57 (6<3) 0.0107 -2.98 (6<4) 0.0412 -4.80 (6<5) 0.0011
	6PM - Midnight	0.02	0.04	0.05	0.12	0.54 ↑	NA	0.0084 *	-0.52 (1<5) 0.0079 -0.50 (2<5) 0.0114 -0.49 (3<5) 0.0128

Table 9. (Continued)

Temperature									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
		1	2	3	4	5	6		
5	Midnight - 6AM	0.01	0.01	0.03	0.03	-0.08	NA	0.9962	
	6AM - Noon	-0.01	0.01	0.03	0.04	-0.80 ↓	NA	0.0108 *	-0.79 (5<1) 0.0194 -0.82 (5<2) 0.0154 -0.83 (5<3) 0.0138 -0.84 (5<4) 0.0173
	Noon - 6PM	-0.07	-0.12	0.03	0.04	-2.26 ↓	NA	0.0027 *	-2.19 (5<1) 0.0050 -2.14 (5<2) 0.0061 -2.29 (5<3) 0.0034 -2.30 (5<4) 0.0048
	6PM - Midnight	-0.02	0.03	0.04	0.05	0.08	NA	0.9382	
6	Midnight - 6AM	-0.06	-0.04	-0.03	0.01	-0.08	NA	0.7980	
	6AM - Noon	-0.12 ↓	-0.16 ↓	-0.09 ↓	-0.08	-0.09	NA	0.5666	
	Noon - 6PM	-0.09	-0.23	-0.12	0.39	-0.06	NA	0.3240	
	6PM - Midnight	-0.05	-0.05	-0.04	0.53	0.04	NA	0.4099	
7	Midnight - 6AM	-0.07	-0.14	-0.03	0.37	0.00	NA	0.3235	
	6AM - Noon	-0.04	-0.18 ↓	-0.07	0.21 ↑	-0.07	NA	0.0858	-0.38 (2<4) 0.0481
	Noon - 6PM	-0.04	-0.29 ↓	-0.16	-0.07	-0.07	NA	0.1967	
	6PM - Midnight	-0.11	-0.13	-0.09	0.00	0.05	NA	0.8877	
8	Midnight - 6AM	-0.12	-0.09	-0.13	0.16	-0.02	NA	0.4590	
	6AM - Noon	-0.03	-0.12	-0.09	0.54 ↑	-0.02	NA	0.3058	
	Noon - 6PM	-0.05	-0.07	-0.03	0.43	-0.05	NA	0.4042	
	6PM - Midnight	-0.08	0.08	0.07	0.70 ↑	-0.06	NA	0.4330	
9	Midnight - 6AM	-0.07	0.07	0.01	0.58	-0.03	NA	0.4842	
	6AM - Noon	0.01	0.08	0.02	0.64 ↑	0.06	NA	0.4569	
	Noon - 6PM	-0.13	-0.10	-0.07	0.34	-0.04	NA	0.5217	
	6PM - Midnight	-0.15	-0.05	-0.07	0.30	-0.05	NA	0.3076	

Table 9. (Continued)

Temperature									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value [#]
		1	2	3	4	5	6		
10	Midnight - 6AM	-0.15 ↓	0.04	-0.07	0.00	-0.06	NA	0.1684	
	6AM - Noon	-0.08	0.07	-0.01	-0.42 ↓	-0.05	NA	0.4244	
	Noon - 6PM	-0.10	-0.12	0.00	-0.93 ↓	-0.01	NA	0.4623	
	6PM - Midnight	-0.15	-0.08	-0.03	-2.47 ↓	-0.07	NA	0.4942	
11	Midnight - 6AM	-0.10	-0.03	-0.07	-3.85 ↓	-0.02	NA	0.4563	
	6AM - Noon	-0.05	0.01	-0.02	-4.09 ↓	-0.01	NA	0.4337	
	Noon - 6PM	-0.08	-0.19 ↓	-0.09	-0.08	-0.02	NA	0.7444	
	6PM - Midnight	-0.12	-0.02	-0.01	0.06	-0.07	NA	0.4544	
12	Midnight - 6AM	-0.14 ↓	-0.01	-0.07	0.06	-0.12	NA	0.3074	
	6AM - Noon	0.02	0.06	0.00	0.06	-0.06	NA	0.6222	
	Noon - 6PM	-0.02	-0.15 ↓	-0.04	-0.03	-0.03	NA	0.4517	
	6PM - Midnight	-0.14 ↓	-0.06	-0.01	0.13	-0.16	NA	0.1897	
13	Midnight - 6AM	-0.18 ↓	-0.05	-0.02	0.05	-0.07	NA	0.3537	
	6AM - Noon	-0.04	0.03	0.02	0.03	-0.06	NA	0.6696	
	Noon - 6PM	-0.08	-0.14	0.03	-0.03	0.03	NA	0.5769	
	6PM - Midnight	-0.19 ↓	-0.11	-0.06	0.01	0.02	NA	0.3118	
14	Midnight - 6AM	-0.20 ↓	-0.08	-0.07	-0.01	0.03	NA	0.2490	
	6AM - Noon	0.03	0.07	0.03	0.14 ↑	0.07	NA	0.6757	
	Noon - 6PM	-0.07	-0.10	-0.01	0.00	-0.04	NA	0.9268	
	6PM - Midnight	-0.11	-0.08	-0.02	-0.03	0.03	NA	0.8349	
15	Midnight - 6AM	-0.13	-0.04	0.00	0.02	-0.02	NA	0.5560	
	6AM - Noon	0.00	0.03	0.02	-0.06	-0.03	NA	0.9247	
	Noon - 6PM	-0.11	-0.12	0.07	-0.02	-0.03	NA	0.3484	
	6PM - Midnight	-0.17	-0.11	0.04	0.05	0.03	NA	0.4481	

Table 9. (Continued)

Temperature									
Study Day	Time	Mean Baseline Adjusted Value, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
		1	2	3	4	5	6		
16	Midnight - 6AM	-0.13	-0.03	0.02	0.00	-0.05	NA	0.6024	
	6AM - Noon	-0.06	0.02	0.07	-0.01	-0.10	NA	0.6122	
	Noon - 6PM	-0.12	-0.09	0.02	0.01	-0.12	NA	0.7182	
	6PM - Midnight	-0.19 ↓	-0.07	0.00	0.04	-0.03	NA	0.4142	
17	Midnight - 6AM	-0.18 ↓	0.00	-0.03	0.05	-0.04	NA	0.1124	
	6AM - Noon	-0.01	0.10	-0.04	-0.02	-0.02	NA	0.4603	
	Noon - 6PM	-0.05	-0.07	-0.04	0.02	-0.04	NA	0.9732	
	6PM - Midnight	-0.08	-0.08	0.00	-0.01	0.04	NA	0.9265	
18	Midnight - 6AM	-0.12	-0.04	-0.09	0.02	-0.04	NA	0.7082	
	6AM - Noon	0.01	0.04	-0.02	-0.02	-0.05	NA	0.8193	
	Noon - 6PM	-0.01	-0.06	0.04	-0.10	0.07	NA	0.8422	
	6PM - Midnight	-0.11	-0.07	0.03	-0.01	0.00	NA	0.8641	
19	Midnight - 6AM	-0.16	-0.05	-0.05	0.05	0.01	NA	0.5508	
	6AM - Noon	0.01	-0.01	0.05	0.00	0.05	NA	0.9691	
	Noon - 6PM	-0.07	-0.08	0.00	-0.01	0.07	NA	0.8634	
	6PM - Midnight	-0.17 ↓	-0.09	-0.04	0.04	-0.03	NA	0.4749	
20	Midnight - 6AM	-0.18 ↓	-0.07	-0.13	0.12	-0.04	NA	0.1707	
	6AM - Noon	-0.04	0.07	-0.01	-0.02	0.01	NA	0.5116	
	Noon - 6PM	-0.04	-0.05	0.00	0.02	0.10	NA	0.6889	
	6PM - Midnight	-0.13	-0.07	-0.04	0.09	0.07	NA	0.4001	
21	Midnight - 6AM	-0.17 ↓	-0.06	-0.13	0.08	-0.07	NA	0.2912	
	6AM - Noon	-0.13 ↓	0.10 ↑	-0.02	-0.04	0.04	NA	0.0275 *	-0.22 (1<2) 0.0136

Cells contain all pairwise comparisons that are statistically significant at the 0.05 level. The format within each cell is (1) the difference of means, (2) the relationship between the corresponding pair of Group means shown in parentheses [For example, "(1<6)" indicates that the mean baseline adjusted value for Group 6 was significantly greater than that for Group 1], and (3) the Tukey-adjusted p-value.

↑, ↓ Indicate that the mean baseline adjusted value was significantly different from zero (at the 0.05 level). "↑" indicates that the mean at the study time was greater than that at baseline**,** while "↓" indicates that the mean at the study time was less than that at baseline.

* Group effect was significant at the 0.05 level.

NA Data not available for this Group at this study time.

Table 10. Abnormality Summaries by Parameter and Group Along with Fisher's Exact Tests Comparing the Proportion Abnormal in Each Group by Parameter

Parameter	Group	Number Abnormal/ N	Proportion Abnormal (95% Confidence Interval)	Mean Duration of Abnormal (Days)[#]	Fisher's Group Effect P-Value
Activity	1	0/5	0.00 (0.00, 0.52)	NA	0.0558
	2	1/5	0.20 (0.01, 0.72)	0.00	
	3	0/5	0.00 (0.00, 0.52)	NA	
	4	0/5	0.00 (0.00, 0.52)	NA	
	5	3/5	0.60 (0.15, 0.95)	0.00	
	6	0/5	0.00 (0.00, 0.52)	NA	
Heart Rate	1	4/5	0.80 (0.28, 0.99)	8.69	1.0000
	2	4/5	0.80 (0.28, 0.99)	11.63	
	3	3/5	0.60 (0.15, 0.95)	0.42	
	4	4/5	0.80 (0.28, 0.99)	8.81	
	5	4/5	0.80 (0.28, 0.99)	1.31	
	6	4/5	0.80 (0.28, 0.99)	1.31	
RP Expiratory Time	1	2/5	0.40 (0.05, 0.85)	8.38	0.8253
	2	4/5	0.80 (0.28, 0.99)	2.75	
	3	4/5	0.80 (0.28, 0.99)	11.19	
	4	4/5	0.80 (0.28, 0.99)	3.75	
	5	3/5	0.60 (0.15, 0.95)	0.75	
	6	4/5	0.80 (0.28, 0.99)	1.25	
RP Inspiratory Time	1	2/5	0.40 (0.05, 0.85)	10.63	0.4096
	2	4/5	0.80 (0.28, 0.99)	5.38	
	3	3/5	0.60 (0.15, 0.95)	6.00	
	4	2/5	0.40 (0.05, 0.85)	7.75	
	5	3/5	0.60 (0.15, 0.95)	2.08	
	6	5/5	1.00 (0.48, 1.00)	1.05	
RP Integral	1	4/5	0.80 (0.28, 0.99)	14.94	0.0586
	2	3/5	0.60 (0.15, 0.95)	7.42	
	3	5/5	1.00 (0.48, 1.00)	4.20	
	4	3/5	0.60 (0.15, 0.95)	2.75	
	5	1/5	0.20 (0.01, 0.72)	1.00	
	6	5/5	1.00 (0.48, 1.00)	1.10	
RP Peak Amplitude	1	4/5	0.80 (0.28, 0.99)	16.00	0.9079
	2	4/5	0.80 (0.28, 0.99)	7.44	
	3	4/5	0.80 (0.28, 0.99)	8.81	
	4	3/5	0.60 (0.15, 0.95)	3.33	
	5	3/5	0.60 (0.15, 0.95)	0.58	
	6	5/5	1.00 (0.48, 1.00)	0.70	

Table10. (Continued)

Parameter	Group	Number Abnormal/ N	Proportion Abnormal (95% Confidence Interval)	Mean Duration of Abnormal (Days)#	Fisher's Group Effect P-Value
RP Respiratory Rate	1	2/5	0.40 (0.05, 0.85)	0.00	0.2832
	2	4/5	0.80 (0.28, 0.99)	1.06	
	3	2/5	0.40 (0.05, 0.85)	5.38	
	4	2/5	0.40 (0.05, 0.85)	2.25	
	5	3/5	0.60 (0.15, 0.95)	2.08	
	6	5/5	1.00 (0.48, 1.00)	1.50	
Temperature	1	4/5	0.80 (0.28, 0.99)	6.19	0.8752
	2	3/5	0.60 (0.15, 0.95)	4.17	
	3	3/5	0.60 (0.15, 0.95)	1.67	
	4	2/5	0.40 (0.05, 0.85)	3.63	
	5	4/5	0.80, (0.28, 0.99)	2.00	
	6	4/5	0.80 (0.28, 0.99)	2.06	

Means exclude those animals that were never abnormal.
NA There were no abnormal animlas for this group.

Table 11. Results of Overall Log-Rank Tests Comparing the Time to Abnormality Between Groups by Parameter

Parameter	Group Effect P-Value
Activity	0.0307 *
Heart Rate	0.2572
RP Expiratory Time	0.0083 *
RP Inspiratory Time	<0.0001 *
RP Integral	0.0002 *
RP Peak Amplitude	0.0388 *
RP Respiratory Rate	0.0252 *
Temperature	0.0259 *

* Group effect was significant at the 0.05 level.

Table 12. Results of Pairwise Log-Rank Tests Comparing the Time to Abnormality Between Groups by Parameter

Parameter	Group	Pairwise Log-Rank Test P-Values									
		Unadjusted P-Value					Bonferroni-Holm Adjusted P-Value				
		2	3	4	5	6	2	3	4	5	6
Activity	1	0.3173	1.0000	1.0000	0.0486 *	1.0000	1.0000	1.0000	1.0000	0.7286	1.0000
	2		0.3173	0.3173	0.3186	0.3173		1.0000	1.0000	1.0000	1.0000
	3			1.0000	0.0486 *	1.0000			1.0000	0.7286	1.0000
	4				0.0486 *	1.0000				0.7286	1.0000
	5					0.0584					0.7286
RP Expiratory Time	1	0.0720	0.1599	0.1740	0.1662	0.0033 *	0.7917	1.0000	1.0000	1.0000	0.0495 *
	2		0.6724	0.5276	0.4664	0.0033 *		1.0000	1.0000	1.0000	0.0495 *
	3			0.8701	0.8226	0.0615			1.0000	1.0000	0.7379
	4				0.8226	0.0483 *				1.0000	0.6278
	5					0.1086					1.0000
RP Inspiratory Time	1	0.1991	0.4713	0.7649	0.1955	0.0021 *	1.0000	1.0000	1.0000	1.0000	0.0309 *
	2		0.6201	0.1435	0.4719	0.0021 *		1.0000	1.0000	1.0000	0.0309 *
	3			0.6714	0.6249	0.0374 *			1.0000	1.0000	0.4110
	4				0.2361	0.0021 *				1.0000	0.0309 *
	5					0.0072 *					0.0869
RP Integral	1	0.5784	0.9629	0.8285	0.2851	0.0021 *	1.0000	1.0000	1.0000	1.0000	0.0309 *
	2		0.3589	0.9143	0.5822	0.0112 *		1.0000	1.0000	1.0000	0.1345
	3			0.4661	0.2846	0.0021 *			1.0000	1.0000	0.0309 *
	4				0.4046	0.0391 *				1.0000	0.4299
	5					0.0072 *					0.0941
RP Peak Amplitude	1	0.5447	0.6820	0.5336	0.9896	0.1689	1.0000	1.0000	1.0000	1.0000	1.0000
	2		0.8190	0.9885	0.2348	0.0018 *		1.0000	1.0000	1.0000	0.0276 *
	3			0.7704	0.5178	0.0377 *			1.0000	1.0000	0.5280
	4				0.6889	0.0471 *				1.0000	0.6122
	5					0.0578					0.6935

Table 12. (Continued)

Parameter	Group	Pairwise Log-Rank Test P-Values									
		Unadjusted P-Value					Bonferroni-Holm Adjusted P-Value				
		2	3	4	5	6	2	3	4	5	6
RP Respiratory Rate	1	0.5057	0.8381	0.8381	0.7793	0.2257	1.0000	1.0000	1.0000	1.0000	1.0000
	2		0.5476	0.3286	0.4400	0.0020 *		1.0000	1.0000	1.0000	0.0303 *
	3			0.8985	0.4471	0.0399 *			1.0000	1.0000	0.4783
	4				0.4471	0.0128 *				1.0000	0.1668
	5					0.0052 *					0.0727
Temperature	1	0.6187	0.6398	0.3272	0.4681	0.0572	1.0000	1.0000	1.0000	1.0000	0.8012
	2		0.9702	0.6819	0.1359	0.0070 *		1.0000	1.0000	1.0000	0.1056
	3			0.6084	0.3240	0.0907			1.0000	1.0000	0.9972
	4				0.1809	0.0572				1.0000	0.8012
	5					0.0572					0.8012

* Significant at the 0.05 level.

Table 13. Results of Overall Log-Rank Tests Comparing the Duration of Abnormality Between Groups by Parameter

Parameter	Group Effect P-Value
Activity	NA
Heart Rate	0.0278 *
RP Expiratory Time	0.0032 *
RP Inspiratory Time	0.0213 *
RP Integral	0.1414
RP Peak Amplitude	0.4127
RP Respiratory Rate	0.0074 *
Temperature	0.2958

NA No animals had a duration of abnormality greater than zero days.
* Group effect was significant at the 0.05 level.

Table 14. Results of Pairwise Log-Rank Tests Comparing the Duration of Abnormality Between Groups by Parameter

Parameter	Group	Pairwise Log-Rank Test P-Values									
		Unadjusted P-Value					Bonferroni-Holm Adjusted P-Value				
		2	3	4	5	6	2	3	4	5	6
Heart Rate	1	0.6787	0.0515	0.7419	0.6490	0.3393	1.0000	0.6178	1.0000	1.0000	1.0000
	2		0.0100 *	0.6361	0.2971	0.0423 *		0.1493	1.0000	1.0000	0.5503
	3			0.0100 *	0.2590	0.0515			0.1493	1.0000	0.6178
	4				0.2971	0.1161				1.0000	1.0000
	5					0.8606					1.0000
RP Expiratory Time	1	0.0772	0.5151	0.1036	0.0634	0.4795	0.7722	1.0000	0.9327	0.6974	1.0000
	2		0.0266 *	0.1585	0.4000	0.5904		0.3456	1.0000	1.0000	1.0000
	3			0.0476 *	0.0101 *	0.6394			0.5709	0.1509	1.0000
	4				0.0101 *	0.9183				0.1509	1.0000
	5					0.1897					1.0000
RP Inspiratory Time	1	0.1051	0.2807	0.3173	0.4142	0.1095	1.0000	1.0000	1.0000	1.0000	1.0000
	2		0.5269	0.1051	0.5936	0.1435		1.0000	1.0000	1.0000	1.0000
	3			0.2807	0.7055	0.1325			1.0000	1.0000	1.0000
	4				0.4142	0.1095				1.0000	1.0000
	5					0.1325					1.0000
RP Respiratory Rate	1	0.1138	0.0833	0.0833	0.0455 *	0.0143 *	1.0000	0.9159	0.9159	0.6370	0.2146
	2		0.0520	0.0520	0.1986	0.2314		0.6757	0.6757	1.0000	1.0000
	3			0.3173	0.4142	0.3431			1.0000	1.0000	1.0000
	4				0.8864	0.8849				1.0000	1.0000
	5					0.8702					1.0000

* Significant at the 0.05 level.

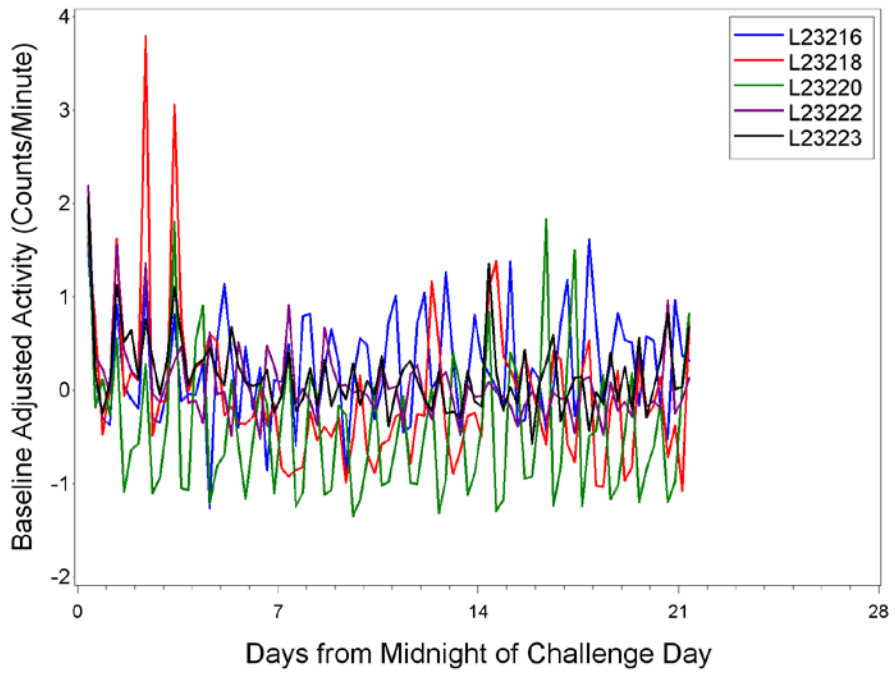

Figure 1a. Plot of baseline adjusted activity (counts/minute) for each animal in Group 1.

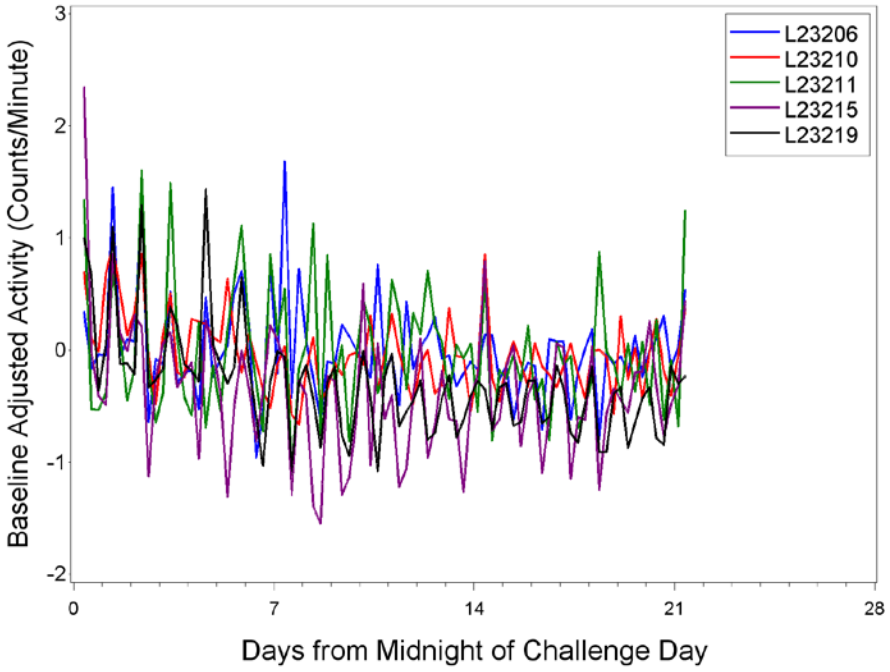

Figure 1b. Plot of baseline adjusted activity (counts/minute) for each animal in Group 2.

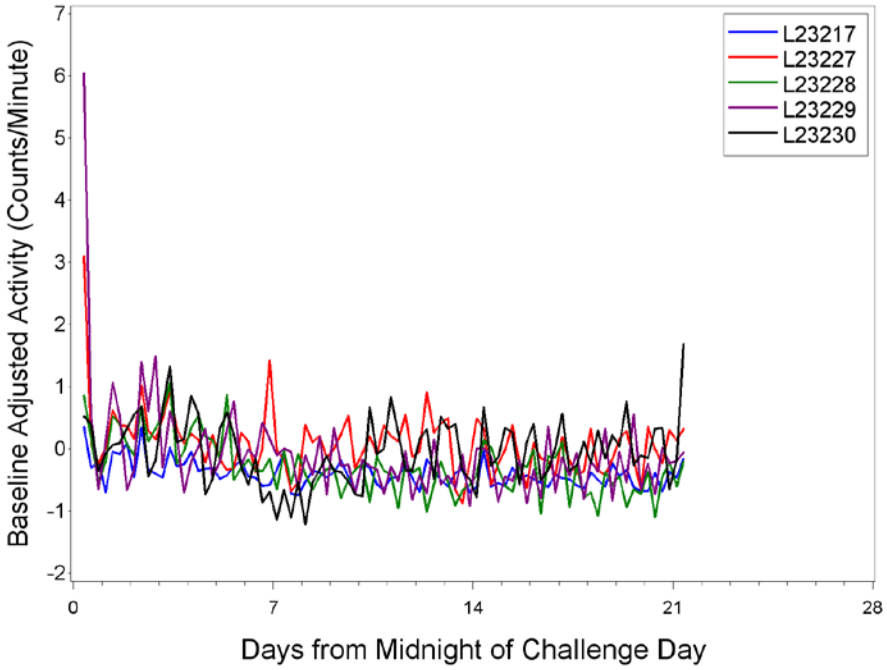

Figure 1c. Plot of baseline adjusted activity (counts/minute) for each animal in Group 3.

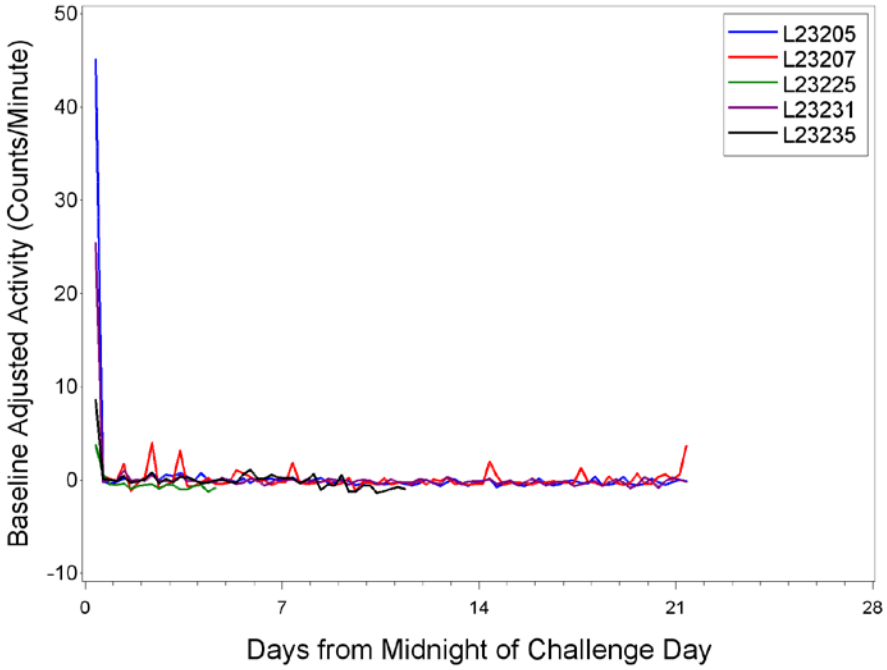

Figure 1d. Plot of baseline adjusted activity (counts/minute) for each animal in Group 4.

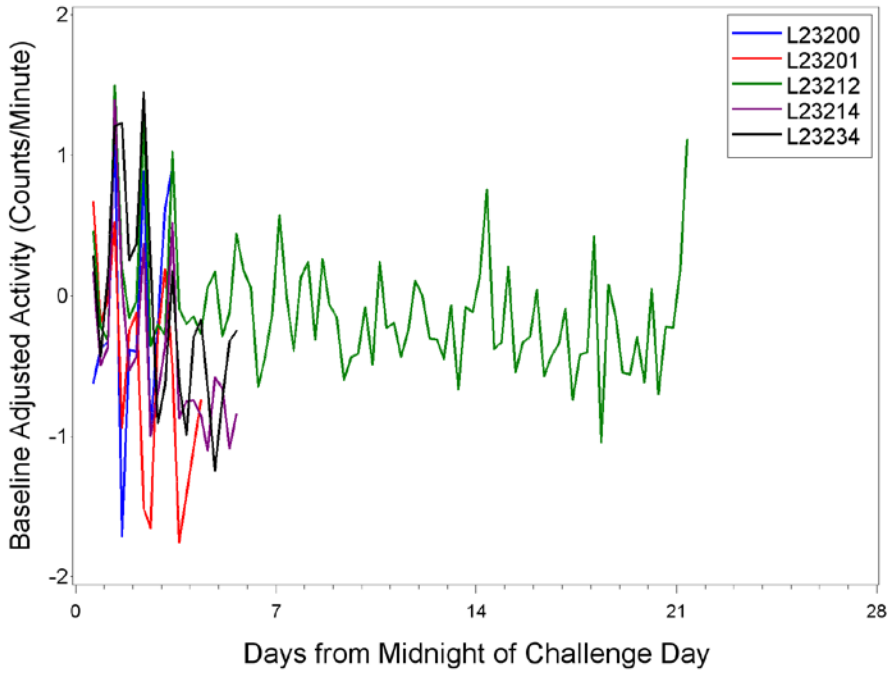

Figure 1e. Plot of baseline adjusted activity (counts/minute) for each animal in Group 5.

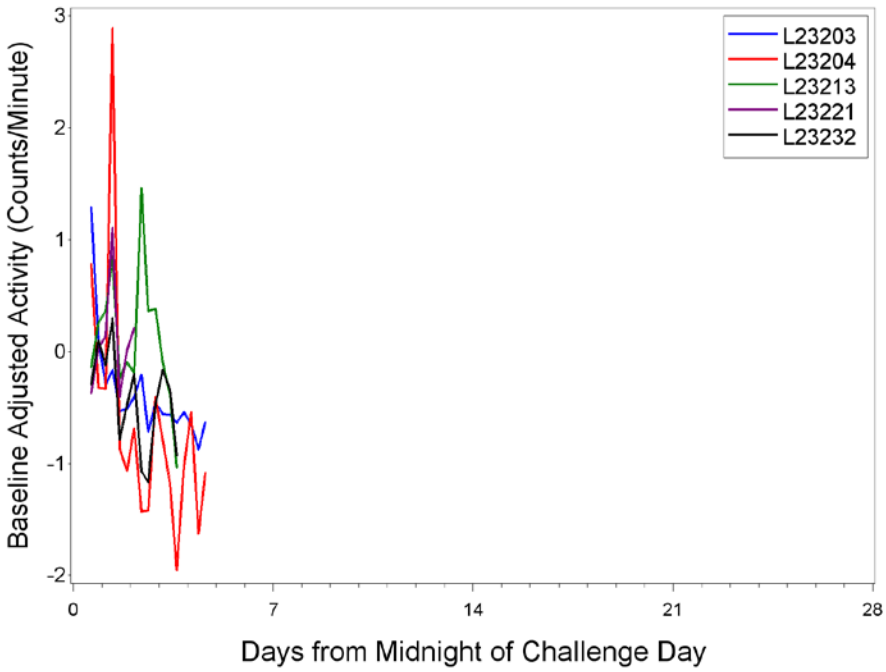

Figure 1f. Plot of baseline adjusted activity (counts/minute) for each animal in Group 6.

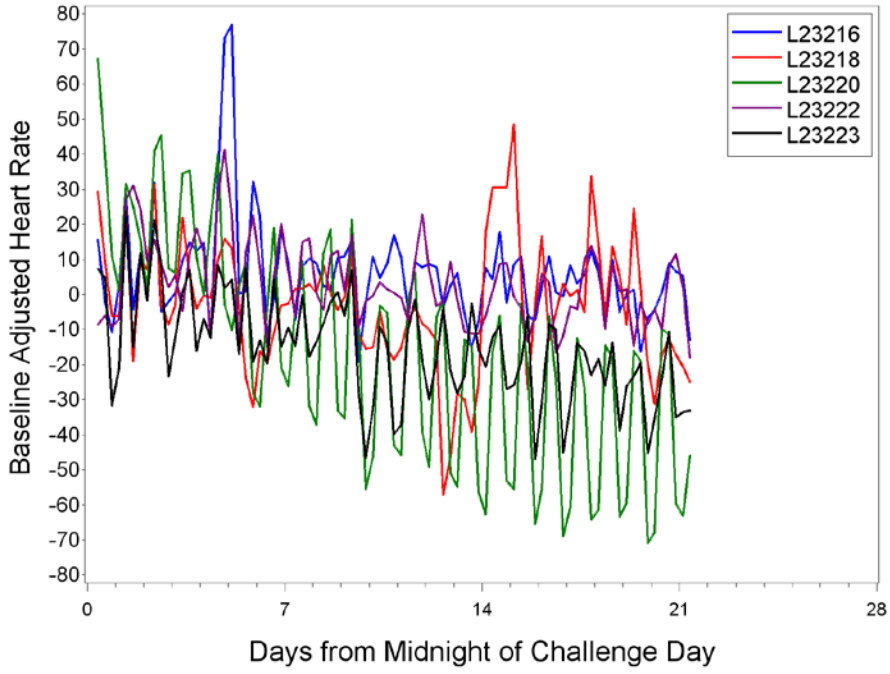

Figure 2a. Plot of baseline adjusted heart rate (BPM) for each animal in Group 1.

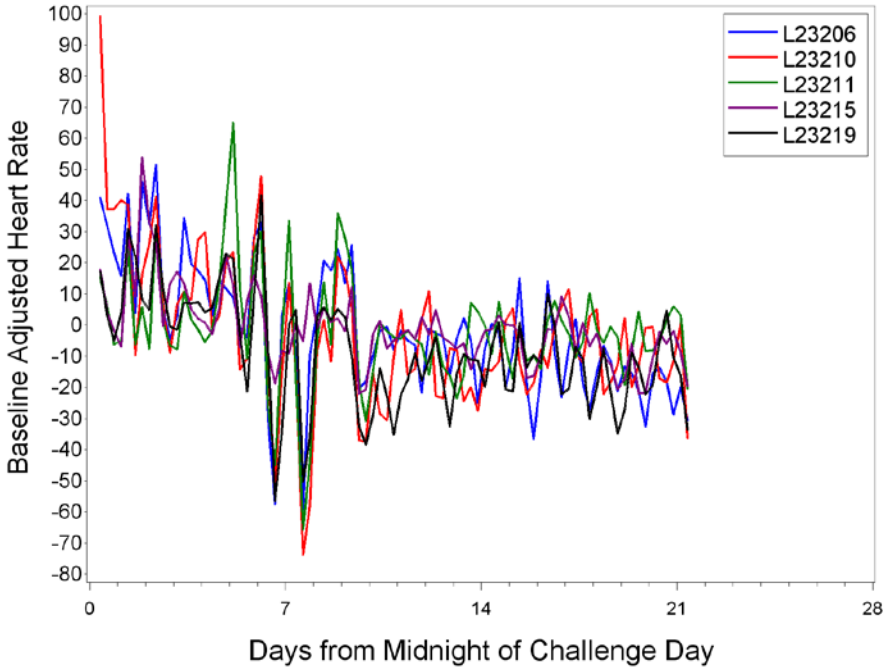

Figure 2b. Plot of baseline adjusted heart rate (BPM) for each animal in Group 2.

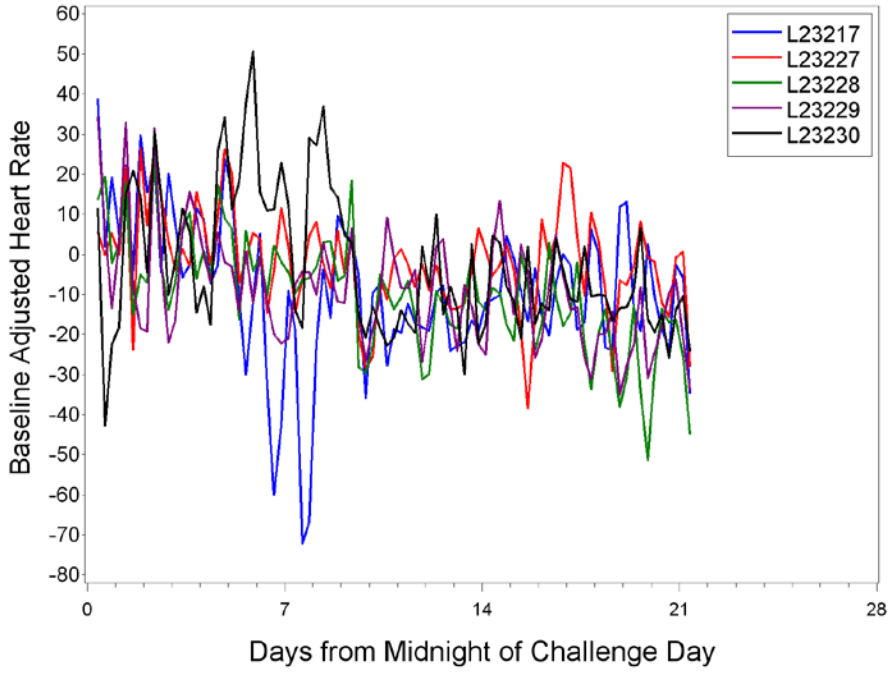

Figure 2c. Plot of baseline adjusted heart rate (BPM) for each animal in Group 3.

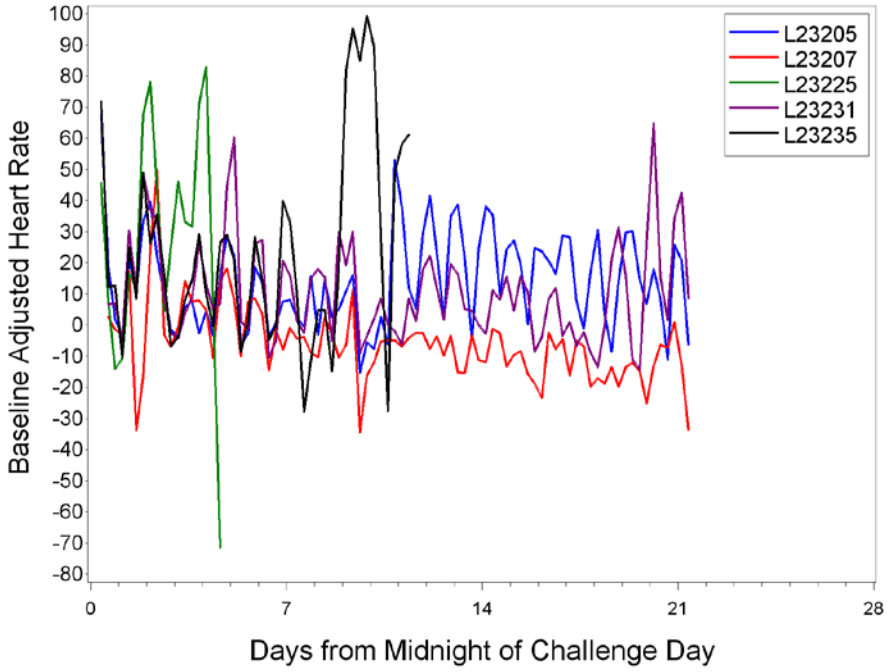

Figure 2d. Plot of baseline adjusted heart rate (BPM) for each animal in Group 4.

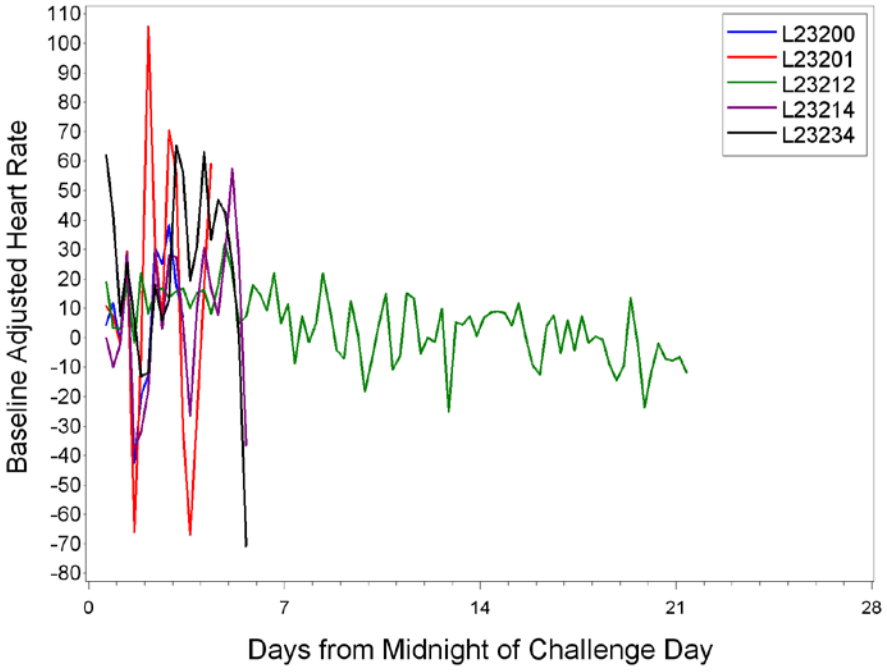

Figure 2e. Plot of baseline adjusted heart rate (BPM) for each animal in Group 5.

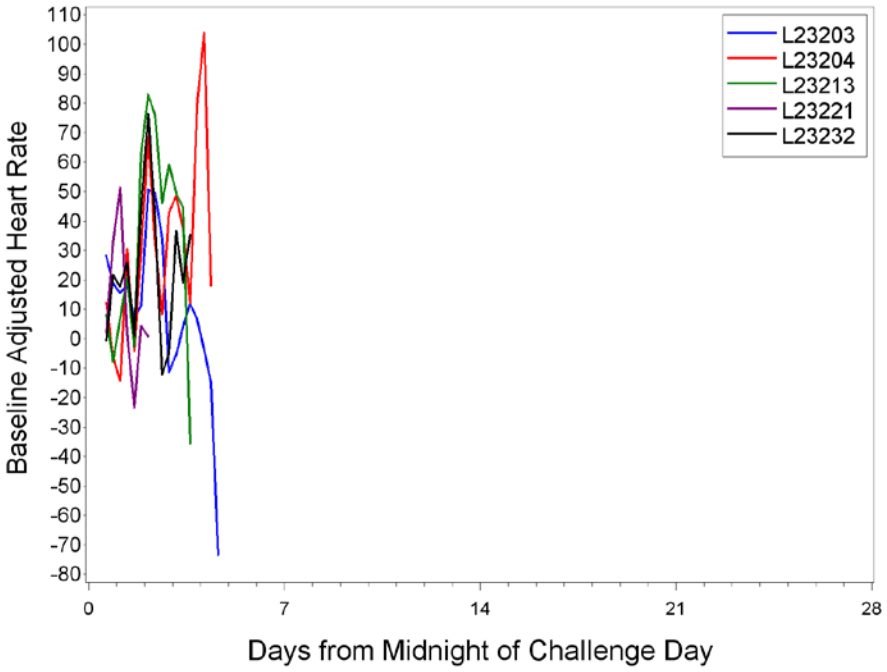

Figure 2f. Plot of baseline adjusted heart rate (BPM) for each animal in Group 6.

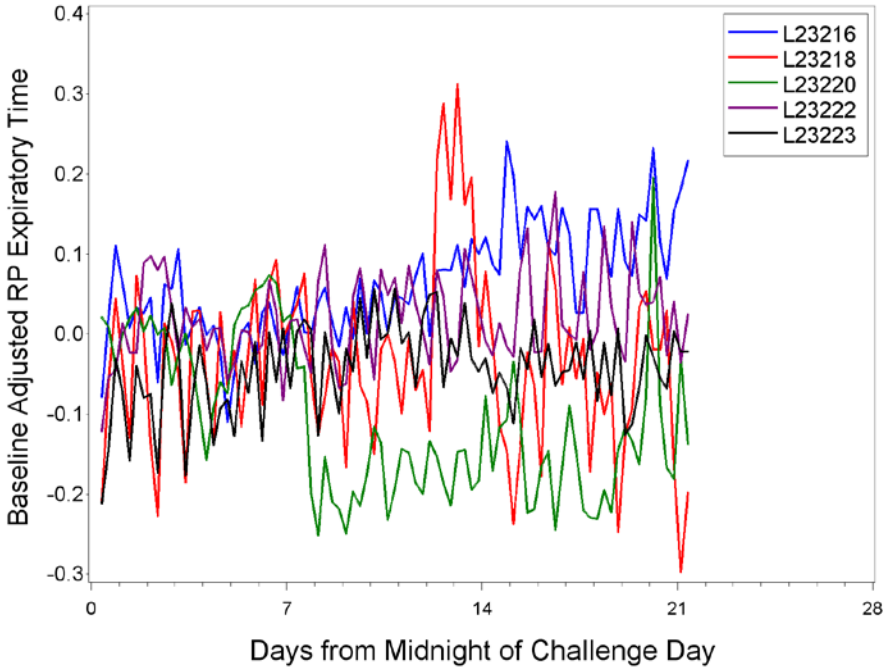

Figure 3a. **Plot of baseline adjusted RP expiratory time (seconds) for each animal in Group 1.**

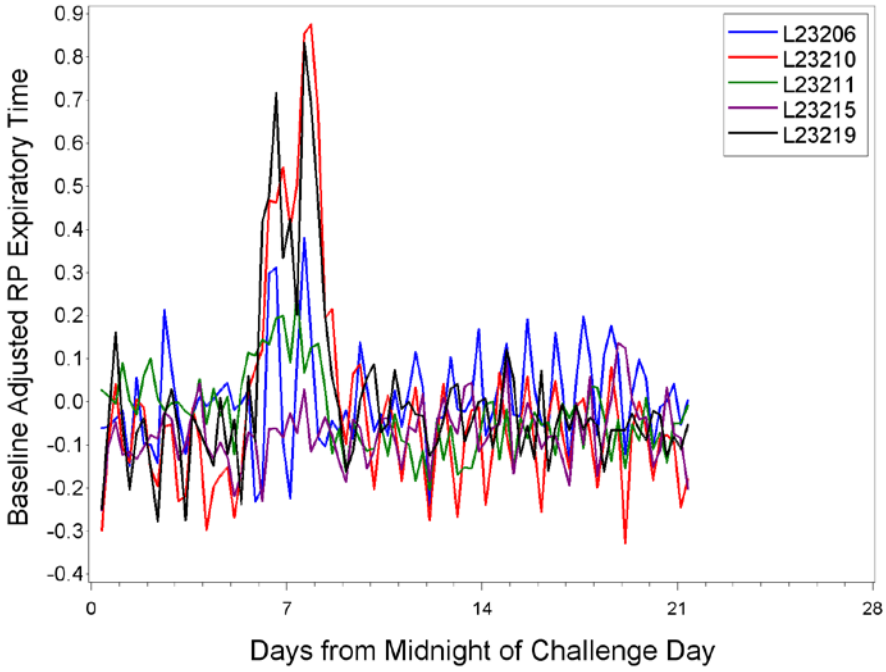

Figure 3b. **Plot of baseline adjusted RP expiratory time (seconds) for each animal in Group 2.**

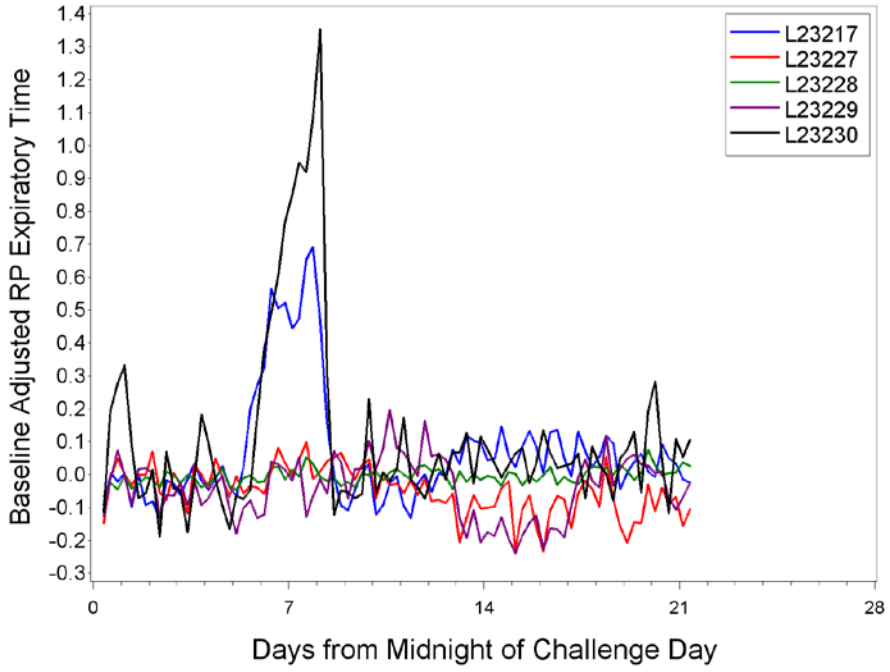

Figure 3c. **Plot of baseline adjusted RP expiratory time (seconds) for each animal in Group 3.**

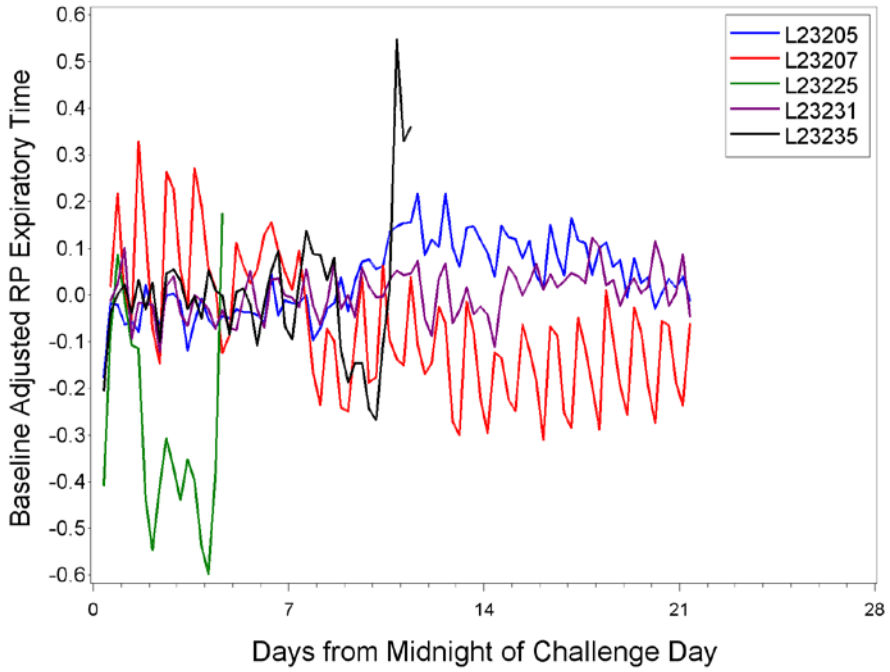

Figure 3d. **Plot of baseline adjusted RP expiratory time (seconds) for each animal in Group 4.**

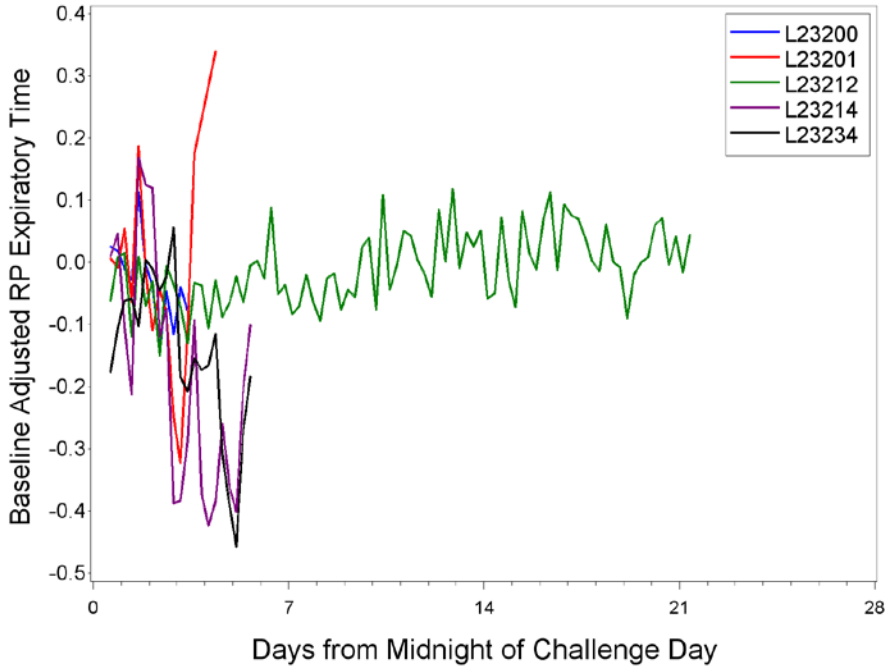

Figure 3e. **Plot of baseline adjusted RP expiratory time (seconds) for each animal in Group 5.**

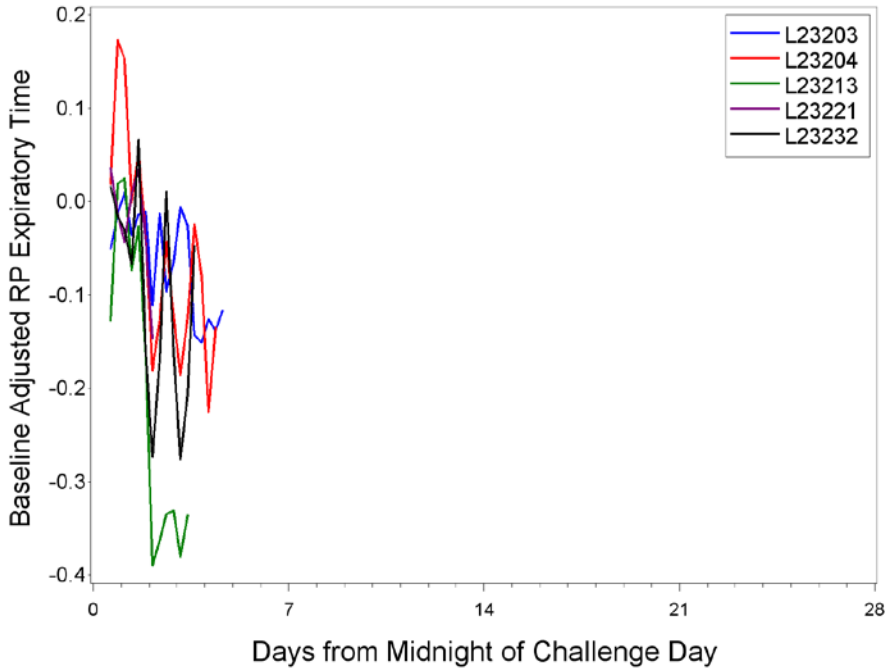

Figure 3f. **Plot of baseline adjusted RP expiratory time (seconds) for each animal in Group 6.**

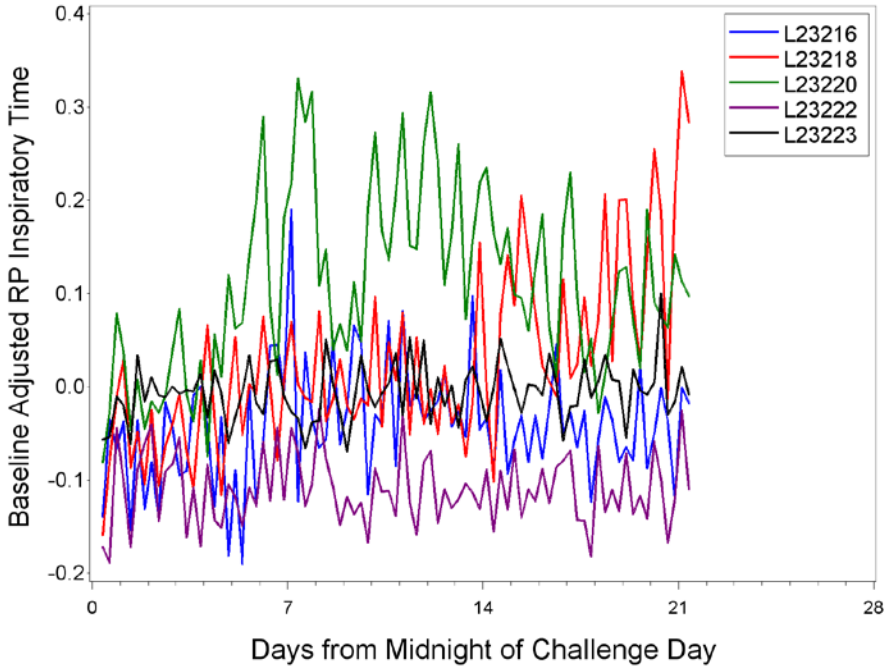

Figure 4a. **Plot of baseline adjusted RP inspiratory time (seconds) for each animal in Group 1.**

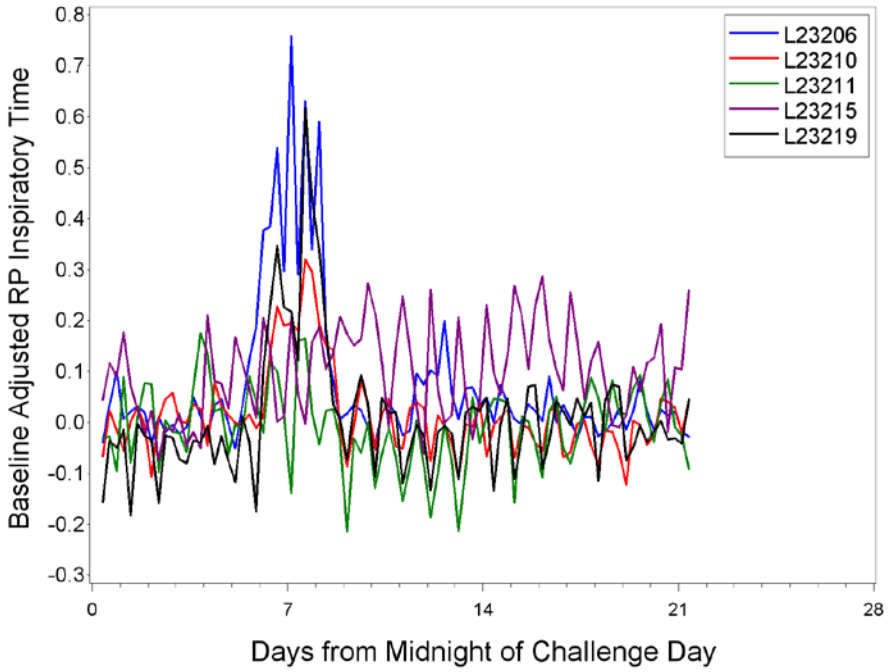

Figure 4b. **Plot of baseline adjusted RP inspiratory time (seconds) for each animal in Group 2.**

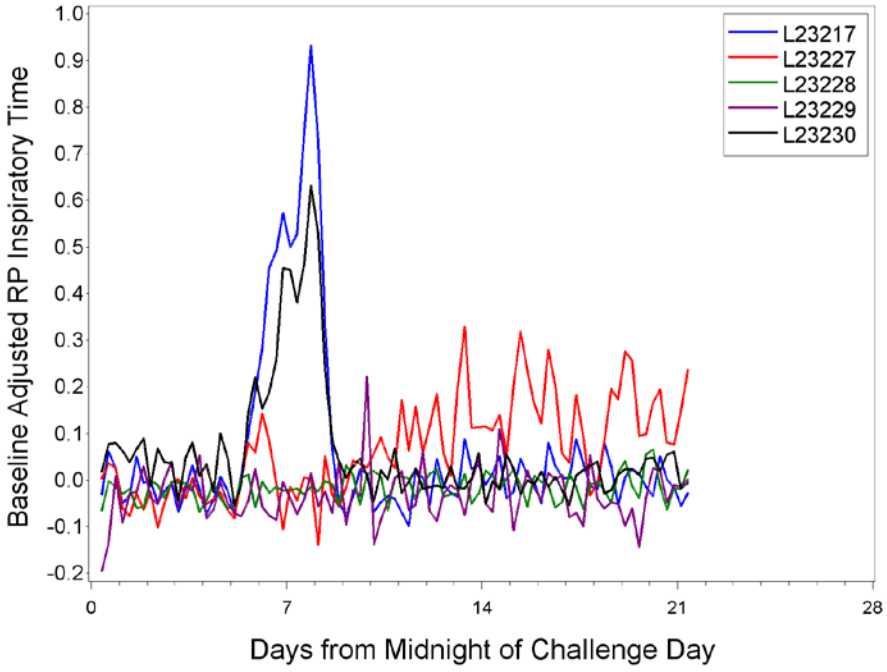

Figure 4c. Plot of baseline adjusted RP inspiratory time (seconds) for each animal in Group 3.

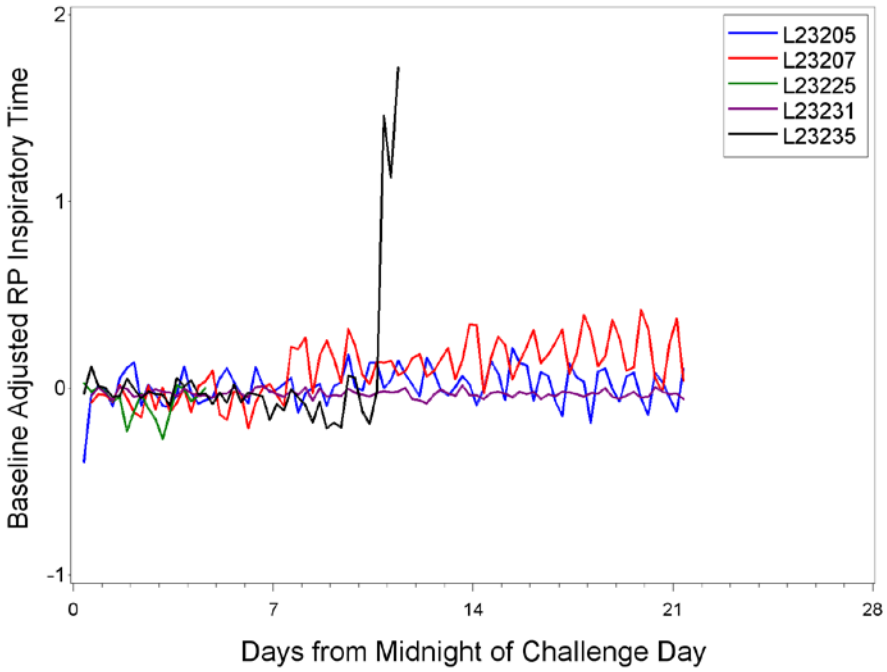

Figure 4d. Plot of baseline adjusted RP inspiratory time (seconds) for each animal in Group 4.

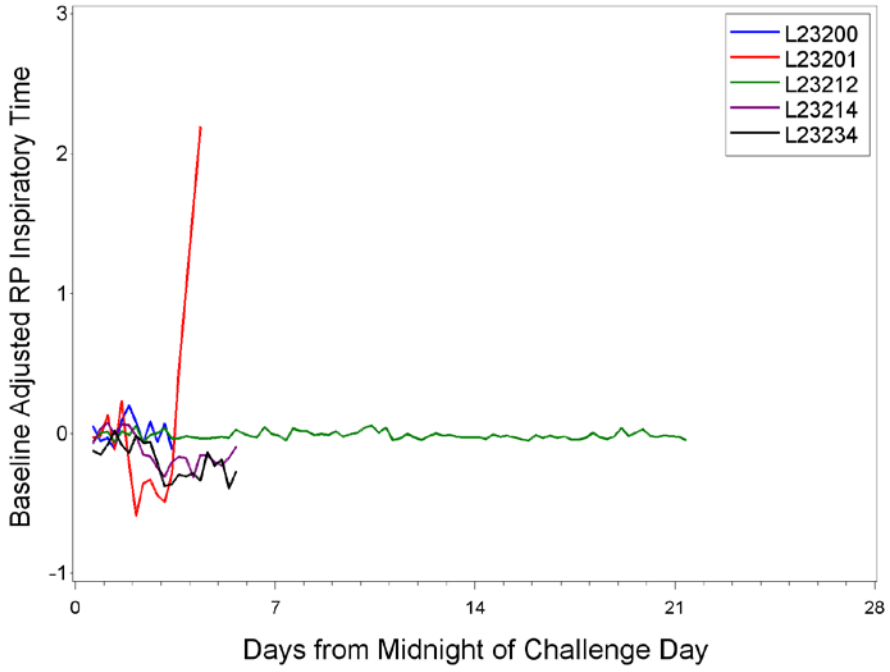

Figure 4e. Plot of baseline adjusted RP inspiratory time (seconds) for each animal in Group 5.

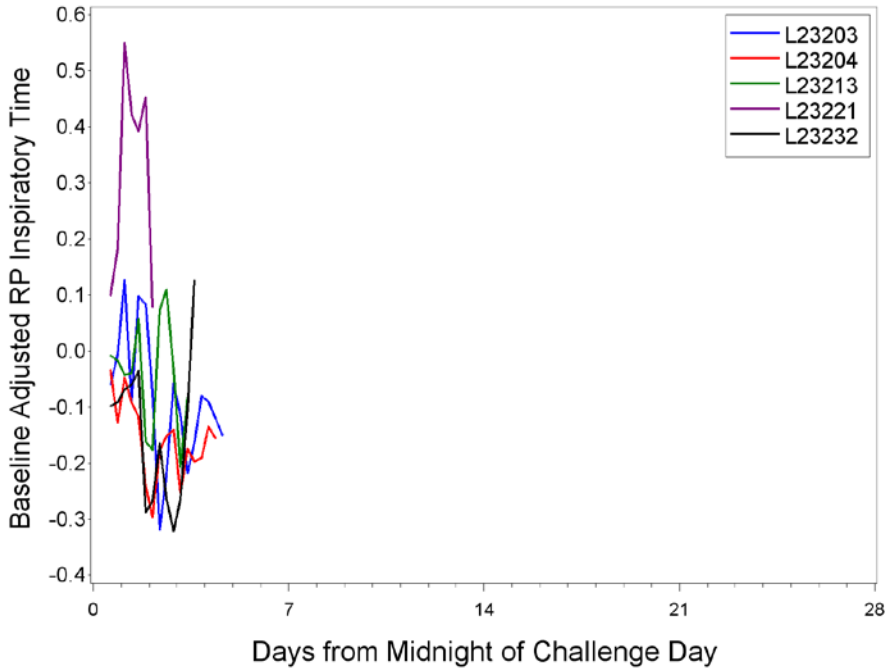

Figure 4f. Plot of baseline adjusted RP inspiratory time (seconds) for each animal in Group 6.

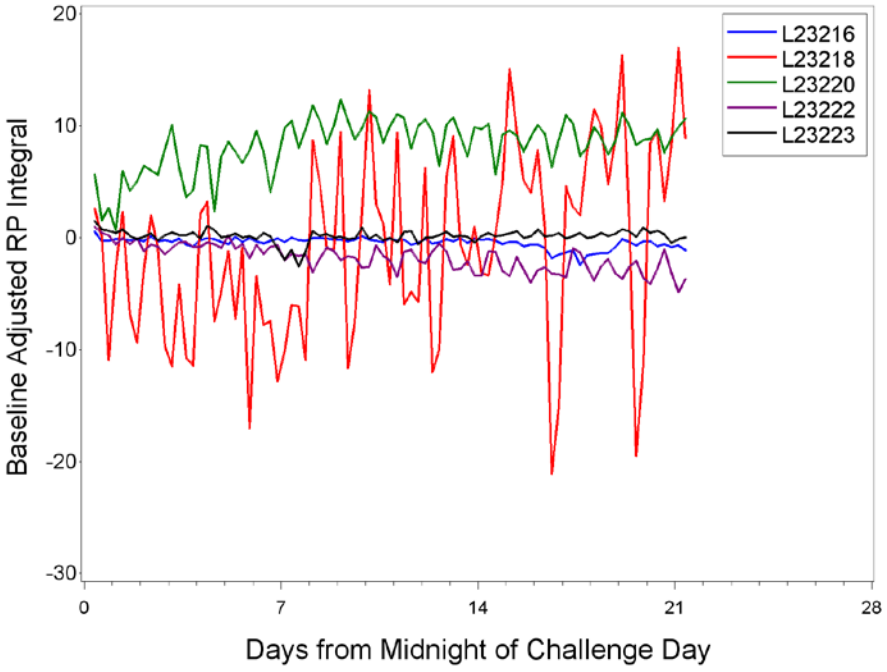

Figure 5a. Plot of baseline adjusted RP integral (mmHg-seconds) for each animal in Group 1.

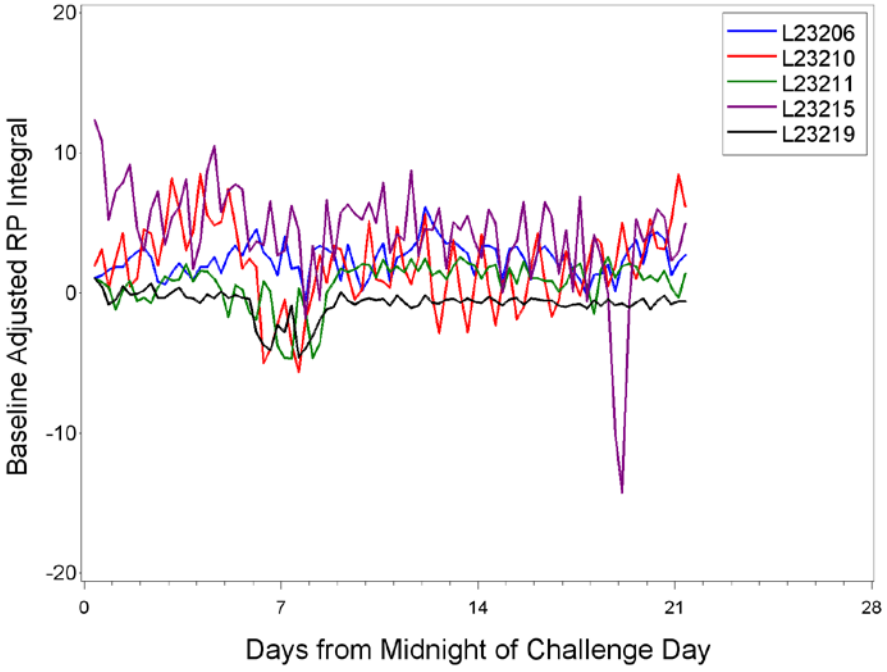

Figure 5b. Plot of baseline adjusted RP integral (mmHg-seconds) for each animal in Group 2.

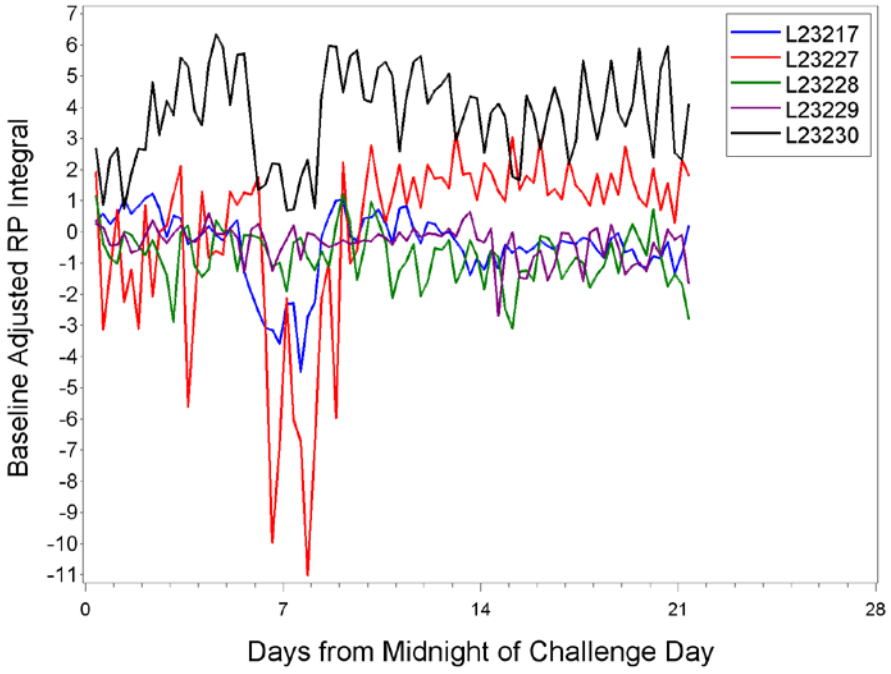

Figure 5c. Plot of baseline adjusted RP integral (mmHg-seconds) for each animal in Group 3.

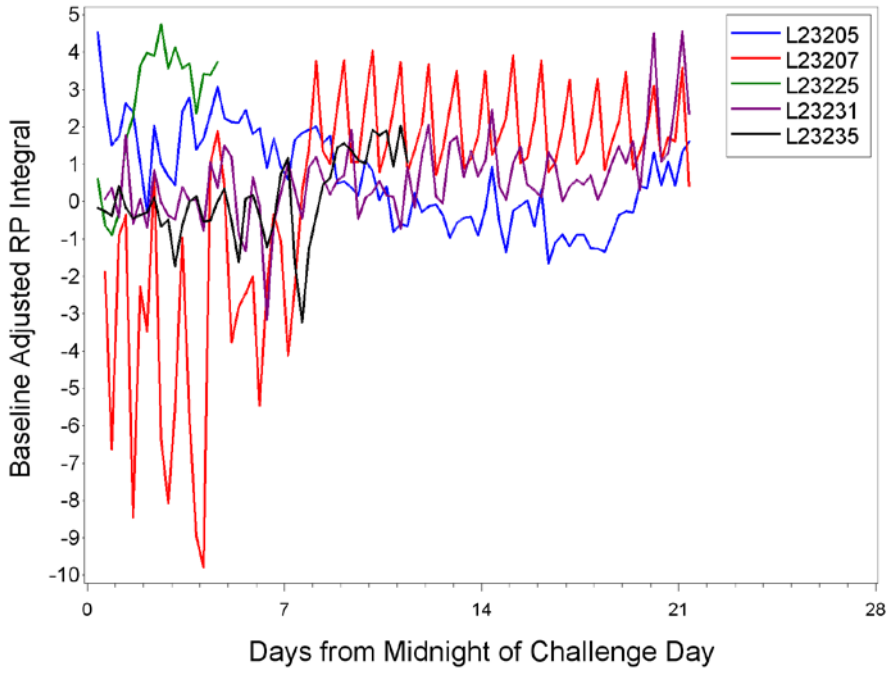

Figure 5d. Plot of baseline adjusted RP integral (mmHg-seconds) for each animal in Group 4.

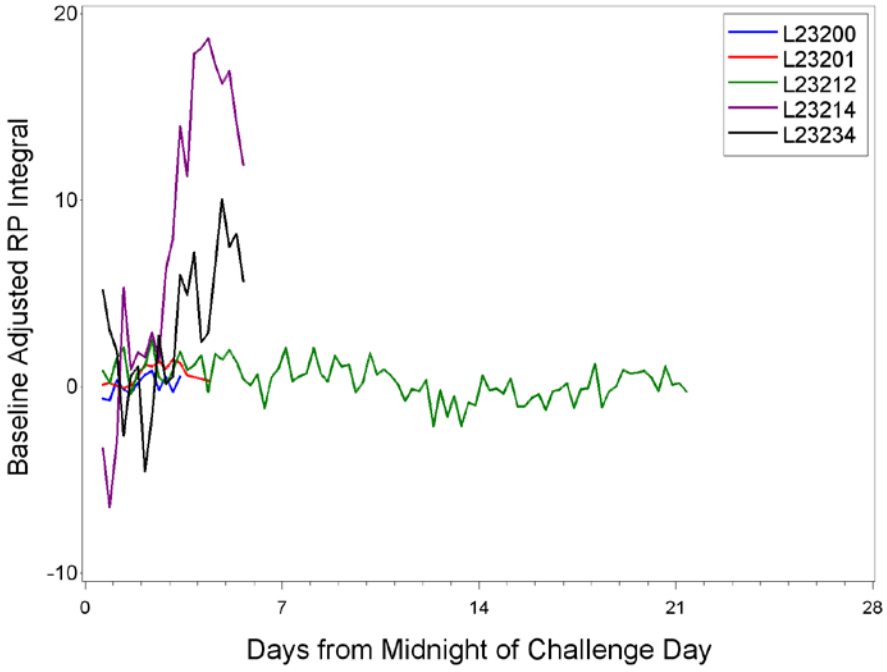

Figure 5e. Plot of baseline adjusted RP integral (mmHg-seconds) for each animal in Group 5.

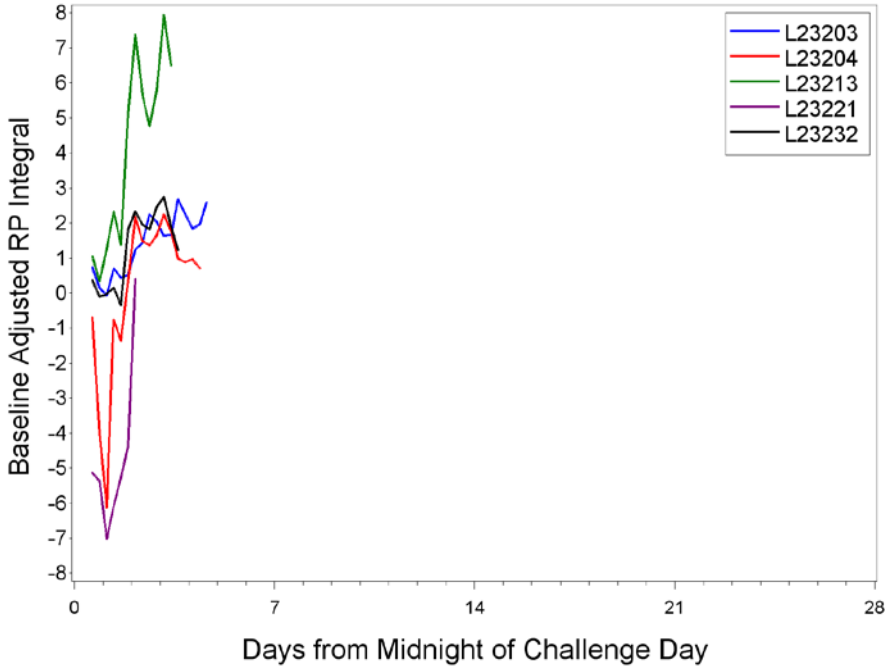

Figure 5f. Plot of baseline adjusted RP integral (mmHg-seconds) for each animal in Group 6.

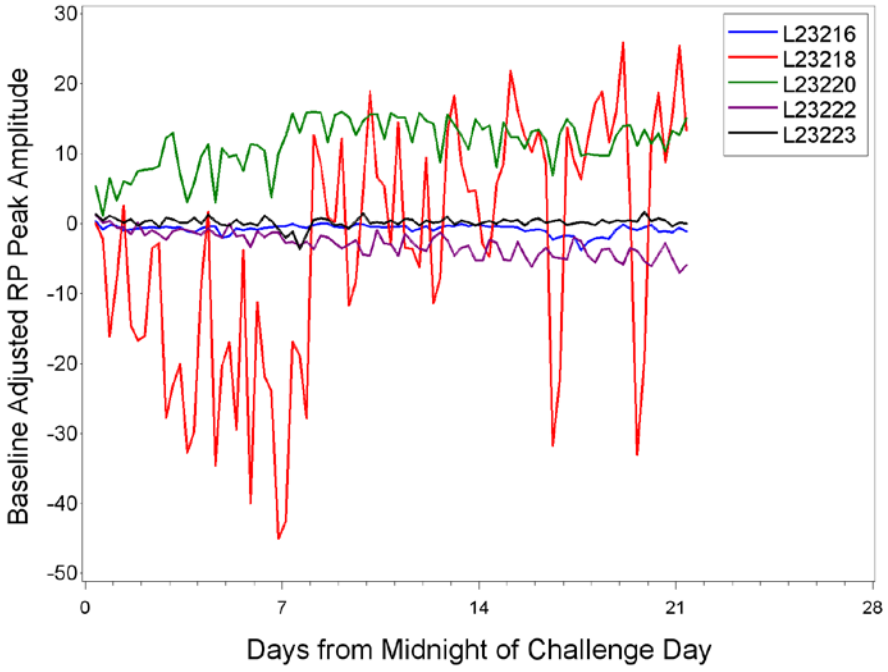

Figure 6a. Plot of baseline adjusted RP peak amplitude (mmHg) for each animal in Group 1.

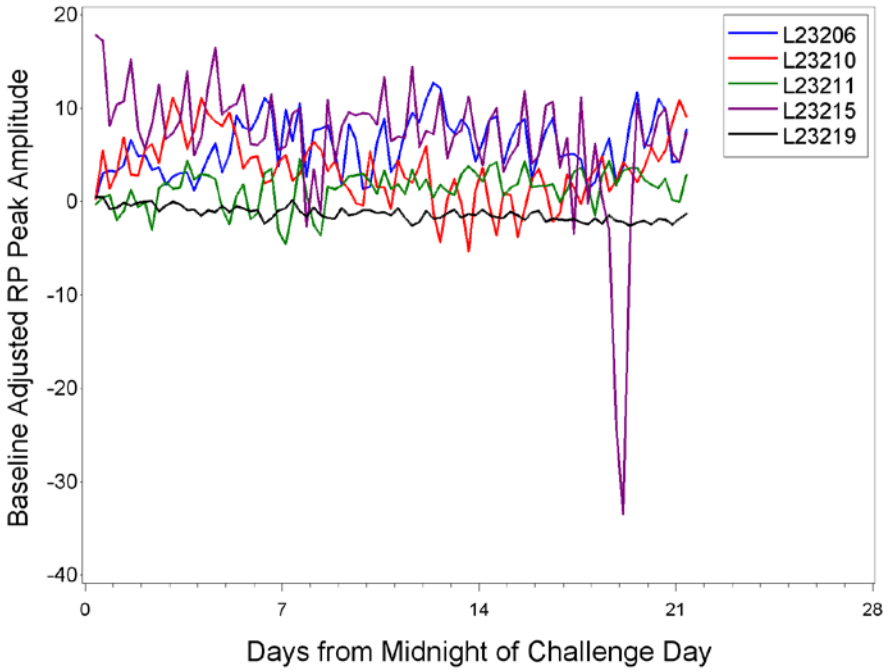

Figure 6b. Plot of baseline adjusted RP peak amplitude (mmHg) for each animal in Group 2.

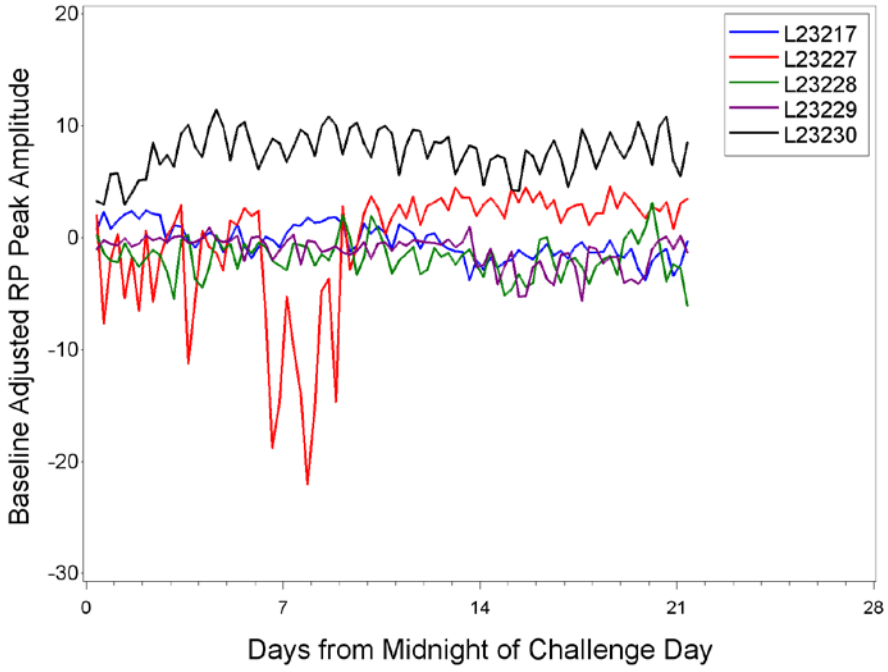

Figure 6c. Plot of baseline adjusted RP peak amplitude (mmHg) for each animal in Group 3.

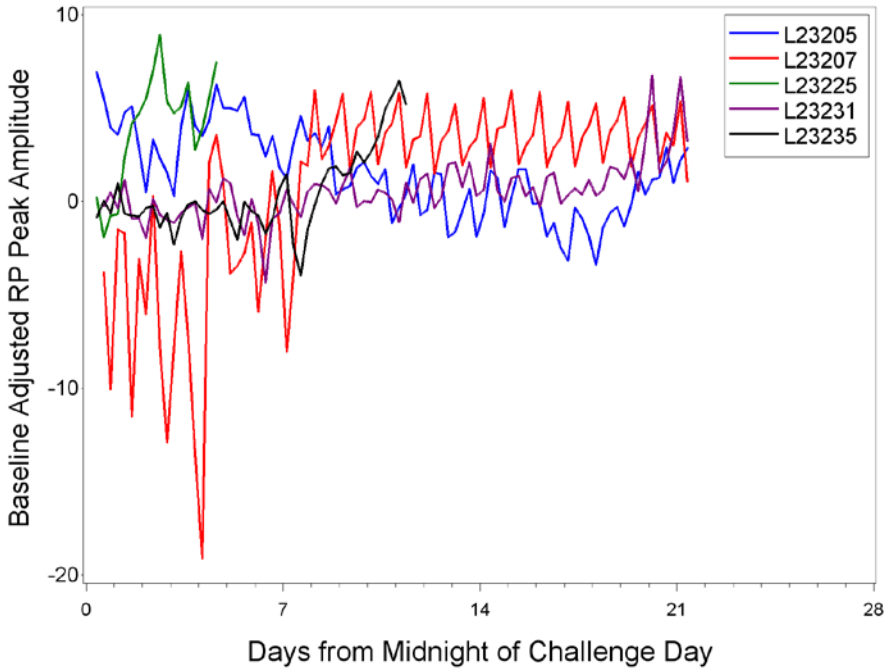

Figure 6d. Plot of baseline adjusted RP peak amplitude (mmHg) for each animal in Group 4.

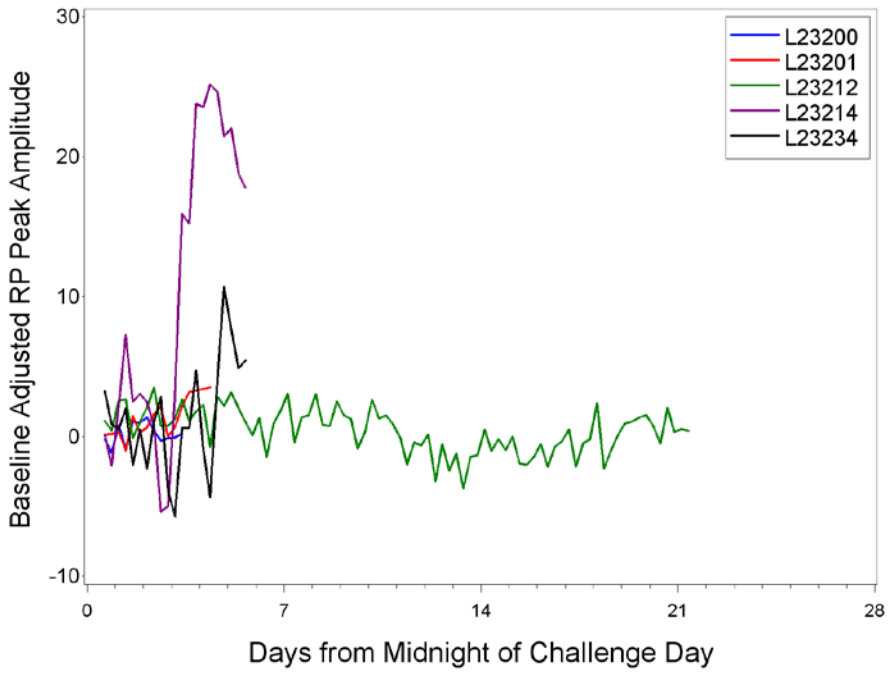

Figure 6e. Plot of baseline adjusted RP peak amplitude (mmHg) for each animal in Group 5.

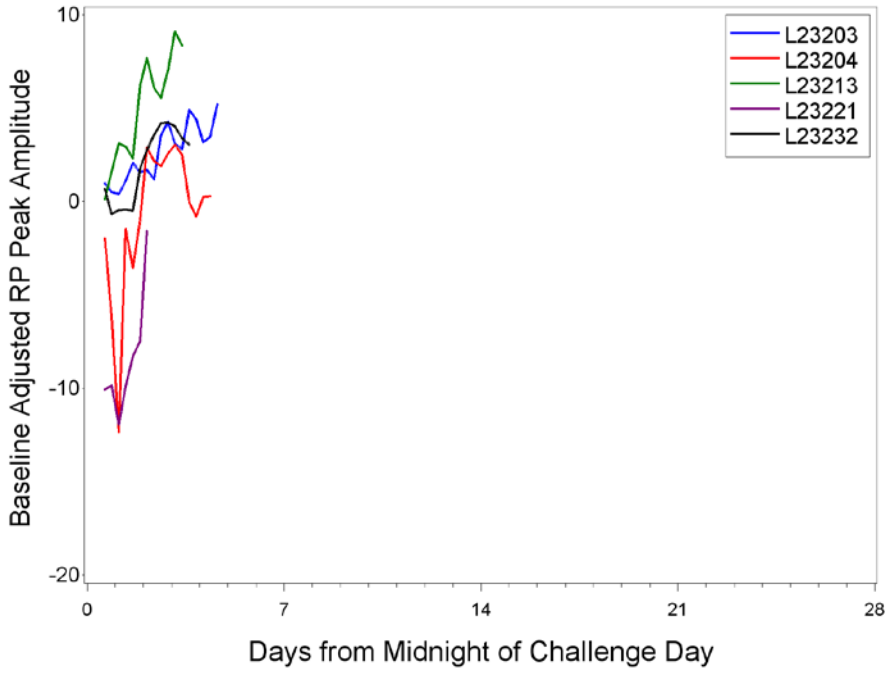

Figure 6f. Plot of baseline adjusted RP peak amplitude (mmHg) for each animal in Group 6.

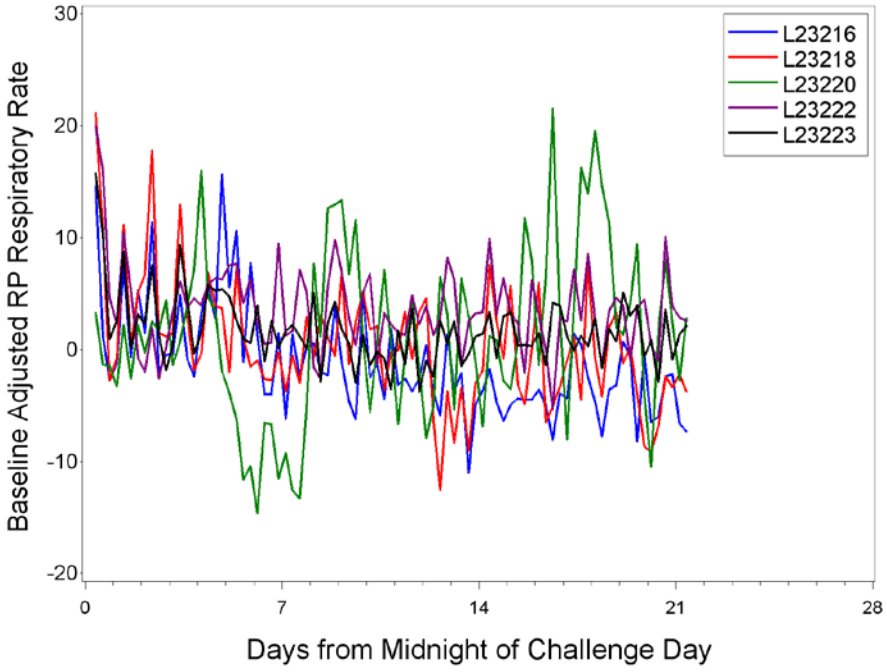

Figure 7a. Plot of baseline adjusted RP respiratory rate (RCPM) for each animal in Group 1.

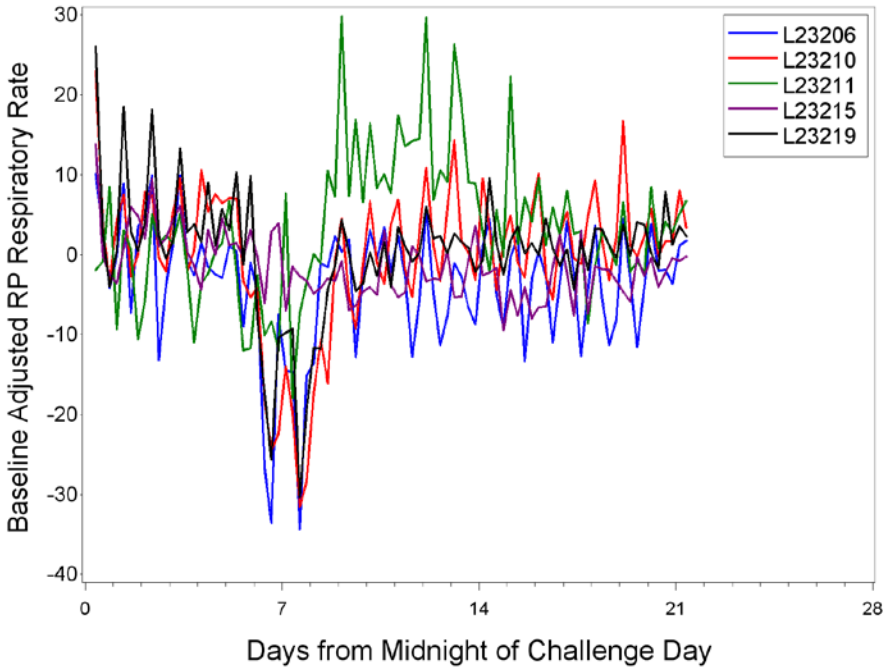

Figure 7b. Plot of baseline adjusted RP respiratory rate (RCPM) for each animal in Group 2.

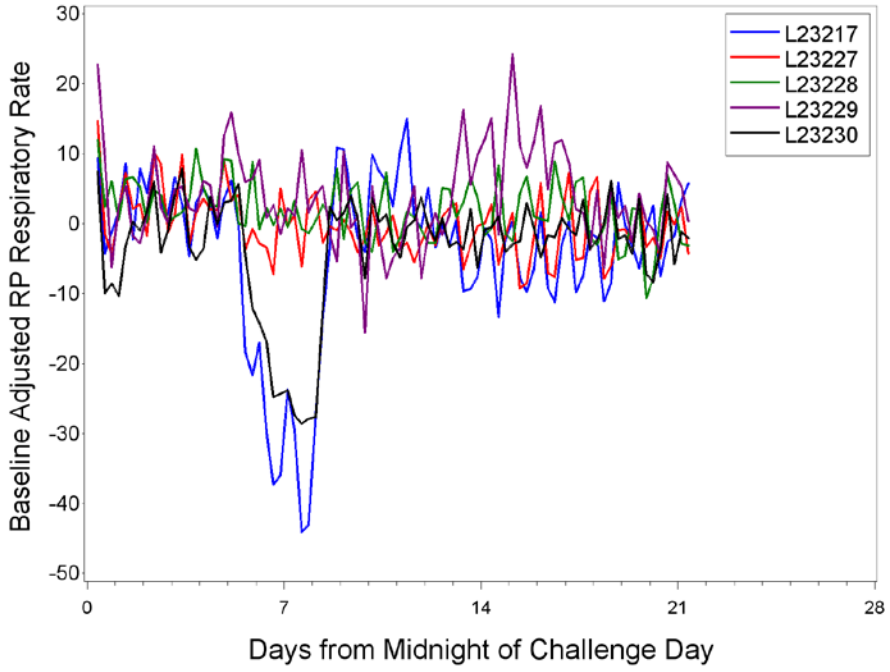

Figure 7c. Plot of baseline adjusted RP respiratory rate (RCPM) for each animal in Group 3.

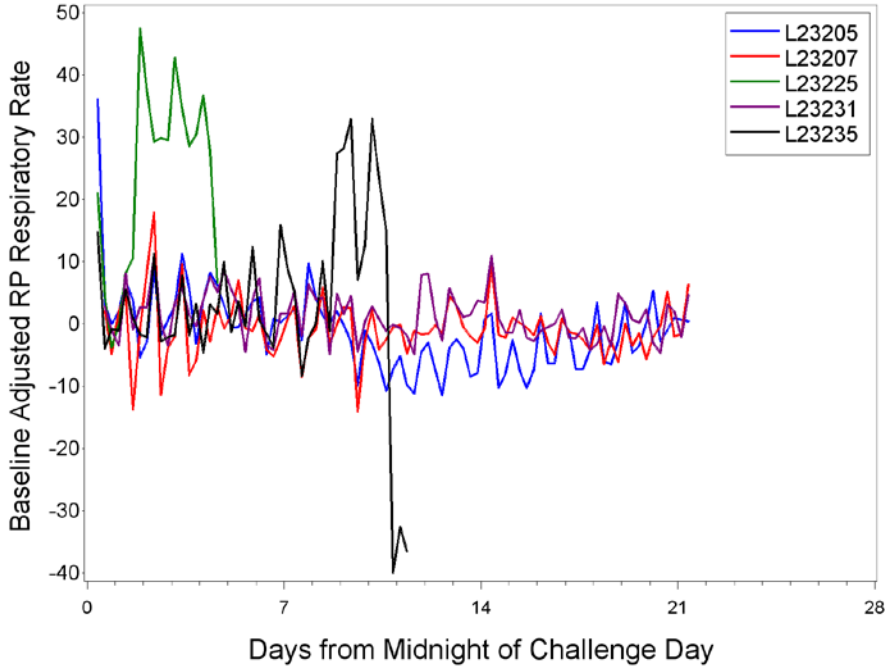

Figure 7d. Plot of baseline adjusted RP respiratory rate (RCPM) for each animal in Group 4.

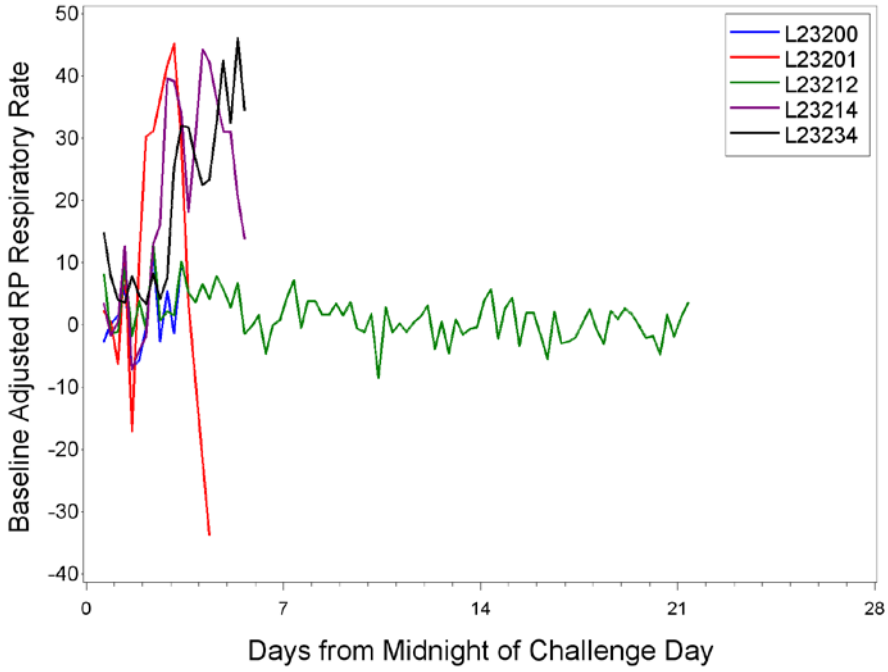

Figure 7e. Plot of baseline adjusted RP respiratory rate (RCPM) for each animal in Group 5.

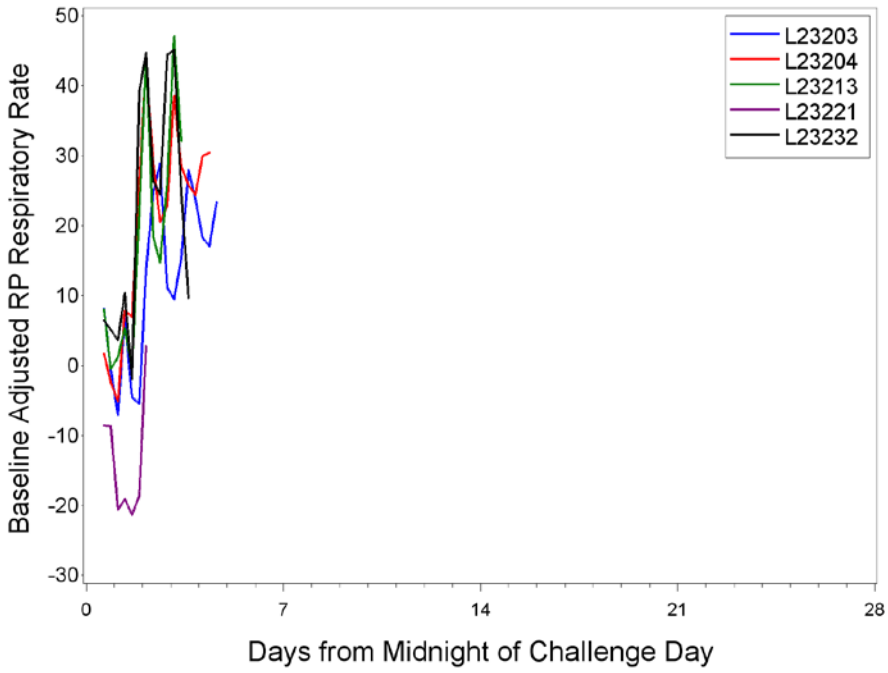

Figure 7f. Plot of baseline adjusted RP respiratory rate (RCPM) for each animal in Group 6.

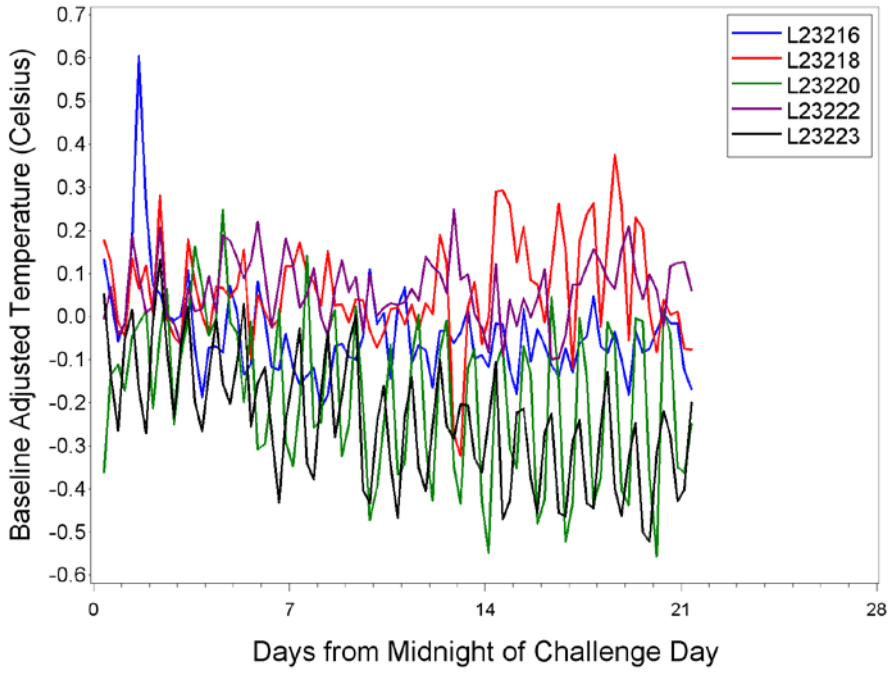

Figure 8a. Plot of baseline adjusted temperature (Celsius) for each animal in Group 1.

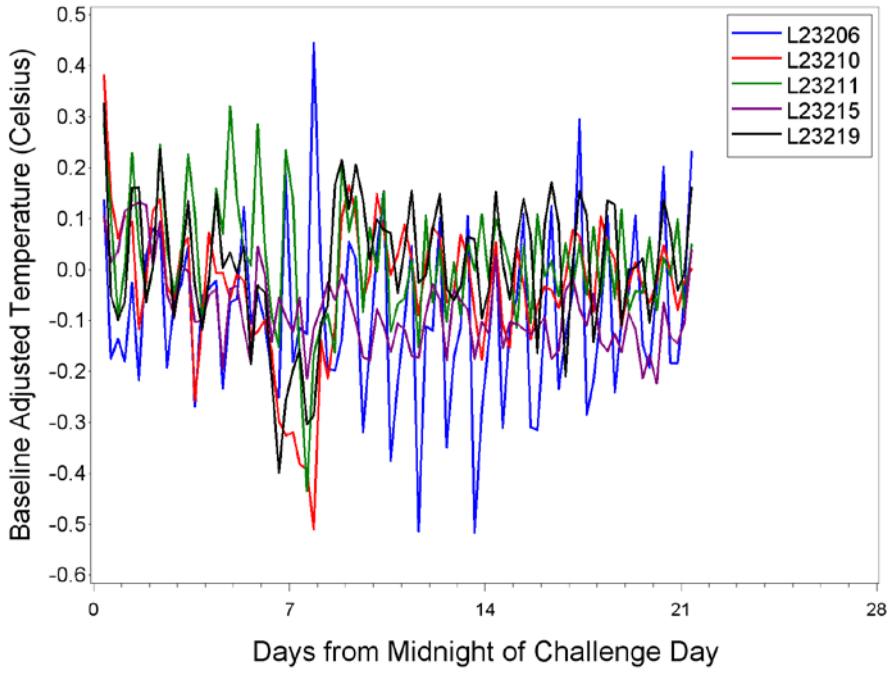

Figure 8b. Plot of baseline adjusted temperature (Celsius) for each animal in Group 2.

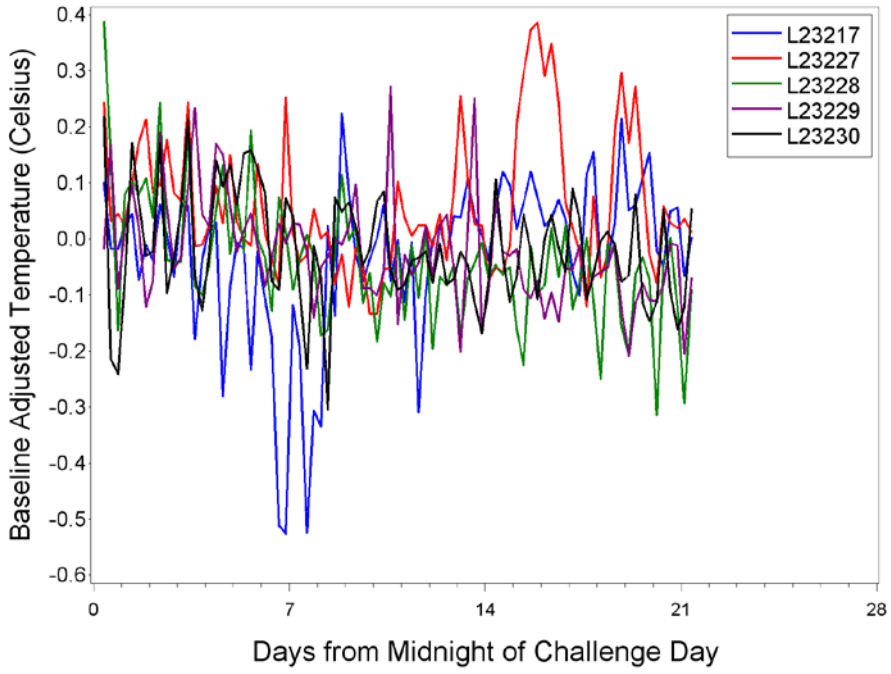

Figure 8c. Plot of baseline adjusted temperature (Celsius) for each animal in Group 3.

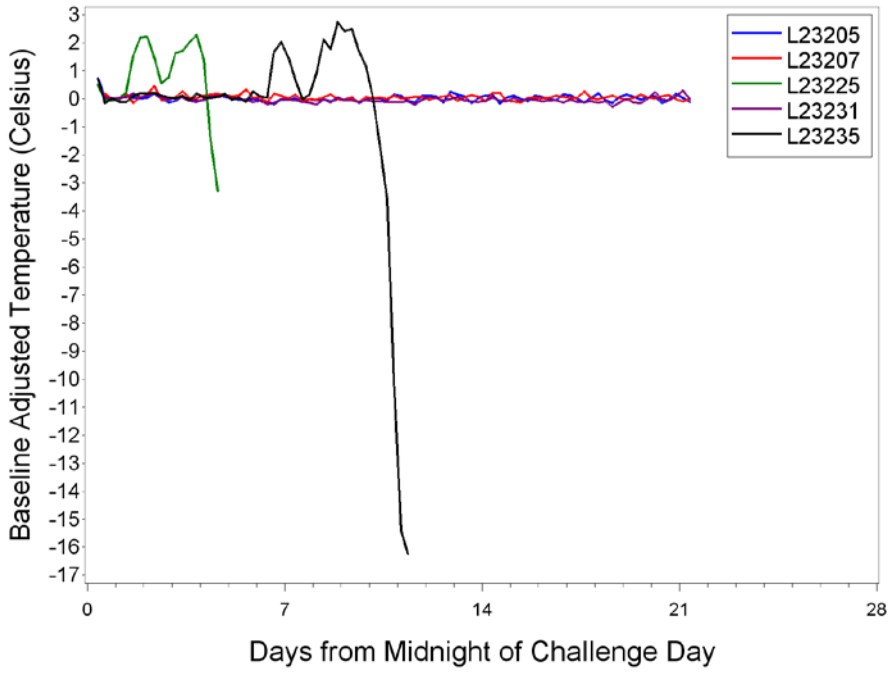

Figure 8d. Plot of baseline adjusted temperature (Celsius) for each animal in Group 4.

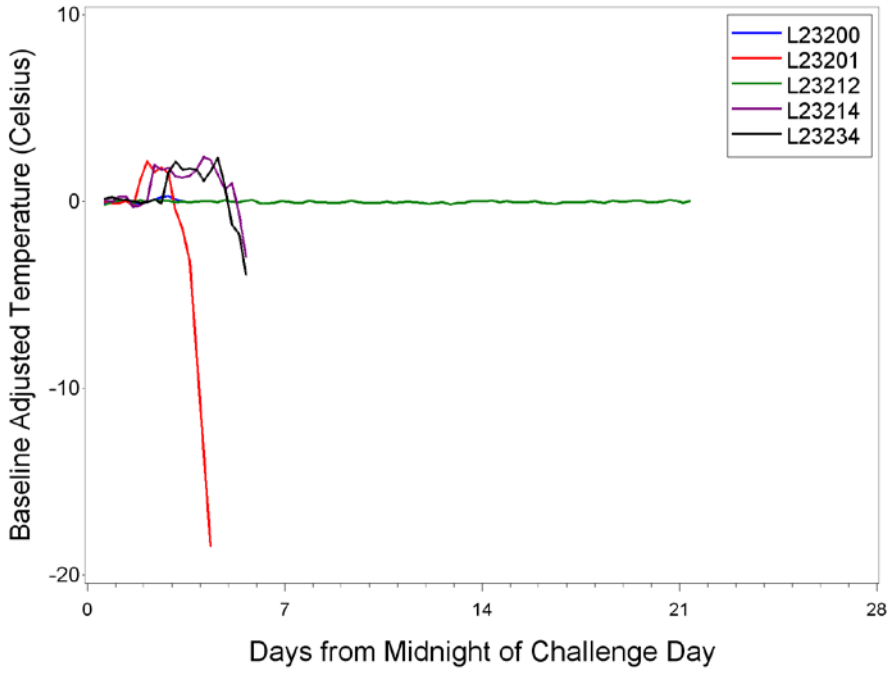

Figure 8e. Plot of baseline adjusted temperature (Celsius) for each animal in Group 5.

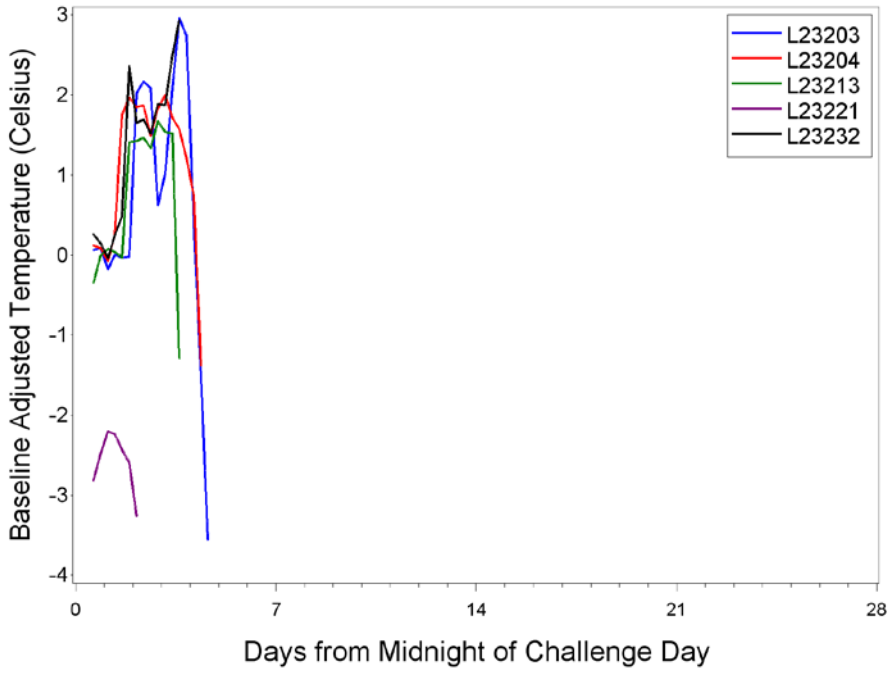

Figure 8f. Plot of baseline adjusted temperature (Celsius) for each animal in Group 6.

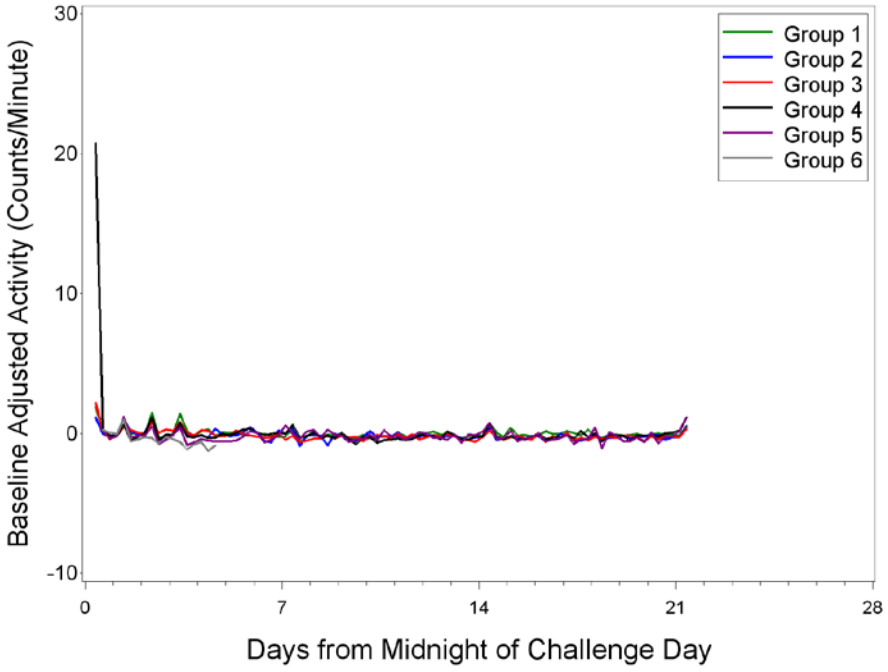

Figure 9. Plot of mean baseline adjusted activity (counts/minute) for each group.

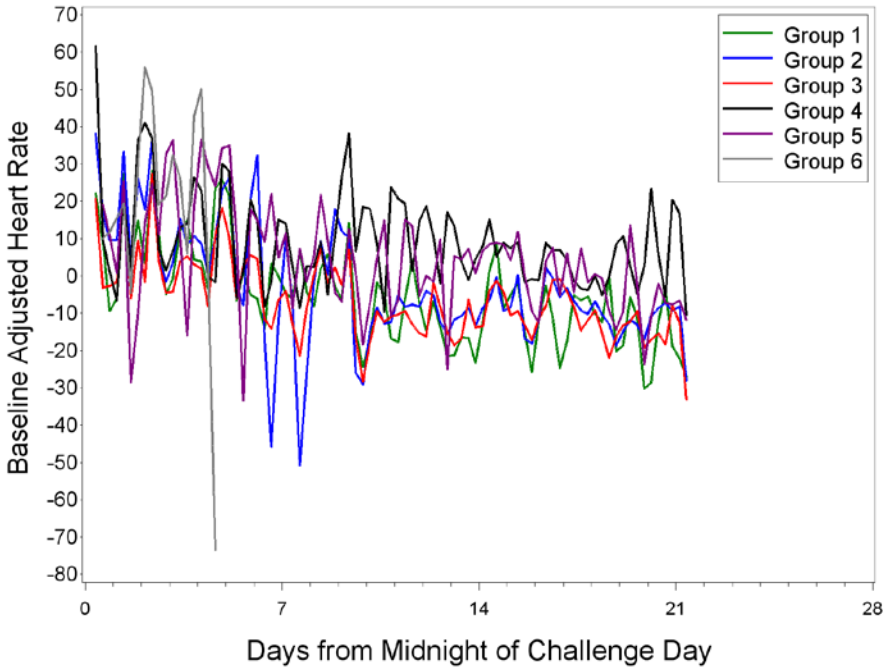

Figure 10. Plot of mean baseline adjusted heart rate (BPM) for each group.

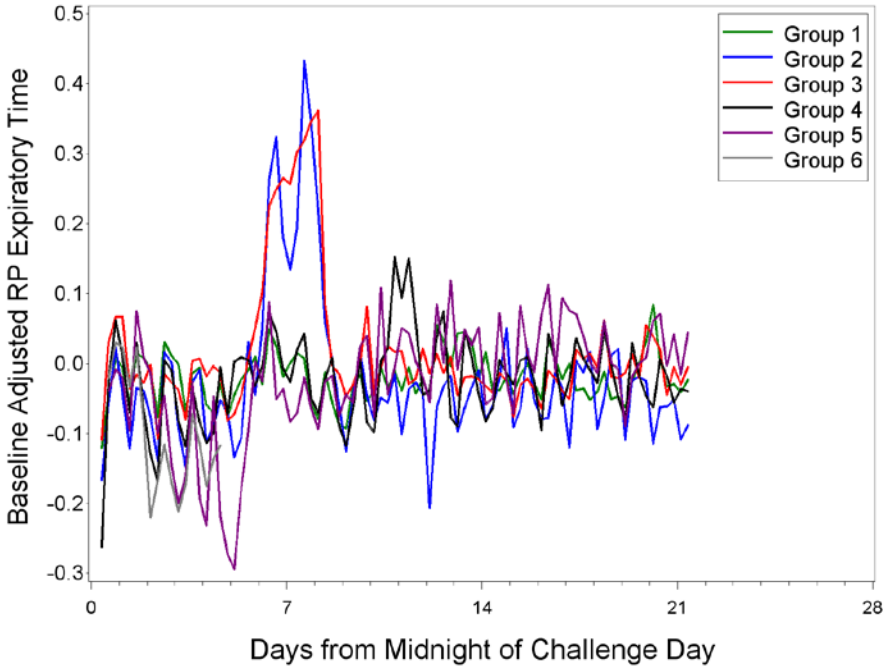

Figure 11. Plot of mean baseline adjusted RP expiratory time (seconds) for each group.

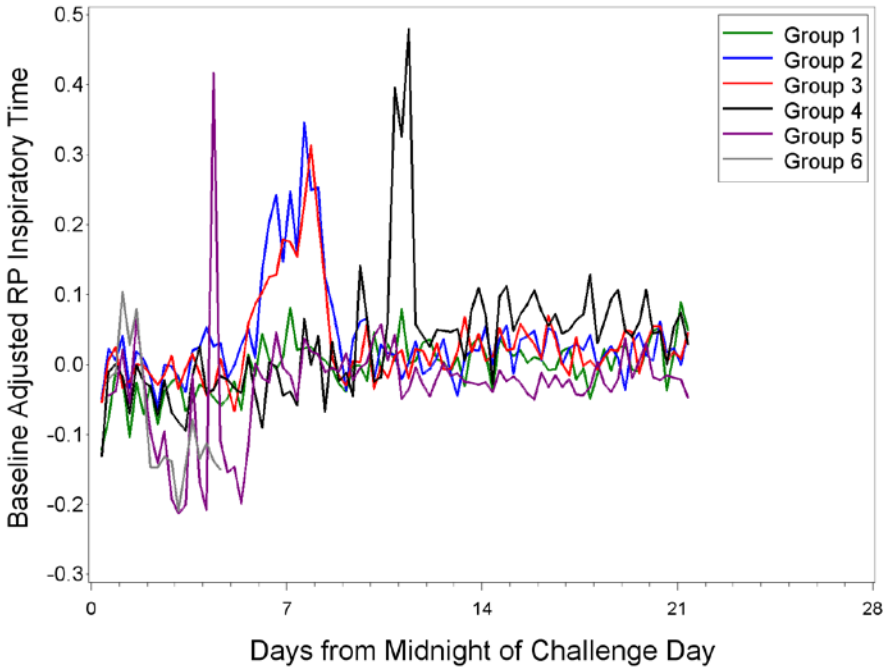

Figure 12. Plot of mean baseline adjusted RP inspiratory time (seconds) for each group.

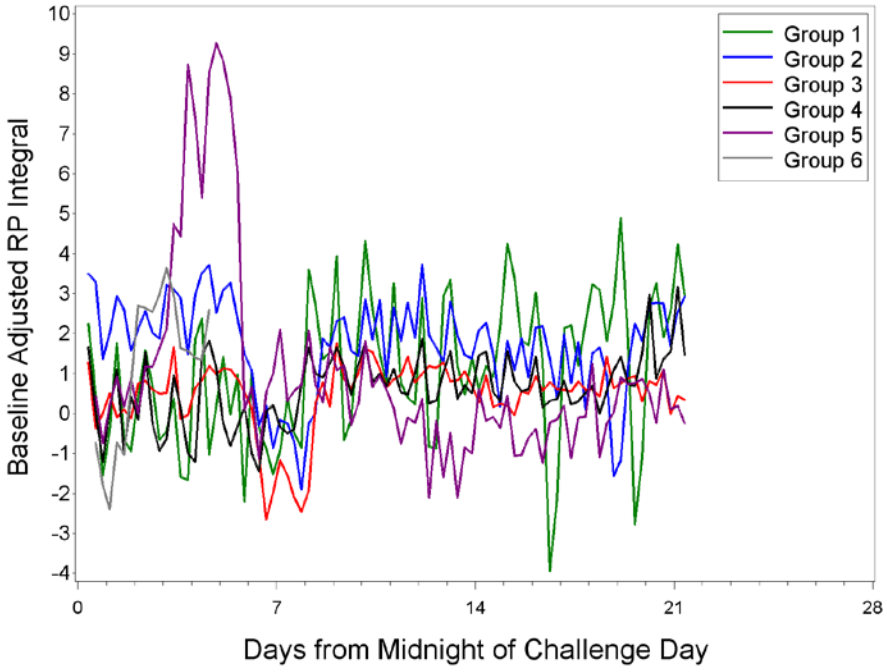

Figure 13. Plot of mean baseline adjusted RP integral (mmHg-seconds) for each group.

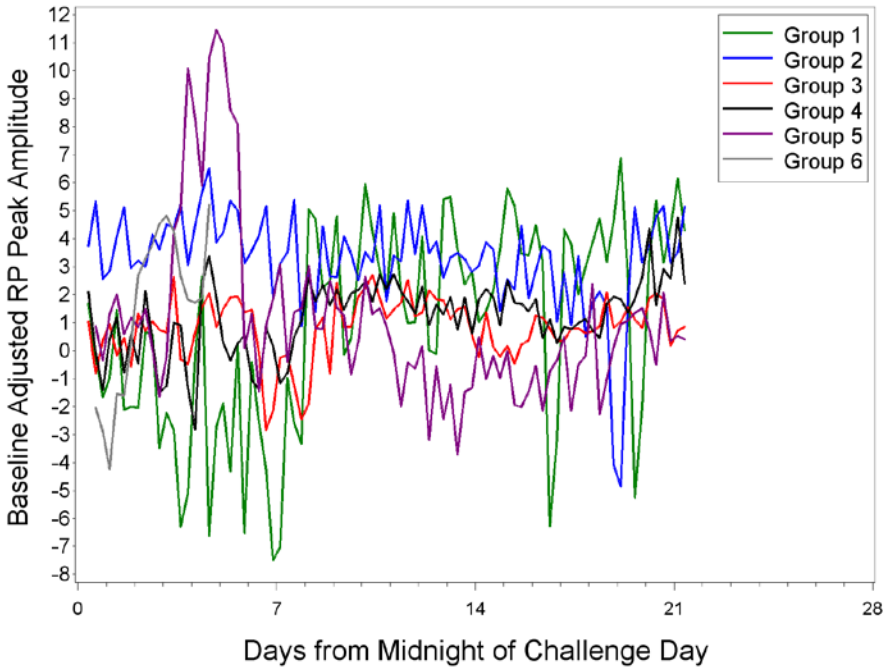

Figure 14. Plot of mean baseline adjusted RP peak amplitude (mmHg) for each group.

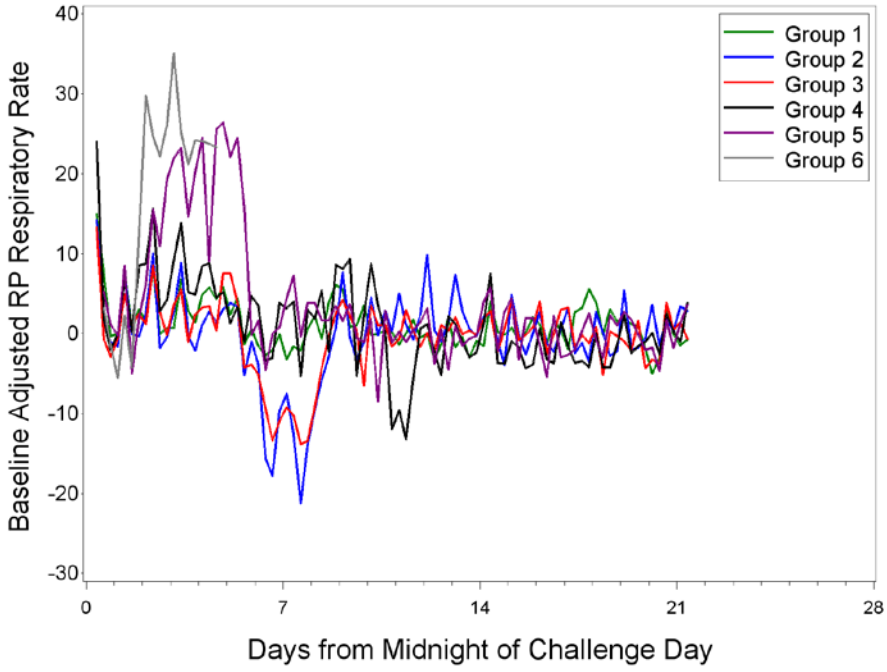

Figure 15. Plot of mean baseline adjusted RP respiratory rate (RCPM) for each group.

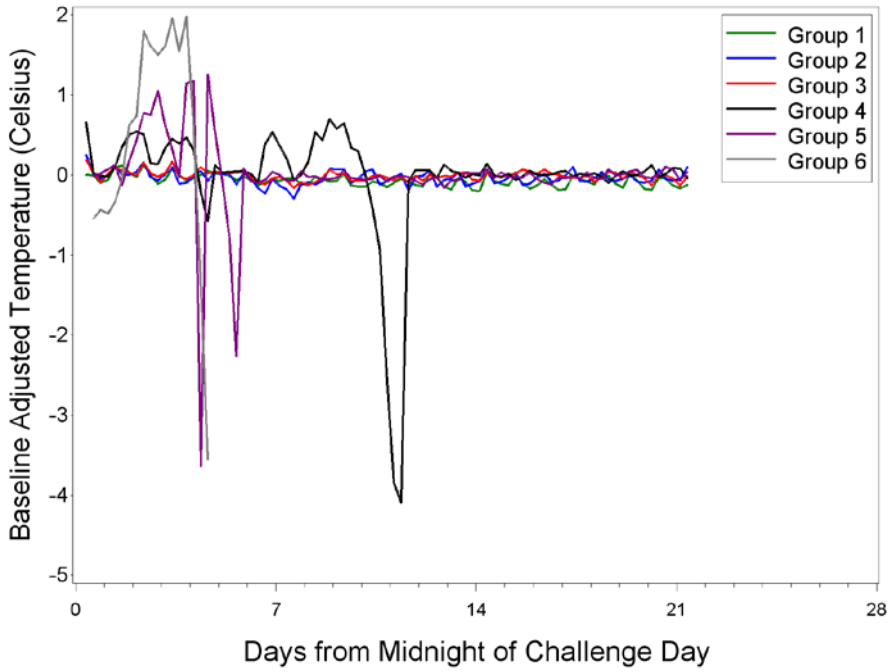

Figure 16. Plot of mean baseline adjusted temperature (Celsius) for each group.

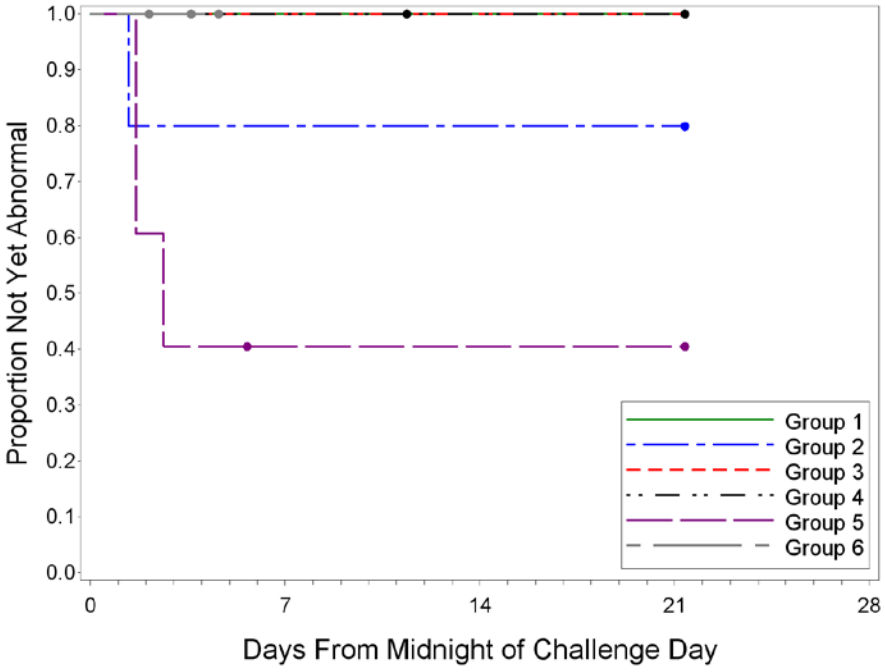

Figure 17. Kaplan-Meier curves for time to abnormality based on activity.

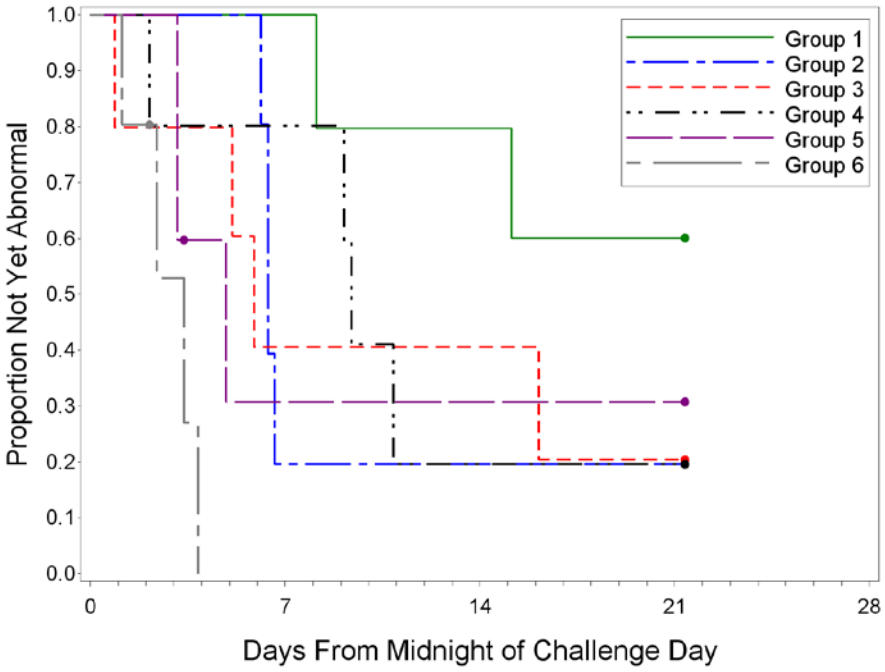

Figure 18. Kaplan-Meier curves for time to abnormality based on RP expiratory time.

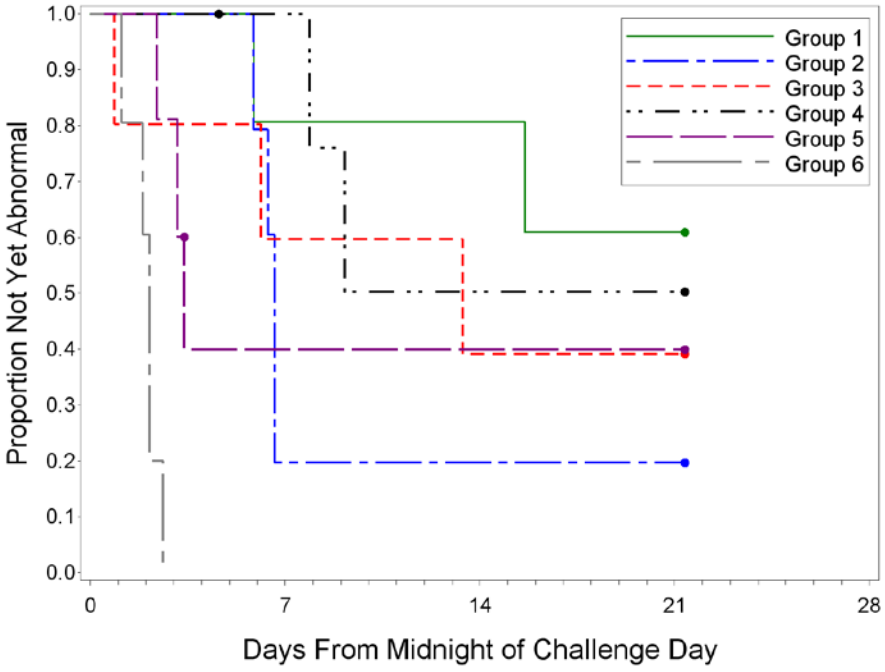

Figure 19. Kaplan-Meier curves for time to abnormality based on RP inspiratory time.

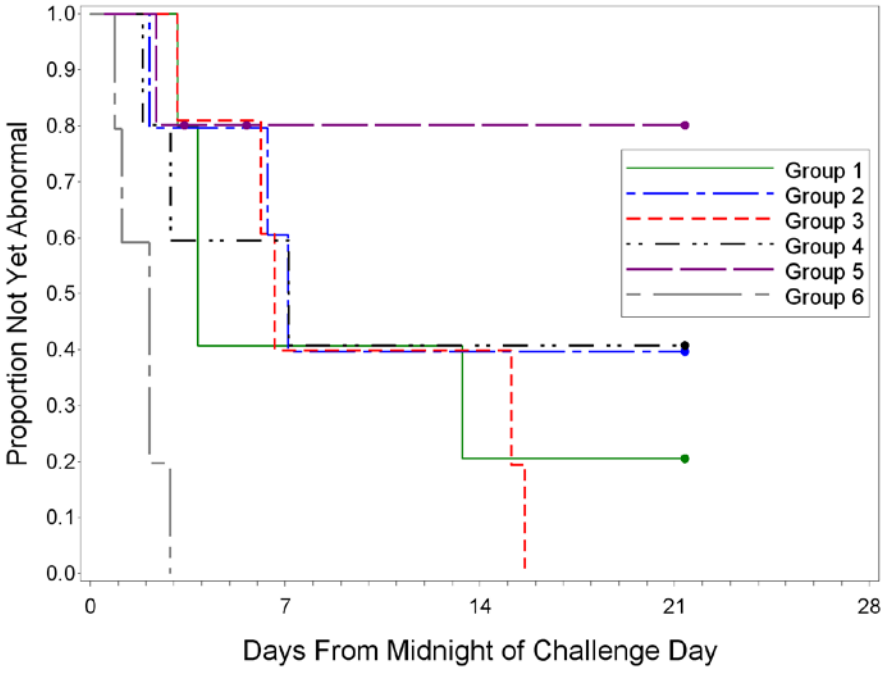

Figure 20. Kaplan-Meier curves for time to abnormality based on RP integral.

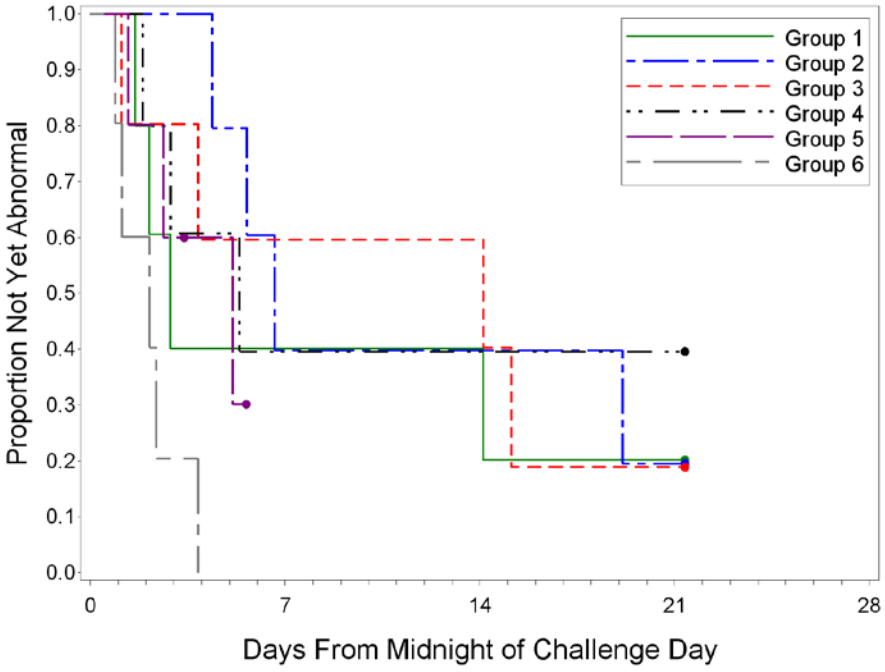

Figure 21. Kaplan-Meier curves for time to abnormality based on RP peak amplitude.

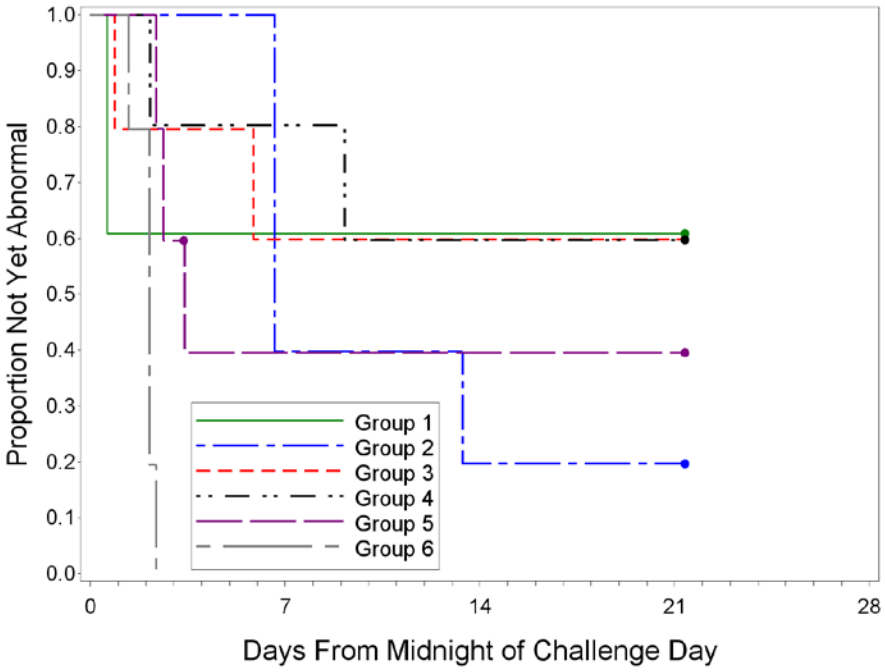

Figure 22. Kaplan-Meier curves for time to abnormality based on RP respiratory rate.

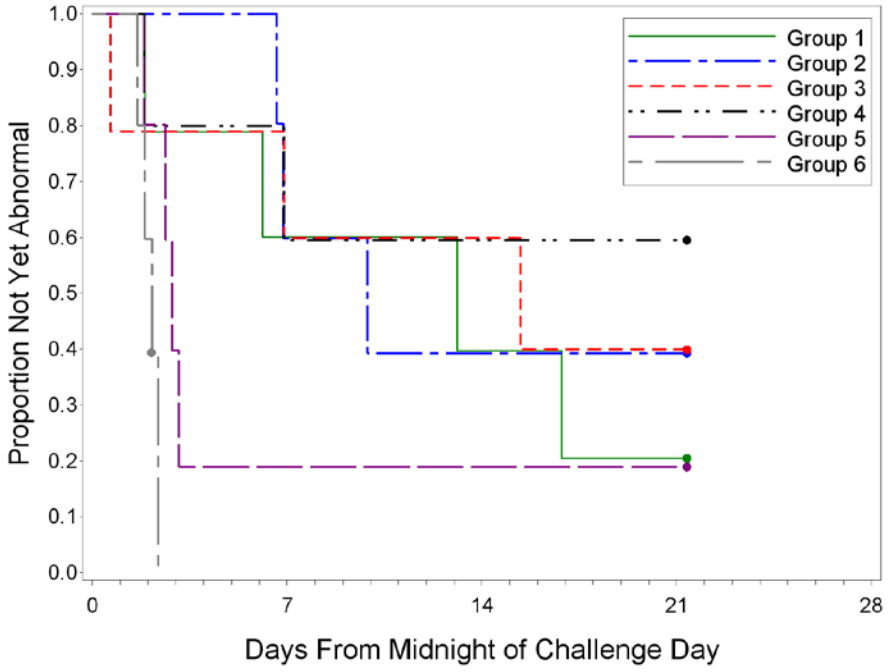

Figure 23. Kaplan-Meier curves for time to abnormality based on temperature.

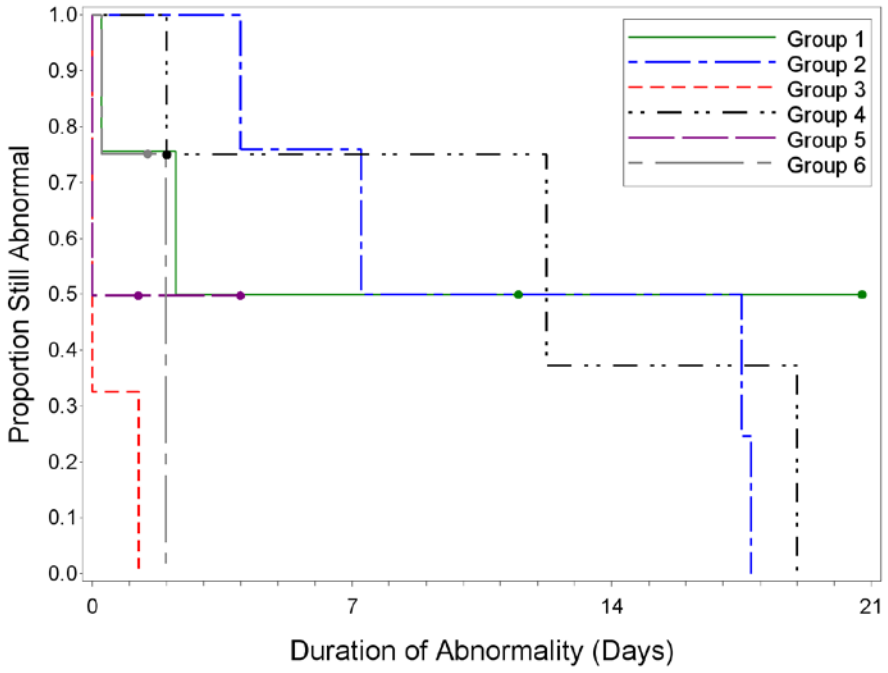

Figure 24. Kaplan-Meier curves for duration of abnormality based on heart rate.

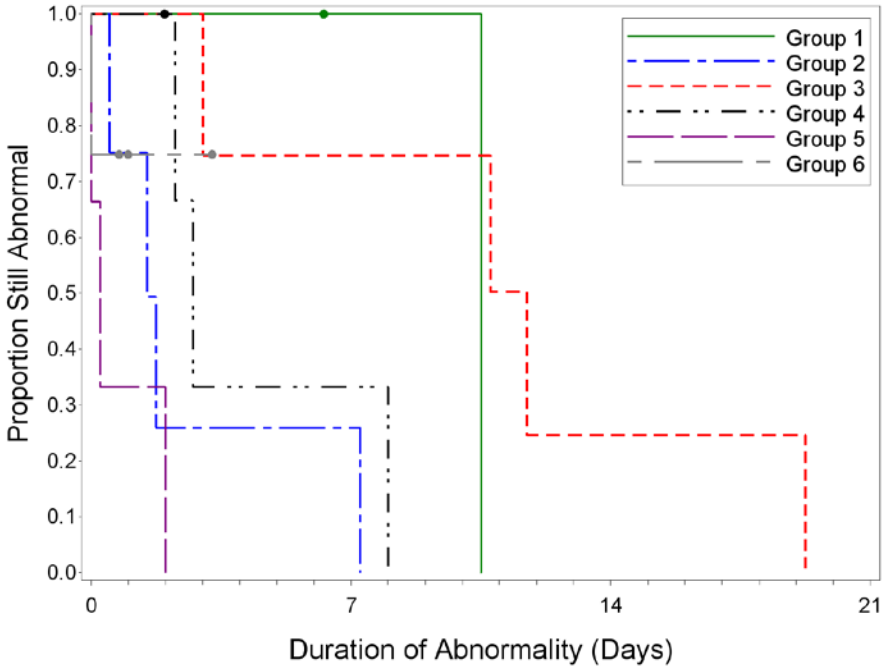

Figure 25. Kaplan-Meier Curves for duration of abnormality based on RP expiratory time.

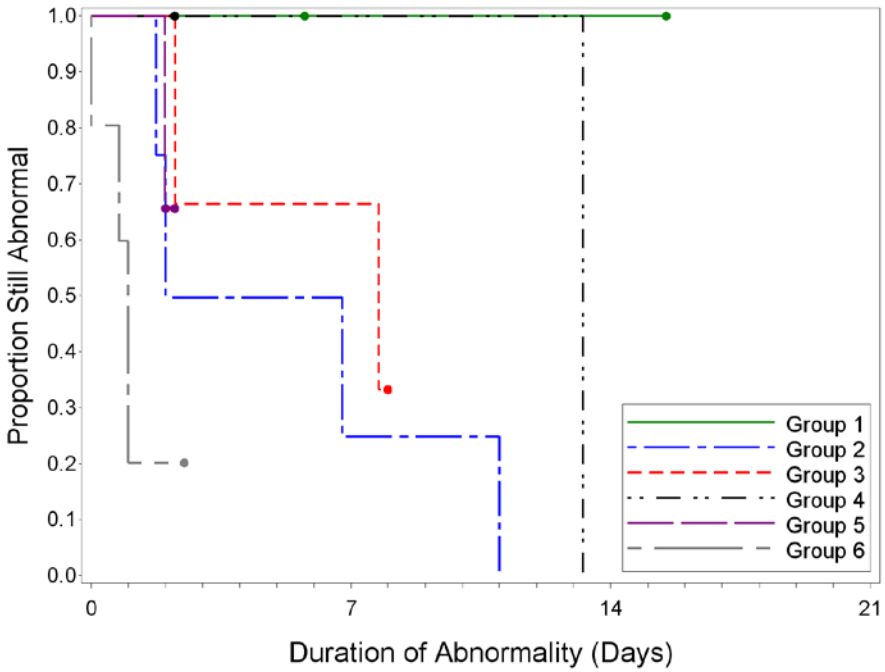

Figure 26. Kaplan-Meier curves for duration of abnormality based on RP inspiratory time.

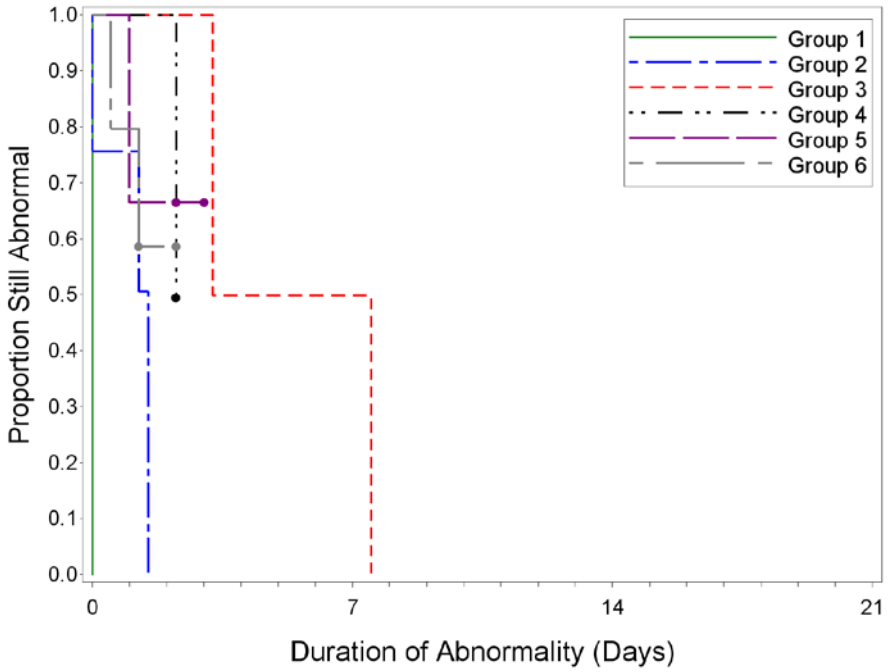

Figure 27. Kaplan-Meier curves for duration of abnormality based on RP respiratory rate.

APPENDIX G
BLOOD DRAW TIMES

1020-CG920503

Blood Draw Times

Animal ID	Group #	Day -3 Date	Day -3 Time	Day 1 Date	Day 1 Time	Day 2 Date	Day 2 Time
L23220	1	9/15/2009	1006	9/19/2009	0822	9/20/2009	0842
L23216	1	9/15/2009	0930	9/19/2009	0816	9/20/2009	0823
L23218	1	9/15/2009	0940	9/19/2009	0818	9/20/2009	0828
L23223	1	9/15/2009	1031	9/19/2009	0830	9/20/2009	0846
L23222	1	9/15/2009	1017	9/19/2009	0845	9/20/2009	0902
L23215	2	9/15/2009	0948	9/19/2009	0834	9/20/2009	0835
L23206	2	9/15/2009	1028	9/19/2009	0840	9/20/2009	0855
L23210	2	9/15/2009	1040	9/19/2009	0900	9/20/2009	0910
L23219	2	9/15/2009	1051	9/19/2009	0849	9/20/2009	0835
L23211	2	9/15/2009	1047	9/19/2009	0855	9/20/2009	0840
L23217	3	9/15/2009	1056	9/19/2009	0857	9/20/2009	0914
L23230	3	9/15/2009	1057	9/19/2009	0902	9/20/2009	0915
L23228	3	9/15/2009	1100	9/19/2009	0901	9/20/2009	0917
L23227	3	9/15/2009	1230	9/19/2009	0910	9/20/2009	0920
L23229	3	9/15/2009	1107	9/19/2009	0908	9/20/2009	0920
L23235	4	9/15/2009	1112	9/19/2009	1040	9/20/2009	1019
L23205	4	9/15/2009	1217	9/19/2009	1045	9/20/2009	1020
L23225	4	9/15/2009	1130	9/19/2009	1042	9/20/2009	1024
L23231	4	9/15/2009	1145	9/19/2009	1047	9/20/2009	1024
L23207	4	9/15/2009	1149	9/19/2009	1050	9/20/2009	1027
L23201	5	9/15/2009	1148	9/19/2009	1051	9/20/2009	1042
L23234	5	9/15/2009	1154	9/19/2009	1054	9/20/2009	1042
L23212	5	9/15/2009	1156	9/19/2009	1056	9/20/2009	1047
L23200	5	9/15/2009	1206	9/19/2009	1100	9/20/2009	1056
L23214	5	9/15/2009	1207	9/19/2009	1104	9/20/2009	1050
L23204	6	9/15/2009	1208	9/19/2009	1132	9/20/2009	1128
L23203	6	9/15/2009	1211	9/19/2009	1133	9/20/2009	1128
L23213	6	9/15/2009	β	9/19/2009	1138	9/20/2009	****α
L23221	6	9/15/2009	1224	9/19/2009	1209		
L23232	6	9/15/2009	β	9/19/2009	1200	9/20/2009	1137

Not Applicable

**** No time recorded for sample, see DR # 8476 JSM 1/26/10

^ = See DR# 8476 JEC 1/27/10

α = Unable to obtain EDTA sample

β = Unable to obtain EDTA and SST samples

G-2

Prepared By: JSM 1/7/10

QC/Tech Reviewed By: JC 1-7-10

QA Audit Completed By/Date: MM 1/27/10

1020-CG920503

Blood Draw Times

Animal ID	Group #	Day 3 Date	Day 3 Time	Day 7 Date	Day 7 Time	Day 14 Date	Day 14 Time
L23220	1	9/21/2009	0912	9/25/2009	0903	10/2/2009	0945
L23216	1	9/21/2009	0855	9/25/2009	0908	10/2/2009	0934
L23218	1	9/21/2009	0905	9/25/2009	0912	10/2/2009	0951
L23223	1	9/21/2009	0928	9/25/2009	0917	10/2/2009	1000
L23222	1	9/21/2009	0936	9/25/2009	0940	10/2/2009	0955
L23215	2	9/21/2009	0913	9/25/2009	0926	10/2/2009	1005
L23206	2	9/21/2009	1011	9/25/2009	0934	10/2/2009	1004
L23210	2	9/21/2009	0953	9/25/2009	1005	10/2/2009	1010
L23219	2	9/21/2009	0922	9/25/2009	0948	10/2/2009	1019
L23211	2	9/21/2009	0927	9/25/2009	1000	10/2/2009	1017
L23217	3	9/21/2009	0934	9/25/2009	1019	10/2/2009	1019
L23230	3	9/21/2009	0940	9/25/2009	1023	10/2/2009	1032
L23228	3	9/21/2009	0945	9/25/2009	1020	10/2/2009	1031
L23227	3	9/21/2009	0949	9/25/2009	1024	10/2/2009	1026
L23229	3	9/21/2009	0955	9/25/2009	1024	10/2/2009	1039
L23235	4	9/21/2009	1000	9/25/2009	1036		
L23205	4	9/21/2009	1143	9/25/2009	1033	10/2/2009	1105
L23225	4	9/21/2009	1007				
L23231	4	9/21/2009	1018	9/25/2009	1040	10/2/2009	1041
L23207	4	9/21/2009	1019	9/25/2009	1041	10/2/2009	1043
L23201	5	9/21/2009	1047				
L23234	5	9/21/2009	1048				
L23212	5	9/21/2009	1128	9/25/2009	1050	10/2/2009	1054
L23200	5	9/21/2009					
L23214	5	9/21/2009	1110α				
L23204	6	9/21/2009	1132				
L23203	6	9/21/2009	1137				
L23213	6	9/21/2009	β				
L23221	6						
L23232	6	9/21/2009	1154				

Not Applicable

**** No time recorded for sample, see DR # 8476 SSR 1/11/10

^ = See DR# 8476 SEC 1/27/10

α = Unable to obtain EDTA sample

β = Unable to obtain EDTA and SST samples

G-3

Prepared By: SSR 1/7/10

QC/Tech Reviewed By: DC 1-7-10

QA Audit Completed By/Date: [illegible] 1/7/10

1020-CG920503

Blood Draw Times

Animal ID	Group #	Day 21 Date	Day 21 Time	Terminal Date	Terminal Time
L23220	1	10/9/2009	0920	NA	NA
L23216	1	10/9/2009	0918	NA	NA
L23218	1	10/9/2009	0926	NA	NA
L23223	1	10/9/2009	0937	NA	NA
L23222	1	10/9/2009	0945	NA	NA
L23215	2	10/9/2009	0952	NA	NA
L23206	2	10/9/2009	1002	NA	NA
L23210	2	10/9/2009	1003	NA	NA
L23219	2	10/9/2009	1011	NA	NA
L23211	2	10/9/2009	1016	NA	NA
L23217	3	10/9/2009	1022	NA	NA
L23230	3	10/9/2009	1042	NA	NA
L23228	3	10/9/2009	1040	NA	NA
L23227	3	10/9/2009	1058	NA	NA
L23229	3	10/9/2009	1112	NA	NA
L23235	4			9/29/2009	0822
L23205	4	10/9/2009	1132	NA	NA
L23225	4			9/22/2009	1443
L23231	4	10/9/2009	1125	NA	NA
L23207	4	10/9/2009	1119	NA	NA
L23201	5			9/22/2009	0838^
L23234	5			9/24/2009	0901
L23212	5	10/9/2009	1136α	NA	NA
L23200	5			9/21/2009	1116
L23214	5			9/24/2009	0907
L23204	6			9/22/2009	1506
L23203	6			9/23/2009	****
L23213	6			9/21/2009	1252
L23221	6			9/20/2009	****
L23232	6			9/22/2009	0900^

Not Applicable

**** No time recorded for sample, see DR # 8476 JRC 1.26.10

^ = See DR# 8476 JRC 1.27.10

α = Unable to obtain EDTA sample

β = Unable to obtain EDTA and SST samples

G-4

Prepared By: JRC 1/7/10

QC/Tech Reviewed By: JC 1-7-10

QA Audit Completed By/Date: [illegible] 1/27/10

APPENDIX H
FINAL REPORT ON STATISTICAL ANALYSIS OF PA-ELISA, QPCR, AND QUANTITATIVE BACTEREMIA DATA

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List of Acronyms

BBRC ..... Battelle Biomedical Research Center
BD ..... Below detection limit
CFU ..... Colony forming unit
DNA ..... Deoxyribonucleic acid
LD_{50} ..... Median lethal dose
LOD ..... limit of detection
LOQ ..... limit of quantification
N ..... Number of animals
PA-ELISA ..... Protective antigen enzyme-linked immunosorbent assay
qPCR ..... Quantitative real-time polymerase chain reaction

1. Introduction

This report summarizes the statistical analysis of survival data collected under Battelle Biomedical Research Center (BBRC) Study No. 1020-CG920503. Thirty (30) male pathogen-free New Zealand White rabbits *(Oryctolagus cuniculus)* were randomized into six Groups, with each Group having five animals. Animals were aerosol challenged on Study Day zero with *Bacillus anthracis* (Ames strain) spores as indicated in Table 1. Blood samples were analyzed on the Study Days listed in Table 1 for circulating protective antigen via the quantitative protective antigen enzyme-linked immunosorbent assay (PA-ELISA), quantitative real-time polymerase chain reaction (qPCR), and bacteremia via the quantitative spread plate techniques.

Table 1. Study Design

Group	Number of Animals per Group	Spore Dose (CFU)	Blood Collection Study Days
1[a]	5	100 x LD_{50}	-3, 1, 2, 3, 7, 14, 21, and Terminal
2	5	100	
3	5	1,000	
4	5	10,000	
5	5	100,000	
6[b]	5	100 x LD_{50}	

CFU Colony forming units.
a Spores are gamma-irradiated (negative control).
LD_{50} Median lethal dose.
b High dose control.

2. Statistical Methods

All three parameters (PA-ELISA, qPCR, and quantitative bacteremia) were log-transformed for the statistical analyses. The limit of detection (LOD) for the PA-ELISA assay was 2 ng/mL. All PA-ELISA measurements less than the LOD or recorded as "BD" (below detection limit) were replaced with one half of the LOD. The LOD for the qPCR assay was two copies/μL of deoxyribonucleic acid (DNA). All qPCR measurements equal to zero copies/μL were replaced with 0.5 copies/μL and all measurements recorded as "<2" were replaced with one half of the LOD. The LOD and limit of quantification (LOQ) for the quantitative bacteremia assay were 100 CFU/mL and 2,500 CFU/mL, respectively. All quantitative bacteremia measurements equal to zero CFU/mL were replaced with one half of the LOD. Furthermore, if an observation was positive for *Bacillus anthracis* but was less than the LOQ, then it was replaced with one half of the LOQ.

For each parameter (PA-ELISA, qPCR, and quantitative bacteremia), summary statistics including geometric means with 95% confidence intervals were computed for each Group and Study Day. Additionally, the proportion of PA-ELISA observations greater than the LOD, the proportion of qPCR observations greater than zero, and the proportion of quantitative bacteremia observations that were positive for *Bacillus anthracis* were computed for each Group and Study Day. Furthermore, a t-test was performed to determine if the geometric mean was significantly greater than the LOD for each parameter, Group, and Study Day. All analyses were performed in SAS® (SAS Institute Inc.; Cary, NC; version 9.1) or Stata (StataCorp LP; College Station, TX; version 11.1).

3. Results

Tables 2, 3, and 4 contain summary statistics and test results for PA-ELISA, qPCR, and quantitative bacteremia, respectively. For each parameter, Group, and Study Day, the summary statistics include the sample size, the geometric mean with corresponding 95% confidence interval, and the p-value associated with a t-test used to determine if the geometric mean was significantly greater than the LOD. The results on some Study Days were based on smaller sample sizes due to missing data or due to animal deaths prior to the end of the study. Additionally for each Group and Study Day, Table 2 contains the proportion of PA-ELISA observations greater than the LOD, Table 3 contains the proportion of qPCR observations greater than zero, and Table 4 contains the proportion of observations that were positive for quantitative bacteremia. All animals in the high dose control Group (Group 6) died prior to Study Day 7.

For PA-ELISA, all animals in Groups 1 through 3 had observations less than the LOD on all Study Days. One animal in Group 4 had an observation above the LOD on Study Day 3 and on the terminal day. Some animals in Group 5 had observations greater than the LOD on Study Days 2 and 3, while some animals in Group 6 had observations greater than the LOD on Study Days 2 and 3 and on the terminal day. The geometric mean PA-ELISA measurements were not significantly greater than the LOD for any Group on any Study Day.

For qPCR, one animal in each of Groups 1 and 2 had an observation greater than zero on Study Days 1 and 3, respectively. All animals in Group 3 had observations equal to zero on all Study Days. One animal in Group 4 had observations greater than zero on Study Days -3 through 3. Some animals in Groups 5 and 6 had observations greater than zero on Study Days 1, 2, and 3 and on the terminal day. The geometric mean qPCR measurements in Groups 5 and 6 were significantly greater than the LOD at the terminal sample and Study Day 3, respectively.

For quantitative bacteremia, all animals in Groups 1 through 3 had observations negative for *Bacillus anthracis* on all Study Days. At least one animal in Group 4 had observations that were positive for *Bacillus anthracis* on Study Days 2 and 3 and on the terminal day. Some animals in Groups 5 and 6 had observations that were positive for *Bacillus anthracis* on Study Days 1, 2, and 3 and on the terminal day. The geometric mean quantitative bacteremia measurement in Group 4 was significantly greater than the LOD at the terminal sample, and the geometric mean

quantitative bacteremia measurement in Group 6 was significantly greater than the LOD on Study Day 3 and at the terminal sample.

4. Conclusions

All animals in the high dose control Group (Group 6) died prior to Study Day 7. All animals in the three lowest dose Groups (Groups 1 through 3) had PA-ELISA observations less than the LOD, and quantitative bacteremia observations negative for *Bacillus anthracis* on all Study Days. The geometric mean PA-ELISA measurements were not significantly greater than the LOD for any Group on any Study Day. The geometric mean qPCR measurements were significantly greater than the LOD in the 100,000 colony forming unit (CFU) dose Group (Group 5) at the terminal sample and in the high dose control Group (Group 6) on Study Day 3. The geometric mean quantitative bacteremia measurement in the 10,000 CFU dose Group (Group 4) was significantly greater than the LOD at the terminal sample, and the geometric mean quantitative bacteremia measurement in the high dose control Group (Group 6) was significantly greater than the LOD on Study Day 3 and at the terminal sample.

Table 2. Descriptive Statistics and Test Results for PA-ELISA (ng/mL) by Group and Study Day

Group	Study Day	N	Proportion Greater Than LOD	Geometric Mean (95% Confidence Interval)	P-Value[a]
1	-3	5	0.00	<LOD (<LOD, <LOD)	NA
	1	5	0.00	<LOD (<LOD, <LOD)	NA
	2	5	0.00	<LOD (<LOD, <LOD)	NA
	3	5	0.00	<LOD (<LOD, <LOD)	NA
	7	5	0.00	<LOD (<LOD, <LOD)	NA
	14	5	0.00	<LOD (<LOD, <LOD)	NA
	21	5	0.00	<LOD (<LOD, <LOD)	NA
	Terminal	0	NA	NA	NA
2	-3	5	0.00	<LOD (<LOD, <LOD)	NA
	1	5	0.00	<LOD (<LOD, <LOD)	NA
	2	5	0.00	<LOD (<LOD, <LOD)	NA
	3	5	0.00	<LOD (<LOD, <LOD)	NA
	7	5	0.00	<LOD (<LOD, <LOD)	NA
	14	5	0.00	<LOD (<LOD, <LOD)	NA
	21	5	0.00	<LOD (<LOD, <LOD)	NA
	Terminal	0	NA	NA	NA
3	-3	5	0.00	<LOD (<LOD, <LOD)	NA
	1	5	0.00	<LOD (<LOD, <LOD)	NA
	2	5	0.00	<LOD (<LOD, <LOD)	NA
	3	5	0.00	<LOD (<LOD, <LOD)	NA
	7	5	0.00	<LOD (<LOD, <LOD)	NA
	14	5	0.00	<LOD (<LOD, <LOD)	NA
	21	5	0.00	<LOD (<LOD, <LOD)	NA
	Terminal	0	NA	NA	NA

Table 2. (Continued)

Group	Study Day	N	Proportion Greater Than LOD	Geometric Mean (95% Confidence Interval)	P-Value[a]
4	-3	5	0.00	<LOD (<LOD, <LOD)	NA
	1	5	0.00	<LOD (<LOD, <LOD)	NA
	2	5	0.00	<LOD (<LOD, <LOD)	NA
	3	5	0.20	2.20 (<LOD, 19.54)	0.4554
	7	4	0.00	<LOD (<LOD, <LOD)	NA
	14	3	0.00	<LOD (<LOD, <LOD)	NA
	21	3	0.00	<LOD (<LOD, <LOD)	NA
	Terminal	1	1.00	32964 (--)	NA
5	-3	5	0.00	<LOD (<LOD, <LOD)	NA
	1	5	0.00	<LOD (<LOD, <LOD)	NA
	2	5	0.40	4.50 (<LOD, 59.68)	0.2161
	3	3	0.67	21.15 (<LOD, 44235.52)	0.1578
	7	1	0.00	<LOD (--)	NA
	14	1	0.00	<LOD (--)	NA
	21	1	0.00	<LOD (--)	NA
	Terminal	1	0.00	<LOD (--)	NA
6	-3	3	0.00	<LOD (<LOD, <LOD)	NA
	1	5	0.00	<LOD (<LOD, <LOD)	NA
	2	4	0.50	4.34 (<LOD, 167.01)	0.2740
	3	3	0.67	2.73 (<LOD, 48.04)	0.3436
	7	0	NA	NA	NA
	14	0	NA	NA	NA
	21	0	NA	NA	NA
	Terminal	2	1.00	3117.72 (<LOD, 1.14×10^{15})	0.0884

N Number of animals.
a P-value testing that the geometric mean was significantly greater than the LOD. The test was not performed when all observations were the same.
LOD Limit of detection (2 ng/mL).
NA No measurements available or all measurements were less than the LOD.
-- Confidence interval could not be calculated since only one measurement was available for this Study Day.

Table 3. Descriptive Statistics and Test Results for Quantitative PCR (qPCR, copies/µL of DNA) by Group and Study Day

Group	Study Day	N	Proportion Greater Than Zero	Geometric Mean (95% Confidence Interval)	P-Value[a]
1	-3	5	0.00	<LOD (<LOD, <LOD)	NA
	1	5	0.20	<LOD (<LOD, <LOD)	0.9996
	2	5	0.00	<LOD (<LOD, <LOD)	NA
	3	5	0.00	<LOD (<LOD, <LOD)	NA
	7	5	0.00	<LOD (<LOD, <LOD)	NA
	14	5	0.00	<LOD (<LOD, <LOD)	NA
	21	5	0.00	<LOD (<LOD, <LOD)	NA
	Terminal	0	NA	NA	NA
2	-3	5	0.00	<LOD (<LOD, <LOD)	NA
	1	5	0.00	<LOD (<LOD, <LOD)	NA
	2	5	0.00	<LOD (<LOD, <LOD)	NA
	3	5	0.20	<LOD (<LOD, <LOD)	0.9996
	7	5	0.00	<LOD (<LOD, <LOD)	NA
	14	5	0.00	<LOD (<LOD, <LOD)	NA
	21	5	0.00	<LOD (<LOD, <LOD)	NA
	Terminal	0	NA	NA	NA
3	-3	5	0.00	<LOD (<LOD, <LOD)	NA
	1	5	0.00	<LOD (<LOD, <LOD)	NA
	2	5	0.00	<LOD (<LOD, <LOD)	NA
	3	5	0.00	<LOD (<LOD, <LOD)	NA
	7	5	0.00	<LOD (<LOD, <LOD)	NA
	14	5	0.00	<LOD (<LOD, <LOD)	NA
	21	5	0.00	<LOD (<LOD, <LOD)	NA
	Terminal	0	NA	NA	NA
4	-3	5	0.20	<LOD (<LOD, 51.97)	0.5476
	1	5	0.20	<LOD (<LOD, 8.41)	0.7795
	2	5	0.20	<LOD (<LOD, 7.49)	0.7985
	3	5	0.20	<LOD (<LOD, 29.19)	0.6059
	7	4	0.00	<LOD (<LOD, <LOD)	NA
	14	3	0.00	<LOD (<LOD, <LOD)	NA
	21	3	0.00	<LOD (<LOD, <LOD)	NA
	Terminal	1	1.00	7394 (--)	NA
5	-3	5	0.00	<LOD (<LOD, <LOD)	NA
	1	5	0.20	<LOD (<LOD, 4.44)	0.8827
	2	5	0.60	7.22 (<LOD, 149.54)	0.1524
	3	3	0.67	12.61 (<LOD, 219027.95)	0.2511
	7	1	0.00	<LOD (--)	NA
	14	1	0.00	<LOD (--)	NA
	21	1	0.00	<LOD (--)	NA
	Terminal	4	1.00	2449.93 (<LOD, 5276302.37)	0.0300*

Table 3. (Continued)

Group	Study Day	N	Proportion Greater Than Zero	Geometric Mean (95% Confidence Interval)	P-Value[a]
5	-3	5	0.00	<LOD (<LOD, <LOD)	NA
	1	5	0.20	<LOD (<LOD, 4.44)	0.8827
	2	5	0.60	7.22 (<LOD, 149.54)	0.1524
	3	3	0.67	12.61 (<LOD, 219027.95)	0.2511
	7	1	0.00	<LOD (--)	NA
	14	1	0.00	<LOD (--)	NA
	21	1	0.00	<LOD (--)	NA
	Terminal	4	1.00	2449.93 (<LOD, 5276302.37)	0.0300*
6	-3	3	0.00	<LOD (<LOD, <LOD)	NA
	1	4	0.25	<LOD (<LOD, <LOD)	0.9970
	2	3	1.00	2.47 (<LOD, 119.9)	0.4190
	3	3	1.00	20.29 (5.05, 81.53)	0.0095*
	7	0	NA	NA	NA
	14	0	NA	NA	NA
	21	0	NA	NA	NA
	Terminal	3	0.67	272.43 (<LOD, 1743367169.9)	0.1549

N Number of animals.
LOD Limit of detection (2 copies/mL of DNA).
a P-value testing that the geometric mean was significantly greater than the LOD. The test was not performed when all observations were the same.
NA No measurements available or all measurements were less than the LOD.
-- Confidence interval could not be calculated since only one measurement was available for this Study Day.
* Group geometric mean was significantly greater than the LOD at the 0.05 level of significance.

Table 4. Descriptive Statistics and Test Results for Quantitative Bacteremia (CFU/mL) by Group and Study Day

Group	Study Day	N	Proportion Positive for *Bacillus anthracis*	Geometric Mean (95% Confidence Interval)	P-Value[a]
1	-3	5	0.00	<LOD (<LOD, <LOD)	NA
	1	5	0.00	<LOD (<LOD, <LOD)	NA
	2	5	0.00	<LOD (<LOD, <LOD)	NA
	3	5	0.00	<LOD (<LOD, <LOD)	NA
	7	5	0.00	<LOD (<LOD, <LOD)	NA
	14	5	0.00	<LOD (<LOD, <LOD)	NA
	21	5	0.00	<LOD (<LOD, <LOD)	NA
	Terminal	0	NA	NA	NA
2	-3	5	0.00	<LOD (<LOD, <LOD)	NA
	1	5	0.00	<LOD (<LOD, <LOD)	NA
	2	5	0.00	<LOD (<LOD, <LOD)	NA
	3	5	0.00	<LOD (<LOD, <LOD)	NA
	7	5	0.00	<LOD (<LOD, <LOD)	NA
	14	5	0.00	<LOD (<LOD, <LOD)	NA
	21	5	0.00	<LOD (<LOD, <LOD)	NA
	Terminal	0	NA	NA	NA
3	-3	5	0.00	<LOD (<LOD, <LOD)	NA
	1	5	0.00	<LOD (<LOD, <LOD)	NA
	2	5	0.00	<LOD (<LOD, <LOD)	NA
	3	5	0.00	<LOD (<LOD, <LOD)	NA
	7	5	0.00	<LOD (<LOD, <LOD)	NA
	14	5	0.00	<LOD (<LOD, <LOD)	NA
	21	5	0.00	<LOD (<LOD, <LOD)	NA
	Terminal	0	NA	NA	NA
4	-3	5	0.00	<LOD (<LOD, <LOD)	NA
	1	5	0.00	<LOD (<LOD, <LOD)	NA
	2	5	0.20	123.14 (<LOD, 1503.72)	0.4143
	3	5	0.20	<LOD (<LOD, 568.62)	0.5287
	7	4	0.00	<LOD (<LOD, <LOD)	NA
	14	3	0.00	<LOD (<LOD, <LOD)	NA
	21	3	0.00	<LOD (<LOD, <LOD)	NA
	Terminal	2	1.00	1168374.94 (9957.83, 137088087.09)	0.0127*

Table 4. (Continued)

Group	Study Day	N	Proportion Positive for *Bacillus anthracis*	Geometric Mean (95% Confidence Interval)	P-Value[a]
5	-3	5	0.00	<LOD (<LOD, <LOD)	NA
	1	5	0.20	<LOD (<LOD, 568.62)	0.5287
	2	5	0.60	502.84 (<LOD, 8148.55)	0.0913
	3	4	0.50	1320.51 (<LOD, 2178552.62)	0.1743
	7	1	0.00	<LOD (--)	NA
	14	1	0.00	<LOD (--)	NA
	21	1	0.00	<LOD (--)	NA
	Terminal	4	0.75	24014.66 (<LOD, 146420048.48)	0.0696
6	-3	3	0.00	<LOD (<LOD, <LOD)	NA
	1	4	0.50	250.00 (<LOD, 4810.81)	0.1984
	2	4	0.75	559.02 (<LOD, 7238.39)	0.0610
	3	3	1.00	2132.30 (675.83, 6727.60)	0.0038*
	7	0	NA	NA	NA
	14	0	NA	NA	NA
	21	0	NA	NA	NA
	Terminal	5	0.80	142560.86 (282.95, 71826977.59)	0.0158*

N Number of animals.
a P-value testing that the geometric mean was significantly greater than the LOD. The test was not performed when all observations were the same.
LOD Limit of detection (100 CFU/mL).
NA No measurements available or all measurements were less than the LOD.
* Group geometric mean was significantly greater than the LOD at the 0.05 level of significance.
-- Confidence interval could not be calculated since only one measurement was available for this Study Day.

APPENDIX I
STATISTICAL ANALYSIS OF HEMATOLOGY DATA

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List of Acronyms

ANOVA ..... Analysis of Variance
BBRC ..... Battelle Biomedical Research Center
CFU ..... Colony forming unit
dL ..... deciliter
fL ..... femotoliter
g ..... gram
HCT ..... Hematocrit
HGB ..... Hemoglobin
LD_{50} ..... Median lethal dose
MCH ..... Mean corpuscular hemoglobin
MCHC ..... Mean corpuscular hemoglobin concentration
MCV ..... Mean corpuscular volume
MPV ..... Mean platelet volume
N ..... Number of animals
N/L ratio ..... Neutrophils/lymphocytes ratio
PLT ..... Platelet count
pg ..... picogram
RBC ..... red blood cell count
RDW ..... red cell distribution width
WBC ..... white blood cell count
µL ..... microliter
µm ..... micrometer

1 Introduction

This report summarizes the statistical analysis of hematology data collected under Battelle Biomedical Research Center (BBRC) Study No. 1020-CG920503. Thirty (30) male pathogen-free New Zealand White rabbits *(Oryctolagus cuniculus)* were randomized into six Groups, with each Group having five animals. Animals were aerosol challenged on Study Day zero with *Bacillus anthracis* (Ames strain) spores as indicated in Table 1.

Table 1. Study Design

Group	Number of Animals per Group	Spore Dose (CFU)	Hematology Blood Collection Study Days
1[a]	5	100 x LD_{50}	-3, 1, 2, 3, 7, 14, 21, and Terminal
2	5	100	
3	5	1,000	
4	5	10,000	
5	5	100,000	
6[b]	5	100 x LD_{50}	

CFU Colony forming units.
a Spores are gamma-irradiated (negative control).
b High-dose control.
LD_{50} Median lethal dose.

Blood samples were collected for hematology analysis as indicated in Table 1. Blood collection on Study Day -3 served as a pre-challenge baseline for each animal. There were only two animals (from different Groups) with terminal samples; therefore, all terminal measurements were excluded from the statistical analysis.

The hematology parameters that were included in this analysis are:

- Red blood cell count (RBC, 10^6 cells/μL)
- Hemoglobin (HGB, g/dL)
- Hematocrit (HCT, %)
- Mean corpuscular volume (MCV, fL)
- Mean corpuscular hemoglobin (MCH, pg)
- Mean corpuscular hemoglobin concentration (MCHC, g/dL)
- Red cell distribution width (RDW, %)

- Platelet count (PLT, 10^3 cells/μL)
- Mean platelet volume (MPV, fL)
- White blood cell count (WBC, 10^3 cells/μL)
- Neutrophils (10^3 cells/μL)
- Lymphocytes (10^3 cells/μL)
- Monocytes (10^3 cells/μL)
- Eosinophils (10^3 cells/μL)
- Basophils (10^3 cells/μL)
- Neutrophils/lymphocytes ratio (N/L ratio)

Hematology analysis was performed at six levels:

1. Descriptive statistics (arithmetic or geometric means with 95% confidence intervals) were calculated for each parameter, by Group and Study Day.

2. A baseline analysis, using the measurements from Study Day -3, was performed for each parameter in order to determine if there were significant differences between the Groups prior to the administration of challenge.

3. Estimates for the mean shift from baseline (the measurement on Study Day -3) were obtained for each parameter, Group, and Study Day. These shifts were evaluated to determine if they were significantly different from "no shift."

4. The mean shifts from baseline for each parameter and Study Day were compared between the Groups. Those Groups having mean shifts that were significantly different from each other were reported.

5. For each parameter, the proportion abnormal in each Group was obtained and evaluated to identify significant differences between the Groups.

6. For each parameter, the time to an abnormal measurement for each animal was obtained and evaluated to identify significant differences between the Groups.

2 Statistical Methods

Analysis of variance (ANOVA) models fitted separately to each hematology parameter with effects for Group, Study Day, and the interaction between Group and Study Day were used to assess the model assumption of normality and to identify potential outliers. Standardized residuals from these ANOVA models were obtained and a hypothesis test was performed for each parameter in order to assess the model assumption of normality for the untransformed data. Each parameter was then transformed by taking the base-10 logarithm of the parameter values. However, prior to taking the base-10 logarithm, parameter values recorded as zero were replaced by one half of the smallest observed non-zero value associated with the respective parameter. The ANOVA models were then refitted using the base-10 log-transformed values, and a hypothesis test was again performed for each parameter in order to assess the model assumption of normality for the log-transformed data. If the assumption of normality was more reasonable for the log-transformed data than it was for the untransformed data, then the log-transformed values were used throughout the analysis for this parameter. The deleted studentized residuals, which are the standardized residuals from the model fitted to the data having the current observation removed, were computed for each observation. If the absolute value of the deleted studentized residual was greater than 4, then the observation was considered a potential outlier. If any potential outliers were identified, then the following analyses were performed, both with and without these observations, in order to evaluate their effect on the results.

For each hematology parameter, the following ANOVA model was fitted to the data at Study Day -3 in order to determine if there were significant differences between the Groups at baseline:

$$Y_{ij} = \mu + Group_i + \varepsilon_{ij} \quad (1)$$

where Y_{ij} is the observed hematology result for the jth animal in Group i (i=1 to 6) at the baseline, μ is an overall constant, and ε_{ij} is the random error left unexplained by the model. Tukey's multiple comparisons procedure was also performed for each parameter in order to determine which pairs of Groups had baseline means that were significantly different from each other; however, the results will only be presented if significant differences are identified. If the

parameter was log-transformed for analysis, then the same model was used with Y_{ij} replaced by $Log(Y_{ij})$, the base-10 log-transformed parameter value for the *j*th animal in Group *i* (*i*=1 to 6). In order to determine if the mean shifts from baseline were significantly different between the Groups, the following ANOVA model was fitted separately for each hematology parameter on each post-challenge Study Day:

$$Y_{dij} - Y_{bij} = \mu + Group_i + \varepsilon_{ij} \quad (2)$$

where Y_{dij} is the observed hematology result for the *j*th animal in Group *i* (*i*=1 to 6) on Study Day *d* (*d*=1, 2, 3, 7, 14, and 21), Y_{bij} is the observed hematology result for the *j*th animal in Group *i* (*i*=1 to 6) at baseline, μ is an overall constant, and ε_{ij} is the random error left unexplained by the model. If a parameter was log-transformed for the analysis, then the same model was used with Y_{dij} and Y_{bij} replaced by their base-10 log-transformed counterparts $Log(Y_{dij})$ and $Log(Y_{bij})$, respectively.

Least square mean estimates from the above shift from baseline ANOVA models were calculated and approximate t-tests were performed to determine if, for each Group, there was a significant shift in hematology results between baseline and each post-challenge Study Day. For untransformed data, this tests whether the difference in means is significantly different from zero. For log-transformed data, this tests whether the ratio of geometric means is significantly different from one. Additionally, Tukey's multiple comparisons procedure was performed to determine which pairs of Groups had mean shifts from baseline that were significantly different from each other. Under the Tukey procedure, the set of all comparisons within each parameter and Study Day combination are made at a joint 95% confidence level.

For each parameter, the threshold for an abnormal parameter value was defined as each individual animal's baseline (Study Day -3) parameter value plus or minus two standard deviations. Since each animal had only one baseline value for each parameter, the standard deviation associated with each parameter was calculated using the baseline values of all animals. Animals were determined to have an abnormal parameter value when their observed value was above the upper threshold or below the lower threshold for that respective parameter.

For each parameter, an overall two-sided Fisher's exact test was performed to determine if there was a significant difference between the abnormal rates in each Group. If the overall Fisher's exact test was significant, then one-sided pairwise Fisher's exact tests were performed to evaluate all pairwise Group comparisons. The Bonferroni-Holm adjustment was used to maintain an overall 0.05 significance level among the multiple pairwise comparisons made within each parameter.

For each parameter, an overall log-rank test was performed to determine if there was a significant difference between the times to abnormality in each Group. If the overall log-rank test was significant, then pairwise log-rank tests were performed to evaluate all pairwise Group comparisons. The Bonferroni-Holm adjustment was used to maintain an overall 0.05 significance level among the multiple pairwise comparisons made within each parameter. For those parameters that had significant overall log-rank test, Kaplan-Meier curves were plotted by Group.

All statistical analyses were conducted using SAS® (SAS Institute Inc., Cary, NC, version 9.1) and all results are reported at the 0.05 level of significance. All ANOVA models were fitted using the MIXED or GLM procedure. All Fisher's exact tests were performed using the FREQ procedure, and all log-rank tests were performed using the LIFETEST procedure. The MULTTEST procedure was used to maintain an overall 0.05 significance level among the multiple pairwise comparisons made within each parameter.

3 Results

The model assumption of normality was more reasonable for 6 of the 16 parameters when models were fitted to the base-10 log-transformed data. These parameters were: RDW, MPV, N/L ratio, monocytes, eosinophils, and basophils. Therefore, models were fitted to base-10 log-transformed values in the final analysis for these parameters. Table 2 contains a list of nine hematology values that were identified as potential outliers using the procedure described above. The statistical analyses of the hematology data were performed both with and without the potential outliers in order to evaluate their effect on the results. Attachment A contains the parameters that experienced changes in significance after the potential outliers were excluded.

Table 3 contains the results of the ANOVA models fitted at baseline (Study Day -3). The Group effect was not significant at the baseline for any of the hematology parameters. This is important to the interpretation of subsequent analysis results of differing shifts from baseline in the Groups. The lack of differences between Groups at baseline implies that differing shifts from baseline for different Groups are entirely associated with the effects of treatment after challenge and not with inherent differences between the Groups prior to challenge.

Descriptive statistics, Group comparisons, and abnormal counts for each parameter are presented in pairs of tables, where Tables 4 through 19 are associated with the parameter of interest. For each parameter, Table “a” contains the descriptive statistics, Table “b” contains the test results for comparing the mean shifts from baseline within each Group at each Study Day.

Tables 4a through 19a contain descriptive statistics (means with 95% confidence intervals for untransformed data, or geometric means with 95% confidence intervals for base-10 log-transformed data) for the hematology parameter results within each Group at each Study Day. The results on some Study Days were based on smaller sample sizes due to missing data or due to animal deaths prior to the end of the study. Figures 1 through 16 display the means and confidence intervals over the course of the study for each hematology parameter.

Tables 4b through 19b contain test results for the mean shift from baseline within each Group at each Study Day. These tables contain test results that indicate if the mean difference between the Study Day and baseline was significantly different from zero for untransformed parameters, or if

the ratio of geometric means was significantly different from one for base-10 log-transformed parameters (at the 0.05 level). In each cell, the estimate of the shift from baseline (difference or ratio) is shown for that parameter, Group, and Study Day. Following the shift estimate, an up arrow (↑) indicates a significant increase from baseline, while a down arrow (↓) indicates a significant decrease from baseline. These tables also contain Group effect p-values for each Study Day, as well as test results from the Tukey's pairwise comparisons procedure that was used to identify pairs of Groups with significantly different shifts from baseline. Under the Tukey procedure, the set of comparisons within each parameter and Study Day is made at a joint 95% confidence level. Each significant difference is shown as the estimated comparison between the shifts from baseline for the pair of Groups under consideration, the direction of the comparison (i.e., which Group experienced a larger shift from baseline), and the corresponding Tukey-adjusted p-value. P-values less than 0.05 provide evidence of a significant difference.

The results from Tables 4b through 19b are discussed below in Groups of related parameters.

3.1 Red blood cell parameters

- **RBC (Tables 4a-b, Figure 1)**: RBC levels were significantly decreased from baseline for Group 1 on Study Day 2. Group 2 had significant decreases from baseline on Study Days 2, 3, and 21. Group 4 had significant decreases from baseline on Study Days 2 and 3. Group 5 had significant decreases from baseline on Study Days 1 through 3. Group 6 had significant decreases from baseline on Study Days 2 and 3. There were no significant differences in the shifts from baseline for any pair of Groups on any Study Day.

- **HGB (Tables 5a-b, Figure 2)**: HGB levels were significantly decreased from baseline for Group 1 on Study Days 2, 3, and 21. Group 2 had significant decreases from baseline on Study Days 1 through 3 and 21. Group 3 had significant decreases from baseline on Study Days 2, 3, and 21. Group 4 had a significant decrease from baseline on Study Days 1 through 3 and on Study Day 21. Groups 5 and 6 had significant decreases from baseline on Study Days 1 through 3. There were no significant differences in the shifts from baseline for any pair of Groups on any Study Day.

- **HCT (Tables 6a-b, Figure 3)**: HCT levels were significantly decreased from baseline for Groups 1 and 4 on Study Day 2. Group 2 had significant decreases from baseline on Study Days 2 and 21. Group 3 had a significant decrease from baseline on Study Day 21. Group 5 had significant decreases from baseline on Study Days 1 through 3. Group 6 had significant decreases from baseline on Study Days 2 and 3. There were no significant differences in the shifts from baseline for any pair of Groups on any Study Day.

- **MCV (Tables 7a-b, Figure 4)**: There were significant decreases from baseline for Group 1 on Study Day 21. Groups 2 and 3 had a significant increase from baseline on Study Day 7. Group 4 had significant decreases from baseline on Study Days 1, 2, and 21. Group 5 had significant decreases from baseline on Study Days 1 through 3. Group 6 had significant decreases from baseline on Study Days 2 and 3. There was a significant Group effect on Study Day 2; however, Tukey's multiple pairwise comparisons procedure did not identify significant differences between any pair of Groups.

- **MCH (Tables 8a-b, Figure 5)**: There were significant decreases from baseline for Groups 1, 3, and 4 on all Study Days. Group 2 had significant decreases from baseline on Study Days 1 through 3 and Study Day 14. Group 5 had significant decreases from baseline on Study Days 1 through 3 and on Study Day 14. Group 6 had significant decreases from baseline on Study Days 1 through 3. There were no significant differences in the shifts from baseline for any pair of Groups on any Study Day.

- **MCHC (Tables 9a-b, Figure 6)**: MCHC levels were significantly decreased from baseline for Groups 1 and 2 on Study Days 1 through 7. Groups 3 and 4 had significant decreases from baseline on Study Days 1 through 14. Group 5 and 6 had significant decreases from baseline on Study Day 1. There were no significant differences in the shifts from baseline for any pair of Groups on any Study Day.

- **RDW (Tables 10a-b, Figure 7)**: RDW levels were significantly decreased as a proportion of baseline for Group 1 on Study Day 21. Group 2 had significant increases as a proportion of baseline on Study Days 2 through 7 and a significant decrease as a proportion of baseline on Study Day 21. Group 3 had significant increases as a proportion of baseline on Study Days 3 and 7 and a significant decrease as a proportion of baseline on Study Day 21. Group 4 had significant increases as a proportion of baseline on Study Days 3 and 7. On Study Day 3, the mean increase as a proportion of baseline for Group 2 was significantly different than the mean decrease as a proportion of baseline for Group 6.

3.2 Platelet counts and volume

- **PLT (Tables 11a-b, Figure 8)**: PLT levels were significantly decreased for Group 1 on Study Days 3 and 14. Group 2 had a significant decrease from baseline on Study Day 14. Group 3 had a significant decrease from baseline on Study Day 21. Group 5 had significant decreases from baseline on Study Days 2, 3, and 21. Group 6 had a significant decrease from baseline on Study Day 2. There were no significant differences in the shifts from baseline for any pair of Groups on any Study Day.

- **MPV (Tables 12a-b, Figure 9)**: There were significant decreases as a proportion of baseline for Group 1 on Study Days 1, 2, 7, and 21. Groups 2, 3, and 4 had significant decreases as a proportion of baseline on Study Days 1, 2, and 21. Group 5 had significant decreases as a proportion of baseline on Study Days 1 and 2. Group 6 had significant decreases as a proportion of baseline on Study Days 1 and 2 and a significant increase as a proportion of baseline on Study Day 3. On Study Day 3, the mean increase as a proportion of baseline for Group 6 was significantly different than the mean changes as a proportion of baseline for Groups 1, 2, 3 and 4. On Study Day 7, the mean decrease as a proportion of baseline for Group 1 was significantly different than the mean increase as a proportion of baseline for Group 4.

3.3 Total and differential white blood cell parameters

- **WBC (Tables 13a-b, Figure 10)**: WBC counts were significantly decreased for Group 1 on all Study Days. Groups 2, 3, 4, and 5 had a significant decrease from

baseline on Study Day 21. Group 6 had a significant decrease from baseline on Study Day 3. There was a significant Group effect on Study Day 1; however, Tukey's multiple pairwise comparisons procedure did not identify significant differences between any pair of Groups. On Study Day 2, the mean decrease from baseline for Group 1 was significantly different from the mean increase for Group 4. On Study Day 3, the mean decrease from baseline for Group 6 was significantly different from the mean increase for Group 5.

- **Neutrophils (Tables 14a-b, Figure 11)**: Neutrophil counts were significantly decreased for Group 1 on Study Days 1 through 3 and Study Days 14 and 21. Groups 2 and 3 had a significant decrease from baseline on Study Day 21. Group 4 had a significant decrease from baseline on Study Day 21. Group 5 had a significant decrease from baseline on Study Day 1. Group 6 had a significant increase from baseline on Study Day 2. On Study Day 1 the mean decrease from baseline for Group 1 was significantly different from the mean increase for Groups 4 and 6. On Study Day 2,the mean increase from baseline for Group 6 was significantly different from the mean change from baseline for Groups 1, 2, 3, 4, and 5. On Study Day 3 the mean decrease from baseline for Group 1 was significantly different from the mean increase for Group 5.

- **Lymphocytes (Tables 15a-b, Figure 12)**: Lymphocyte counts were significantly decreased for Group 1 on Study Days 14 and 21. Group 2 had significant decreases from baseline on Study Days 7 and 21. Groups 3, 4, and 5 had a significant decrease from baseline on Study Day 21. Group 6 had significant decreases from baseline on Study Days 2 and 3. There was a significant Group effect on Study Day 2; however, Tukey's multiple pairwise comparisons procedure did not identify significant differences between any pair of Groups. On Study Day 3, the mean decrease from baseline for Group 6 was significantly different from the mean increase for Groups 2, 3, 4, and 5.

- **N/L Ratio (Tables 16a-b, Figure 13)**: There were significant decreases as a proportion of baseline for Group 1 on Study Days 1, 3, 14, and 21. Group 2 had

significant decreases as a proportion of baseline on Study Days 3 and 21. Group 4 had a significant decrease as a proportion of baseline on Study Days 3 and 21. Group 5 had a significant decrease as a proportion of baseline on Study Day 1. Group 6 had a significant increase as a proportion of baseline on Study Day 2. On Study Day 2, the mean increase as a proportion of baseline for Group 6 was significantly different than the mean decrease as a proportion of baseline for Groups 1, 2, 3, 4, and 5. On Study Day 3, the mean decrease as a proportion of baseline for Group 1 was significantly different than the mean increase as a proportion of baseline for Group 6.

- **Monocytes (Tables 17a-b, Figure 14)**: There were significant decreases as a proportion of baseline for Group 1 on Study Days 1, 2, and 21. Groups 2, 3, and 4 had a significant decrease as a proportion of baseline on Study Day 21. Group 5 had a significant decrease as a proportion of baseline on Study Day 3. Groups 6 had significant decreases as a proportion of baseline on Study Days 2 and 3. On Study Day 2, the mean decrease as a proportion of baseline for Group 6 was significantly different than the mean increases as a proportion of baseline for Groups 2 and 3.

- **Eosinophils (Tables 18a-b, Figure 15)**: There was a significant decrease as a proportion of baseline for Group 1 on Study Day 21. Group 3 had significant increases as a proportion of baseline on Study Days 1 and 7. Group 4 had a significant increase as a proportion of baseline on Study Day 7. Group 5 had significant increases as a proportion of baseline on Study Days 2 and 3. Group 6 had a significant decrease as a proportion of baseline on Study Day 2. On Study Day 2, the mean decrease as a proportion of baseline for Group 5 was significantly different than the mean increase as a proportion of baseline for Group 3. On Study Day 7, the mean increase as a proportion of baseline for Group 4 was significantly different than the mean decrease as a proportion of baseline for Group1.

- **Basophils (Tables 19a-b, Figure 16)**: There were significant decreases as a proportion of baseline for Group 1 on Study Days 7 through 21. Groups 2 and 3 had a significant decrease as a proportion of baseline on Study Day 21. Group 4 had a significant increase as a proportion of baseline on Study Day 7. Group 5 had a

significant decrease as a proportion of baseline on Study Day 2 and a significant increase as a proportion of baseline on Study Day 7. Group 6 had significant increases as a proportion of baseline on Study Days 2 and 3. On Study Days 2 and 3, the mean decrease as a proportion of baseline for Group 6 was significantly different than the mean increase as a proportion of baseline for Group 3. On Study Day 7, the mean decrease as a proportion of baseline for Group 1 was significantly different than the mean increase as a proportion of baseline for Group 4.

Table 20 contains descriptive statistics associated with the proportion of animals that were abnormal at any point during the study by Group. Table 21 contains the results of the overall two-sided Fisher's exact tests for each parameter and Table 22 contains the pairwise Fisher's exact tests comparing the abnormal rates for each pair of Groups with respect to those parameters that had significant overall tests. The pairwise comparisons are not dependent upon the ordering of the Groups being compared (e.g., comparing Group 1 to Group 2 is equivalent to comparing Group 2 to Group 1); therefore, the cells in the lower left portion of Table 22 are shaded out. A significant Group effect was identified for PLT, with the proportion of abnormal animals in Group 5 being significantly greater than that in Group 4; however, the comparison between Groups 4 and 5 was no longer significant after the Bonferroni-Holm adjustment for multiple comparisons.

Table 23 contains the results of the overall log-rank tests for each parameter and Table 24 contains the results of the pairwise log-rank tests comparing the time to abnormality for each pair of Groups with respect to those parameters that had significant overall tests. Again, the pairwise comparisons are not dependent upon the ordering of the Groups being compared (e.g., comparing Group 1 to Group 2 is equivalent to comparing Group 2 to Group 1); therefore, the cells in the lower left portion of Table 24 are shaded out. A significant Group effect was identified for PLT. For PLT, the times to abnormality in Groups 3, 4, and 6 were significantly greater than that in Group 5 and the time to abnormality in Group 4 was also significantly greater than those in Groups 1 and 2. After the Bonferroni-Holm adjustment for multiple comparisons, only the time to abnormality in Group 4 was significantly greater than that in Group 5. Figure 17 displays the Kaplan-Meier curves associated with time to abnormality for PLT in each Group.

4 Conclusions

All animals in the high dose control Group (Group 6), and all but one animal in the 100,000 colony forming unit (CFU) Group (Group 5), died prior to Study Day 7. Among the red blood cell parameters, MCH and MCHC had similar results in terms of significant shifts from baseline with the lowest dose Groups (Groups 1 through 4) all experiencing significant decreases from baseline on Study Days 1 through 3. Only RDW had a significant result in terms of the pairwise Group comparisons, with the mean increase from baseline in the 100 CFU dose Group (Group 2) being significantly different than mean decrease from baseline in the high dose control Group (Group 6) on Study Day 3.

Among the platelet counts and volume, the mean shifts as a proportion of baseline for MPV were significantly different between the Groups on Study Days 3 and 7. On Study Day 3, the mean increase as a proportion of baseline in the high dose control Group (Group 6) was significantly different than the mean changes as a proportion of baseline in the lowest dose Groups (Groups 1 through 4). On Study Day 7, the mean decrease as a proportion of baseline in the negative control Group (Group 1) was significantly different than the mean change as a proportion of baseline in the 10,000 CFU dose Group (Group 4). For PLT, the proportion of abnormal animals in the 100,000 CFU dose Group (Group 5) was significantly greater than that in the 10,000 CFU dose Group (Group 4); however, this comparison was no longer significant after the Bonferroni-Holm adjustment for multiple comparisons. Also for PLT, the times to abnormality in the 1,000 CFU and 10,000 CFU dose Groups (Groups 3 and 4) and in the high dose control Group (Group 6) were significantly greater than that in the 100,000 CFU dose Group (Group 5), and the time to abnormality in the 10,000 CFU dose Group (Group 4) was significantly greater than those in the two lowest dose Groups (Groups 1 and 2). However, after the Bonferroni-Holm adjustment for multiple comparisons, only the time to abnormality in the 10,000 CFU dose Group (Group 4) was significantly greater than that in the 100,000 CFU dose Group (Group 5).

Among the white blood cell parameters, the mean increases from baseline in the high dose control Group (Group 6) were significantly different than the mean changes from baseline in all other Groups (Groups 1 through 5) on Study Day 2 for neutrophils and N/L ratio. For lymphocytes, the mean decrease from baseline in the high dose control Group (Group 6) was

significantly different than the mean changes from baseline in most of the lower dose Groups (Groups 2 through 5) on Study Day 3.

Table 2. List of Potential Hematology Outliers

Parameter	Animal	Group	Study Day	Parameter Value	Deleted Studentized Residual
Red Blood Cell Count	L23218	1	2	3.67	-4.979
Hemoglobin	L23218	1	2	7.60	-5.289
Hematocrit	L23218	1	2	23.80	-4.787
Mean Platelet Volume †	L23223	1	3	13.10	4.375
	L23230	3	14	13.10	4.398
	L23225	4	3	15.20	4.437
Neutrophils	L23212	5	3	1.06	-4.177
Neutrophils/Lymphocytes Ratio†	L23200	5	1	0.03	-4.503
	L23214	5	2	3.00	4.363

† Distribution was log-normal for this parameter. Parameter values are reported on the original scale, while the residuals are reported on the log-transformed scale.

Table 3. Summary of ANOVA Results for Baseline Data (Study Day -3)

Hematology Parameter	Group Effect P-Value
Red Blood Cell Count	0.4155
Hemoglobin	0.2456
Hematocrit	0.1475
Mean Corpuscular Volume	0.4034
Mean Corpuscular Hemoglobin	0.6842
Mean Corpuscular Hemoglobin Concentration	0.5803
Red Cell Distribution Width †	0.3691
Platelet Count	0.3199
Mean Platelet Volume †	0.4595
White Blood Cell Count	0.5139
Neutrophils	0.2069
Lymphocytes	0.9845
Neutrophils/Lymphocytes Ratio †	0.7187
Monocytes †	0.2357
Eosinophils †	0.1076
Basophils †	0.8744

† Indicates that values for this parameter were log-transformed for the analysis.

Table 4a. Descriptive Statistics for Red Blood Cell Count (RBC, 10^6 cells/µL), by Group and Study Day

Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)
1	-3	5	5.80 (5.18, 6.42)	2	-3	5	6.27 (5.76, 6.78)	3	-3	5	6.02 (5.71, 6.32)
	1	5	5.87 (5.66, 6.08)		1	5	6.06 (5.64, 6.48)		1	5	5.93 (5.54, 6.32)
	2	5	5.24 (4.10, 6.38)		2	5	5.85 (5.59, 6.12)		2	5	5.68 (5.43, 5.93)
	3	5	5.45 (4.93, 5.98)		3	4	5.85 (5.29, 6.42)		3	5	5.75 (5.55, 5.94)
	7	5	5.83 (5.52, 6.13)		7	4	6.23 (5.33, 7.12)		7	5	6.22 (5.80, 6.64)
	14	5	5.94 (5.38, 6.51)		14	5	6.29 (5.84, 6.74)		14	5	6.15 (5.69, 6.60)
	21	5	5.79 (5.51, 6.08)		21	4	5.77 (5.18, 6.36)		21	5	5.72 (5.15, 6.28)

Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)
4	-3	5	5.87 (5.10, 6.64)	5	-3	5	6.05 (5.86, 6.24)	6	-3	3	5.66 (4.71, 6.61)
	1	5	5.69 (5.20, 6.18)		1	5	5.77 (5.55, 5.99)		1	4	5.82 (5.41, 6.24)
	2	5	5.46 (4.89, 6.04)		2	5	5.49 (5.22, 5.77)		2	3	5.22 (5.06, 5.38)
	3	5	5.38 (4.74, 6.02)		3	3	5.34 (4.72, 5.96)		3	3	5.06 (4.38, 5.75)
	7	4	5.69 (5.40, 5.98)		7	1	5.86 (--)		7	NA	NA
	14	3	5.98 (4.90, 7.07)		14	1	6.07 (--)		14	NA	NA
	21	3	5.46 (4.92, 6.01)		21	1	5.90 (--)		21	NA	NA

N Number of animals.
-- Confidence interval could not be calculated since only one observation was available for this Group on this Study Day.
NA Data not available for this Group at this Study Day.

Table 4b. Test Results for Red Blood Cell Count (RBC, 10^6 cells/µL)

Red Blood Cell Count								
Study Day	Mean Shift from Baseline, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
	1	2	3	4	5	6		
1	0.07	-0.20	-0.09	-0.18	-0.28 ↓	-0.09	0.5495	
2	-0.56 ↓	-0.41 ↓	-0.33	-0.41 ↓	-0.56 ↓	-0.68 ↓	0.7742	
3	-0.34	-0.52 ↓	-0.27	-0.49 ↓	-0.72 ↓	-0.84 ↓	0.5576	
7	0.03	-0.14	0.20	-0.03	-0.10	NA	0.7840	
14	0.14	0.02	0.13	0.41	0.11	NA	0.7823	
21	0.00	-0.60 ↓	-0.30	-0.11	-0.06	NA	0.1734	

Cells contain all significant pairwise Group comparisons at the 0.05 level. The format within each cell is: difference of shifts (relationship between corresponding Group mean shifts) Tukey-adjusted P-value.

↑, ↓ "↑" indicates the mean at the Study Day was significantly greater than that at baseline; "↓" indicates the mean at the Study Day was significantly less than that at baseline (at the 0.05 level).

NA Data not available for this Group at this Study Day.

Table 5a. Descriptive Statistics for Hemoglobin (HGB, g/dL) by Group and Study Day

Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)
1	-3	5	12.2 (11.3, 13.1)	2	-3	5	13.0 (12.5, 13.5)	3	-3	5	12.5 (12.0, 13.1)
	1	5	11.8 (11.3, 12.3)		1	5	12.2 (11.6, 12.7)		1	5	12.0 (11.3, 12.7)
	2	5	10.5 (8.4, 12.6)		2	5	11.8 (11.3, 12.3)		2	5	11.5 (11.0, 12.0)
	3	5	11.0 (10.0, 11.9)		3	4	11.7 (10.9, 12.6)		3	5	11.6 (11.0, 12.2)
	7	5	11.8 (11.3, 12.3)		7	4	12.7 (10.6, 14.7)		7	5	12.7 (12.1, 13.3)
	14	5	12.0 (11.0, 13.1)		14	5	12.8 (12.1, 13.5)		14	5	12.5 (11.5, 13.5)
	21	5	11.6 (11.1, 12.1)		21	4	11.6 (10.6, 12.5)		21	5	11.5 (10.6, 12.4)

Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)
4	-3	5	12.6 (11.4, 13.9)	5	-3	5	12.9 (12.3, 13.4)	6	-3	3	12.0 (10.6, 13.3)
	1	5	11.7 (11.3, 12.2)		1	5	11.8 (11.4, 12.2)		1	4	11.7 (10.3, 13.1)
	2	5	11.3 (10.4, 12.1)		2	5	11.2 (10.6, 11.8)		2	3	10.5 (9.1, 11.8)
	3	5	11.2 (10.0, 12.4)		3	3	11.1 (9.7, 12.5)		3	3	10.3 (8.0, 12.5)
	7	4	12.0 (11.1, 12.9)		7	1	12.4 (--)		7	NA	NA
	14	3	12.5 (12.2, 12.9)		14	1	12.7 (--)		14	NA	NA
	21	3	11.1 (10.3, 11.8)		21	1	12.3 (--)		21	NA	NA

N Number of animals.
-- Confidence interval could not be calculated since only one observation was available for this Group on this Study Day.
NA Data not available for this Group at this Study Day.

Table 5b. Test Results for Hemoglobin (HGB, g/dL)

Hemoglobin								
Study Day	Mean Shift from Baseline, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
	1	2	3	4	5	6		
1	-0.4	-0.8 ↓	-0.6	-0.9 ↓	-1.1 ↓	-0.6 ↓	0.5533	
2	-1.7 ↓	-1.2 ↓	-1.1 ↓	-1.4 ↓	-1.7 ↓	-1.8 ↓	0.6658	
3	-1.2 ↓	-1.4 ↓	-0.9 ↓	-1.4 ↓	-2.0 ↓	-2.0 ↓	0.5408	
7	-0.4	-0.5	0.1	-0.5	-0.5	NA	0.8298	
14	-0.2	-0.2	0.0	0.5	-0.2	NA	0.7165	
21	-0.6 ↓	-1.6 ↓	-1.0 ↓	-0.9 ↓	-0.6	NA	0.1997	

Cells contain all significant pairwise Group comparisons at the 0.05 level. The format within each cell is: difference of shifts (relationship between corresponding Group mean shifts) Tukey-adjusted P-value.

↑, ↓ "↑" indicates the mean at the Study Day was significantly greater than that at baseline; "↓" indicates the mean at the Study Day was significantly less than that at baseline (at the 0.05 level).

NA Data not available for this Group at this Study Day.

Table 6a. Descriptive Statistics for Hematocrit (HCT, %) by Group and Study Day

Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)
1	-3	5	36.8 (34.5, 39.1)	2	-3	5	39.4 (37.2, 41.7)	3	-3	5	37.7 (36.5, 38.9)
	1	5	37.2 (35.0, 39.4)		1	5	38.2 (36.6, 39.7)		1	5	37.5 (34.5, 40.5)
	2	5	33.2 (26.6, 39.7)		2	5	36.5 (34.2, 38.8)		2	5	35.6 (34.0, 37.2)
	3	5	34.4 (30.8, 38.1)		3	4	36.7 (33.0, 40.3)		3	5	36.0 (33.8, 38.3)
	7	5	37.3 (34.8, 39.8)		7	4	40.3 (33.1, 47.4)		7	5	40.5 (38.9, 42.1)
	14	5	37.3 (33.4, 41.1)		14	5	39.6 (37.8, 41.4)		14	5	38.5 (34.9, 42.0)
	21	5	35.7 (33.8, 37.6)		21	4	35.5 (31.8, 39.1)		21	5	35.2 (32.0, 38.4)

Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)
4	-3	5	38.1 (34.0, 42.1)	5	-3	5	39.4 (37.9, 41.0)	6	-3	3	36.1 (32.0, 40.2)
	1	5	36.2 (34.9, 37.5)		1	5	36.9 (34.8, 39.1)		1	4	36.5 (32.2, 40.7)
	2	5	34.8 (31.4, 38.2)		2	5	34.4 (32.2, 36.7)		2	3	32.0 (27.4, 36.6)
	3	5	35.2 (30.3, 40.0)		3	3	33.6 (29.4, 37.7)		3	3	30.9 (25.3, 36.5)
	7	4	38.2 (36.0, 40.4)		7	1	37.9 (--)		7	NA	NA
	14	3	38.9 (37.3, 40.5)		14	1	38.5 (--)		14	NA	NA
	21	3	34.1 (31.8, 36.3)		21	1	37.3 (--)		21	NA	NA

N Number of animals.
-- Confidence interval could not be calculated since only one observation was available for this Group on this Study Day.
NA Data not available for this Group at this Study Day.

Table 6b. Test Results for Hematocrit (HCT, %)

Hematocrit								
Study Day	Mean Shift from Baseline, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
	1	2	3	4	5	6		
1	0.4	-1.3	-0.2	-1.9	-2.5 ↓	-1.0	0.3718	
2	-3.6 ↓	-2.9 ↓	-2.1	-3.3 ↓	-5.0 ↓	-5.2 ↓	0.5290	
3	-2.4	-3.0	-1.7	-2.9	-6.2 ↓	-6.3 ↓	0.3582	
7	0.5	0.6	2.8	0.7	-1.0	NA	0.6659	
14	0.5	0.2	0.8	2.8	-0.4	NA	0.7256	
21	-1.1	-4.2 ↓	-2.5 ↓	-2.0	-1.6	NA	0.4060	

Cells contain all significant pairwise Group comparisons at the 0.05 level. The format within each cell is: difference of shifts (relationship between corresponding Group mean shifts) Tukey-adjusted P-value.

↑, ↓ "↑" indicates the mean at the Study Day was significantly greater than that at baseline; "↓" indicates the mean at the Study Day was significantly less than that at baseline (at the 0.05 level).

NA Data not available for this Group at this Study Day.

Table 7a. Descriptive Statistics for Mean Corpuscular Volume (MCV, fL) by Group and Study Day

Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)
1	-3	5	63.7 (60.6, 66.8)	2	-3	5	63.0 (60.2, 65.7)	3	-3	5	62.8 (59.8, 65.8)
	1	5	63.3 (60.0, 66.5)		1	5	63.1 (59.9, 66.4)		1	5	63.3 (60.0, 66.6)
	2	5	63.4 (61.4, 65.5)		2	5	62.3 (59.3, 65.4)		2	5	62.7 (60.0, 65.3)
	3	5	63.1 (59.6, 66.5)		3	4	62.6 (58.0, 67.2)		3	5	62.7 (60.2, 65.2)
	7	5	64.1 (60.2, 68.0)		7	4	64.6 (61.2, 68.1)		7	5	65.2 (62.6, 67.9)
	14	5	62.7 (60.3, 65.1)		14	5	63.0 (60.6, 65.4)		14	5	62.6 (60.1, 65.0)
	21	5	61.6 (59.7, 63.6)		21	4	61.5 (58.4, 64.7)		21	5	61.7 (59.7, 63.7)

Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)
4	-3	5	65.0 (61.4, 68.5)	5	-3	5	65.2 (64.4, 66.0)	6	-3	3	63.9 (58.4, 69.4)
	1	5	63.9 (60.0, 67.8)		1	5	64.0 (62.4, 65.6)		1	4	62.6 (59.5, 65.6)
	2	5	63.7 (59.9, 67.5)		2	5	62.7 (61.3, 64.1)		2	3	61.4 (54.3, 68.5)
	3	5	65.4 (60.7, 70.0)		3	3	62.9 (59.8, 65.9)		3	3	61.1 (56.3, 65.8)
	7	4	67.1 (62.5, 71.8)		7	1	64.7 (--)		7	NA	NA
	14	3	65.2 (56.2, 74.2)		14	1	63.5 (--)		14	NA	NA
	21	3	62.4 (52.0, 72.9)		21	1	63.2 (--)		21	NA	NA

N Number of animals.
-- Confidence interval could not be calculated since only one observation was available for this Group on this Study Day.
NA Data not available for this Group at this Study Day.

Table 7b. Test Results for Mean Corpuscular Volume (MCV, fL)

Mean Corpuscular Volume								
Study Day	Mean Shift from Baseline, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
	1	2	3	4	5	6		
1	-0.4	0.2	0.5	-1.1 ↓	-1.2 ↓	-0.8	0.0650	
2	-0.2	-0.6	-0.1	-1.3 ↓	-2.5 ↓	-1.7 ↓	0.0485 *	
3	-0.6	0.3	-0.1	0.4	-2.8 ↓	-2.0 ↓	0.0572	
7	0.4	2.3 ↑	2.4 ↑	1.5	-0.7	NA	0.1821	
14	-1.0	0.0	-0.2	0.3	-1.9	NA	0.4295	
21	-2.1 ↓	-0.8	-1.1	-2.5 ↓	-2.2	NA	0.6018	

Cells contain all significant pairwise Group comparisons at the 0.05 level. The format within each cell is: difference of shifts (relationship between corresponding Group mean shifts) Tukey-adjusted P-value.

↑, ↓ "↑" indicates the mean at the Study Day was significantly greater than that at baseline; "↓" indicates the mean at the Study Day was significantly less than that at baseline (at the 0.05 level).

* The overall Group effect was significant at the 0.05 level.

NA Data not available for this Group at this Study Day.

Table 8a. Descriptive Statistics for Mean Corpuscular Hemoglobin (MCH, pg) by Group and Study Day

Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)
1	-3	5	21.1 (20.2, 21.9)	2	-3	5	20.8 (19.9, 21.7)	3	-3	5	20.9 (19.7, 22.1)
	1	5	20.1 (19.4, 20.7)		1	5	20.1 (19.4, 20.8)		1	5	20.2 (19.1, 21.3)
	2	5	20.1 (19.4, 20.9)		2	5	20.1 (19.5, 20.8)		2	5	20.2 (19.4, 21.1)
	3	5	20.2 (19.3, 21.0)		3	4	20.1 (19.5, 20.6)		3	5	20.3 (19.5, 21.1)
	7	5	20.3 (19.4, 21.1)		7	4	20.4 (19.5, 21.3)		7	5	20.4 (19.5, 21.2)
	14	5	20.3 (19.6, 20.9)		14	5	20.3 (19.8, 20.9)		14	5	20.3 (19.4, 21.2)
	21	5	20.1 (19.5, 20.6)		21	4	20.1 (19.4, 20.9)		21	5	20.2 (19.3, 21.0)

Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)
4	-3	5	21.6 (20.3, 22.9)	5	-3	5	21.3 (20.6, 21.9)	6	-3	3	21.2 (18.7, 23.7)
	1	5	20.7 (19.2, 22.2)		1	5	20.4 (19.9, 21.0)		1	4	20.1 (19.0, 21.2)
	2	5	20.7 (19.3, 22.0)		2	5	20.3 (19.8, 20.8)		2	3	20.1 (18.0, 22.2)
	3	5	20.9 (19.5, 22.3)		3	3	20.8 (20.5, 21.1)		3	3	20.3 (17.6, 22.9)
	7	4	21.1 (19.1, 23.2)		7	1	21.2 (--)		7	NA	NA
	14	3	21.0 (17.4, 24.5)		14	1	20.9 (--)		14	NA	NA
	21	3	20.3 (16.9, 23.7)		21	1	20.9 (--)		21	NA	NA

N Number of animals.
-- Confidence interval could not be calculated since only one observation was available for this Group on this Study Day.
NA Data not available for this Group at this Study Day.

Table 8b. Test Results for Mean Corpuscular Hemoglobin (MCH, pg)

Mean Corpuscular Hemoglobin								
Study Day	Mean Shift from Baseline, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
	1	2	3	4	5	6		
1	-1.0 ↓	-0.7 ↓	-0.8 ↓	-0.9 ↓	-0.8 ↓	-0.8 ↓	0.6054	
2	-0.9 ↓	-0.7 ↓	-0.7 ↓	-0.9 ↓	-1.0 ↓	-0.7 ↓	0.5213	
3	-0.9 ↓	-0.6 ↓	-0.6 ↓	-0.7 ↓	-0.9 ↓	-0.6 ↓	0.6367	
7	-0.8 ↓	-0.3	-0.6 ↓	-0.7 ↓	-0.5	NA	0.4259	
14	-0.8 ↓	-0.4 ↓	-0.6 ↓	-0.6 ↓	-0.8 ↓	NA	0.4576	
21	-1.0 ↓	-0.5	-0.8 ↓	-1.3 ↓	-0.8	NA	0.5371	

Cells contain all significant pairwise Group comparisons at the 0.05 level. The format within each cell is: difference of shifts (relationship between corresponding Group mean shifts) Tukey-adjusted P-value.

↑, ↓ "↑" indicates the mean at the Study Day was significantly greater than that at baseline; "↓" indicates the mean at the Study Day was significantly less than that at baseline (at the 0.05 level).

NA Data not available for this Group at this Study Day.

Table 9a. Descriptive Statistics for Mean Corpuscular Hemoglobin Concentration (MCHC, g/dL) by Group and Study Day

Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)
1	-3	5	33.1 (32.5, 33.8)	2	-3	5	33.0 (31.8, 34.2)	3	-3	5	33.3 (32.5, 34.1)
	1	5	31.8 (31.0, 32.6)		1	5	31.9 (31.2, 32.7)		1	5	32.0 (30.6, 33.3)
	2	5	31.7 (31.1, 32.4)		2	5	32.3 (31.6, 33.0)		2	5	32.3 (31.7, 32.9)
	3	5	32.0 (31.0, 33.0)		3	4	32.1 (30.6, 33.6)		3	5	32.4 (31.6, 33.2)
	7	5	31.7 (30.8, 32.5)		7	4	31.6 (31.0, 32.1)		7	5	31.2 (30.1, 32.4)
	14	5	32.3 (31.7, 33.0)		14	5	32.3 (31.7, 32.9)		14	5	32.5 (31.3, 33.6)
	21	5	32.6 (32.1, 33.0)		21	4	32.7 (31.2, 34.2)		21	5	32.7 (31.9, 33.6)

Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)
4	-3	5	33.2 (32.8, 33.7)	5	-3	5	32.6 (31.9, 33.3)	6	-3	3	33.2 (32.1, 34.2)
	1	5	32.4 (31.8, 33.0)		1	5	31.9 (30.8, 33.0)		1	4	32.2 (31.6, 32.7)
	2	5	32.5 (31.6, 33.3)		2	5	32.4 (31.7, 33.1)		2	3	32.7 (32.4, 33.1)
	3	5	32.0 (30.5, 33.5)		3	3	33.0 (31.4, 34.6)		3	3	33.2 (31.5, 34.9)
	7	4	31.5 (29.8, 33.1)		7	1	32.7 (--)		7	NA	NA
	14	3	32.1 (30.7, 33.5)		14	1	32.9 (--)		14	NA	NA
	21	3	32.5 (32.5, 32.5)		21	1	33.0 (--)		21	NA	NA

N Number of animals.
-- Confidence interval could not be calculated since only one observation was available for this Group on this Study Day.
NA Data not available for this Group at this Study Day.

Table 9b. Test Results for Mean Corpuscular Hemoglobin Concentration (MCHC, g/dL)

Mean Corpuscular Hemoglobin Concentration								
Study Day	Mean Shift from Baseline, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
	1	2	3	4	5	6		
1	-1.4 ↓	-1.1 ↓	-1.3 ↓	-0.8 ↓	-0.7 ↓	-0.9 ↓	0.4264	
2	-1.4 ↓	-0.7 ↓	-1.0 ↓	-0.8 ↓	-0.3	-0.3	0.0904	
3	-1.1 ↓	-1.1 ↓	-0.9 ↓	-1.3 ↓	0.0	0.2	0.1866	
7	-1.5 ↓	-1.6 ↓	-2.1 ↓	-1.8 ↓	-0.5	NA	0.5186	
14	-0.8	-0.7	-0.8 ↓	-1.1 ↓	-0.3	NA	0.9328	
21	-0.6	-0.5	-0.6	-0.8	-0.2	NA	0.9873	

Cells contain all significant pairwise Group comparisons at the 0.05 level. The format within each cell is: difference of shifts (relationship between corresponding Group mean shifts) Tukey-adjusted P-value.

↑, ↓ "↑" indicates the mean at the Study Day was significantly greater than that at baseline; "↓" indicates the mean at the Study Day was significantly less than that at baseline (at the 0.05 level).

NA Data not available for this Group at this Study Day.

Table 10a. Descriptive Statistics for Red Cell Distribution Width (RDW, %) by Group and Study Day

Group	Study Day	N	Geometric Mean (95% Confidence Interval)	Group	Study Day	N	Geometric Mean (95% Confidence Interval)	Group	Study Day	N	Geometric Mean (95% Confidence Interval)
1	-3	5	12.2 (11.3, 13.1)	2	-3	5	12.7 (12.2, 13.2)	3	-3	5	12.8 (12.3, 13.4)
	1	5	12.2 (11.4, 13.1)		1	5	12.9 (12.0, 14.0)		1	5	12.9 (12.4, 13.4)
	2	5	12.1 (11.4, 12.9)		2	5	13.1 (12.2, 14.0)		2	5	13.1 (12.5, 13.7)
	3	5	12.3 (11.4, 13.3)		3	4	13.6 (12.5, 14.7)		3	5	13.5 (12.9, 14.1)
	7	5	12.7 (12.4, 13.0)		7	4	13.7 (12.5, 15.0)		7	5	14.0 (13.4, 14.6)
	14	5	11.8 (11.4, 12.2)		14	5	12.4 (11.9, 13.0)		14	5	12.6 (12.1, 13.2)
	21	5	11.4 (11.0, 11.9)		21	4	11.6 (10.9, 12.3)		21	5	12.1 (11.7, 12.4)

Group	Study Day	N	Geometric Mean (95% Confidence Interval)	Group	Study Day	N	Geometric Mean (95% Confidence Interval)	Group	Study Day	N	Geometric Mean (95% Confidence Interval)
4	-3	5	12.5 (11.9, 13.1)	5	-3	5	12.8 (12.2, 13.5)	6	-3	3	12.8 (11.5, 14.3)
	1	5	12.6 (12.2, 12.9)		1	5	12.9 (12.1, 13.6)		1	4	12.9 (12.1, 13.8)
	2	5	12.6 (12.1, 13.1)		2	5	13.0 (12.1, 13.8)		2	3	12.9 (11.7, 14.2)
	3	5	12.9 (12.5, 13.3)		3	3	13.3 (11.6, 15.2)		3	3	12.7 (12.1, 13.4)
	7	4	13.5 (12.5, 14.5)		7	1	13.8 (--)		7	NA	NA
	14	3	12.4 (11.8, 13.1)		14	1	12.3 (--)		14	NA	NA
	21	3	12.1 (11.6, 12.7)		21	1	12.0 (--)		21	NA	NA

N Number of animals.
-- Confidence interval could not be calculated since only one observation was available for this Group on this Study Day.
NA Data not available for this Group at this Study Day.

Table 10b. Test Results for Red Cell Distribution Width (RDW, %)

Red Cell Distribution Width †								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value #
	1	2	3	4	5	6		
1	1.00	1.02	1.00	1.01	1.00	1.01	0.9304	
2	0.99	1.03 ↑	1.02	1.01	1.01	1.00	0.2352	
3	1.01	1.07 ↑	1.05 ↑	1.03 ↑	1.02	0.98	0.0227 *	0.92 (6<2) 0.0261
7	1.04	1.08 ↑	1.09 ↑	1.06 ↑	1.10	NA	0.6415	
14	0.97	0.98	0.98	0.98	0.98	NA	0.9513	
21	0.94 ↓	0.92 ↓	0.94 ↓	0.96	0.95	NA	0.4860	

† Indicates that values for this parameter were log-transformed for the analysis.

Cells contain all significant pairwise Group comparisons at the 0.05 level. The format within each cell is: ratio of shifts (relationship between corresponding Group mean shifts) Tukey-adjusted P-value.

↑, ↓ "↑" indicates the geometric mean at the Study Day was significantly greater than that at baseline; "↓" indicates the geometric mean at the Study Day was significantly less than that at baseline (at the 0.05 level).

* The overall Group effect was significant at the 0.05 level.

NA Data not available for this Group at this Study Day.

Table 11a. Descriptive Statistics for Platelet Count (PLT, 10^3 cells/µL) by Group and Study Day

Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)
1	-3	5	545 (331, 759)	2	-3	5	473 (312, 635)	3	-3	5	437 (341, 534)
	1	5	628 (175, 1082)		1	5	472 (280, 664)		1	5	396 (348, 445)
	2	5	540 (53, 1027)		2	5	397 (232, 561)		2	5	355 (325, 384)
	3	5	372 (15, 729)		3	4	422 (205, 638)		3	5	415 (373, 457)
	7	5	484 (187, 782)		7	4	265 (5, 525)		7	5	353 (265, 441)
	14	5	191 (0*, 445)		14	5	263 (68, 459)		14	5	284 (81, 488)
	21	5	473 (245, 702)		21	4	401 (49, 753)		21	5	284 (154, 413)

Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)
4	-3	5	457 (337, 577)	5	-3	5	580 (468, 692)	6	-3	3	493 (336, 649)
	1	5	451 (261, 641)		1	5	504 (373, 634)		1	4	509 (404, 614)
	2	5	403 (262, 544)		2	5	360 (206, 514)		2	3	249 (124, 373)
	3	5	473 (342, 605)		3	3	307 (13, 601)		3	3	290 (0*, 749)
	7	4	384 (144, 624)		7	1	251 (--)		7	NA	NA
	14	3	524 (439, 610)		14	1	325 (--)		14	NA	NA
	21	3	453 (442, 463)		21	1	170 (--)		21	NA	NA

N Number of animals.
-- Confidence interval could not be calculated since only one observation was available for this Group on this Study Day.
* Lower bound of confidence interval set to zero since a negative parameter value is not possible.
NA Data not available for this Group at this Study Day.

Table 11b. Test Results for Platelet Count (PLT, 10^3 cells/µL)

Platelet Count								
Study Day	Mean Shift from Baseline, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
	1	2	3	4	5	6		
1	83	-1	-41	-6	-77	20	0.3600	
2	-5	-77	-83	-54	-220 ↓	-237 ↓	0.1052	
3	-173 ↓	-17	-23	16	-242 ↓	-196	0.1998	
7	-61	-174	-85	-93	-175	NA	0.9493	
14	-354 ↓	-210 ↓	-153	4	-101	NA	0.2296	
21	-72	-38	-154 ↓	-67	-256 ↓	NA	0.3360	

Cells contain all significant pairwise Group comparisons at the 0.05 level. The format within each cell is: difference of shifts (relationship between corresponding Group mean shifts) Tukey-adjusted P-value.

↑, ↓ "↑" indicates the mean at the Study Day was significantly greater than that at baseline; "↓" indicates the mean at the Study Day was significantly less than that at baseline (at the 0.05 level).

NA Data not available for this Group at this Study Day.

Table 12a.Descriptive Statistics for Mean Platelet Volume (MPV, fL) by Group and Study Day

Group	Study Day	N	Geometric Mean (95% Confidence Interval)	Group	Study Day	N	Geometric Mean (95% Confidence Interval)	Group	Study Day	N	Geometric Mean (95% Confidence Interval)
1	-3	5	8.4 (7.3, 9.7)	2	-3	5	8.1 (7.4, 8.9)	3	-3	5	8.2 (7.3, 9.3)
	1	5	7.2 (6.4, 8.2)		1	5	7.1 (6.6, 7.7)		1	5	7.0 (6.5, 7.5)
	2	5	6.8 (6.5, 7.0)		2	5	6.8 (5.9, 7.7)		2	5	6.3 (6.1, 6.4)
	3	5	7.8 (5.3, 11.3)		3	4	6.9 (5.6, 8.4)		3	5	7.2 (6.4, 8.0)
	7	5	6.8 (5.5, 8.4)		7	4	7.8 (6.7, 9.1)		7	5	8.2 (7.7, 8.7)
	14	5	8.5 (6.2, 11.7)		14	5	7.2 (6.7, 7.8)		14	5	7.7 (5.3, 11.2)
	21	5	6.5 (5.9, 7.2)		21	4	6.6 (5.8, 7.4)		21	5	6.6 (5.9, 7.4)

Group	Study Day	N	Geometric Mean (95% Confidence Interval)	Group	Study Day	N	Geometric Mean (95% Confidence Interval)	Group	Study Day	N	Geometric Mean (95% Confidence Interval)
4	-3	5	8.0 (7.1, 8.9)	5	-3	5	7.5 (6.8, 8.3)	6	-3	3	8.2 (7.1, 9.5)
	1	5	6.2 (5.8, 6.7)		1	5	6.4 (5.7, 7.1)		1	4	6.1 (5.5, 6.9)
	2	5	6.6 (5.5, 8.0)		2	5	6.6 (6.0, 7.4)		2	3	6.9 (5.8, 8.1)
	3	5	8.9 (6.1, 13.2)		3	3	9.2 (5.2, 16.3)		3	3	16.0 (8.2, 31.2)
	7	4	8.2 (5.9, 11.5)		7	1	8.4 (--)		7	NA	NA
	14	3	6.3 (5.1, 7.8)		14	1	7.6 (--)		14	NA	NA
	21	3	5.7 (5.2, 6.2)		21	1	7.9 (--)		21	NA	NA

N Number of animals.
-- Confidence interval could not be calculated since only one observation was available for this Group on this Study Day.
NA Data not available for this Group at this Study Day.

Table 12b. Test Results for Mean Platelet Volume (MPV, fL)

Mean Platelet Volume †								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value #
	1	2	3	4	5	6		
1	0.86 ↓	0.88 ↓	0.85 ↓	0.78 ↓	0.85 ↓	0.77 ↓	0.0642	
2	0.81 ↓	0.83 ↓	0.76 ↓	0.83 ↓	0.88 ↓	0.86 ↓	0.3934	
3	0.92	0.86	0.87	1.12	1.23	2.00 ↑	0.0002 *	0.46 (1<6) 0.0007 0.43 (2<6) 0.0004 0.43 (3<6) 0.0003 0.56 (4<6) 0.0114
7	0.81 ↓	0.98	0.99	1.06	1.11	NA	0.0431 *	0.76 (1<4) 0.0462
14	1.02	0.89	0.94	0.80	1.00	NA	0.5882	
21	0.78 ↓	0.82 ↓	0.80 ↓	0.72 ↓	1.04	NA	0.2611	

† Indicates that values for this parameter were log-transformed for the analysis.

Cells contain all significant pairwise Group comparisons at the 0.05 level. The format within each cell is: ratio of shifts (relationship between corresponding Group mean shifts) Tukey-adjusted P-value.

↑, ↓ "↑" indicates the geometric mean at the Study Day was significantly greater than that at baseline; "↓" indicates the geometric mean at the Study Day was significantly less than that at baseline (at the 0.05 level).

* The overall Group effect was significant at the 0.05 level.

NA Data not available for this Group at this Study Day.

Table 13a. Descriptive Statistics for White Blood Cell Count (WBC, 10^3 cells/µL) by Group and Study Day

Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)
1	-3	5	8.05 (4.84, 11.26)	2	-3	5	6.77 (5.37, 8.16)	3	-3	5	7.20 (5.34, 9.07)
	1	5	6.38 (4.00, 8.76)		1	5	6.45 (5.06, 7.83)		1	5	7.81 (5.95, 9.66)
	2	5	5.55 (3.48, 7.62)		2	5	6.67 (5.31, 8.02)		2	5	7.73 (5.41, 10.05)
	3	5	6.46 (3.39, 9.54)		3	4	6.57 (4.96, 8.17)		3	5	7.66 (5.08, 10.25)
	7	5	5.94 (3.24, 8.65)		7	4	4.97 (3.56, 6.38)		7	5	6.48 (4.82, 8.15)
	14	5	5.03 (2.13, 7.92)		14	5	5.70 (4.27, 7.13)		14	5	6.59 (3.97, 9.21)
	21	5	4.14 (3.23, 5.05)		21	4	3.90 (1.98, 5.82)		21	5	4.40 (2.77, 6.04)

Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)
4	-3	5	6.78 (5.67, 7.89)	5	-3	5	8.31 (6.58, 10.03)	6	-3	3	6.84 (1.85, 11.83)
	1	5	7.34 (6.58, 8.11)		1	5	7.69 (4.58, 10.79)		1	4	7.87 (4.56, 11.19)
	2	5	7.59 (6.18, 9.00)		2	5	7.60 (2.94, 12.26)		2	3	7.51 (4.76, 10.27)
	3	5	6.56 (4.36, 8.76)		3	3	10.10 (2.86, 17.35)		3	3	3.89 (0.00 *, 9.00)
	7	4	7.99 (3.48, 12.51)		7	1	6.88 (--)		7	NA	NA
	14	3	6.70 (2.60, 10.80)		14	1	5.25 (--)		14	NA	NA
	21	3	3.61 (0.41, 6.80)		21	1	4.25 (--)		21	NA	NA

N Number of animals.
-- Confidence interval could not be calculated since only one observation was available for this Group on this Study Day.
* Lower bound of confidence interval set to zero since a negative parameter value is not possible.
NA Data not available for this Group at this Study Day.

Table 13b. Test Results for White Blood Cell Count (WBC, 10^3 cells/µL)

White Blood Cell Count								
Study Day	Mean Shift from Baseline, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
	1	2	3	4	5	6		
1	-1.67 ↓	-0.32	0.61	0.56	-0.62	0.57	0.0444 *	
2	-2.50 ↓	-0.10	0.53	0.81	-0.71	0.23	0.0530	-3.31 (1<4) 0.0447
3	-1.59 ↓	-0.13	0.46	-0.22	1.20	-3.40 ↓	0.0292 *	-4.60 (6<5) 0.0342
7	-2.11 ↓	-1.73	-0.72	0.97	-0.87	NA	0.2541	
14	-3.03 ↓	-1.07	-0.61	-0.37	-2.50	NA	0.1939	
21	-3.91 ↓	-2.79 ↓	-2.80 ↓	-3.47 ↓	-3.50 ↓	NA	0.5997	

Cells contain all significant pairwise Group comparisons at the 0.05 level. The format within each cell is: difference of shifts (relationship between corresponding Group mean shifts) Tukey-adjusted P-value.

↑, ↓ "↑" indicates the mean at the Study Day was significantly greater than that at baseline; "↓" indicates the mean at the Study Day was significantly less than that at baseline (at the 0.05 level).

* The overall Group effect was significant at the 0.05 level.

NA Data not available for this Group at this Study Day.

Table 14a. Descriptive Statistics for Neutrophils (10^3 cells/µL) by Group and Study Day

Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)
1	-3	5	2.50 (0.81, 4.20)	2	-3	5	1.73 (1.31, 2.15)	3	-3	5	1.64 (1.19, 2.09)
	1	5	1.34 (0.38, 2.30)		1	5	1.48 (1.22, 1.73)		1	5	1.52 (1.07, 1.98)
	2	5	1.30 (0.66, 1.93)		2	5	1.40 (0.99, 1.81)		2	5	1.46 (1.00, 1.92)
	3	5	1.30 (0.48, 2.13)		3	4	1.08 (0.77, 1.39)		3	5	1.40 (0.93, 1.87)
	7	5	1.50 (0.62, 2.39)		7	4	1.09 (0.80, 1.37)		7	5	1.44 (1.00, 1.89)
	14	5	1.17 (0.22, 2.11)		14	5	1.34 (0.75, 1.94)		14	5	1.45 (0.69, 2.21)
	21	5	0.91 (0.68, 1.14)		21	4	0.60 (0.38, 0.83)		21	5	0.75 (0.54, 0.96)

Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)
4	-3	5	1.80 (1.36, 2.23)	5	-3	5	2.59 (1.30, 3.89)	6	-3	3	1.68 (1.01, 2.35)
	1	5	1.87 (1.04, 2.69)		1	5	1.94 (0.41, 3.47)		1	4	2.07 (1.67, 2.46)
	2	5	1.99 (0.66, 3.32)		2	5	2.17 (0.23, 4.11)		2	3	4.37 (0.56, 8.18)
	3	5	1.22 (0.33, 2.11)		3	3	3.48 (0.00*, 8.71)		3	3	1.28 (0.00*, 3.00)
	7	4	2.32 (0.34, 4.30)		7	1	1.32 (--)		7	NA	NA
	14	3	1.40 (1.12, 1.69)		14	1	0.82 (--)		14	NA	NA
	21	3	0.57 (0.11, 1.03)		21	1	0.56 (--)		21	NA	NA

N Number of animals.

-- Confidence interval could not be calculated since only one observation was available for this Group on this Study Day.

* Lower bound of confidence interval set to zero since a negative parameter value is not possible.

NA Data not available for this Group at this Study Day.

Table 14b. Test Results for Neutrophils (10^3 cells/µL)

Neutrophils								
Study Day	Mean Shift from Baseline, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
	1	2	3	4	5	6		
1	-1.16 ↓	-0.25	-0.12	0.07	-0.66 ↓	0.29	0.0061 *	-1.23 (1<4) 0.0185 -1.45 (1<6) 0.0075
2	-1.21 ↓	-0.33	-0.18	0.19	-0.42	2.68 ↑	0.0002 *	-3.88 (1<6) <0.0001 -3.01 (2<6) 0.0011 -2.86 (3<6) 0.0019 -2.48 (4<6) 0.0077 -3.10 (5<6) 0.0008
3	-1.20 ↓	-0.53	-0.24	-0.58	0.91	-0.41	0.0459 *	-2.12 (1<5) 0.0171
7	-1.00	-0.52	-0.20	0.61	0.06	NA	0.4099	
14	-1.34 ↓	-0.39	-0.19	-0.40	-0.44	NA	0.4795	
21	-1.59 ↓	-1.00 ↓	-0.89 ↓	-1.24 ↓	-0.70	NA	0.5883	

Cells contain all significant pairwise Group comparisons at the 0.05 level. The format within each cell is: difference of shifts (relationship between corresponding Group mean shifts) Tukey-adjusted P-value.

↑, ↓ "↑" indicates the mean at the Study Day was significantly greater than that at baseline; "↓" indicates the mean at the Study Day was significantly less than that at baseline (at the 0.05 level).

* The overall Group effect was significant at the 0.05 level.

NA Data not available for this Group at this Study Day.

Table 15a. Descriptive Statistics for Lymphocytes (10^3 cells/µL) by Group and Study Day

Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)
1	-3	5	4.82 (1.93, 7.72)	2	-3	5	4.48 (3.37, 5.59)	3	-3	5	4.91 (3.40, 6.43)
	1	5	4.40 (2.77, 6.03)		1	5	4.49 (3.29, 5.69)		1	5	5.57 (3.89, 7.25)
	2	5	3.77 (2.00, 5.54)		2	5	4.75 (3.61, 5.89)		2	5	5.51 (3.61, 7.42)
	3	5	4.63 (2.43, 6.83)		3	4	5.07 (3.32, 6.81)		3	5	5.54 (3.19, 7.90)
	7	5	3.90 (2.05, 5.75)		7	4	3.42 (2.52, 4.32)		7	5	4.27 (3.08, 5.46)
	14	5	3.42 (1.66, 5.18)		14	5	3.82 (3.08, 4.56)		14	5	4.50 (2.61, 6.39)
	21	5	2.82 (2.11, 3.54)		21	4	2.95 (1.35, 4.55)		21	5	3.20 (2.06, 4.35)

Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)	Group	Study Day	N	Mean (95% Confidence Interval)
4	-3	5	4.39 (3.20, 5.59)	5	-3	5	4.94 (3.54, 6.35)	6	-3	3	4.61 (0.00 *, 10.20)
	1	5	4.93 (4.02, 5.85)		1	5	5.13 (3.31, 6.96)		1	4	5.13 (2.39, 7.87)
	2	5	5.04 (3.14, 6.93)		2	5	4.98 (1.54, 8.42)		2	3	2.89 (1.86, 3.92)
	3	5	4.75 (3.07, 6.43)		3	3	6.21 (0.64, 11.79)		3	3	2.33 (0.00 *, 5.75)
	7	4	4.93 (2.48, 7.37)		7	1	4.67 (--)		7	NA	NA
	14	3	4.70 (1.21, 8.19)		14	1	3.93 (--)		14	NA	NA
	21	3	2.69 (0.17, 5.21)		21	1	3.23 (--)		21	NA	NA

N Number of animals.

-- Confidence interval could not be calculated since only one observation was available for this Group on this Study Day.

* Lower bound of confidence interval set to zero since a negative parameter value is not possible.

NA Data not available for this Group at this Study Day.

Table 15b. Test Results for Lymphocytes (10^3 cells/µL)

Lymphocytes								
Study Day	Mean Shift from Baseline, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
	1	2	3	4	5	6		
1	-0.42	0.01	0.65	0.54	0.19	0.22	0.6180	
2	-1.06	0.27	0.60	0.64	0.04	-2.09 ↓	0.0396 *	
3	-0.20	0.51	0.63	0.36	0.56	-2.65 ↓	0.0206 *	-3.15 (6<2) 0.0304 -3.27 (6<3) 0.0164 -3.00 (6<4) 0.0314 -3.21 (6<5) 0.0425
7	-0.92	-1.14 ↓	-0.64	0.18	-1.3	NA	0.3713	
14	-1.40 ↓	-0.66	-0.42	-0.01	-2.04	NA	0.2958	
21	-2.00 ↓	-1.61 ↓	-1.71 ↓	-2.03 ↓	-2.74 ↓	NA	0.8896	

Cells contain all significant pairwise Group comparisons at the 0.05 level. The format within each cell is: difference of shifts (relationship between corresponding Group mean shifts) Tukey-adjusted P-value.

↑, ↓ "↑" indicates the mean at the Study Day was significantly greater than that at baseline; "↓" indicates the mean at the Study Day was significantly less than that at baseline (at the 0.05 level).

* The overall Group effect was significant at the 0.05 level.

NA Data not available for this Group at this Study Day.

Table 16a. Descriptive Statistics for Neutrophils/Lymphocytes Ratio by Group and Study Day

Group	Study Day	N	Geometric Mean (95% Confidence Interval)	Group	Study Day	N	Geometric Mean (95% Confidence Interval)	Group	Study Day	N	Geometric Mean (95% Confidence Interval)
1	-3	5	0.511 (0.198, 1.319)	2	-3	5	0.387 (0.299, 0.500)	3	-3	5	0.334 (0.230, 0.486)
	1	5	0.260 (0.111, 0.606)		1	5	0.332 (0.244, 0.453)		1	5	0.273 (0.166, 0.448)
	2	5	0.342 (0.195, 0.602)		2	5	0.293 (0.202, 0.424)		2	5	0.266 (0.164, 0.430)
	3	5	0.231 (0.092, 0.580)		3	4	0.214 (0.124, 0.367)		3	5	0.257 (0.137, 0.480)
	7	5	0.378 (0.239, 0.598)		7	4	0.317 (0.266, 0.378)		7	5	0.336 (0.231, 0.488)
	14	5	0.280 (0.139, 0.563)		14	5	0.338 (0.245, 0.464)		14	5	0.310 (0.188, 0.513)
	21	5	0.321 (0.232, 0.443)		21	4	0.211 (0.153, 0.291)		21	5	0.236 (0.172, 0.325)

Group	Study Day	N	Geometric Mean (95% Confidence Interval)	Group	Study Day	N	Geometric Mean (95% Confidence Interval)	Group	Study Day	N	Geometric Mean (95% Confidence Interval)
4	-3	5	0.412 (0.264, 0.642)	5	-3	5	0.497 (0.247, 1.001)	6	-3	3	0.388 (0.090, 1.674)
	1	5	0.363 (0.203, 0.651)		1	5	0.241 (0.051, 1.132)		1	4	0.418 (0.278, 0.628)
	2	5	0.370 (0.142, 0.967)		2	5	0.394 (0.070, 2.228)		2	3	1.468 (0.447, 4.823)
	3	5	0.226 (0.116, 0.441)		3	3	0.484 (0.037, 6.349)		3	3	0.596 (0.201, 1.772)
	7	4	0.438 (0.267, 0.720)		7	1	0.283 (--)		7	NA	NA
	14	3	0.307 (0.178, 0.530)		14	1	0.209 (--)		14	NA	NA
	21	3	0.214 (0.183, 0.251)		21	1	0.173 (--)		21	NA	NA

N Number of animals.

-- Confidence interval could not be calculated since only one observation was available for this Group on this Study Day.

NA Data not available for this Group at this Study Day.

Table 16b. Test Results for Neutrophils/Lymphocytes Ratio

Neutrophils/Lymphocytes Ratio †								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value #
	1	2	3	4	5	6		
1	0.51 ↓	0.86	0.82	0.88	0.49 ↓	1.06	0.2663	
2	0.67	0.76	0.79	0.90	0.79	3.95 ↑	0.0076 *	0.17 (1<6) 0.0050 0.19 (2<6) 0.0097 0.20 (3<6) 0.0126 0.23 (4<6) 0.0241 0.20 (5<6) 0.0125
3	0.45 ↓	0.60 ↓	0.77	0.55 ↓	1.16	1.60	0.0200 *	0.28 (1<6) 0.0224
7	0.74	0.89	1.01	1.23	1.34	NA	0.5146	
14	0.55 ↓	0.87	0.93	0.80	0.99	NA	0.6374	
21	0.63 ↓	0.59 ↓	0.71	0.56 ↓	0.82	NA	0.8576	

† Indicates that values for this parameter were log-transformed for the analysis.

Cells contain all significant pairwise Group comparisons at the 0.05 level. The format within each cell is: ratio of shifts (relationship between corresponding Group mean shifts) Tukey-adjusted P-value.

↑, ↓ "↑" indicates the geometric mean at the Study Day was significantly greater than that at baseline; "↓" indicates the geometric mean at the Study Day was significantly less than that at baseline (at the 0.05 level).

* The overall Group effect was significant at the 0.05 level.

NA Data not available for this Group at this Study Day.

Table 17a. Descriptive Statistics for Monocytes (10^3 cells/µL) by Group and Study Day

Group	Study Day	N	Geometric Mean (95% Confidence Interval)	Group	Study Day	N	Geometric Mean (95% Confidence Interval)	Group	Study Day	N	Geometric Mean (95% Confidence Interval)
1	-3	5	0.15 (0.08, 0.29)	2	-3	5	0.09 (0.08, 0.11)	3	-3	5	0.11 (0.06, 0.22)
	1	5	0.07 (0.02, 0.25)		1	5	0.08 (0.06, 0.10)		1	5	0.11 (0.06, 0.22)
	2	5	0.06 (0.02, 0.19)		2	5	0.12 (0.06, 0.22)		2	5	0.13 (0.07, 0.24)
	3	5	0.08 (0.02, 0.38)		3	4	0.09 (0.06, 0.15)		3	5	0.11 (0.06, 0.20)
	7	5	0.08 (0.02, 0.25)		7	4	0.08 (0.03, 0.21)		7	5	0.13 (0.06, 0.25)
	14	5	0.06 (0.02, 0.20)		14	5	0.06 (0.03, 0.16)		14	5	0.09 (0.03, 0.26)
	21	5	0.04 (0.03, 0.07)		21	4	0.03 (0.02, 0.05)		21	5	0.05 (0.02, 0.10)

Group	Study Day	N	Geometric Mean (95% Confidence Interval)	Group	Study Day	N	Geometric Mean (95% Confidence Interval)	Group	Study Day	N	Geometric Mean (95% Confidence Interval)
4	-3	5	0.18 (0.12, 0.27)	5	-3	5	0.18 (0.07, 0.43)	6	-3	3	0.14 (0.04, 0.46)
	1	5	0.13 (0.08, 0.20)		1	5	0.09 (0.03, 0.30)		1	4	0.14 (0.10, 0.18)
	2	5	0.11 (0.04, 0.31)		2	5	0.11 (0.04, 0.30)		2	3	0.03 (0.01, 0.09)
	3	5	0.20 (0.11, 0.37)		3	3	0.05 (0.00, 1.53)		3	3	0.04 (0.01, 0.21)
	7	4	0.16 (0.09, 0.29)		7	1	0.08 (--)		7	NA	NA
	14	3	0.14 (0.01, 1.36)		14	1	0.07 (--)		14	NA	NA
	21	3	0.05 (0.01, 0.17)		21	1	0.03 (--)		21	NA	NA

N Number of animals.
-- Confidence interval could not be calculated since only one observation was available for this Group on this Study Day.
NA Data not available for this Group at this Study Day.

Table 17b. Test Results for Monocytes (103 cells/µL)

Monocytes †								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value #
	1	2	3	4	5	6		
1	0.48 ↓	0.85	0.98	0.73	0.54	0.87	0.5115	
2	0.38 ↓	1.27	1.17	0.63	0.61	0.20 ↓	0.0211 *	0.16 (6<2) 0.0291 0.17 (6<3) 0.0408
3	0.53	1.08	0.99	1.14	0.24 ↓	0.23 ↓	0.1599	
7	0.51	0.90	1.13	1.02	1.14	NA	0.5768	
14	0.40	0.69	0.78	0.82	1.00	NA	0.7679	
21	0.29 ↓	0.39 ↓	0.44 ↓	0.29 ↓	0.43	NA	0.7359	

† Indicates that values for this parameter were log-transformed for the analysis.

Cells contain all significant pairwise Group comparisons at the 0.05 level. The format within each cell is: ratio of shifts (relationship between corresponding Group mean shifts) Tukey-adjusted P-value.

↑, ↓ "↑" indicates the geometric mean at the Study Day was significantly greater than that at baseline; "↓" indicates the geometric mean at the Study Day was significantly less than that at baseline (at the 0.05 level).

* The overall Group effect was significant at the 0.05 level.

NA Data not available for this Group at this Study Day.

Table 18a. Descriptive Statistics for Eosinophils (103 cells/µL) by Group and Study Day

Group	Study Day	N	Geometric Mean (95% Confidence Interval)	Group	Study Day	N	Geometric Mean (95% Confidence Interval)	Group	Study Day	N	Geometric Mean (95% Confidence Interval)
1	-3	5	0.16 (0.14, 0.18)	2	-3	5	0.13 (0.10, 0.16)	3	-3	5	0.11 (0.06, 0.19)
	1	5	0.18 (0.15, 0.21)		1	5	0.14 (0.10, 0.20)		1	5	0.15 (0.09, 0.24)
	2	5	0.13 (0.10, 0.17)		2	5	0.11 (0.09, 0.14)		2	5	0.14 (0.07, 0.25)
	3	5	0.12 (0.06, 0.25)		3	4	0.10 (0.08, 0.14)		3	5	0.14 (0.08, 0.22)
	7	5	0.15 (0.10, 0.24)		7	4	0.13 (0.10, 0.17)		7	5	0.16 (0.09, 0.28)
	14	5	0.12 (0.07, 0.20)		14	5	0.15 (0.12, 0.20)		14	5	0.13 (0.08, 0.23)
	21	5	0.12 (0.10, 0.15)		21	4	0.11 (0.07, 0.17)		21	5	0.09 (0.05, 0.17)

Group	Study Day	N	Geometric Mean (95% Confidence Interval)	Group	Study Day	N	Geometric Mean (95% Confidence Interval)	Group	Study Day	N	Geometric Mean (95% Confidence Interval)
4	-3	5	0.10 (0.08, 0.13)	5	-3	5	0.15 (0.10, 0.23)	6	-3	3	0.10 (0.03, 0.28)
	1	5	0.11 (0.09, 0.14)		1	5	0.16 (0.11, 0.25)		1	4	0.13 (0.08, 0.21)
	2	5	0.13 (0.09, 0.17)		2	5	0.09 (0.05, 0.16)		2	3	0.06 (0.03, 0.11)
	3	5	0.10 (0.07, 0.14)		3	3	0.09 (0.03, 0.29)		3	3	0.07 (0.02, 0.26)
	7	4	0.17 (0.13, 0.22)		7	1	0.27 (--)		7	NA	NA
	14	3	0.10 (0.06, 0.19)		14	1	0.14 (--)		14	NA	NA
	21	3	0.10 (0.06, 0.19)		21	1	0.15 (--)		21	NA	NA

N Number of animals.
-- Confidence interval could not be calculated since only one observation was available for this Group on this Study Day.
NA Data not available for this Group at this Study Day.

Table 18b. Test Results for Eosinophils (10^3 cells/µL)

Eosinophils †								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value #
	1	2	3	4	5	6		
1	1.12	1.1	1.41 ↑	1.04	1.05	1.24	0.3456	
2	0.83	0.88	1.30	1.22	0.57 ↓	0.58 ↓	0.0270 *	0.44 (5<3) 0.0495
3	0.77	0.76	1.27	0.97	0.58 ↓	0.73	0.2324	
7	0.96	0.94	1.54 ↑	1.75 ↑	1.80	NA	0.0153 *	0.55 (1<4) 0.0470
14	0.76	1.20	1.27	0.99	0.93	NA	0.1025	
21	0.79 ↓	0.82	0.84	0.75	1.00	NA	0.8583	

† Indicates that values for this parameter were log-transformed for the analysis.

Cells contain all significant pairwise Group comparisons at the 0.05 level. The format within each cell is: ratio of shifts (relationship between corresponding Group mean shifts) Tukey-adjusted P-value.

↑, ↓ "↑" indicates the geometric mean at the Study Day was significantly greater than that at baseline; "↓" indicates the geometric mean at the Study Day was significantly less than that at baseline (at the 0.05 level).

* The overall Group effect was significant at the 0.05 level.

NA Data not available for this Group at this Study Day.

Table 19a. Descriptive Statistics for Basophils (10^3 cells/µL) by Group and Study Day

Group	Study Day	N	Geometric Mean (95% Confidence Interval)	Group	Study Day	N	Geometric Mean (95% Confidence Interval)	Group	Study Day	N	Geometric Mean (95% Confidence Interval)
1	-3	5	0.30 (0.17, 0.55)	2	-3	5	0.27 (0.17, 0.41)	3	-3	5	0.33 (0.16, 0.65)
	1	5	0.33 (0.19, 0.56)		1	5	0.24 (0.19, 0.30)		1	5	0.37 (0.17, 0.78)
	2	5	0.25 (0.18, 0.36)		2	5	0.26 (0.19, 0.34)		2	5	0.37 (0.15, 0.91)
	3	5	0.21 (0.07, 0.60)		3	4	0.21 (0.15, 0.29)		3	5	0.36 (0.15, 0.89)
	7	5	0.23 (0.12, 0.44)		7	4	0.21 (0.12, 0.38)		7	5	0.39 (0.19, 0.78)
	14	5	0.17 (0.07, 0.46)		14	5	0.28 (0.18, 0.42)		14	5	0.29 (0.11, 0.80)
	21	5	0.21 (0.13, 0.37)		21	4	0.18 (0.10, 0.34)		21	5	0.22 (0.08, 0.62)

Group	Study Day	N	Geometric Mean (95% Confidence Interval)	Group	Study Day	N	Geometric Mean (95% Confidence Interval)	Group	Study Day	N	Geometric Mean (95% Confidence Interval)
4	-3	5	0.24 (0.18, 0.33)	5	-3	5	0.29 (0.19, 0.44)	6	-3	3	0.27 (0.18, 0.41)
	1	5	0.27 (0.18, 0.41)		1	5	0.29 (0.18, 0.45)		1	4	0.37 (0.20, 0.68)
	2	5	0.25 (0.14, 0.45)		2	5	0.20 (0.14, 0.28)		2	3	0.14 (0.07, 0.29)
	3	5	0.24 (0.16, 0.38)		3	3	0.18 (0.07, 0.50)		3	3	0.11 (0.03, 0.42)
	7	4	0.33 (0.20, 0.55)		7	1	0.52 (--)		7	NA	NA
	14	3	0.30 (0.19, 0.47)		14	1	0.28 (--)		14	NA	NA
	21	3	0.19 (0.11, 0.34)		21	1	0.28 (--)		21	NA	NA

N Number of animals.
-- Confidence interval could not be calculated since only one observation was available for this Group on this Study Day.
NA Data not available for this Group at this Study Day.

Table 19b. Test Results for Basophils (10^3 cells/µL)

Basophils †								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value #
	1	2	3	4	5	6		
1	1.09	0.89	1.12	1.10	1.00	1.23	0.4322	
2	0.84	0.96	1.12	1.02	0.68 ↓	0.47 ↓	0.0488 *	0.42 (6<3) 0.0476
3	0.70	0.80	1.10	1.00	0.72	0.37 ↓	0.0611	0.34 (6<3) 0.0385
7	0.76 ↓	0.80	1.19	1.38 ↑	1.86 ↑	NA	0.0091 *	0.55 (1<4) 0.0382
14	0.58 ↓	1.04	0.89	1.31	1.00	NA	0.1333	
21	0.71 ↓	0.70 ↓	0.68 ↓	0.84	1.00	NA	0.6564	

† Indicates that values for this parameter were log-transformed for the analysis.

Cells contain all significant pairwise Group comparisons at the 0.05 level. The format within each cell is: ratio of shifts (relationship between corresponding Group mean shifts) Tukey-adjusted P-value.

↑, ↓ "↑" indicates the geometric mean at the Study Day was significantly greater than that at baseline; "↓" indicates the geometric mean at the Study Day was significantly less than that at baseline (at the 0.05 level).

* The overall Group effect was significant at the 0.05 level.

NA Data not available for this Group at this Study Day.

Table 20. Proportion of Animals that were Abnormal with Exact 95 % Confidence Interval by Parameter and Group

Parameter	Group	Number Abnormal / N	Proportion Abnormal (95% Confidence Interval)
Red Blood Cell Count	1	2/5	0.40 (0.05, 0.85)
	2	0/5	0.00 (0.00, 0.52)
	3	0/5	0.00 (0.00, 0.52)
	4	1/5	0.20 (0.01, 0.72)
	5	1/5	0.20 (0.01, 0.72)
	6	1/4	0.25 (0.01, 0.81)
Hemoglobin	1	5/5	1.00 (0.48, 1.00)
	2	4/5	0.80 (0.28, 0.99)
	3	2/5	0.40 (0.05, 0.85)
	4	3/5	0.60 (0.15, 0.95)
	5	4/5	0.80 (0.28, 0.99)
	6	2/4	0.50 (0.07, 0.93)
Hematocrit	1	3/5	0.60 (0.15, 0.95)
	2	2/5	0.40 (0.05, 0.85)
	3	3/5	0.60 (0.15, 0.95)
	4	4/5	0.80 (0.28, 0.99)
	5	3/5	0.60 (0.15, 0.95)
	6	2/4	0.50 (0.07, 0.93)
Mean Corpuscular Volume	1	0/5	0.00 (0.00, 0.52)
	2	0/5	0.00 (0.00, 0.52)
	3	0/5	0.00 (0.00, 0.52)
	4	1/5	0.20 (0.01, 0.72)
	5	1/5	0.20 (0.01, 0.72)
	6	0/4	0.00 (0.00, 0.60)
Mean Corpuscular Hemoglobin	1	0/5	0.00 (0.00, 0.52)
	2	0/5	0.00 (0.00, 0.52)
	3	0/5	0.00 (0.00, 0.52)
	4	1/5	0.20 (0.01, 0.72)
	5	0/5	0.00 (0.00, 0.52)
	6	0/4	0.00 (0.00, 0.60)
Mean Corpuscular Hemoglobin Concentration	1	4/5	0.80 (0.28, 0.99)
	2	3/5	0.60 (0.15, 0.95)
	3	5/5	1.00 (0.48, 1.00)
	4	3/5	0.60 (0.15, 0.95)
	5	2/5	0.40 (0.05, 0.85)
	6	0/4	0.00 (0.00, 0.60)
Red Cell Distribution Width	1	4/5	0.80 (0.28, 0.99)
	2	4/5	0.80 (0.28, 0.99)
	3	3/5	0.60 (0.15, 0.95)
	4	2/5	0.40 (0.05, 0.85)
	5	1/5	0.20 (0.01, 0.72)
	6	0/4	0.00 (0.00, 0.60)
Platelet Count	1	4/5	0.80 (0.28, 0.99)
	2	4/5	0.80 (0.28, 0.99)
	3	2/5	0.40 (0.05, 0.85)
	4	0/5	0.00 (0.00, 0.52)
	5	5/5	1.00 (0.48, 1.00)
	6	1/4	0.25 (0.01, 0.81)

Table 20. (Continued)

Parameter	Group	Number Abnormal / N	Proportion Abnormal (95% Confidence Interval)
Mean Platelet Volume	1	5/5	1.00 (0.48, 1.00)
	2	5/5	1.00 (0.48, 1.00)
	3	4/5	0.80 (0.28, 0.99)
	4	5/5	1.00 (0.48, 1.00)
	5	3/5	0.60 (0.15, 0.95)
	6	2/4	0.50 (0.07, 0.93)
White Blood Cell Count	1	4/5	0.80 (0.28, 0.99)
	2	0/5	0.00 (0.00, 0.52)
	3	1/5	0.20 (0.01, 0.72)
	4	2/5	0.40 (0.05, 0.85)
	5	3/5	0.60 (0.15, 0.95)
	6	1/4	0.25 (0.01, 0.81)
Neutrophils	1	2/5	0.40 (0.05, 0.85)
	2	0/5	0.00 (0.00, 0.52)
	3	0/5	0.00 (0.00, 0.52)
	4	3/5	0.60 (0.15, 0.95)
	5	2/5	0.40 (0.05, 0.85)
	6	2/4	0.50 (0.07, 0.93)
Lymphocytes	1	1/5	0.20 (0.01, 0.72)
	2	0/5	0.00 (0.00, 0.52)
	3	0/5	0.00 (0.00, 0.52)
	4	1/5	0.20 (0.01, 0.72)
	5	2/5	0.40 (0.05, 0.85)
	6	1/4	0.25 (0.01, 0.81)
Neutrophils/Lymphocytes Ratio	1	3/5	0.60 (0.15, 0.95)
	2	0/5	0.00 (0.00, 0.52)
	3	0/5	0.00 (0.00, 0.52)
	4	1/5	0.20 (0.01, 0.72)
	5	2/5	0.40 (0.05, 0.85)
	6	1/4	0.25 (0.01, 0.81)
Monocytes	1	5/5	1.00 (0.48, 1.00)
	2	2/5	0.40 (0.05, 0.85)
	3	2/5	0.40 (0.05, 0.85)
	4	3/5	0.60 (0.15, 0.95)
	5	3/5	0.60 (0.15, 0.95)
	6	2/4	0.50 (0.07, 0.93)
Eosinophils	1	3/5	0.60 (0.15, 0.95)
	2	0/5	0.00 (0.00, 0.52)
	3	2/5	0.40 (0.05, 0.85)
	4	1/5	0.20 (0.01, 0.72)
	5	3/5	0.60 (0.15, 0.95)
	6	0/4	0.00 (0.00, 0.60)
Basophils	1	2/5	0.40 (0.05, 0.85)
	2	0/5	0.00 (0.00, 0.52)
	3	1/5	0.20 (0.01, 0.72)
	4	0/5	0.00 (0.00, 0.52)
	5	1/5	0.20 (0.01, 0.72)
	6	1/4	0.25 (0.01, 0.81)

N = number of animals

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Table 21. Results of Overall Two-sided Fisher's Exact Tests for the Proportion of Animals that were Abnormal by Parameter

Parameter	Group Effect P-Value
Red Blood Cell Count	0.6579
Hemoglobin	0.3947
Hematocrit	0.9499
Mean Corpuscular Volume	1.0000
Mean Corpuscular Hemoglobin	1.0000
Mean Corpuscular Hemoglobin Concentration	0.0638
Red Cell Distribution Width	0.1029
Platelet Count	0.0081 *
Mean Platelet Volume	0.1821
White Blood Cell Count	0.1361
Neutrophils	0.1937
Lymphocytes	0.6579
Neutrophils/Lymphocytes Ratio	0.2039
Monocytes	0.4344
Eosinophils	0.1757
Basophils	0.6579

* Comparison significant at the 0.05 level.

Table 22. Results of One-sided Pairwise Fisher's Exact Tests for the Proportion of Animals with Abnormal Platelet Counts

Parameter	Group	One-Sided Pairwise Fisher's Exact Test P-Values									
		Unadjusted P-Values					Bonferroni-Holm Adjusted P-Values				
		2	3	4	5	6	2	3	4	5	6
Platelet Count	1	0.7778	0.9762	1.0000	0.5000	0.9921	1.0000	1.0000	1.0000	1.0000	1.0000
	2		0.9762	1.0000	0.5000	0.9921		1.0000	1.0000	1.0000	1.0000
	3			1.0000	0.0833	0.8810			1.0000	1.0000	1.0000
	4				0.0040 *	0.4444				0.0595	1.0000
	5					1.0000					1.0000

a A p-value of 1.0000 was substituted when all animals in both Groups experienced the same abnormality result.

* Comparison significant at the 0.05 level.

Table 23. Results of Overall Log-rank Tests for Time to Abnormality by Parameter

Parameter	Group Effect P-value
Red Blood Cell Count	0.5648
Hemoglobin	0.2279
Hematocrit	0.8633
Mean Corpuscular Volume	0.5460
Mean Corpuscular Hemoglobin	0.4408
Mean Corpuscular Hemoglobin Concentration	0.0830
Red Cell Distribution Width	0.1375
Platelet Count	0.0027 *
Mean Platelet Volume	0.4142
White Blood Cell Count	0.0685
Neutrophils	0.2612
Lymphocytes	0.5447
Neutrophils/Lymphocytes Ratio	0.1539
Monocytes	0.0921
Eosinophils	0.0817
Basophils	0.5524

* Comparison significant at the 0.05 level.

Table 24. Results of Pairwise Log-rank Tests for Time to Abnormal Platelet Count

Parameter	Group	Pairwise Log-rank Test P-values									
		Unadjusted P-Values					Bonferroni-Holm Adjusted P-Values				
		2	3	4	5	6	2	3	4	5	6
Platelet Count	1	0.4305	0.0854	0.0133 *	0.4221	0.1844	1.0000	1.0000	0.3851	1.0000	1.0000
	2		0.1639	0.0135 *	0.0927	0.2297		1.0000	0.3851	1.0000	1.0000
	3			0.1343	0.0142 *	0.7710			1.0000	0.3851	1.0000
	4				0.0015 *	0.2636				0.0463 *	1.0000
	5					0.0374 *					0.9343

* Comparison significant at the 0.05 level.

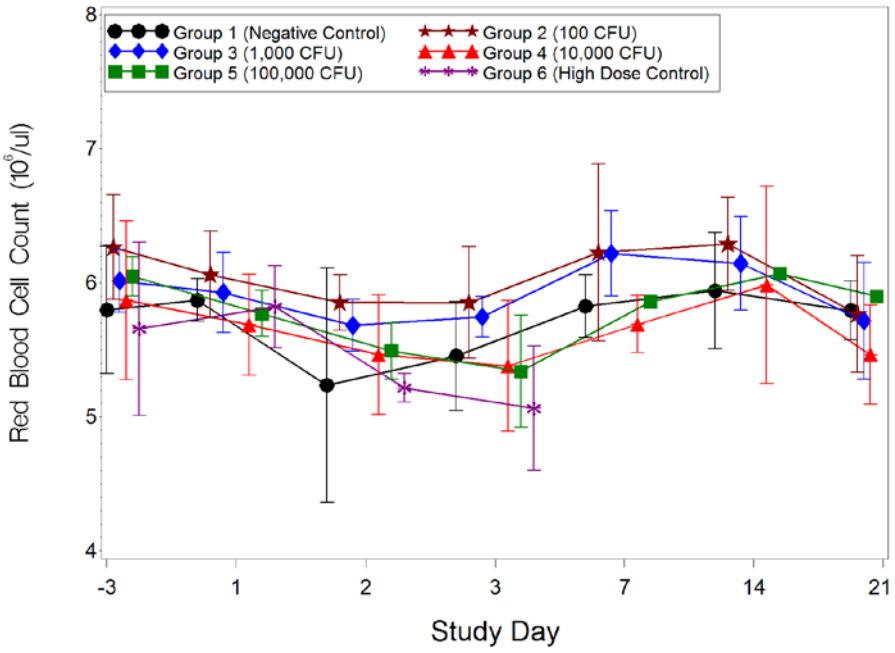

Figure 1. Plot of Red Blood Cell Count over time.

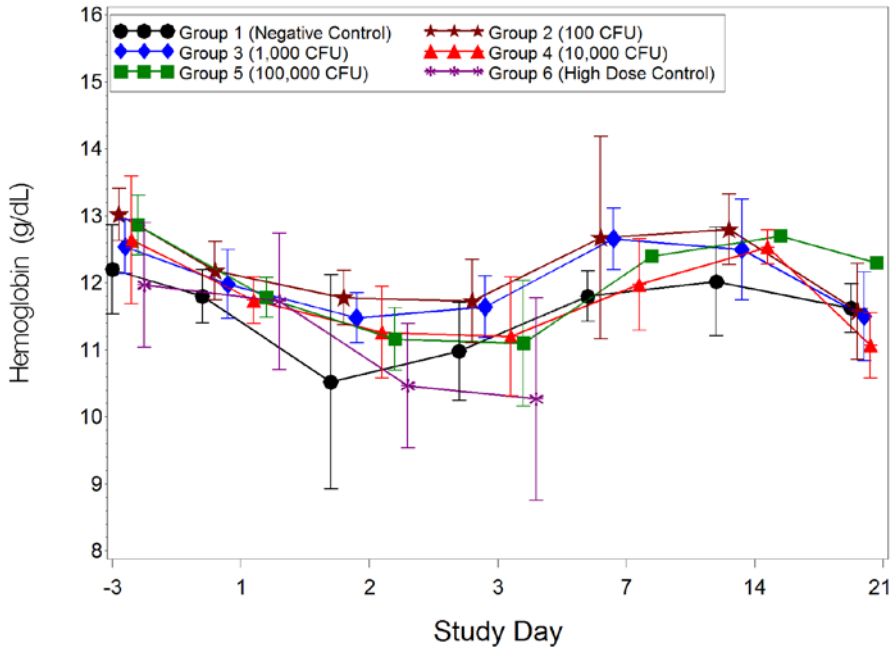

Figure 2. Plot of Hemoglobin over time.

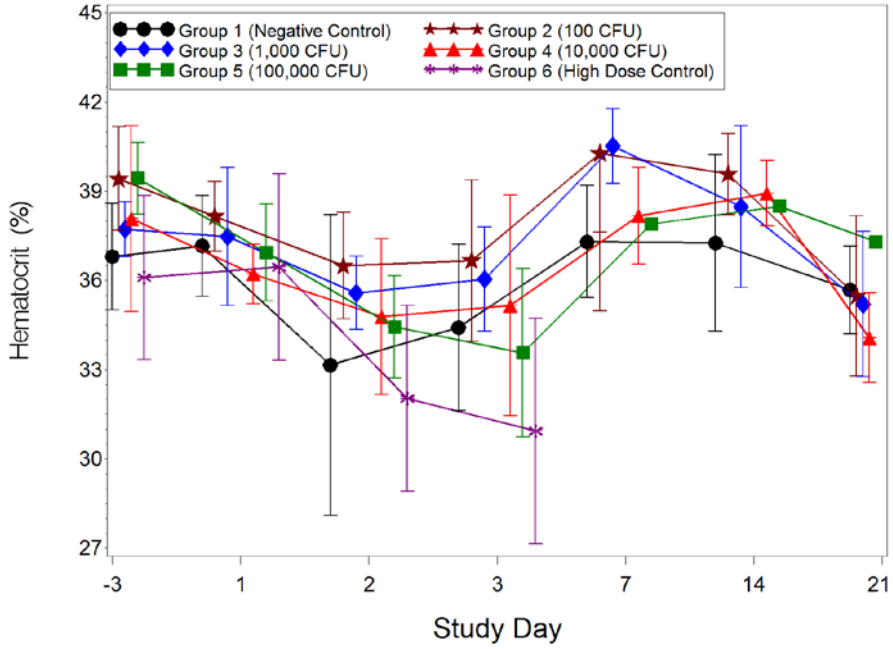

Figure 3. Plot of Hematocrit over time.

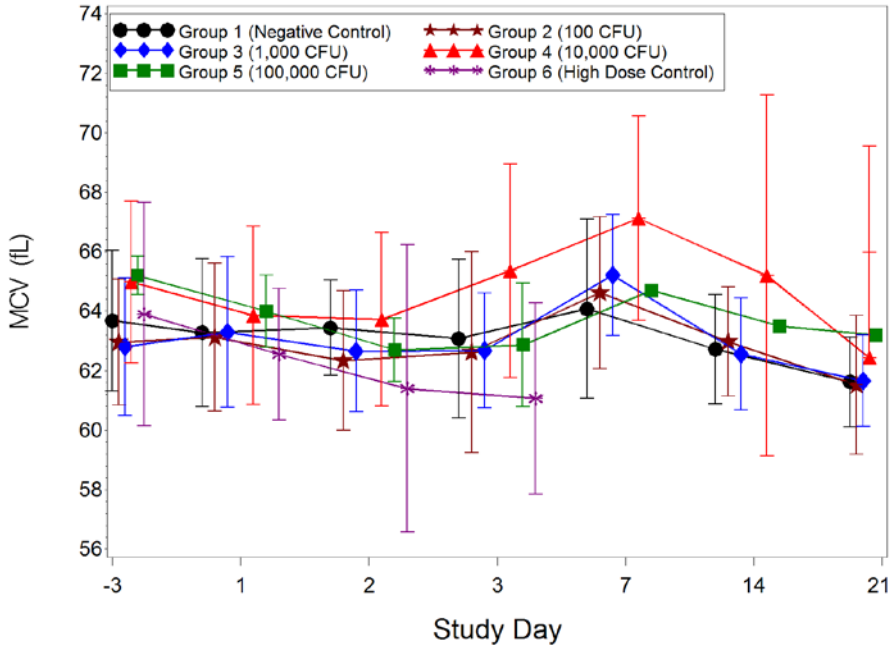

Figure 4. Plot of Mean Corpuscular Volume (MCV) over time.

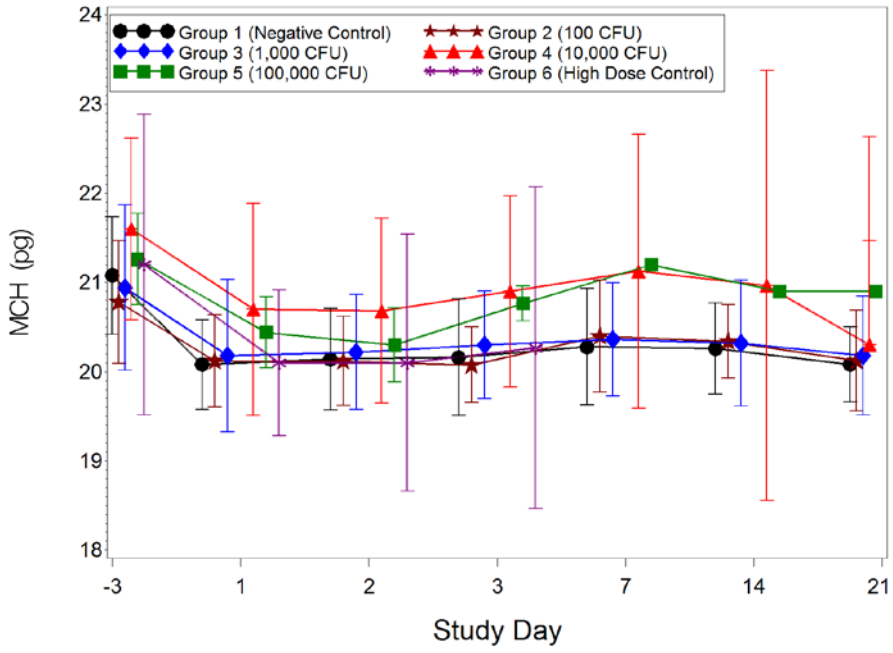

Figure 5. Plot of Mean Corpuscular Hemoglobin (MCH) over time.

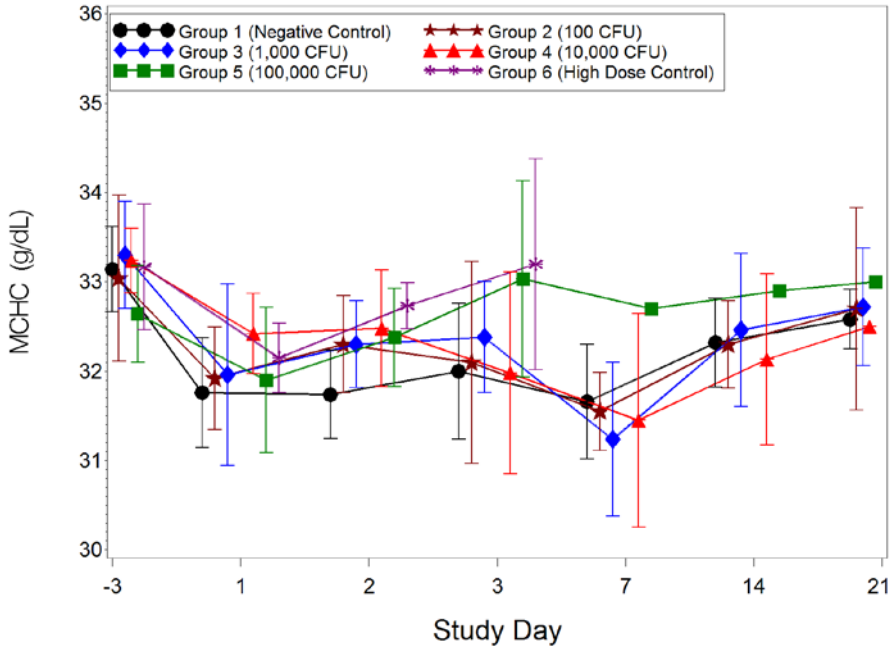

Figure 6. Plot of Mean Corpuscular Hemoglobin Concentration (MCHC) over time.

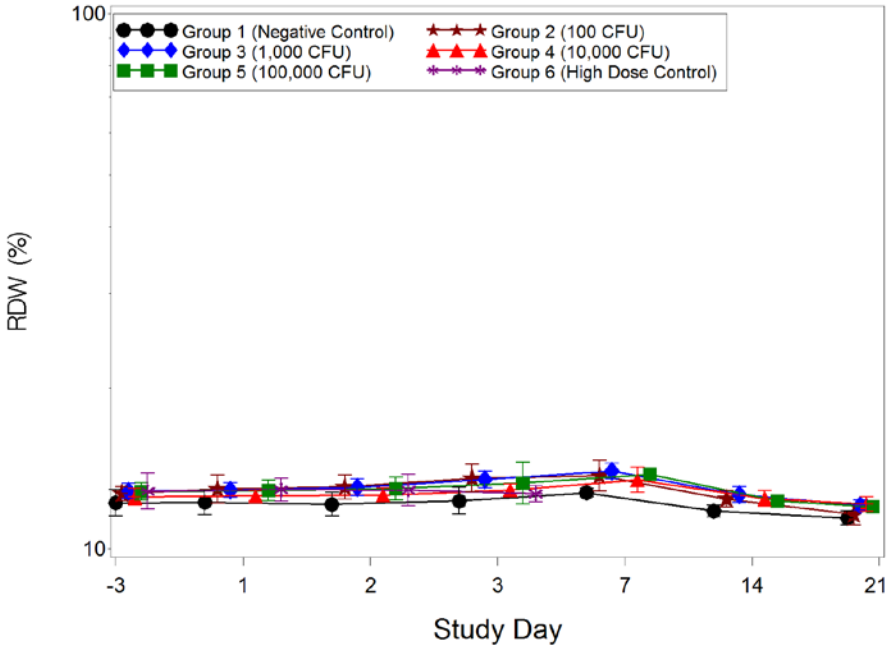
Group 1 (Negative Control)
Group 2 (100 CFU)
Group 3 (1,000 CFU)
Group 4 (10,000 CFU)
Group 5 (100,000 CFU)
Group 6 (High Dose Control)
RDW (%)
100
10
-3
1
2
3
7
14
21
Study Day

Figure 7. Plot of Red Cell Distribution Width (RDW) over time.

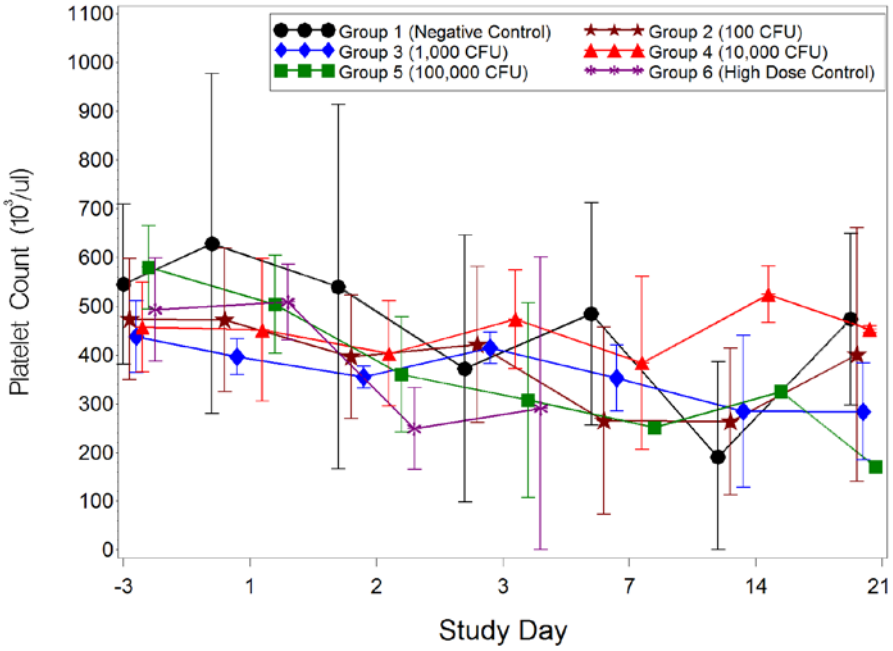

Figure 8. Plot of Platelet Count over time.

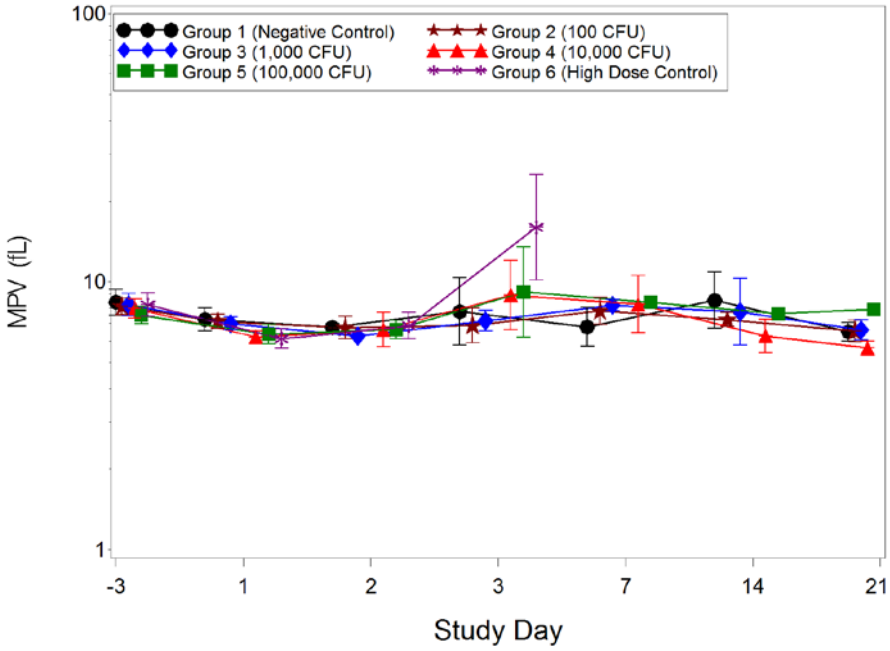

Figure 9. Plot of Mean Platelet Volume (MPV) over time.

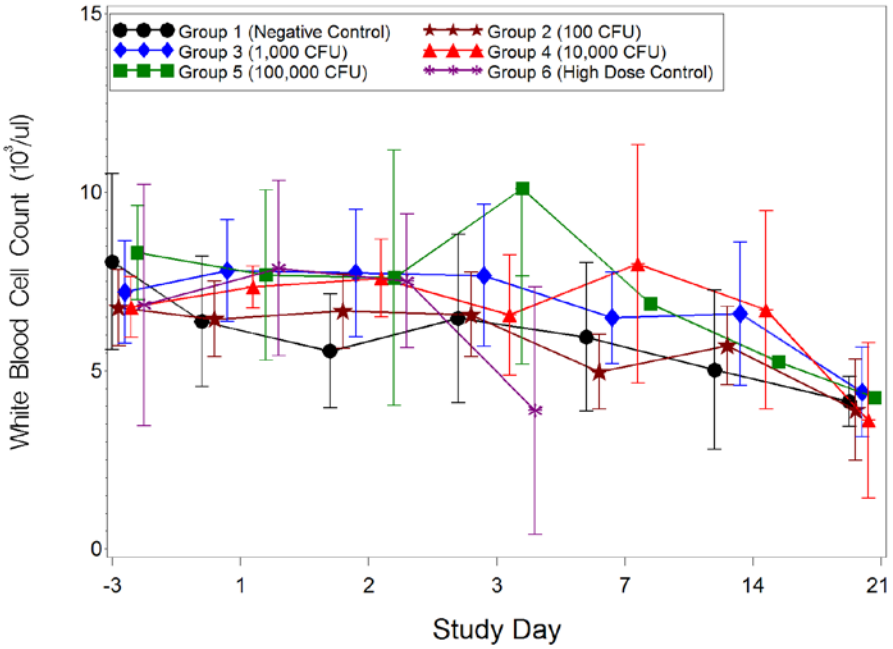

Figure 10. Plot of White Blood Cell Count over time.

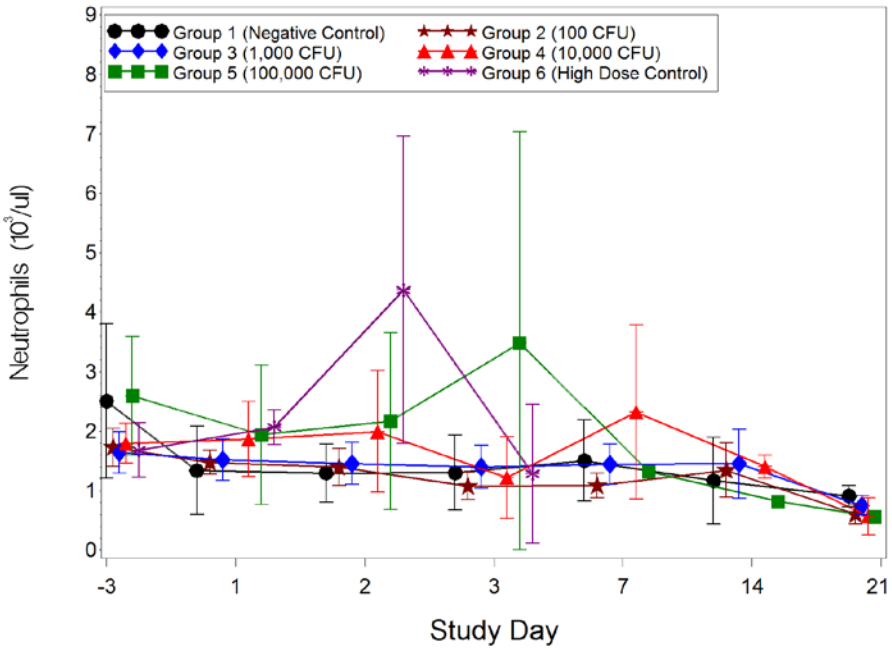

Figure 11. Plot of Neutrophils over time.

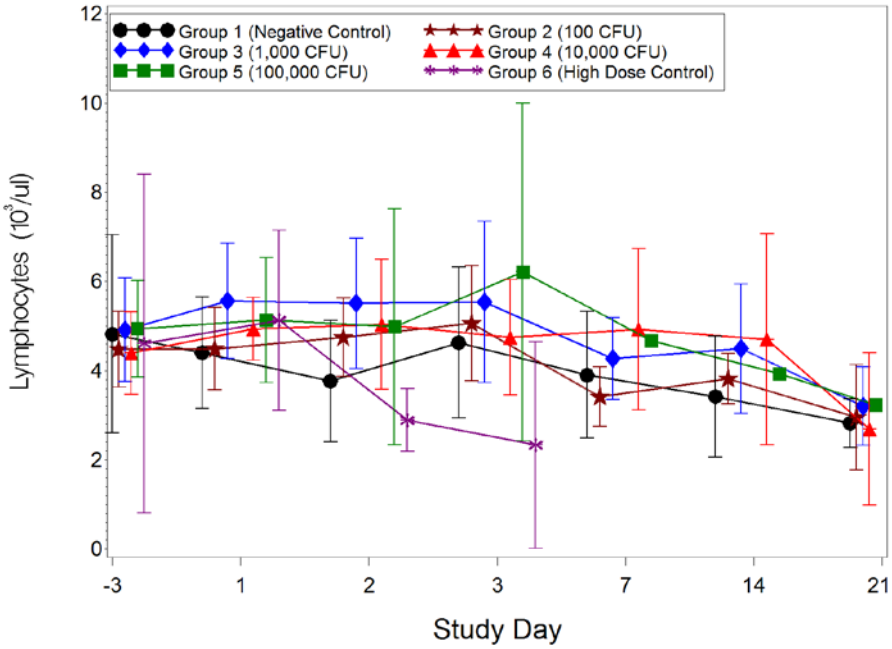

Figure 12. Plot of Lymphocytes over time.

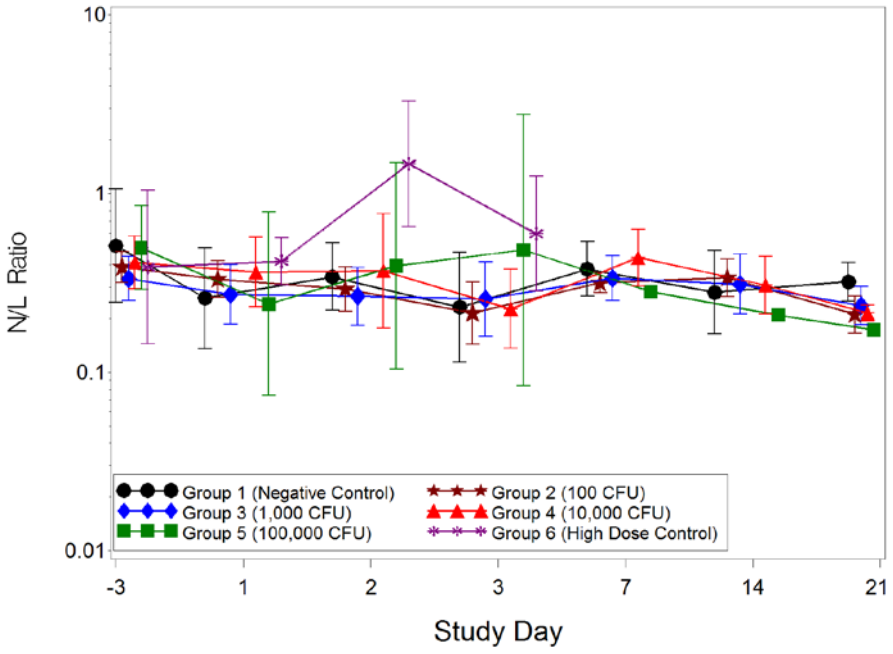

Figure 13. Plot of Neutrophils/Lymphocytes (N/L) Ratio over time.

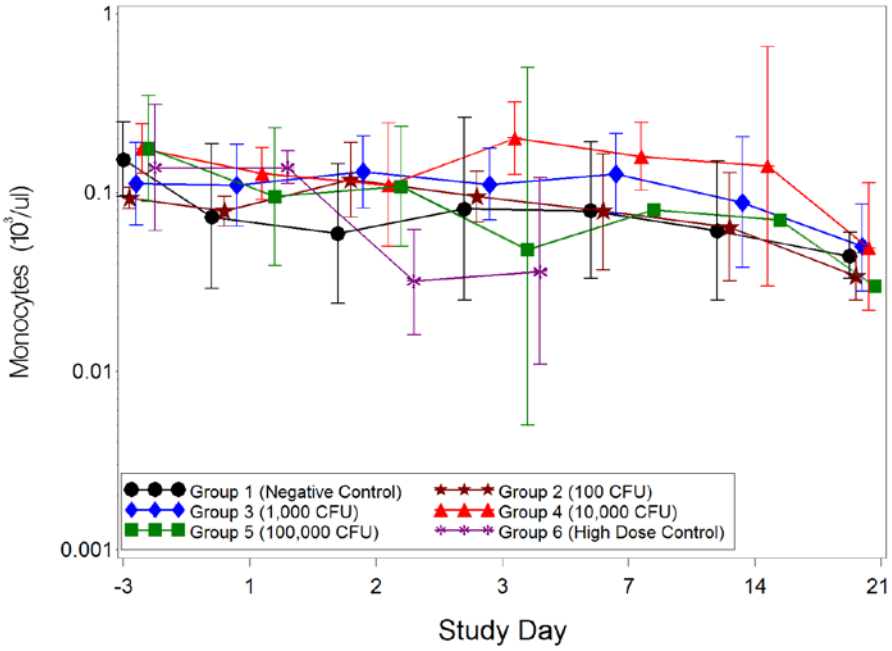

Figure 14. Plot of Monocytes over time.

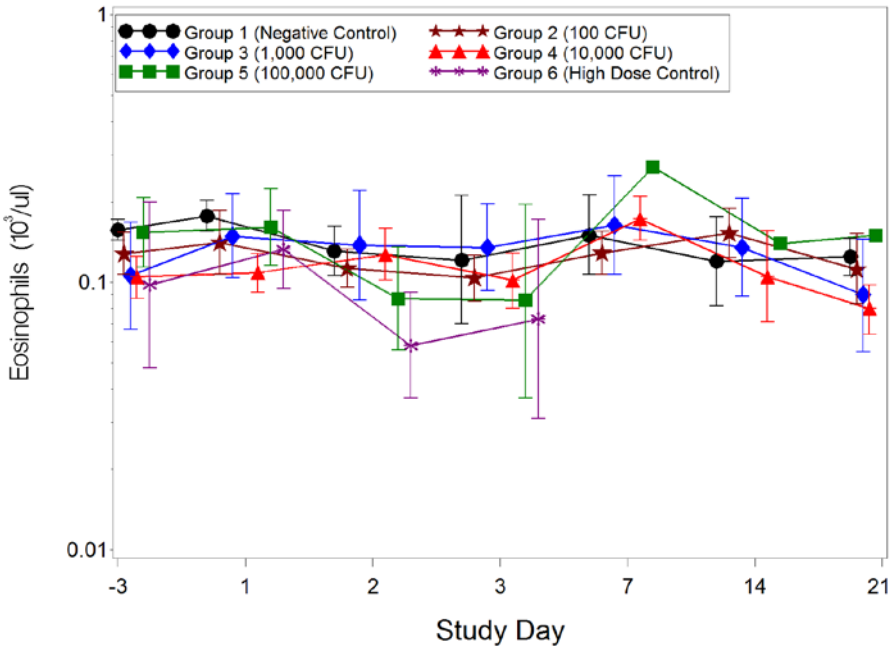

Figure 15. Plot of Eosinophils over time.

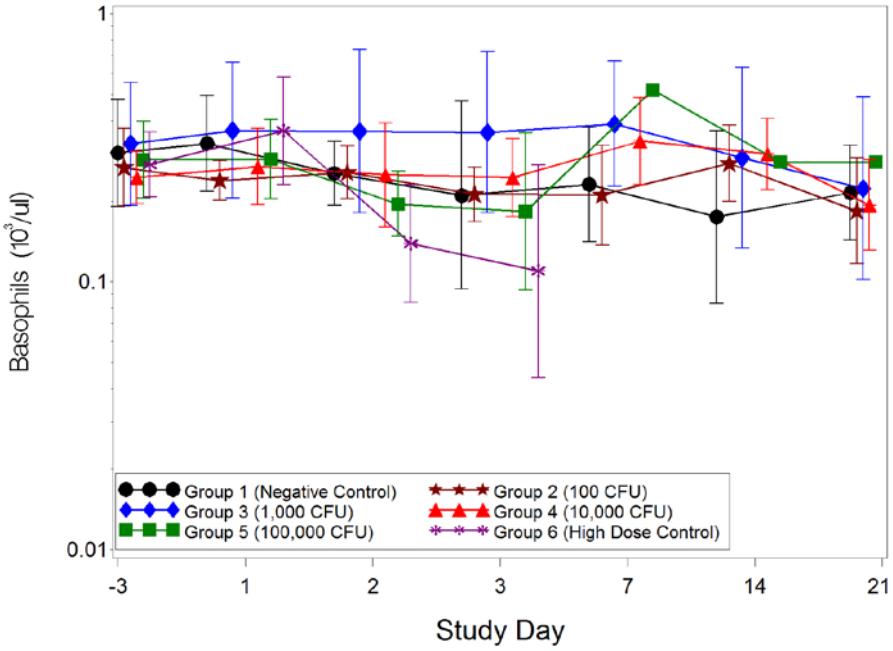

Figure 16. Plot of Basophils over time.

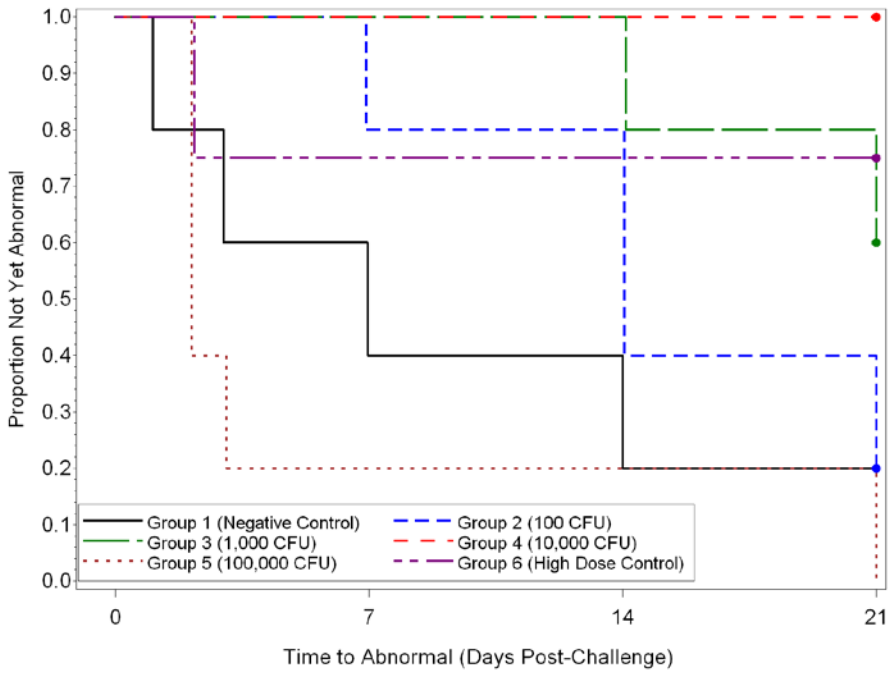

Figure 17. Kaplan-Meier curves representing time to abnormal Platelet Count for each Group.

ATTACHMENT 1:

RESULTS OF ANALYSIS REPEATED WITH POTENTIAL OUTLIERS EXCLUDED

ATTACHMENT 1: RESULTS OF ANALYSIS REPEATED WITH POTENTIAL OUTLIERS EXCLUDED

Nine potential outliers were identified in Table 2 of the report. To determine the effect of the potential outliers on the statistical analysis, the analysis was performed on the data with these observations excluded. The results that had a change in significance after excluding the potential outliers are presented below.

Tables 1-1 through 1-5 contain test results for those parameters that experienced changes in significance due to the exclusion of the potential outliers, when compared to the corresponding results shown in Tables 4b through 19b where the potential outliers were not excluded. Table entries are shown in bold if the significance changed in comparison to the corresponding results shown in Tables 4b through 19b. With the potential outliers excluded, the following changes in significance were noted:

- **RBC (Table 1-1)**: On Study Day 3 there was a significant decrease from baseline in Group 3.

- **HCT (Table 1-2)**: On Study Day 2 there was a significant decrease from baseline in Group 3 and there was no longer a significant decrease from baseline in Group 1.

- **MPV (Table 1-3)**: On Study Day 3 there was a significant decrease as a proportion of baseline in Group 1 and a significant increase as a proportion in Group 5. Additionally, the mean increase as a proportion of baseline for Group 5 was significantly different from the mean decrease as a proportion of baseline for Groups 1 and 3, and the mean increase as a proportion of baseline for Group 6 was significantly greater than that for Group 5. On Study Day 14 there was a significant decrease as a proportion of baseline in Groups 2, 3, and 4.

- **Neutrophils (Table 1-4)**: On Study Day 3 there was a significant increase from baseline in Group 5, and the mean increase from baseline in Group 5 was significantly different from the mean decrease from baseline in Group 4.

- **N/L Ratio (Table 1-5)**: On Study Day 1 there was no longer a significant decrease as a proportion of baseline in Groups. Additionally, the mean decrease as a proportion of baseline for Group 1 was significantly different from the mean increase as a proportion of baseline for Group 6. On Study Day 2, Group 5 had a significant decrease as a proportion of baseline.

Table 1-1. Test Results for Red Blood Cell Count (RBC, 10^6 cells/µL) with Potential Outliers Excluded

Red Blood Cell Count								
Study Day	Mean Shift from Baseline, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
	1	2	3	4	5	6		
2	-0.33 ↓	-0.41 ↓	**-0.33** ↓	-0.41 ↓	-0.56 ↓	-0.68 ↓	0.5729	

Cells contain all significant pairwise Group comparisons at the 0.05 level. The format within each cell is: difference of shifts (relationship between corresponding Group mean shifts) Tukey-adjusted P-value.

↑, ↓ "↑" indicates the mean at the Study Day was significantly greater than that at baseline; "↓" indicates the mean at the Study Day was significantly less than that at baseline (at the 0.05 level).

Table 1-2. Test Results for Hematocrit (HCT, %) with Potential Outliers Excluded

Hematocrit								
Study Day	Mean Shift from Baseline, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
	1	2	3	4	5	6		
2	**-2.0**	-2.9 ↓	**-2.1** ↓	-3.3 ↓	-5.0 ↓	-5.2 ↓	0.2033	

Cells contain all significant pairwise Group comparisons at the 0.05 level. The format within each cell is: difference of shifts (relationship between corresponding Group mean shifts) Tukey-adjusted P-value.

↑, ↓ "↑" indicates the mean at the Study Day was significantly greater than that at baseline; "↓" indicates the mean at the Study Day was significantly less than that at baseline (at the 0.05 level).

Table 1-3. Test Results for Mean Platelet Volume (MPV, fL) with Potential Outliers Excluded

Mean Platelet Volume †								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value #
	1	2	3	4	5	6		
3	**0.84** ↓	0.86	0.87	1.01	**1.23** ↑	2.00 ↑	<0.0001*	**0.68 (1<5) 0.0376** 0.42 (1<6) <0.0001 0.43 (2<6) <0.0001 **0.70 (3<5) 0.0491** 0.43 (3<6) <0.0001 0.50 (4<6) 0.0002 **0.62 (5<6) 0.0107**
14	1.02	**0.89** ↓	**0.82** ↓	**0.80** ↓	1.00	NA	0.0605	

† Indicates that values for this parameter were log-transformed for the analysis.

Cells contain all significant pairwise Group comparisons at the 0.05 level. The format within each cell is: ratio of shifts (relationship between corresponding Group mean shifts) Tukey-adjusted P-value.

↑, ↓ "↑" indicates the geometric mean at the Study Day was significantly greater than that at baseline; "↓" indicates the geometric mean at the Study Day was significantly less than that at baseline (at the 0.05 level).

* The overall Group effect was significant at the 0.05 level.

NA Data not available for this Group at this Study Day.

Table 1-4. Test Results for Neutrophils (10^3 cells/µL) with Potential Outliers Excluded

Neutrophils								
Study Day	Mean Shift from Baseline, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
	1	2	3	4	5	6		
3	-1.20 ↓	-0.53	-0.24	-0.58	**1.47** ↑	-0.41	0.0156 *	-2.67 (1<5) 0.0049 **-2.05 (4<5) 0.0398**

Cells contain all significant pairwise Group comparisons at the 0.05 level. The format within each cell is: difference of shifts (relationship between corresponding Group mean shifts) Tukey-adjusted P-value.

↑, ↓ "↑" indicates the mean at the Study Day was significantly greater than that at baseline; "↓" indicates the mean at the Study Day was significantly less than that at baseline (at the 0.05 level).

* The overall Group effect was significant at the 0.05 level.

Table 1-5. Test Results for Neutrophils/Lymphocytes Ratio with Potential Outliers Excluded

Neutrophils/Lymphocytes Ratio †								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value #
	1	2	3	4	5	6		
1	0.51 ↓	0.86	0.82	0.88	**0.82**	1.06	0.0684	**0.48 (1<6) 0.0429**
2	0.67	0.76	0.79	0.90	**0.55** ↓	3.95 ↑	0.0005 *	0.17 (1<6) 0.0006 0.19 (2<6) 0.0014 0.20 (3<6) 0.0019 0.23 (4<6) 0.0043 0.14 (5<6) 0.0003

† Indicates that values for this parameter were log-transformed for the analysis.

Cells contain all significant pairwise Group comparisons at the 0.05 level. The format within each cell is: ratio of shifts (relationship between corresponding Group mean shifts) Tukey-adjusted P-value.

↑, ↓ "↑" indicates the geometric mean at the Study Day was significantly greater than that at baseline; "↓" indicates the geometric mean at the Study Day was significantly less than that at baseline (at the 0.05 level).

* The overall Group effect was significant at the 0.05 level.

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CLINICAL CHEMISTRY STATISTICAL REPORT

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List of Acronyms

ALT ..... Alanine Aminotransferase
ANOVA ..... Analysis of variance
AST ..... Aspartate Aminotransferase
BBRC ..... Battelle Biomedical Research Center
BUN ..... Blood Urea Nitrogen
CFU ..... Colony forming unit
CRP ..... C-Reactive Protein
dL ..... deciliter
g ..... gram
LD_{50} ..... Median lethal dose
LDH ..... Lactate Dehydrogenase
L ..... liter
LOD ..... limit of detection
mEq ..... milliequivalents
mg ..... milligram
N ..... Number of animals
SDH ..... Sorbitol Dehydrogenase
U/L ..... units/liter

1. Introduction

This report summarizes the statistical analysis of clinical chemistry data collected under Battelle Biomedical Research Center (BBRC) Study No. 1020-CG920503. Thirty (30) male pathogen-free New Zealand White rabbits *(Oryctolagus cuniculus)* were randomized into six Groups, with each Group having five animals. Animals were aerosol challenged on Study Day 0 with *Bacillus anthracis* (Ames strain) spores as indicated in Table 1.

Table 1. Study Design

Group	Number of Animals per Group	Spore Dose Colony Forming Units (CFU)	Clinical Chemistry Blood Collection Study Days
1[a]	5	100 x LD_{50}	-3, 1, 2, 3, 7, 14, 21, and Terminal
2	5	100	
3	5	1,000	
4	5	10,000	
5	5	100,000	
6[b]	5	100 x LD_{50}	

a Spores are gamma-irradiated (negative control)
b High dose control
LD_{50} Median lethal dose

Blood samples were collected for clinical chemistry analysis as indicated in Table 1. Blood collection on study Day -3 served as a pre-challenge baseline for each animal.

The clinical chemistry parameters that were included in this analysis are:

- C-reactive protein (CRP, mg/dL)
- Total bilirubin (mg/dL)
- Aspartate aminotransferase (AST, U/L)
- Alanine aminotransferase (ALT, U/L)
- Lactate dehydrogenase (LDH, U/L)
- Sorbitol dehydrogenase (SDH, U/L)
- Total protein (g/dL)
- Blood urea nitrogen (BUN, mg/dL)
- Creatinine (mg/dL)

- BUN/creatinine ratio
- Sodium (mEq/L)
- Potassium (mEq/L)
- Chloride (mEq/L)
- Calcium (mg/dL)
- Phosphorus (mg/dL)

Clinical chemistry analysis was performed at six levels:

1. Descriptive statistics (arithmetic or geometric means and 95% confidence intervals) were calculated for each parameter, by Group and Study Day.
2. A baseline analysis, using the measurements from Study Day -3, was performed for each parameter in order to determine if there were significant differences between the Groups prior to the administration of challenge.
3. Estimates for the mean shift from baseline (the measurement on Study Day -3) were obtained for each parameter, Group, and Study Day. These shifts were evaluated to determine if they were significantly different from "no shift."
4. The mean shifts from baseline for each parameter and Study Day were compared between the Groups. Those Groups having mean shifts that were significantly different from each other were reported.
5. For each parameter, the proportion abnormal in each Group was obtained and evaluated to identify significant differences between the Groups.
6. For each parameter, the time to an abnormal measurement for each animal was obtained and evaluated to identify significant differences between the Groups.

2. Statistical Methods

Analysis of variance (ANOVA) models fitted separately to each clinical chemistry parameter with effects for Group, Study Day, and the interaction between Group and Study Day were used to assess the model assumption of normality and to identify potential outliers. Standardized residuals from these ANOVA models were obtained and a hypothesis test was performed for each parameter in order to assess the model assumption of normality for the untransformed data. Each parameter was then transformed by taking the base-10 logarithm of the parameter values. However, prior to taking the base-10 logarithm, parameter values recorded as zero were replaced by one half of the smallest observed non-zero value associated with the respective parameter. The ANOVA models were then refitted using the base-10 log-transformed values, and a hypothesis test was again performed for each parameter in order to assess the model assumption of normality for the log-transformed data. If the assumption of normality was more reasonable for the log-transformed data than it was for the untransformed data, then the log-transformed values were used throughout the analysis for this parameter. The deleted studentized residuals, which are the standardized residuals from the model fitted to the data having the current observation removed, were computed for each observation. If the absolute value of the deleted studentized residual was greater than 4, then the observation was considered a potential outlier. If any potential outliers were identified, then the following analyses were performed, both with and without these observations, in order to evaluate their effect on the results.

For each clinical chemistry parameter, the following ANOVA model was fitted to the data at Study Day -3 in order to determine if there were significant differences between the Groups at baseline:

$$Y_{ij} = \mu + Group_i + \varepsilon_{ij} \qquad (1)$$

where Y_{ij} is the observed clinical chemistry result for the *j*th animal in Group *i* (*i*=1 to 6) at the baseline, μ is an overall constant, and ε_{ij} is the random error left unexplained by the model. Tukey's multiple comparisons procedure was also performed for each parameter in order to determine which pairs of Groups had baseline means that were significantly different from each other; however, the results will only be presented if significant differences are identified. If the

parameter was log-transformed for analysis, then the same model was used with Y_{ij} replaced by $Log(Y_{ij})$, the base-10 log-transformed parameter value for the *j*th animal in Group *i* (*i*=1 to 6).

In order to determine if the mean shifts from baseline were significantly different between the Groups, the following ANOVA model was fitted separately for each clinical chemistry parameter on each Study Day:

$$Y_{dij} - Y_{bij} = \mu + Group_i + \varepsilon_{ij} \quad (2)$$

where Y_{dij} is the observed clinical chemistry result for the *j*th animal in Group *i* (*i*=1 to 6) on Study Day *d* (*d*=1, 2, 3, 7, and 14), Y_{bij} is the observed clinical chemistry result for the *j*th animal in Group *i* (*i*=1 to 6) at the baseline, μ is an overall constant, and ε_{ij} is the random error left unexplained by the model. If a parameter was log-transformed for the analysis, then the same model was used with Y_{dij} and Y_{bij} replaced by their base-10 log-transformed counterparts $Log(Y_{dij})$ and $Log(Y_{bij})$, respectively.

Least square mean estimates from the above shift from baseline ANOVA models were calculated and approximate t-tests were performed to determine if, for each Group, there was a significant shift in clinical chemistry results between baseline and each Study Day. For untransformed data, this tests whether the difference in means is significantly different from zero. For log-transformed data, this tests whether the ratio of geometric means is significantly different from one. Additionally, Tukey's multiple comparisons procedure was performed to determine which pairs of Groups had mean shifts from baseline that were significantly different from each other. Under the Tukey procedure, the set of all comparisons within each parameter and Study Day combination are made at a joint 95% confidence level.

For each parameter, excluding CRP, the threshold for an abnormal parameter value was defined as each individual animal's baseline (Study Day -3) parameter value plus or minus two standard deviations. Since each animal had only one baseline value for each parameter, the standard deviation associated with each parameter was calculated using the baseline values of all animals. Animals were determined to have an abnormal parameter value when their observed value was above the upper threshold or below the lower threshold for that respective parameter. For CRP,

an animal was considered abnormal if it had a value above the limit of detection (LOD), which was 0.5.

For each parameter, an overall two-sided Fisher's exact test was performed to determine if there was a significant difference between the abnormal rates in each Group. If the overall Fisher's exact test was significant, then one-sided pairwise Fisher's exact tests were performed to evaluate all pairwise Group comparisons. The Bonferroni-Holm adjustment was used to maintain an overall 0.05 significance level among the multiple pairwise comparisons made within each parameter.

For each parameter, an overall log-rank test was performed to determine if there was a significant difference between the times to abnormality in each Group. If the overall log-rank test was significant, then pairwise log-rank tests were performed to evaluate all pairwise Group comparisons. The Bonferroni-Holm adjustment was used to maintain an overall 0.05 significance level among the multiple pairwise comparisons made within each parameter. For those parameters that had significant overall log-rank test, Kaplan-Meier curves were plotted by Group.

All statistical analyses were conducted using SAS® (SAS Institute, Inc.; Cary, NC; version 9.1) and all results are reported at the 0.05 level of significance. All ANOVA models were fitted using the MIXED or GLM procedure. All Fisher's exact tests were performed using the FREQ procedure, and all log-rank tests were performed using the LIFETEST procedure. The MULTTEST procedure was used to maintain an overall 0.05 significance level among the multiple pairwise comparisons made within each parameter.

3. Results

The model assumption of normality was more reasonable for 10 of the 15 parameters when models were fitted to the base-10 log-transformed data. These parameters were: CRP, AST, ALT, LDH, SDH, total protein, BUN, creatinine, potassium, and phosphorus. Therefore, models were fitted to base-10 log-transformed values in the final analysis for these parameters. Since the total bilirubin measurements for each animal were equal to zero at all Study Days, total bilirubin was removed from the analysis. Table 2 contains a list of 18 measurements that were identified as potential outliers using the procedure described above. The statistical analyses of the clinical chemistry data were performed both with and without the potential outliers in order to evaluate their effect on the results. Attachment 1 contains the parameters that experienced changes in significance after the potential outliers were excluded.

Table 3 contains the results of the ANOVA models fitted at baseline (Study Day -3). There was a significant Group effect for AST (p-value=0.0038), with the negative control Group (Group 1) having significantly higher levels than Groups 2, 3, 4, and 6. There was a significant group effect for ALT (p-value=0.0057), with Group 5 having significantly higher levels than Group 4. There was a significant group effect for LDH (p-value=0.0074), with the negative control group (Group 1) having significantly higher levels than Groups 3, 4, 5, and 6. There was a significant group effect for total protein (p-value=0.0096), with Group 3 having significantly lower levels than Groups 1 and 5. There was a significant group effect for calcium (p-value=0.0268), with Group 5 having significantly higher levels than Group 2. There was a significant group effect for phosphorus (p-value=0.0011), with the negative control group (Group 1) having significantly higher levels than all other groups (Groups 2 through 6). Significant group effects at baseline are not necessarily detrimental to the analysis since using the shift from baseline accounts for any differences between the groups at baseline. However, if the significant differences between the groups at baseline are systematically related to how the groups were treated, then the significant group effects at other days throughout the study could be attributed to the differences at baseline.

Descriptive statistics and group comparisons for each parameter are presented in pairs of tables, where Tables 4 through 17 are associated with the parameter of interest. For each parameter,

Table “a” contains the descriptive statistics, while Table “b” contains the test results for comparing the mean shifts from baseline within each Group at each Study Day.

Tables 4a through 17a contain descriptive statistics (means with 95% confidence intervals for untransformed data, or geometric means with 95% confidence intervals for base-10 log-transformed data) for the clinical chemistry parameter results within each group at each Study Day. The results on some Study Days were based on smaller sample sizes due to missing data or due to animal deaths prior to the end of the study. Figures 1 through 14 display the means and confidence intervals over the course of the study for each clinical chemistry parameter.

Tables 4b through 17b contain test results for the mean shift from baseline within each group at each Study Day. These tables contain test results that indicate if the mean difference between the Study Day and baseline was significantly different from zero for untransformed parameters, or if the ratio of geometric means was significantly different from one for base-10 log-transformed parameters (at the 0.05 level). In each cell, the estimate of the shift from baseline (difference or ratio) is shown for that parameter, Group, and Study Day. Following the shift estimate, an up arrow (↑) indicates a significant increase from baseline, while a down arrow (↓) indicates a significant decrease from baseline. These tables also contain group effect p-values for each Study Day, as well as test results from the Tukey’s pairwise comparisons procedure that was used to identify pairs of groups with significantly different shifts from baseline. Under the Tukey procedure, the set of comparisons within each parameter and Study Day is made at a joint 95% confidence level. Each significant difference is shown as the estimated comparison between the shifts from baseline for the pair of groups under consideration, the direction of the comparison (i.e., which group experienced a larger shift from baseline), and the corresponding Tukey-adjusted p-value. P-values less than 0.05 provide evidence of a significant difference. The results from Tables 4b through 17b are discussed below in groups of related parameters.

3.1 Liver Function Parameters

- **CRP (Tables 4a-b, Figure 1)**: There were significant increases as a proportion of baseline in Groups 2, 3, 5, and 6 at Study Day 1, and the increases in Groups 2 and 5 were both significantly different than the decrease in Group 1. As a proportion of baseline, Groups 5 and 6 had significant increases at Study Day 2, and the increase in

Group 6 was significantly greater than those in Groups 1, 2, 3, and 4. There were significant increases as a proportion of baseline in Groups 5 and 6 at Study Day 3. The increase in Group 6 was significantly different than the changes in Groups 1, 2, 3, and 4, and the increase in Group 5 was significantly different than the decrease in Group 1. There was a significant decrease as a proportion of baseline in Group 1 at Study Day 21.

- **AST (Tables 5a-b, Figure 2)**: There was a significant increase as a proportion of baseline in Groups 5 and 6 at Study Day 2, and in Group 5 at Study Day 3. The increase in Group 5 at Study Day 3 was significantly different than the changes in Groups 1, 2, 3, 4, and 6. There were significant increases as a proportion of baseline in Groups 2, 3, 4, and 5 at Study Day 3, and the increase in Group 4 was significantly different than the decrease in Group 1. As a proportion of baseline, there was a significant increase in Group 3 at Study Day 14, and in Groups 2 and 3 at Study Day 21.

- **ALT (Tables 6a-b, Figure 3)**: There were significant increases as a proportion of baseline in Groups 2, 3, 4, and 5 at Study Day 1, in Groups 5 and 6 at Study Day 2, and in Groups 4 and 5 at Study Day 3. The increase in Group 5 at Study Day 3 was significantly greater than those in Groups 1, 2, 3, 4, and 6. As a proportion of baseline, Groups 2, 3, and 4 had significant increases at Study Day 7, as did Groups 2 and 3 at Study Day 14.

- **LDH (Tables 7a-b, Figure 4**): As a proportion of baseline, there was a significant increase in Group 5 at Study Day 1. There was a significant decrease as a proportion of baseline in Group 1, and significant increases as a proportion of baseline in Groups 5 and 6 at Study Day 2. The increase in Group 5 at Study Day 2 was significantly different than the decrease in Group 1. There was a significant decrease as a proportion of baseline in Group 1 and significant increases as a proportion of baseline in Groups 5 and 6 at Study Day 3. The increase in Groups 5 was significantly different than the decreases in Groups 1, 2, 3, and 4, and the increase in Group 6 was significantly different than the decreases in Groups 1 and 3. There was a

significant decrease as a proportion of baseline in Group 1 and significant increases as a proportion of baseline in Groups 4 and 5 at Study Day 7. The decrease in Group 1 was significantly different than the increases in Groups 2, 3, 4, and 5. As a proportion of baseline, there was a significant increase in Group 4 at Study Day 21.

- **SDH (Tables 8a-b, Figure 5)**: There was a significant increase as a proportion of baseline in Group 6 at Study Day 1. There were significant increases as a proportion of baseline in Groups 5 and 6 at Study Day 2, and the increase in Group 6 was significantly different than the decrease in Group 1. At Study Day 3, there was a significant increase as a proportion of baseline in Group 5 that was significantly different than the changes in all other groups. There were significant increases as a proportion of baseline in Groups 2, 3, 4, and 5 at Study Day 7. The increases in Groups 2, 3, and 4 were all significantly greater than that in Group 1. There were significant increases as a proportion of baseline in Group 3 at Study Day 14.

3.2 Serum Protein Parameters

- **Total Protein (Tables 9a-b, Figure 6)**: There were significant increases as a proportion of baseline in Groups 2, 3, and 4 at Study Day 1, and the increase in Group 2 was significantly greater than that in Group 5. There were significant increases as a proportion of baseline in Groups 2 and 3 at Study Days 2 and 3, and the increase in Group 2 at Study Day 3 was significantly different than the decrease in Group 5. As a proportion of baseline, there were significant increases in Groups 1, 2, 3, and 4 at Study Days 7 and 14. There were significant decreases as a proportion of baseline in Groups 1 and 4 at Study Day 21, and the decrease in Group 1 was significantly different than the increases in Groups 2 and 3.

3.3 Kidney Function Parameters

- **BUN (Tables 10a-b, Figure 7)**: There were significant increases as a proportion of baseline in Groups 1, 2, and 3 at Study Day 1, and the increases in Groups 1 and 2 were both significantly different than the decrease in Group 6. There were significant decreases as a proportion of baseline in Groups 4 and 6 at Study Day 2, both of which

were significantly different than the increases in Groups 1 and 2. Group 4 had a significant decrease as a proportion of baseline again at Study Day 3. There was a significant increase as a proportion of baseline in Group 2 at Study Day 7, and in Groups 1 and 2 at Study Day 14.

- **Creatinine (Tables 11a-b, Figure 8)**: There was a significant decrease as a proportion of baseline in Group 4 at Study Day 1, and in Group 3 at Study Day 2. There was a significant increase as a proportion of baseline in Group 5 at Study Day 3, and in Group 2 at Study Days 7 and 21.

- **BUN/Creatinine Ratio (Tables 12a-b, Figure 9)**: There were significant increases from baseline in Groups 3, 4, and 5 at Study Day 1. There was a significant increase from baseline in Group 1 and a significant decrease from baseline in Group 6 at Study Day 2, and the decrease from baseline in Group 6 was significantly different than the increases in Groups 1, 2, and 3. There was a significant decrease from baseline in Group 6 at Study Day 3, and in Group 4 at Study Day 21.

3.4 Electrolytes, Ions

- **Sodium (Tables 13a-b, Figure 10)**: There were significant increases from baseline in Groups 3 and 4 at Study Days 1, 2, and 3, and the increases in both groups at Study Day 3 were significantly different than the decreases in Groups 5 and 6. There were significant increases from baseline in Groups 2 and 3 at Study Day 7, and in Group 3 at Study Day 14.

- **Potassium (Tables 14a-b, Figure 11)**: There were significant increases as a proportion of baseline in Group 1 at Study Days 7 and 14, in Group 2 at Study Days 1, 3, and 14, in Group 3 at Study Days 7 and 14, and in Group 4 at Study Days 7 and 14. There were significant decreases as a proportion of baseline in Groups 1, 2, and 4 at Study Day 21, in Group 5 at Study Day 3, and in Group 6 at Study Day 2. There was a significant group effect at Study Day 2; however, there were no significant differences in shifts as a proportion of baseline between any two groups.

- **Chloride (Tables 15a-b, Figure 12)**: There were significant increases from baseline in Groups 1 and 3 at Study Day 2, and while the group effect was significant, there were no significant differences between any pair of groups. There were significant decreases from baseline in Groups 5 and 6 at Study Day 3, and the decrease in Group 6 was significantly different than the increases in Group 3 and 4. There was a significant increase from baseline in Group 3 at Study Day 14.

- **Calcium (Tables 16a-b, Figure 13)**: There were significant increases from baseline in Groups 2 and 3 at Study Day 1, and the increase in Group 2 was significantly greater than that in Group 5. There was a significant increase from baseline in Group 2 and a significant decrease from baseline in Group 6 at Study Day 2. The decrease in Group 6 was significantly different than the increase in Group 2. There was a significant increase from baseline in Group 2 and a significant decrease from baseline in Group 5 at Study Day 3. The decrease in Group 5 was significantly different than the increases in Groups 2 and 3. There were significant increases from baseline in Groups 1, 2, and 3 at Study Day 7, and in Groups 1, 2, 3, and 4 at Study Day 14. There was a significant decrease from baseline in Group 1 at Study Day 21.

- **Phosphorus (Tables 17a-b, Figure 14)**: There was a significant decrease as a proportion of baseline in Group 1 and a significant increase as a proportion of baseline in Group 3 at Study Day 1. Group 1 had a significant decrease as a proportion of baseline at Study Day 2, while Groups 2, 5, and 6 had significant increases as a proportion of baseline. The increases in Groups 2, 5, and 6 were all significantly different than the decrease in Group 1. There were significant increases as a proportion of baseline in Groups 2, 3, 5, and 6 at Study Day 3, and in Groups 1 through 5 at Study Day 7. The increases in Groups 2 and 3 were both significantly greater than those in Groups 1 and 4 at Study Day 7. There were significant increases as a proportion of baseline in Groups 2, 3, and 4 at Study Day 14, and the increases in Groups 2 and 3 were both significantly greater than that in Group 1. There were significant increases as a proportion of baseline in Groups 1 through 5 at Study Day 21, and the increases in Group 2 and 3 were both significantly greater than that

in Group 1. There were significant increases as a proportion of baseline in Groups 4, 5, and 6 at the terminal blood draw.

For each parameter, Table 18 contains the proportion of animals that were abnormal at any point during the study by Group. Table 19 presents the results of the overall Fisher's exact tests for each parameter. The overall tests identified significant differences between the Groups for LDH (p-value=0.0046) and phosphorus (p-value=0.0020). Table 20 contains the results of pairwise Fisher's exact tests comparing the proportion of abnormal animals associated with each pair of Groups for those parameters that had significant overall tests (LDH and phosphorus). The pairwise comparisons are not dependent upon the ordering of the Groups being compared (e.g. comparing Group 1 to Group 2 is equivalent to comparing Group 2 to Group 1); therefore, the cells in the lower left portion of the table are shaded out for each parameter. For LDH, Group 5 had a significantly greater proportion abnormal than Groups 2 and 3. However, after the Bonferroni-Holm adjustment for multiple comparisons, the proportion abnormal in Group 5 was no longer significantly greater than that in any other Group. For phosphorus, the proportion abnormal in Groups 2, 3, 4, and 5 were significantly greater than that in Group 1. However, these comparisons were no longer significant after the Bonferroni-Holm adjustment for multiple comparisons.

Table 21 contains the results of the overall log-rank tests for each parameter. There were significant differences between the Groups for LDH (p-value=0.0046) and phosphorus (p-value=0.0028); therefore, the results of their pairwise comparisons are displayed in Table 22. Again, the pairwise comparisons are not dependent upon the ordering of the Groups being compared (e.g., comparing Group 1 to Group 2 is equivalent to comparing Group 2 to Group 1); therefore, the cells in the lower left portion of the table are shaded out for each parameter. Prior to adjusting for the multiple pairwise comparisons within each parameter, the time to an abnormal LDH level in Groups 2 and 3 was significantly greater than that in Groups 4 and 5, and the time to an abnormal LDH level in Group 4 was significantly greater than that in Group 5. Prior to adjusting for the multiple pairwise comparisons within each parameter, the time to an abnormal phosphorus level in Group 1 was significantly greater than that in Groups 2, 3, 4 and 5, and the time to an abnormal phosphorus level in Group 4 was significantly greater than that in Groups 2 and 3. After adjusting for the multiple pairwise comparisons made within each

parameter, the times to an abnormal LDH level in Groups 2 and 3 remained significantly greater than that in Group 5. The time to an abnormal phosphorus level in Group 1 remained significantly greater than those in Groups 2 and 3, and the time to an abnormal phosphorus level in Group 4 remained significantly greater than that in Group 3. Figures 15 and 16 display the Kaplan-Meier curves associated with time to abnormality for LDH and phosphorus, respectively.

4. Conclusions

All animals in the high dose control group (Group 6), and all but one animal in the 100,000 CFU group (Group 5), died prior to Study Day 7. Among the liver function parameters, there were similar results in terms of significance at Study Days 2, 3, and 7. At Study Day 2, the increase as a proportion of baseline in the high dose control group (Group 6) was significantly different than the change as a proportion of baseline in the negative control group (Group 1) for CRP, AST, and SDH. At Study Day 3, the increase as a proportion of baseline in the 100,000 CFU group (Group 5) was significantly different than the changes as a proportion of baseline in the lower dose groups (Groups 1 through 4) for AST, ALT, LDH, and SDH. At Study Day 7, the increases as a proportion of baseline in the higher dose groups with surviving animals (Groups 2 through 5) were often significantly different than the change as a proportion of baseline in the negative control group (Group 1) for LDH and SDH.

For total protein, all but two of the significant shifts as a proportion of baseline were increases. In the 100 CFU and 1,000 CFU groups (Groups 2 and 3), there were significant increases as a proportion of baseline on Study Days 1 through 21, while the highest dose groups (Groups 5 and 6) experienced no significant shifts as a proportion of baseline at any Study Day. At Study Days 1 and 3, the increase as a proportion of baseline in the 100 CFU dose group (Group 2) was significantly different than the changes in the 100,000 CFU dose group (Group 5). At Study Day 21, the decrease as a proportion of baseline in the negative control group (Group 1) was significantly different than the increases in the 100 CFU and 1,000 CFU groups (Groups 2 and 3).

Among the kidney function parameters, the decrease as a proportion of baseline in the high dose control group (Group 6) was significantly different than the increases as a proportion of baseline in the negative control group (Group 1) and in the 100 CFU dose group (Group 2) at Study Days 1 and 2 for BUN. The decrease as a proportion of baseline in the 10,000 CFU group (Group 4) was also significantly different than the increases as a proportion of baseline in the negative control group (Group 1) and in the 100 CFU dose group (Group 2) at Study Day 2 for BUN. Furthermore, for BUN/creatinine ratio, the decrease from baseline in the high dose control

group (Group 6) was significantly different than the increases in the lowest dose groups (Groups 1 through 3) at Study Day 2.

Among the electrolytes and ions, sodium, chloride, and calcium exhibited some similarities in terms of significant differences. At Study Day 3, the increases from baseline in the 1,000 CFU and 10,000 CFU groups (Groups 3 and 4) were significantly different than the decrease from baseline in the high dose control group (Group 6) for sodium and chloride. Also at Study Day 3, the increase from baseline in the 1,000 CFU group (Group 3) was significantly different than the decrease from baseline in the 100,000 CFU group (Group 5) for sodium and calcium. For phosphorus, the change as a proportion of baseline in the negative control group (Group 1) was significantly different than the changes in at least two of the higher dose groups (Groups 2 through 6) at Study Days 2 and 7 through 21.

LDH and phosphorus were the only parameters that had significant differences between the groups in terms of the proportion that were ever abnormal during the study. According to the unadjusted pairwise comparisons between groups within LDH, the proportion of abnormal animals in the 100,000 CFU group (Group 5) was significantly greater than that in the 100 CFU and 1,000 CFU groups (Groups 2 and 3). For phosphorus, the proportions of abnormal animals in most of the higher dose groups (Groups 2 through 5) were significantly greater than those in the negative control group (Group 1). After adjusting for the multiple pairwise comparisons made within each parameter, there were no significant differences between any two groups for either parameter.

LDH and phosphorus were also the only two parameters that had significant differences between groups in terms of time to abnormality. For LDH, the times to abnormality in the 100 CFU and 1,000 CFU groups (Groups 2 and 3) were significantly greater than those in the 10,000 CFU and 100,000 CFU groups (Groups 4 and 5), and the 10,000 CFU group (Group 4) had a significantly greater time to abnormality than the 100,000 CFU group (Group 5). For phosphorus, the time to abnormality in the negative control group (Group 1) was significantly greater than that in most of the higher dose groups (Groups 2 through 5), and the 10,000 CFU group (Group 4) had a significantly greater time to abnormality than the 100 CFU and 1,000 CFU groups (Groups 2 and 3). After adjusting for the multiple pairwise comparisons made within each parameter, the

time to an abnormal LDH level in the 100 CFU and 1,000 CFU groups (Groups 2 and 3, respectively) remained significantly greater than that in the 100,000 CFU group (Group 5), the time to an abnormal phosphorous level in negative control group (Group 1) remained significantly greater than those in the 100 CFU and 1,000 CFU groups (Groups 2 and 3, respectively), and the time to an abnormal phosphorous level in the 10,000 CFU group (Group 4) remained significantly greater than that in the 1,000 CFU group (Group 3).

Table 2. List of Potential Clinical Chemistry Outliers

Parameter	Animal	Group	Study Day	Result	Deleted Studentized Residual
C-Reactive Protein†	L23225	4	3	9.59	4.283
Aspartate Aminotransferase†	L23201	5	2	701.80	7.353
	L23212	5	3	21.50	-6.241
Alanine Aminotransferase†	L23201	5	2	290.60	5.749
	L23201	5	3	588.10	4.486
	L23212	5	3	37.00	-5.482
Sorbitol Dehydrogenase†	L23201	5	2	331.10	5.655
	L23212	5	3	24.50	-5.000
Blood Urea Nitrogen†	L23201	5	3	60.30	6.644
Creatinine†	L23204	6	Terminal	5.01	5.232
	L23213	6	Terminal	1.65	-5.232
BUN/Creatinine Ratio	L23201	5	3	31.40	4.206
Sodium	L23204	6	Terminal	121.00	-10.511
	L23213	6	Terminal	150.00	10.511
Chloride	L23204	6	Terminal	77.00	-6.258
	L23213	6	Terminal	94.00	6.258
Calcium	L23201	5	3	6.45	-5.810
	L23212	5	3	13.99	4.030

† Distribution was log-normal for these parameters. Parameter values are reported on the original scale, while the residuals are reported on the log-transformed scale.

Table 3. Summary of ANOVA Results for Baseline Data (Study Day -3)

Parameter	Group Effect P-value	Estimated Difference or Ratio (Relationship) Tukey's P-value[#]
C-Reactive Protein[†]	0.3631	
Aspartate Aminotransferase[†]	0.0038 *	0.55 (2<1) 0.0124 0.51 (3<1) 0.0051 0.58 (4<1) 0.0286 0.53 (6<1) 0.0091
Alanine Aminotransferase[†]	0.0057 *	0.57 (4<5) 0.0380
Lactate Dehydrogenase[†]	0.0074 *	0.45 (3<1) 0.0351 0.42 (4<1) 0.0202 0.39 (5<1) 0.0110 0.40 (6<1) 0.0137
Sorbitol Dehydrogenase[†]	0.1322	
Total Protein[†]	0.0096 *	0.90 (3<1) 0.0261 0.89 (3<5) 0.0119
Blood Urea Nitrogen[†]	0.1073	
Creatinine[†]	0.4196	
BUN/Creatinine Ratio	0.1397	
Sodium	0.1059	
Potassium[†]	0.3524	
Chloride	0.3057	
Calcium	0.0268 *	-1.41 (2<5) 0.0435
Phosphorus[†]	0.0011 *	0.71 (2<1) 0.0035 0.68 (3<1) 0.0011 0.73 (4<1) 0.0082 0.72 (5<1) 0.0049 0.76 (6<1) 0.0279

Cells contain all pairwise comparisons that are significant at the 0.05 level. The format within each cell is: (1) the difference of means (or ratio of geometric means if parameter is log-transformed), (2) the relationship between the corresponding pair of group means shown in parentheses, and (3) the Tukey-adjusted p-value.

† Indicates that values for this parameter were log-transformed for the analysis.

* Group effect was significant at the 0.05 level.

Table 4a. Descriptive Statistics for C-Reactive Protein (mg/dL) by Group and Study Day

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
1	-3	5	0.75 (0.11, 5.02)
	1	5	0.60 (0.18, 2.01)
	2	5	0.89 (0.23, 3.40)
	3	5	0.46 (0.15, 1.38)
	7	5	0.41 (0.10, 1.61)
	14	5	0.40 (0.11, 1.47)
	21	5	0.25 (--)
	Terminal	NA	NA
2	-3	5	0.25 (--)
	1	5	1.29 (0.79, 2.11)
	2	5	0.45 (0.22, 0.95)
	3	5	0.33 (0.15, 0.73)
	7	4	0.32 (0.14, 0.71)
	14	3	0.25 (--)
	21	5	0.25 (--)
	Terminal	NA	NA
3	-3	5	0.32 (0.16, 0.61)
	1	5	0.73 (0.27, 1.96)
	2	5	0.33 (0.15, 0.72)
	3	5	0.29 (0.19, 0.44)
	7	5	0.25 (--)
	14	5	0.32 (0.16, 0.64)
	21	5	0.29 (0.19, 0.45)
	Terminal	NA	NA
4	-3	5	0.36 (0.13, 0.95)
	1	5	0.76 (0.24, 2.35)
	2	5	0.68 (0.12, 4.01)
	3	5	0.52 (0.07, 3.93)
	7	4	0.58 (0.04, 8.73)
	14	3	0.25 (--)
	21	3	0.25 (--)
	Terminal	1	6.28 (--)
5	-3	5	0.33 (0.15, 0.71)
	1	5	1.65 (0.92, 2.97)
	2	5	1.15 (0.48, 2.78)
	3	4	1.92 (0.22, 16.66)
	7	1	0.25 (--)
	14	1	0.25 (--)
	21	1	0.25 (--)
	Terminal	1	3.27 (--)

Table 4a. (Continued)

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
6	-3	5	0.33 (0.23, 0.48)
	1	4	1.62 (0.64, 4.11)
	2	3	6.22 (2.68, 14.45)
	3	3	11.14 (7.76, 16.01)
	7	NA	NA
	14	NA	NA
	21	NA	NA
	Terminal	2	7.86 (3.08, 20.06)

N Number of animals.

-- Confidence intervals could not be calculated since only one observation was available.

NA Data not available for this Group at this Study Day.

Table 4b. Summary of C-Reactive Protein Test Results for the Shifts between Study Days and Baseline (Study Day -3)

C-Reactive Protein†								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-value	Estimated Ratio (Relationship) Tukey's P-value#
	1	2	3	4	5	6		
1	0.80	5.15 ↑	2.29 ↑	2.13	5.01 ↑	4.09 ↑	0.0255 *	0.15 (1<2) 0.0316 0.16 (1<5) 0.0351
2	1.18	1.81	1.04	1.91	3.49 ↑	18.29 ↑	0.0056 *	0.06 (1<6) 0.0064 0.10 (2<6) 0.0268 0.06 (3<6) 0.0043 0.10 (4<6) 0.0318
3	0.61	1.33	0.92	1.46	5.43 ↑	32.76 ↑	0.0002 *	0.11 (1<5) 0.0288 0.02 (1<6) 0.0002 0.04 (2<6) 0.0021 0.03 (3<6) 0.0006 0.04 (4<6) 0.0028
7	0.54	1.28	0.79	2.34	1.00	NA	0.6596	
14	0.53	1.00	1.01	1.00	1.00	NA	0.9172	
21	0.33 ↓	1.00	0.92	1.00	1.00	NA	0.2318	
Terminal	NA	NA	NA	4.27	13.08	19.80	0.4009	

† Indicates that values for this parameter were log-transformed for the analysis.

Cells contain all pairwise comparisons that are significant at the 0.05 level. The format within each cell is: (1) the ratio of shifts, (2) the relationship between the corresponding pair of group mean shifts shown in parentheses [For example, "(1<2)" indicates that the mean shift as a proportion of baseline for Group 1 was less than that for Group 2], and (3) the Tukey-adjusted p-value.

↑, ↓ Indicate that the ratio of the geometric mean at baseline and the geometric mean at the Study Day was significantly different from one (at the 0.05 level). "↑" indicates that the geometric mean at the Study Day was greater than that at baseline, while "↓" indicates that the geometric mean at the Study Day was less than that at baseline.

* Group effect was significant at the 0.05 level.

NA Data not available for this Group at this Study Day.

Table 5a. Descriptive Statistics for Aspartate Aminotransferase (AST, U/L) by Group and Study Day

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
1	-3	5	24.1 (15.0, 38.5)
	1	5	19.1 (15.2, 24.0)
	2	5	19.9 (15.2, 26.0)
	3	5	20.0 (16.3, 24.6)
	7	5	21.7 (16.3, 28.9)
	14	5	28.5 (17.7, 46.1)
	21	5	24.3 (17.2, 34.2)
	Terminal	NA	NA
2	-3	5	13.1 (10.4, 16.6)
	1	5	15.7 (12.8, 19.1)
	2	5	14.8 (12.7, 17.2)
	3	5	17.5 (14.6, 20.9)
	7	4	18.4 (12.3, 27.4)
	14	3	18.1 (14.3, 22.9)
	21	5	17.7 (14.6, 21.4)
	Terminal	NA	NA
3	-3	5	12.4 (10.2, 15.0)
	1	5	14.3 (10.9, 18.8)
	2	5	15.6 (12.1, 20.1)
	3	5	13.1 (8.9, 19.3)
	7	5	16.2 (12.4, 21.3)
	14	5	30.8 (8.7, 109.5)
	21	5	16.6 (11.8, 23.3)
	Terminal	NA	NA
4	-3	5	13.9 (10.9, 17.8)
	1	5	15.7 (11.1, 22.2)
	2	5	19.0 (7.0, 51.4)
	3	5	20.2 (16.2, 25.3)
	7	4	22.9 (16.3, 32.2)
	14	3	19.0 (11.5, 31.2)
	21	3	19.6 (9.6, 40.3)
	Terminal	1	944.0 (--)
5	-3	5	16.3 (10.6, 25.3)
	1	5	19.1 (12.8, 28.6)
	2	5	36.1 (4.6, 286.1)
	3	4	278.8 (16.4, 4735.3)
	7	1	23.7 (--)
	14	1	20.5 (--)
	21	1	21.8 (--)
	Terminal	1	121.2 (--)

Table 5a. (Continued)

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
6	-3	5	12.9 (10.3, 16.1)
	1	4	15.1 (12.2, 18.6)
	2	3	66.8 (27.2, 164.1)
	3	3	22.0 (13.1, 37.2)
	7	NA	NA
	14	NA	NA
	21	NA	NA
	Terminal	2	301.4 (4.9, 18510.0)

N Number of animals.
-- Confidence intervals could not be calculated since only one observation was available.
NA Data not available for this Group at this Study Day.

Table 5b. Summary of AST Test Results for the Shifts between Study Days and Baseline (Study Day -3)

Aspartate Aminotransferase†								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-value	Estimated Ratio (Relationship) Tukey's P-value#
	1	2	3	4	5	6		
1	0.79	1.19	1.16	1.13	1.17	1.10	0.2086	
2	0.83	1.13	1.26	1.36	2.21 ↑	5.13 ↑	0.0233 *	0.16 (1<6) 0.0155
3	0.83	1.33	1.06	1.45	17.20 ↑	1.69	<0.0001*	0.05 (1<5) <0.0001* 0.08 (2<5) <0.0001* 0.06 (3<5) <0.0001* 0.08 (4<5) 0.0001 0.10 (6<5) 0.0014
7	0.90	1.37 ↑	1.31 ↑	1.69 ↑	1.71 ↑	NA	0.0133 *	0.53 (1<4) 0.0097
14	1.19	1.24	2.49 ↑	1.32	1.47	NA	0.4649	
21	1.01	1.34 ↑	1.35 ↑	1.36	1.57	NA	0.3843	
Terminal	NA	NA	NA	60.13	7.21	23.83	0.465	

† Indicates that values for this parameter were log-transformed for the analysis.
# Cells contain all pairwise comparisons that are significant at the 0.05 level. The format within each cell is: (1) the ratio of shifts, (2) the relationship between the corresponding pair of Group mean shifts shown in parentheses [For example, "(1<5)" indicates that the mean shift as a proportion of baseline for Group 1 was less than that for Group 5], and (3) the Tukey-adjusted p-value.
↑, ↓ Indicate that the ratio of the geometric mean at baseline and the geometric mean at the Study Day was significantly different from one (at the 0.05 level). "↑" indicates that the geometric mean at the Study Day was greater than that at baseline, while "↓" indicates that the geometric mean at the Study Day was less than that at baseline.
* Group effect was significant at the 0.05 level.
NA Data not available for this Group at this Study Day.

Table 6a. Descriptive Statistics for Alanine Aminotransferase (ALT, U/L) by Group and Study Day

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
1	-3	5	35.1 (21.3, 57.7)
	1	5	35.4 (23.7, 52.7)
	2	5	37.3 (26.1, 53.4)
	3	5	38.0 (28.3, 51.0)
	7	5	37.2 (27.4, 50.4)
	14	5	40.9 (25.3, 66.3)
	21	5	37.6 (28.7, 49.2)
	Terminal	NA	NA
2	-3	5	22.1 (15.9, 30.6)
	1	5	28.3 (21.3, 37.6)
	2	5	27.5 (21.7, 34.7)
	3	5	29.9 (22.3, 40.0)
	7	4	34.5 (26.1, 45.4)
	14	3	28.8 (21.6, 38.3)
	21	5	26.2 (19.9, 34.3)
	Terminal	NA	NA
3	-3	5	22.4 (18.1, 27.6)
	1	5	29.2 (23.0, 36.9)
	2	5	28.7 (22.3, 36.9)
	3	5	28.0 (23.0, 34.0)
	7	5	30.4 (25.4, 36.4)
	14	5	39.2 (24.3, 63.3)
	21	5	26.3 (20.8, 33.3)
	Terminal	NA	NA
4	-3	5	21.5 (16.1, 28.8)
	1	5	27.1 (19.6, 37.5)
	2	5	28.8 (16.8, 49.2)
	3	5	30.3 (22.3, 41.1)
	7	4	31.4 (21.1, 46.8)
	14	3	26.5 (13.5, 52.1)
	21	3	22.3 (15.6, 32.0)
	Terminal	1	143.8 (--)
5	-3	5	38.0 (24.2, 59.7)
	1	5	45.2 (30.7, 66.6)
	2	5	58.6 (18.9, 181.8)
	3	4	165.0 (27.0, 1009.4)
	7	1	45.7 (--)
	14	1	39.1 (--)
	21	1	34.7 (--)
	Terminal	1	56.4 (--)

Table 6a. (Continued)

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
6	-3	5	22.5 (18.6, 27.1)
	1	4	25.3 (19.7, 32.5)
	2	3	43.6 (37.1, 51.3)
	3	3	29.5 (20.7, 42.2)
	7	NA	NA
	14	NA	NA
	21	NA	NA
	Terminal	2	133.0 (56.3, 314.0)

N Number of animals.
-- Confidence intervals could not be calculated since only one observation was available.
NA Data not available for this Group at this Study Day.

Table 6b. Summary of ALT Test Results for the Shifts between Study Days and Baseline (Study Day -3)

Alanine Aminotransferase†								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-value	Estimated Ratio (Relationship) Tukey's P-value#
	1	2	3	4	5	6		
1	1.01	1.28 ↑	1.30 ↑	1.26 ↑	1.19 ↑	1.05	0.1921	
2	1.06	1.24	1.28	1.34	1.54 ↑	1.91 ↑	0.2148	
3	1.08	1.35	1.25	1.41 ↑	4.02 ↑	1.29	0.0005 *	0.27 (1<5) 0.0003 0.34 (2<5) 0.0026 0.31 (3<5) 0.0012 0.35 (4<5) 0.0037 0.32 (6<5) 0.0061
7	1.06	1.44 ↑	1.36 ↑	1.45 ↑	1.38	NA	0.0861	
14	1.17	1.41 ↑	1.76 ↑	1.22	1.18	NA	0.1715	
21	1.07	1.19	1.18	1.03	1.05	NA	0.7789	
Terminal	NA	NA	NA	6.82	2.01	5.76	0.3590	

† Indicates that values for this parameter were log-transformed for the analysis.
# Cells contain all pairwise comparisons that are significant at the 0.05 level. The format within each cell is: (1) the ratio of shifts, (2) the relationship between the corresponding pair of Group mean shifts shown in parentheses [For example, "(1<5)" indicates that the mean shift as a proportion of baseline for Group 1 was less than that for Group 5], and (3) the Tukey-adjusted p-value.
↑, ↓ Indicate that the ratio of the geometric mean at baseline and the geometric mean at the Study Day was significantly different from one (at the 0.05 level). "↑" indicates that the geometric mean at the Study Day was greater than that at baseline, while "↓" indicates that the geometric mean at the Study Day was less than that at baseline.
* Group effect was significant at the 0.05 level.
NA Data not available for this Group at this Study Day.

Table 7a. Descriptive Statistics for Lactate Dehydrogenase (LDH, U/L) by Group and Study Day

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
1	-3	5	134 (78, 230)
	1	5	108 (52, 223)
	2	5	78 (50, 122)
	3	5	51 (33, 77)
	7	5	70 (40, 123)
	14	5	105 (47, 237)
	21	5	118 (79, 178)
	Terminal	NA	NA
2	-3	5	74 (62, 89)
	1	5	76 (63, 92)
	2	5	71 (43, 117)
	3	5	63 (37, 108)
	7	4	76 (56, 103)
	14	3	55 (22, 134)
	21	5	87 (64, 118)
	Terminal	NA	NA
3	-3	5	60 (37, 96)
	1	5	59 (47, 75)
	2	5	57 (50, 66)
	3	5	40 (31, 53)
	7	5	73 (53, 101)
	14	5	74 (37, 146)
	21	5	90 (61, 134)
	Terminal	NA	NA
4	-3	5	56 (26, 120)
	1	5	67 (34, 135)
	2	5	52 (26, 106)
	3	5	53 (31, 91)
	7	4	67 (62, 73)
	14	3	62 (34, 115)
	21	3	112 (41, 306)
	Terminal	1	5189 (--)
5	-3	5	53 (36, 77)
	1	5	93 (66, 132)
	2	5	118 (35, 401)
	3	4	341 (82, 1415)
	7	1	64 (--)
	14	1	113 (--)
	21	1	70 (--)
	Terminal	1	447 (--)

Table 7a. (Continued)

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
6	-3	5	54 (36, 80)
	1	4	62 (33, 118)
	2	3	90 (53, 151)
	3	3	113 (5, 2507)
	7	NA	NA
	14	NA	NA
	21	NA	NA
	Terminal	2	2011 (0, 120932398)

N Observation was available.
NA Data not available for this Group at this Number of animals.
-- Confidence intervals could not be calculated since only one Study Day.

Table 7b. Summary of LDH Test Results for the Shifts between Study Days and Baseline (Study Day -3)

Lactate Dehydrogenase†								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value#
	1	2	3	4	5	6		
1	0.81	1.02	0.99	1.20	1.77 ↑	1.14	0.2734	
2	0.58 ↓	0.95	0.96	0.93	2.24 ↑	1.85 ↑	0.0068 *	0.26 (1<5) 0.0049
3	0.38 ↓	0.85	0.67	0.94	6.58 ↑	2.33 ↑	<0.0001*	0.06 (1<5) <0.0001* 0.16 (1<6) 0.0017 0.13 (2<5) 0.0002 0.10 (3<5) <0.0001* 0.29 (3<6) 0.0463 0.14 (4<5) 0.0003
7	0.52 ↓	1.01	1.22	1.50 ↑	2.00 ↑	NA	0.0007 *	0.52 (1<2) 0.0416 0.43 (1<3) 0.0043 0.35 (1<4) 0.0011 0.26 (1<5) 0.0091
14	0.78	0.68	1.23	1.37	3.53	NA	0.267	
21	0.88	1.18	1.51	2.45 ↑	2.19	NA	0.1799	
Terminal	NA	NA	NA	37.60	7.98	35.37	0.7843	

† Indicates that values for this parameter were log-transformed for the analysis.

Cells contain all pairwise comparisons that are significant at the 0.05 level. The format within each cell is: (1) the ratio of shifts, (2) the relationship between the corresponding pair of Group mean shifts shown in parentheses [For example, "(1<5)" indicates that the mean shift as a proportion of baseline for Group 1 was less than that for Group 5], and (3) the Tukey-adjusted p-value.

↑, ↓ Indicate that the ratio of the geometric mean at baseline and the geometric mean at the Study Day was significantly different from one (at the 0.05 level). "↑" indicates that the geometric mean at the Study Day was greater than that at baseline, while "↓" indicates that the geometric mean at the Study Day was less than that at baseline.

* Group effect was significant at the 0.05 level.

NA Data not available for this Group at this Study Day.

Table 8a. Descriptive Statistics for Sorbitol Dehydrogenase (SDH, U/L) by Group and Study Day

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
1	-3	5	28.6 (22.0, 37.1)
	1	5	23.3 (15.4, 35.2)
	2	5	21.0 (12.8, 34.3)
	3	5	24.8 (18.1, 33.9)
	7	5	28.6 (21.9, 37.2)
	14	5	30.0 (18.0, 50.0)
	21	5	23.1 (15.8, 33.9)
	Terminal	NA	NA
2	-3	5	17.7 (14.1, 22.3)
	1	5	20.8 (15.7, 27.6)
	2	5	20.7 (17.0, 25.2)
	3	5	23.5 (17.8, 31.0)
	7	4	28.7 (19.4, 42.4)
	14	3	22.2 (20.1, 24.4)
	21	5	20.4 (17.6, 23.6)
	Terminal	NA	NA
3	-3	5	21.5 (17.3, 26.7)
	1	5	26.7 (18.3, 39.0)
	2	5	24.9 (14.6, 42.7)
	3	5	21.9 (14.5, 33.0)
	7	5	31.4 (24.1, 40.8)
	14	5	41.0 (18.4, 91.5)
	21	5	20.8 (15.6, 27.7)
	Terminal	NA	NA
4	-3	5	22.7 (14.6, 35.4)
	1	5	25.1 (21.3, 29.6)
	2	5	30.2 (14.2, 64.3)
	3	5	34.1 (21.7, 53.5)
	7	4	35.9 (18.8, 68.7)
	14	3	24.6 (10.0, 60.6)
	21	3	19.4 (6.1, 61.4)
	Terminal	1	210.5 (--)
5	-3	5	24.9 (14.3, 43.2)
	1	5	31.5 (21.9, 45.2)
	2	5	42.6 (10.1, 179.6)
	3	4	148.5 (19.4, 1136.5)
	7	1	26.7 (--)
	14	1	22.9 (--)
	21	1	19.0 (--)
	Terminal	1	135.9 (--)

Table 8a. (Continued)

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
6	-3	5	18.2 (12.4, 26.7)
	1	4	28.5 (20.0, 40.5)
	2	3	51.8 (25.0, 107.3)
	3	2	23.9 (1.1, 517.9)
	7	NA	NA
	14	NA	NA
	21	NA	NA
	Terminal	2	120.8 (0.0, 2780073.5)

N Number of animals.
-- Confidence intervals could not be calculated since only one observation was available.
NA Data not available for this Group at this Study Day.

Table 8b. Summary of SDH Test Results for the Shifts between Study Days and Baseline (Study Day -3)

Sorbitol Dehydrogenase†								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value#
	1	2	3	4	5	6		
1	0.82	1.17	1.24	1.10	1.27	1.45 ↑	0.0969	
2	0.73	1.16	1.16	1.33	1.71 ↑	2.78 ↑	0.0161 *	0.26 (1<6) 0.0084
3	0.87	1.33	1.02	1.5	6.50 ↑	1.02	<0.0001*	0.13 (1<5) <0.0001* 0.20 (2<5) 0.0009 0.16 (3<5) 0.0001 0.23 (4<5) 0.0022 0.16 (6<5) 0.0028
7	1.00	1.50 ↑	1.46 ↑	1.77 ↑	1.63 ↑	NA	0.0031 *	0.67 (1<2) 0.0268 0.69 (1<3) 0.0299 0.57 (1<4) 0.0021
14	1.05	1.24	1.91 ↑	1.37	1.4	NA	0.3296	
21	0.81	1.15	0.97	1.08	1.16	NA	0.2881	
Terminal	NA	NA	NA	5.93	3.88	7.33	0.7737	

† Indicates that values for this parameter were log-transformed for the analysis.
# Cells contain all pairwise comparisons that are significant at the 0.05 level. The format within each cell is: (1) the ratio of shifts, (2) the relationship between the corresponding pair of Group mean shifts shown in parentheses [For example, "(1<5)" indicates that the mean shift as a proportion of baseline for Group 1 was less than that for Group 5], and (3) the Tukey-adjusted p-value.
↑, ↓ Indicate that the ratio of the geometric mean at baseline and the geometric mean at the Study Day was significantly different from one (at the 0.05 level). "↑" indicates that the geometric mean at the Study Day was greater than that at baseline, while "↓" indicates that the geometric mean at the Study Day was less than that at baseline.
* Group effect was significant at the 0.05 level.
NA Data not available for this Group at this Study Day.

Table 9a. Descriptive Statistics for Total Protein (g/dL) by Group and Study Day

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
1	-3	5	5.83 (5.71, 5.95)
	1	5	6.00 (5.61, 6.42)
	2	5	5.86 (5.55, 6.18)
	3	5	6.10 (5.71, 6.53)
	7	5	6.25 (5.84, 6.68)
	14	5	6.19 (5.81, 6.59)
	21	5	5.39 (5.16, 5.63)
	Terminal	NA	NA
2	-3	5	5.46 (5.29, 5.64)
	1	5	6.18 (6.00, 6.36)
	2	5	6.03 (5.80, 6.27)
	3	5	6.40 (6.05, 6.77)
	7	4	6.67 (6.22, 7.15)
	14	3	6.25 (5.59, 6.98)
	21	5	5.53 (5.33, 5.74)
	Terminal	NA	NA
3	-3	5	5.24 (4.97, 5.52)
	1	5	5.72 (5.43, 6.03)
	2	5	5.65 (5.34, 5.97)
	3	5	5.67 (5.27, 6.09)
	7	5	6.05 (5.59, 6.55)
	14	5	6.06 (5.72, 6.41)
	21	5	5.32 (5.18, 5.47)
	Terminal	NA	NA
4	-3	5	5.69 (5.27, 6.16)
	1	5	6.02 (5.80, 6.25)
	2	5	5.92 (5.38, 6.51)
	3	5	5.92 (5.14, 6.83)
	7	4	6.27 (5.91, 6.65)
	14	3	6.32 (5.60, 7.15)
	21	3	5.30 (4.99, 5.63)
	Terminal	1	4.30 (--)
5	-3	5	5.89 (5.36, 6.48)
	1	5	5.99 (5.60, 6.39)
	2	5	5.90 (5.55, 6.27)
	3	4	5.43 (4.67, 6.32)
	7	1	6.08 (--)
	14	1	6.21 (--)
	21	1	5.52 (--)
	Terminal	1	5.96 (--)

Table 9a. (Continued)

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
6	-3	5	5.51 (5.17, 5.87)
	1	4	5.65 (5.24, 6.10)
	2	3	5.74 (4.88, 6.76)
	3	3	5.36 (4.10, 7.00)
	7	NA	NA
	14	NA	NA
	21	NA	NA
	Terminal	2	5.06 (0.91, 28.12)

N Number of animals.
-- Confidence intervals could not be calculated since only one observation was available.
NA Data not available for this Group at this Study Day.

Table 9b. Summary of Total Protein Test Results for the Shifts between Study Days and Baseline (Study Day -3)

Total Protein†								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value#
	1	2	3	4	5	6		
1	1.03	1.13 ↑	1.09 ↑	1.06 ↑	1.02	1.04	0.0216 *	0.90 (5<2) 0.0246
2	1.00	1.10 ↑	1.08 ↑	1.04	1.00	1.07	0.0579	
3	1.05	1.17 ↑	1.08 ↑	1.04	0.92	0.99	0.0096 *	0.78 (5<2) 0.0038
7	1.07 ↑	1.21 ↑	1.16 ↑	1.10 ↑	1.09	NA	0.1346	
14	1.06 ↑	1.15 ↑	1.16 ↑	1.12 ↑	1.11	NA	0.218	
21	0.92 ↓	1.01	1.02	0.94 ↓	0.99	NA	0.0036 *	0.91 (1<2) 0.0110 0.91 (1<3) 0.0086
Terminal	NA	NA	NA	0.76	1.02	0.91	0.6399	

† Indicates that values for this parameter were log-transformed for the analysis.

Cells contain all pairwise comparisons that are significant at the 0.05 level. The format within each cell is: (1) the ratio of shifts, (2) the relationship between the corresponding pair of Group mean shifts shown in parentheses [For example, "(1<2)" indicates that the mean shift as a proportion of baseline for Group 1 was less than that for Group 2], and (3) the Tukey-adjusted p-value.

↑, ↓ Indicate that the ratio of the geometric mean at baseline and the geometric mean at the Study Day was significantly different from one (at the 0.05 level). "↑" indicates that the geometric mean at the Study Day was greater than that at baseline, while "↓" indicates that the geometric mean at the Study Day was less than that at baseline.

* Group effect was significant at the 0.05 level.

NA Data not available for this Group at this Study Day.

Table 10a. Descriptive Statistics for Blood Urea Nitrogen (BUN, mg/dL) by Group and Study Day

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
1	-3	5	17.4 (13.5, 22.6)
	1	5	20.0 (16.4, 24.4)
	2	5	19.2 (16.4, 22.6)
	3	5	15.8 (12.8, 19.4)
	7	5	18.7 (14.7, 23.7)
	14	5	21.3 (16.1, 28.1)
	21	5	18.6 (15.6, 22.0)
	Terminal	NA	NA
2	-3	5	15.1 (12.6, 18.0)
	1	5	17.7 (16.1, 19.4)
	2	5	16.2 (12.7, 20.8)
	3	5	15.1 (11.4, 19.9)
	7	4	18.9 (16.4, 21.8)
	14	3	20.3 (12.6, 32.7)
	21	5	16.6 (13.3, 20.9)
	Terminal	NA	NA
3	-3	5	18.6 (15.7, 22.0)
	1	5	20.6 (17.9, 23.6)
	2	5	17.6 (15.2, 20.4)
	3	5	15.7 (13.7, 18.0)
	7	5	19.1 (16.8, 21.7)
	14	5	21.4 (17.9, 25.5)
	21	5	17.2 (15.5, 19.1)
	Terminal	NA	NA
4	-3	5	20.6 (15.9, 26.5)
	1	5	20.4 (16.6, 25.1)
	2	5	17.8 (14.4, 22.0)
	3	5	16.2 (12.0, 21.9)
	7	4	20.9 (14.4, 30.4)
	14	3	24.1 (12.6, 45.9)
	21	3	19.4 (13.8, 27.1)
	Terminal	1	66.9 (--)
5	-3	5	20.0 (14.9, 26.8)
	1	5	19.7 (15.9, 24.4)
	2	5	18.8 (14.5, 24.4)
	3	4	21.8 (7.3, 65.1)
	7	1	18.0 (--)
	14	1	20.8 (--)
	21	1	17.0 (--)
	Terminal	1	20.5 (--)

Table 10a. (Continued)

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
6	-3	5	19.5 (17.0, 22.4)
	1	4	18.1 (14.1, 23.1)
	2	3	14.5 (10.9, 19.4)
	3	3	15.1 (8.2, 28.0)
	7	NA	NA
	14	NA	NA
	21	NA	NA
	Terminal	2	43.6 (0.2, 8376.0)

N Number of animals.
-- Confidence intervals could not be calculated since only one observation was available.
NA Data not available for this Group at this Study Day.

Table 10b. Summary of BUN Test Results for the Shifts between Study Days and Baseline (Study Day -3)

Blood Urea Nitrogen†								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value#
	1	2	3	4	5	6		
1	1.15 ↑	1.17 ↑	1.11 ↑	0.99	0.98	0.93	0.0058 *	0.81 (6<1) 0.0403 0.79 (6<2) 0.0176
2	1.10	1.08	0.95	0.86 ↓	0.94	0.74 ↓	0.0005 *	0.78 (4<1) 0.0233 0.67 (6<1) 0.0008 0.80 (4<2) 0.0470 0.69 (6<2) 0.0015
3	0.91	1.00	0.84	0.79 ↓	1.12	0.77	0.2116	
7	1.07	1.23 ↑	1.02	0.98	1.03	NA	0.1982	
14	1.22 ↑	1.30 ↑	1.15	1.11	1.19	NA	0.8626	
21	1.06	1.11	0.93	0.89	0.97	NA	0.3365	
Terminal	NA	NA	NA	3.72	0.91	2.10	0.4028	

† Indicates that values for this parameter were log-transformed for the analysis.

Cells contain all pairwise comparisons that are significant at the 0.05 level. The format within each cell is: (1) the ratio of shifts, (2) the relationship between the corresponding pair of Group mean shifts shown in parentheses [For example, "(6<1)" indicates that the mean shift as a proportion of baseline for Group 6 was less than that for Group 1], and (3) the Tukey-adjusted p-value.

↑, ↓ Indicate that the ratio of the geometric mean at baseline and the geometric mean at the Study Day was significantly different from one (at the 0.05 level). "↑" indicates that the geometric mean at the Study Day was greater than that at baseline**,** while "↓" indicates that the geometric mean at the Study Day was less than that at baseline.

* Group effect was significant at the 0.05 level.

NA Data not available for this Group at this Study Day.

Table 11a. Descriptive Statistics for Creatinine (mg/dL) by Group and Study Day

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
1	-3	5	0.96 (0.81, 1.13)
	1	5	0.90 (0.72, 1.11)
	2	5	0.82 (0.75, 0.91)
	3	5	0.98 (0.93, 1.04)
	7	5	1.06 (0.94, 1.19)
	14	5	1.05 (0.90, 1.23)
	21	5	1.11 (0.96, 1.27)
	Terminal	NA	NA
2	-3	5	0.78 (0.65, 0.94)
	1	5	0.81 (0.64, 1.04)
	2	5	0.74 (0.64, 0.86)
	3	5	0.90 (0.72, 1.12)
	7	4	1.15 (0.86, 1.55)
	14	3	0.90 (0.64, 1.27)
	21	5	1.06 (0.92, 1.23)
	Terminal	NA	NA
3	-3	5	1.03 (0.64, 1.68)
	1	5	0.82 (0.73, 0.92)
	2	5	0.79 (0.65, 0.94)
	3	5	0.88 (0.75, 1.04)
	7	5	1.06 (0.79, 1.42)
	14	5	1.00 (0.88, 1.14)
	21	5	1.16 (1.02, 1.32)
	Terminal	NA	NA
4	-3	5	0.98 (0.53, 1.81)
	1	5	0.72 (0.63, 0.83)
	2	5	0.80 (0.64, 0.99)
	3	5	0.87 (0.66, 1.16)
	7	4	0.93 (0.80, 1.07)
	14	3	1.08 (0.98, 1.19)
	21	3	1.14 (0.86, 1.51)
	Terminal	1	4.92 (--)
5	-3	5	0.86 (0.77, 0.95)
	1	5	0.71 (0.69, 0.73)
	2	5	0.88 (0.70, 1.11)
	3	4	1.29 (0.82, 2.03)
	7	1	0.87 (--)
	14	1	0.97 (--)
	21	1	1.14 (--)
	Terminal	1	1.04 (--)

Table 11a. (Continued)

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
6	-3	5	0.75 (0.61, 0.94)
	1	4	0.73 (0.60, 0.89)
	2	3	0.92 (0.64, 1.33)
	3	3	0.96 (0.50, 1.85)
	7	NA	NA
	14	NA	NA
	21	NA	NA
	Terminal	2	2.88 (0.00, 3335.06)

N Number of animals.
-- Confidence intervals could not be calculated since only one observation was available.
NA Data not available for this Group at this Study Day.

Table 11b. Summary of Creatinine Test Results for the Shifts between Study Days and Baseline (Study Day -3)

Creatinine†								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value#
	1	2	3	4	5	6		
1	0.94	1.04	0.79	0.74 ↓	0.83	0.89	0.4206	
2	0.86	0.94	0.76 ↓	0.81	1.03	1.17	0.3001	
3	1.03	1.15	0.85	0.89	1.50 ↑	1.22	0.0777	
7	1.11	1.53 ↑	1.03	1.15	1.10	NA	0.316	
14	1.10	1.17	0.97	1.32	1.23	NA	0.6993	
21	1.16	1.36 ↑	1.13	1.39	1.44	NA	0.6842	
Terminal	NA	NA	NA	2.31	1.22	3.40	0.7401	

† Indicates that values for this parameter were log-transformed for the analysis.
# There were no significant Tukey's pairwise comparisons.
↑, ↓ Indicate that the ratio of the geometric mean at baseline and the geometric mean at the Study Day was significantly different from one (at the 0.05 level). "↑" indicates that the geometric mean at the Study Day was greater than that at baseline, while "↓" indicates that the geometric mean at the Study Day was less than that at baseline.
NA Data not available for this Group at this Study Day.

Table 12a. Descriptive Statistics for BUN/Creatinine Ratio by Group and Study Day

Group	Study Day	N	Mean (95% Confidence Interval)
1	-3	5	18.4 (15.5, 21.3)
	1	5	22.4 (20.1, 24.6)
	2	5	23.4 (21.3, 25.5)
	3	5	16.2 (13.5, 18.9)
	7	5	17.8 (14.6, 21.0)
	14	5	20.7 (14.8, 26.6)
	21	5	16.9 (14.0, 19.8)
	Terminal	NA	NA
2	-3	5	19.5 (14.7, 24.4)
	1	5	21.9 (18.3, 25.6)
	2	5	22.0 (19.4, 24.7)
	3	5	16.8 (14.7, 19.0)
	7	4	16.5 (12.5, 20.5)
	14	3	22.6 (18.5, 26.7)
	21	5	15.7 (13.8, 17.6)
	Terminal	NA	NA
3	-3	5	18.8 (11.5, 26.0)
	1	5	25.3 (19.9, 30.8)
	2	5	22.9 (17.0, 28.8)
	3	5	18.0 (14.5, 21.4)
	7	5	18.4 (12.6, 24.2)
	14	5	21.7 (16.0, 27.4)
	21	5	14.9 (13.2, 16.6)
	Terminal	NA	NA
4	-3	5	22.9 (12.1, 33.8)
	1	5	28.5 (22.2, 34.8)
	2	5	22.5 (17.9, 27.2)
	3	5	19.0 (13.8, 24.2)
	7	4	22.8 (16.4, 29.1)
	14	3	22.8 (8.0, 37.6)
	21	3	17.0 (15.3, 18.7)
	Terminal	1	13.6 (--)
5	-3	5	23.6 (18.7, 28.4)
	1	5	28.2 (21.6, 34.8)
	2	5	21.8 (15.8, 27.8)
	3	4	18.3 (3.9, 32.6)
	7	1	20.7 (--)
	14	1	21.4 (--)
	21	1	14.9 (--)
	Terminal	1	19.7 (--)

Table 12a. (Continued)

Group	Study Day	N	Mean (95% Confidence Interval)
6	-3	5	26.9 (19.6, 34.1)
	1	4	25.2 (14.6, 35.8)
	2	3	15.8 (14.7, 16.9)
	3	3	15.9 (10.3, 21.4)
	7	NA	NA
	14	NA	NA
	21	NA	NA
	Terminal	2	15.4 (0.0[a], 42.7)

N Number of animals.
NA Data not available for this Group at this Study Day.
-- Confidence intervals could not be calculated since only one observation was available.
a Lower bound of confidence interval set to zero as a negative parameter value is not possible.

Table 12b. Summary of BUN/Creatinine Ratio Test Results for the Shifts between Study Days and Baseline (Study Day -3)

BUN/Creatinine Ratio								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value[#]
	1	2	3	4	5	6		
1	4.0	2.4	6.5 ↑	5.6 ↑	4.6 ↑	0.5	0.5081	
2	5.0 ↑	2.5	4.1	-0.4	-1.8	-10.0 ↓	0.0119 *	-15.0 (6<1) 0.0098 -12.4 (6<2) 0.0419 -14.1 (6<3) 0.0168
3	-2.2	-2.7	-0.8	-3.9	-4.6	-9.9 ↓	0.2346	
7	-0.6	-4.2	-0.4	-3.8	-1.5	NA	0.6311	
14	2.3	1.8	2.9	-4.1	-0.8	NA	0.6404	
21	-1.5	-3.8	-3.9	-9.9 ↓	-7.3	NA	0.2624	
Terminal	NA	NA	NA	5.1	-6.9	-10.5	0.5690	

Cells contain all pairwise comparisons that are statistically significant at the 0.05 level. The format within each cell is: (1) the difference of shifts, (2) the relationship between the corresponding pair of Group mean shifts shown in parentheses [For example, "(6<1)" indicates that the mean shift from baseline for Group 6 was less than that for Group 1], and (3) the Tukey-adjusted p-value.
↑, ↓ Indicate that the difference between the mean at baseline and the mean at the Study Day was significantly different from zero (at the 0.05 level). "↑" indicates that the mean at the Study Day was greater than that at baseline, while "↓" indicates that the mean at the Study Day was less than that at baseline.
* Group effect was significant at the 0.05 level.
NA Data not available for this Group at this Study Day.

Table 13a. Descriptive Statistics for Sodium (mEq/L) by Group and Study Day

Group	Study Day	N	Mean (95% Confidence Interval)
1	-3	5	144 (142, 146)
	1	5	145 (144, 147)
	2	5	145 (144, 146)
	3	5	144 (141, 147)
	7	5	145 (142, 148)
	14	5	145 (143, 146)
	21	5	141 (140, 142)
	Terminal	NA	NA
2	-3	5	144 (142, 146)
	1	5	146 (145, 148)
	2	5	145 (144, 147)
	3	5	146 (144, 147)
	7	4	151 (144, 158)
	14	4	145 (141, 148)
	21	5	142 (140, 143)
	Terminal	NA	NA
3	-3	5	138 (128, 148)
	1	5	145 (143, 147)
	2	5	146 (144, 147)
	3	5	146 (144, 147)
	7	5	148 (144, 151)
	14	5	145 (143, 146)
	21	5	142 (141, 142)
	Terminal	NA	NA
4	-3	5	140 (131, 150)
	1	5	146 (145, 147)
	2	5	146 (144, 149)
	3	5	148 (142, 155)
	7	4	146 (145, 147)
	14	3	146 (--)
	21	3	141 (134, 148)
	Terminal	1	139 (--)
5	-3	5	146 (143, 149)
	1	5	146 (144, 147)
	2	5	145 (143, 147)
	3	4	145 (144, 146)
	7	1	143 (--)
	14	1	145 (--)
	21	1	141 (--)
	Terminal	1	143 (--)

Table 13a. (Continued)

Group	Study Day	N	Mean (95% Confidence Interval)
6	-3	5	144 (142, 145)
	1	4	146 (145, 147)
	2	3	143 (136, 151)
	3	3	141 (138, 144)
	7	NA	NA
	14	NA	NA
	21	NA	NA
	Terminal	2	136 (0[a], 320)

N Number of animals.
NA Data not available for this Group at this Study Day.
-- Confidence intervals could not be calculated since only one observation was available.
a Lower bound of confidence interval set to zero as a negative parameter value is not possible.

Table 13b. Summary of Sodium Test Results for the Shifts between Study Days and Baseline (Study Day -3)

Sodium								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value[#]
	1	2	3	4	5	6		
1	1	2	7 ↑	6 ↑	-1	2	0.145	
2	1	2	8 ↑	6 ↑	-1	-1	0.0504	
3	0	2	8 ↑	8 ↑	-2	-3	0.0017 *	-9 (5<3) 0.0256 -10 (6<3) 0.0240 -10 (5<4) 0.0158 -11 (6<4) 0.0154
7	1	7 ↑	10 ↑	3	-1	NA	0.1681	
14	1	1	7 ↑	3	1	NA	0.2442	
21	-3	-2	4	-2	-3	NA	0.1981	
Terminal	NA	NA	NA	12	-2	-9	0.7947	

Cells contain all pairwise comparisons that are statistically significant at the 0.05 level. The format within each cell is: (1) the difference of shifts, (2) the relationship between the corresponding pair of Group mean shifts shown in parentheses [For example, "(5<3)" indicates that the mean shift from baseline for Group 5 was less than that for Group 3], and (3) the Tukey-adjusted p-value.
↑, ↓ Indicate that the difference between the mean at baseline and the mean at the Study Day was significantly different from zero (at the 0.05 level). "↑" indicates that the mean at the Study Day was greater than that at baseline**,** while "↓" indicates that the mean at the Study Day was less than that at baseline.
* Group effect was significant at the 0.05 level.
NA Data not available for this Group at this Study Day.

Table 14a. Descriptive Statistics for Potassium (mEq/L) by Group and Study Day

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
1	-3	5	4.3 (3.5, 5.4)
	1	5	4.9 (4.2, 5.7)
	2	5	4.7 (4.3, 5.2)
	3	5	4.8 (4.3, 5.5)
	7	5	5.3 (4.8, 5.9)
	14	5	5.5 (5.4, 5.6)
	21	5	3.7 (3.4, 3.9)
	Terminal	NA	NA
2	-3	5	4.5 (4.3, 4.8)
	1	5	5.2 (4.4, 6.1)
	2	5	5.1 (4.4, 5.9)
	3	5	5.3 (4.6, 6.0)
	7	4	5.2 (4.6, 5.9)
	14	4	6.0 (4.8, 7.5)
	21	5	3.8 (3.5, 4.1)
	Terminal	NA	NA
3	-3	5	4.2 (3.9, 4.4)
	1	5	4.5 (4.3, 4.8)
	2	5	4.6 (4.1, 5.1)
	3	5	4.4 (4.2, 4.7)
	7	5	5.1 (3.9, 6.9)
	14	5	5.3 (4.9, 5.8)
	21	5	3.8 (3.6, 4.0)
	Terminal	NA	NA
4	-3	5	4.7 (4.1, 5.5)
	1	5	4.7 (4.2, 5.1)
	2	5	4.7 (4.2, 5.3)
	3	5	5.0 (4.0, 6.4)
	7	4	5.8 (4.8, 7.0)
	14	3	6.0 (5.1, 6.9)
	21	3	3.9 (3.5, 4.3)
	Terminal	1	21.2 (--)
5	-3	5	4.8 (3.7, 6.2)
	1	5	4.6 (4.1, 5.3)
	2	5	4.4 (3.6, 5.2)
	3	4	4.0 (3.3, 4.8)
	7	1	4.8 (--)
	14	1	4.9 (--)
	21	1	3.9 (--)
	Terminal	1	11.4 (--)

Table 14a. (Continued)

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
6	-3	5	4.9 (4.5, 5.3)
	1	4	4.8 (4.3, 5.4)
	2	3	3.7 (3.2, 4.3)
	3	3	4.5 (3.0, 6.6)
	7	NA	NA
	14	NA	NA
	21	NA	NA
	Terminal	2	22.5 (0.8, 640.0)

N Number of animals.
NA Data not available for this Group at this Study Day.
-- Confidence intervals could not be calculated since only one observation was available.

Table 14b. Summary of Potassium Test Results for the Shifts between Study Days and Baseline (Study Day -3)

Potassium†								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value#
	1	2	3	4	5	6		
1	1.13	1.14 ↑	1.09	0.99	0.97	1.01	0.2939	
2	1.09	1.12	1.10	0.99	0.91	0.77 ↓	0.0392 *	
3	1.12	1.16 ↑	1.06	1.06	0.85 ↓	0.92	0.0543	
7	1.22 ↑	1.16	1.24 ↑	1.24 ↑	1.14	NA	0.9793	
14	1.27 ↑	1.30 ↑	1.29 ↑	1.28 ↑	1.17	NA	0.9584	
21	0.85 ↓	0.83 ↓	0.91	0.83 ↓	0.93	NA	0.6789	
Terminal	NA	NA	NA	4.24	2.24	4.74	0.4641	

† Indicates that values for this parameter were log-transformed for the analysis.
# There were no significant Tukey's pairwise comparisons.
↑, ↓ Indicate that the ratio of the geometric mean at baseline and the geometric mean at the Study Day was significantly different from one (at the 0.05 level). "↑" indicates that the geometric mean at the Study Day was greater than that at baseline, while "↓" indicates that the geometric mean at the Study Day was less than that at baseline.
* Group effect was significant at the 0.05 level.
NA Data not available for this Group at this Study Day.

Table 15a. Descriptive Statistics for Chloride (mEq/L) by Group and Study Day

Group	Study Day	N	Mean (95% Confidence Interval)
1	-3	5	103 (101, 105)
	1	5	107 (105, 108)
	2	5	108 (106, 110)
	3	5	102 (98, 105)
	7	5	104 (102, 107)
	14	5	106 (104, 108)
	21	5	103 (101, 104)
	Terminal	NA	NA
2	-3	5	107 (105, 109)
	1	5	106 (105, 108)
	2	5	107 (106, 108)
	3	5	105 (103, 106)
	7	4	110 (103, 117)
	14	4	107 (105, 108)
	21	5	105 (101, 108)
	Terminal	NA	NA
3	-3	5	103 (95, 111)
	1	5	107 (105, 109)
	2	5	107 (106, 109)
	3	5	106 (104, 108)
	7	5	108 (104, 113)
	14	5	108 (107, 109)
	21	5	105 (103, 107)
	Terminal	NA	NA
4	-3	5	103 (95, 111)
	1	5	106 (105, 107)
	2	5	105 (103, 108)
	3	5	106 (103, 110)
	7	4	107 (104, 110)
	14	3	109 (105, 113)
	21	3	104 (102, 106)
	Terminal	1	86 (--)
5	-3	5	106 (103, 110)
	1	5	104 (101, 107)
	2	5	104 (103, 106)
	3	4	102 (99, 104)
	7	1	106 (--)
	14	1	108 (--)
	21	1	103 (--)
	Terminal	1	95 (--)

Table 15a. (Continued)

Group	Study Day	N	Mean (95% Confidence Interval)
6	-3	5	107 (103, 112)
	1	4	106 (104, 108)
	2	3	105 (101, 108)
	3	3	102 (99, 105)
	7	NA	NA
	14	NA	NA
	21	NA	NA
	Terminal	2	86 (0[a], 194)

N Number of animals.
NA Data not available for this Group at this Study Day.
-- Confidence intervals could not be calculated since only one observation was available.
a Lower bound of confidence interval set to zero as a negative parameter value is not possible.

Table 15b. Summary of Chloride Test Results for the Shifts between Study Days and Baseline (Study Day -3)

Chloride								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value[#]
	1	2	3	4	5	6		
1	4	-1	4	3	-2	-2	0.1233	
2	5 ↑	0	4 ↑	2	-2	-4	0.0393 *	
3	-1	-2	3	3	-5 ↓	-7 ↓	0.0102 *	-10 (6<3) 0.0451 -10 (6<4) 0.0393
7	1	3	5	2	2	NA	0.8904	
14	3	-1	5 ↑	4	4	NA	0.4181	
21	0	-2	2	-1	-1	NA	0.6619	
Terminal	NA	NA	NA	-6	-10	-23	0.7402	

Cells contain all pairwise comparisons that are statistically significant at the 0.05 level. The format within each cell is: (1) the difference of shifts, (2) the relationship between the corresponding pair of Group mean shifts shown in parentheses [For example, "(6<3)" indicates that the mean shift from baseline for Group 6 was less than that for Group 3], and (3) the Tukey-adjusted p-value.
↑, ↓ Indicate that the difference between the mean at baseline and the mean at the Study Day was significantly different from zero (at the 0.05 level). "↑" indicates that the mean at the Study Day was greater than that at baseline, while "↓" indicates that the mean at the Study Day was less than that at baseline.
* Group effect was significant at the 0.05 level.
NA Data not available for this Group at this Study Day.

Table 16a. Descriptive Statistics for Calcium (mg/dL) by Group and Study Day

Group	Study Day	N	Mean (95% Confidence Interval)
1	-3	5	13.65 (12.42, 14.88)
	1	5	14.20 (13.33, 15.08)
	2	5	14.07 (13.24, 14.91)
	3	5	14.01 (13.15, 14.86)
	7	5	14.72 (14.12, 15.32)
	14	5	15.11 (14.47, 15.75)
	21	5	12.48 (12.08, 12.89)
	Terminal	NA	NA
2	-3	5	12.56 (11.87, 13.26)
	1	5	14.54 (13.60, 15.48)
	2	5	14.11 (13.39, 14.82)
	3	5	14.40 (13.58, 15.22)
	7	4	14.33 (13.29, 15.38)
	14	4	15.02 (13.70, 16.34)
	21	5	12.63 (12.24, 13.02)
	Terminal	NA	NA
3	-3	5	12.72 (11.88, 13.56)
	1	5	13.84 (13.02, 14.66)
	2	5	13.86 (12.74, 14.98)
	3	5	13.55 (12.56, 14.54)
	7	5	14.47 (13.13, 15.80)
	14	5	14.91 (13.80, 16.03)
	21	5	12.45 (12.24, 12.67)
	Terminal	NA	NA
4	-3	5	13.59 (12.76, 14.43)
	1	5	14.35 (13.99, 14.71)
	2	5	14.04 (12.58, 15.49)
	3	5	14.14 (11.95, 16.33)
	7	4	14.86 (13.94, 15.78)
	14	3	15.51 (13.36, 17.66)
	21	3	12.58 (11.19, 13.97)
	Terminal	1	16.09 (--)
5	-3	5	13.97 (13.00, 14.95)
	1	5	14.22 (13.78, 14.66)
	2	5	13.03 (10.52, 15.54)
	3	4	10.78 (5.72, 15.84)
	7	1	13.72 (--)
	14	1	14.48 (--)
	21	1	12.59 (--)
	Terminal	1	15.59 (--)

Table 16a. (Continued)

Group	Study Day	N	Mean (95% Confidence Interval)
6	-3	5	13.41 (12.89, 13.93)
	1	4	13.90 (12.95, 14.84)
	2	3	11.48 (9.50, 13.46)
	3	3	11.36 (10.35, 12.37)
	7	NA	NA
	14	NA	NA
	21	NA	NA
	Terminal	2	12.71 (7.81, 17.60)

N Number of animals.
NA Data not available for this Group at this Study Day.
-- Confidence intervals could not be calculated since only one observation was available.

Table 16b. Summary of Calcium Test Results for the Shifts between Study Days and Baseline (Study Day -3)

Calcium								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value[#]
	1	2	3	4	5	6		
1	0.56	1.97 ↑	1.12 ↑	0.76	0.25	0.64	0.0522	-1.73 (5<2) 0.0351
2	0.42	1.54 ↑	1.14	0.44	-0.95	-1.76 ↓	0.0186 *	-3.30 (6<2) 0.0316
3	0.36	1.84 ↑	0.83	0.55	-3.11 ↓	-1.88	0.0060 *	-4.95 (5<2) 0.0054 -3.94 (5<3) 0.0354
7	1.07 ↑	1.62 ↑	1.74 ↑	1.13	0.6	NA	0.7459	
14	1.46 ↑	2.60 ↑	2.19 ↑	1.88 ↑	1.36	NA	0.5546	
21	-1.16 ↓	0.07	-0.27	-1.05	-0.53	NA	0.2304	
Terminal	NA	NA	NA	3.04	1.29	-0.73	0.1228	

Cells contain all pairwise comparisons that are statistically significant at the 0.05 level. The format within each cell is: (1) the difference of shifts, (2) the relationship between the corresponding pair of Group mean shifts shown in parentheses [For example, "(5<2)" indicates that the mean shift from baseline for Group 5 was less than that for Group 2], and (3) the Tukey-adjusted p-value.
↑, ↓ Indicate that the difference between the mean at baseline and the mean at the Study Day was significantly different from zero (at the 0.05 level). "↑" indicates that the mean at the Study Day was greater than that at baseline, while "↓" indicates that the mean at the Study Day was less than that at baseline.
* Group effect was significant at the 0.05 level.
NA Data not available for this Group at this Study Day.

Table 17a. Descriptive Statistics for Phosphorus (mg/dL) by Group and Study Day

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
1	-3	5	4.38 (4.19, 4.58)
	1	5	3.81 (2.97, 4.87)
	2	5	3.71 (3.00, 4.60)
	3	5	4.90 (4.31, 5.58)
	7	5	5.26 (4.37, 6.34)
	14	5	4.60 (3.71, 5.69)
	21	5	5.59 (5.00, 6.25)
	Terminal	NA	NA
2	-3	5	3.10 (2.35, 4.10)
	1	5	3.53 (3.09, 4.02)
	2	5	3.55 (2.96, 4.27)
	3	5	4.30 (3.59, 5.15)
	7	4	5.57 (4.72, 6.58)
	14	3	4.66 (3.75, 5.80)
	21	5	5.76 (5.03, 6.60)
	Terminal	NA	NA
3	-3	5	2.98 (2.64, 3.36)
	1	5	3.42 (3.07, 3.80)
	2	5	3.24 (2.98, 3.52)
	3	5	3.85 (3.59, 4.12)
	7	5	4.83 (3.95, 5.92)
	14	5	4.33 (3.85, 4.86)
	21	5	5.83 (5.49, 6.19)
	Terminal	NA	NA
4	-3	5	3.19 (2.85, 3.57)
	1	5	3.21 (2.61, 3.95)
	2	5	3.26 (2.45, 4.34)
	3	5	3.58 (2.62, 4.91)
	7	4	3.82 (2.78, 5.25)
	14	3	4.47 (3.52, 5.66)
	21	3	5.57 (5.14, 6.04)
	Terminal	1	16.39 (--)
5	-3	5	3.13 (2.54, 3.86)
	1	5	3.18 (2.68, 3.77)
	2	5	3.68 (2.90, 4.67)
	3	4	4.82 (2.98, 7.81)
	7	1	4.13 (--)
	14	1	4.26 (--)
	21	1	5.36 (--)
	Terminal	1	6.38 (--)

Table 17a. (Continued)

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
6	-3	5	3.33 (3.13, 3.54)
	1	4	3.68 (3.02, 4.49)
	2	3	4.57 (2.83, 7.36)
	3	3	4.44 (3.07, 6.42)
	7	NA	NA
	14	NA	NA
	21	NA	NA
	Terminal	2	18.59 (8.95, 38.61)

N Number of animals.
NA Data not available for this Group at this Study Day.
-- Confidence intervals could not be calculated since only one observation was available.

Table 17b. Summary of Phosphorus Test Results for the Shifts between Study Days and Baseline (Study Day -3)

Phosphorus†								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value#
	1	2	3	4	5	6		
1	0.87 ↓	1.14	1.15 ↑	1.01	1.02	1.09	0.0578	
2	0.85 ↓	1.15 ↑	1.09	1.02	1.17 ↑	1.35 ↑	0.0038 *	0.74 (1<2) 0.0341 0.72 (1<5) 0.0193 0.63 (1<6) 0.0028
3	1.12	1.39 ↑	1.29 ↑	1.12	1.56 ↑	1.31 ↑	0.0935	
7	1.20 ↑	1.64 ↑	1.62 ↑	1.22 ↑	1.30 ↑	NA	0.0021 *	0.73 (1<2) 0.0105 0.74 (1<3) 0.0090 0.74 (4<2) 0.0216 0.75 (4<3) 0.0200
14	1.05	1.53 ↑	1.45 ↑	1.36 ↑	1.34	NA	0.0350 *	0.69 (1<2) 0.0473 0.72 (1<3) 0.0471
21	1.28 ↑	1.86 ↑	1.96 ↑	1.70 ↑	1.69 ↑	NA	0.0011 *	0.69 (1<2) 0.0027 0.65 (1<3) 0.0008
Terminal	NA	NA	NA	4.76 ↑	1.91 ↑	5.68 ↑	0.0398 *	0.34 (5<6) 0.0386

† Indicates that values for this parameter were log-transformed for the analysis.

Cells contain all pairwise comparisons that are significant at the 0.05 level. The format within each cell is: (1) the ratio of shifts, (2) the relationship between the corresponding pair of Group mean shifts shown in parentheses [For example, "(1<2)" indicates that the mean shift as a proportion of baseline for Group 1 was less than that for Group 2], and (3) the Tukey-adjusted p-value.

↑, ↓ Indicate that the ratio of the geometric mean at baseline and the geometric mean at the Study Day was significantly different from one (at the 0.05 level). "↑" indicates that the geometric mean at the Study Day was greater than that at baseline, while "↓" indicates that the geometric mean at the Study Day was less than that at baseline.

* Group effect was significant at the 0.05 level.

NA Data not available for this Group at this Study Day.

Table 18. Proportion of Animals that were Abnormal with Exact 95% Confidence Interval by Parameter and Group

Parameter	Group	Number Abnormal / N	Proportion Abnormal (95% Confidence Interval)
C-Reactive Protein	1	5/5	1.00 (0.48, 1.00)
	2	5/5	1.00 (0.48, 1.00)
	3	4/5	0.80 (0.28, 0.99)
	4	4/5	0.80 (0.28, 0.99)
	5	5/5	1.00 (0.48, 1.00)
	6	2/4	0.50 (0.07, 0.93)
Aspartate Aminotransferase	1	2/5	0.40 (0.05, 0.85)
	2	0/5	0.00 (0.00, 0.52)
	3	2/5	0.40 (0.05, 0.85)
	4	1/5	0.20 (0.01, 0.72)
	5	4/5	0.80 (0.28, 0.99)
	6	2/4	0.50 (0.07, 0.93)
Alanine Aminotransferase	1	0/5	0.00 (0.00, 0.52)
	2	0/5	0.00 (0.00, 0.52)
	3	2/5	0.40 (0.05, 0.85)
	4	1/5	0.20 (0.01, 0.72)
	5	3/5	0.60 (0.15, 0.95)
	6	1/4	0.25 (0.01, 0.81)
Lactate Dehydrogenase	1	2/5	0.40 (0.05, 0.85)
	2	0/5	0.00 (0.00, 0.52)
	3	0/5	0.00 (0.00, 0.52)
	4	3/5	0.60 (0.15, 0.95)
	5	5/5	1.00 (0.48, 1.00)
	6	2/4	0.50 (0.07, 0.93)
Sorbitol Dehydrogenase	1	2/5	0.40 (0.05, 0.85)
	2	0/5	0.00 (0.00, 0.52)
	3	2/5	0.40 (0.05, 0.85)
	4	3/5	0.60 (0.15, 0.95)
	5	4/5	0.80 (0.28, 0.99)
	6	2/4	0.50 (0.07, 0.93)
Total Protein	1	2/5	0.40 (0.05, 0.85)
	2	4/5	0.80 (0.28, 0.99)
	3	4/5	0.80 (0.28, 0.99)
	4	3/5	0.60 (0.15, 0.95)
	5	1/5	0.20 (0.01, 0.72)
	6	1/4	0.25 (0.01, 0.81)
Blood Urea Nitrogen	1	1/5	0.20 (0.01, 0.72)
	2	1/5	0.20 (0.01, 0.72)
	3	1/5	0.20 (0.01, 0.72)
	4	2/5	0.40 (0.05, 0.85)
	5	1/5	0.20 (0.01, 0.72)
	6	1/4	0.25 (0.01, 0.81)

Table 18. (Continued)

Parameter	Group	Number Abnormal / N	Proportion Abnormal (95% Confidence Interval)
Creatinine	1	0/5	0.00 (0.00, 0.52)
	2	1/5	0.20 (0.01, 0.72)
	3	1/5	0.20 (0.01, 0.72)
	4	2/5	0.40 (0.05, 0.85)
	5	1/5	0.20 (0.01, 0.72)
	6	1/4	0.25 (0.01, 0.81)
BUN/Creatinine Ratio	1	0/5	0.00 (0.00, 0.52)
	2	0/5	0.00 (0.00, 0.52)
	3	2/5	0.40 (0.05, 0.85)
	4	2/5	0.40 (0.05, 0.85)
	5	0/5	0.00 (0.00, 0.52)
	6	1/4	0.25 (0.01, 0.81)
Sodium	1	0/5	0.00 (0.00, 0.52)
	2	0/5	0.00 (0.00, 0.52)
	3	2/5	0.40 (0.05, 0.85)
	4	1/5	0.20 (0.01, 0.72)
	5	0/5	0.00 (0.00, 0.52)
	6	1/4	0.25 (0.01, 0.81)
Potassium	1	5/5	1.00 (0.48, 1.00)
	2	4/5	0.80 (0.28, 0.99)
	3	3/5	0.60 (0.15, 0.95)
	4	3/5	0.60 (0.15, 0.95)
	5	2/5	0.40 (0.05, 0.85)
	6	2/4	0.50 (0.07, 0.93)
Chloride	1	0/5	0.00 (0.00, 0.52)
	2	0/5	0.00 (0.00, 0.52)
	3	1/5	0.20 (0.01, 0.72)
	4	1/5	0.20 (0.01, 0.72)
	5	1/5	0.20 (0.01, 0.72)
	6	1/4	0.25 (0.01, 0.81)
Calcium	1	3/5	0.60 (0.15, 0.95)
	2	5/5	1.00 (0.48, 1.00)
	3	4/5	0.80 (0.28, 0.99)
	4	3/5	0.60 (0.15, 0.95)
	5	2/5	0.40 (0.05, 0.85)
	6	2/4	0.50 (0.07, 0.93)
Phosphorus	1	0/5	0.00 (0.00, 0.52)
	2	5/5	1.00 (0.48, 1.00)
	3	5/5	1.00 (0.48, 1.00)
	4	4/5	0.80 (0.28, 0.99)
	5	4/5	0.80 (0.28, 0.99)
	6	2/4	0.50 (0.07, 0.93)

N Number of animals.

Table 19. Results of Overall Two-sided Fisher's Exact Test for the Proportion of Animals that were Abnormal by Parameter

Parameter	Group Effect P-Value
C-Reactive Protein	0.1737
Aspartate Aminotransferase	0.1892
Alanine Aminotransferase	0.2039
Lactate Dehydrogenase	0.0046 *
Sorbitol Dehydrogenase	0.2411
Total Protein	0.3341
Blood Urea Nitrogen	1.0000
Creatinine	0.9079
BUN/Creatinine Ratio	0.2158
Sodium	0.3421
Potassium	0.4908
Chloride	0.8684
Calcium	0.4908
Phosphorus	0.0020 *

*Group effect was significant at the 0.05 level.

Table 20. Results of One-sided Pairwise Fisher's Exact Tests for the Proportion of Animals with Abnormal Lactate Dehydrogenase and Phosphorus

Parameter	Group	One-Sided Pairwise Fisher's Exact Test P-Values									
		Unadjusted P-Values					Bonferroni-Holm Adjusted P-Values				
		2	3	4	5	6	2	3	4	5	6
Lactate Dehydrogenase	1	1.0000	1.0000	0.5000	0.0833	0.6429	1.0000	1.0000	1.0000	1.0000	1.0000
	2		1.0000[a]	0.0833	0.0040*	0.1667		1.0000[a]	1.0000	0.0556	1.0000
	3			0.0833	0.0040*	0.1667			1.0000	0.0556	1.0000
	4				0.2222	0.8333				1.0000	1.0000
	5					1.0000					1.0000
Phosphorus	1	0.0040*	0.0040*	0.0238*	0.0238*	0.1667	0.0556	0.0556	0.2857	0.2857	1.0000
	2		1.0000[a]	1.0000	1.0000	1.0000		1.0000[a]	1.0000	1.0000	1.0000
	3			1.0000	1.0000	1.0000			1.0000	1.0000	1.0000
	4				0.7778	0.9524				1.0000	1.0000
	5					0.9524					1.0000

[a] A p-value of 1.0000 was substituted when all animals in both Groups experienced the same abnormality result.

* Comparison significant at the 0.05 level.

Table 21. Results of Overall Log-rank Tests for Time to Abnormality by Parameter

Parameter	Group Effect P-Value
C-Reactive Protein	0.2267
Aspartate Aminotransferase	0.1726
Alanine Aminotransferase	0.1810
Lactate Dehydrogenase	0.0046 *
Sorbitol Dehydrogenase	0.2478
Total Protein	0.0532
Blood Urea Nitrogen	0.9773
Creatinine	0.7911
BUN/Creatinine Ratio	0.2258
Sodium	0.3132
Potassium	0.2245
Chloride	0.7951
Calcium	0.0764
Phosphorus	0.0028 *

* Group effect was significant at the 0.05 level.

Table 22. Results of the Pairwise Log-rank Tests for Time to Abnormal Lactate Dehydrogenase and Phosphorus

Parameter	Group	Pairwise Log-Rank Test P-Values									
		Unadjusted P-Values					Bonferroni-Holm Adjusted P-Values				
		2	3	4	5	6	2	3	4	5	6
Lactate Dehydrogenase	1	0.1343	0.1343	0.9446	0.1120	0.8348	1.0000	0.9400	1.0000	0.8957	1.0000
	2		1.0000	0.0495*	0.0019*	0.0888		1.0000	0.5944	0.0284*	0.8881
	3			0.0495*	0.0019*	0.0888			0.5944	0.0284*	0.8881
	4				0.0124*	0.7317				0.1618	1.0000
	5					0.1758					0.9400
Phosphorus	1	0.0020*	0.0031*	0.0158*	0.0144*	0.0888	0.0303*	0.0438*	0.1735	0.1734	0.7993
	2		0.3959	0.0363*	0.3253	0.1198		1.0000	0.3631	1.0000	0.8478
	3			0.0031*	0.1558	0.1060			0.0438*	0.9346	0.8478
	4				0.6438	0.5881				1.0000	1.0000
	5					0.5076					1.0000

* Comparison significant at the 0.05 level.

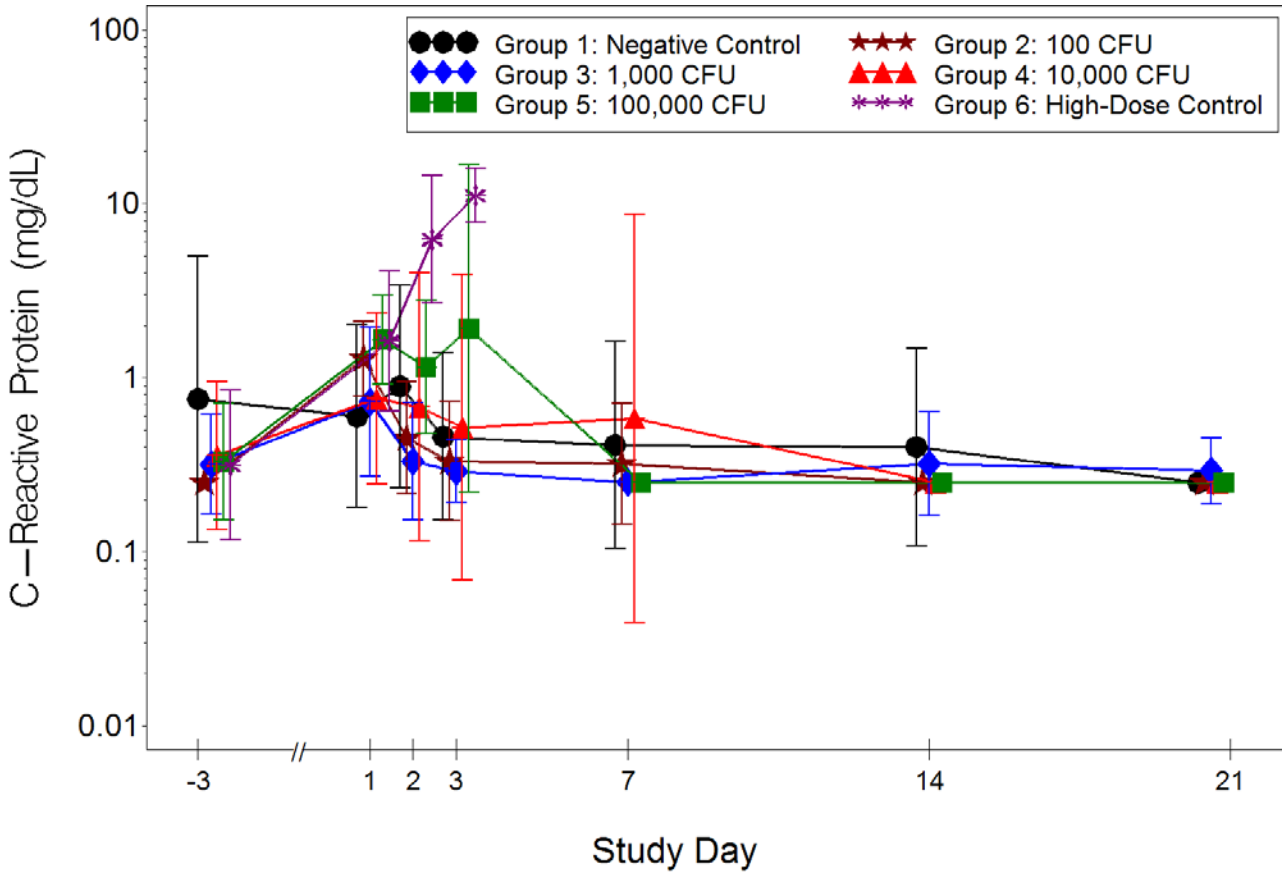

Figure 1. Plot of C-Reactive Protein over time.

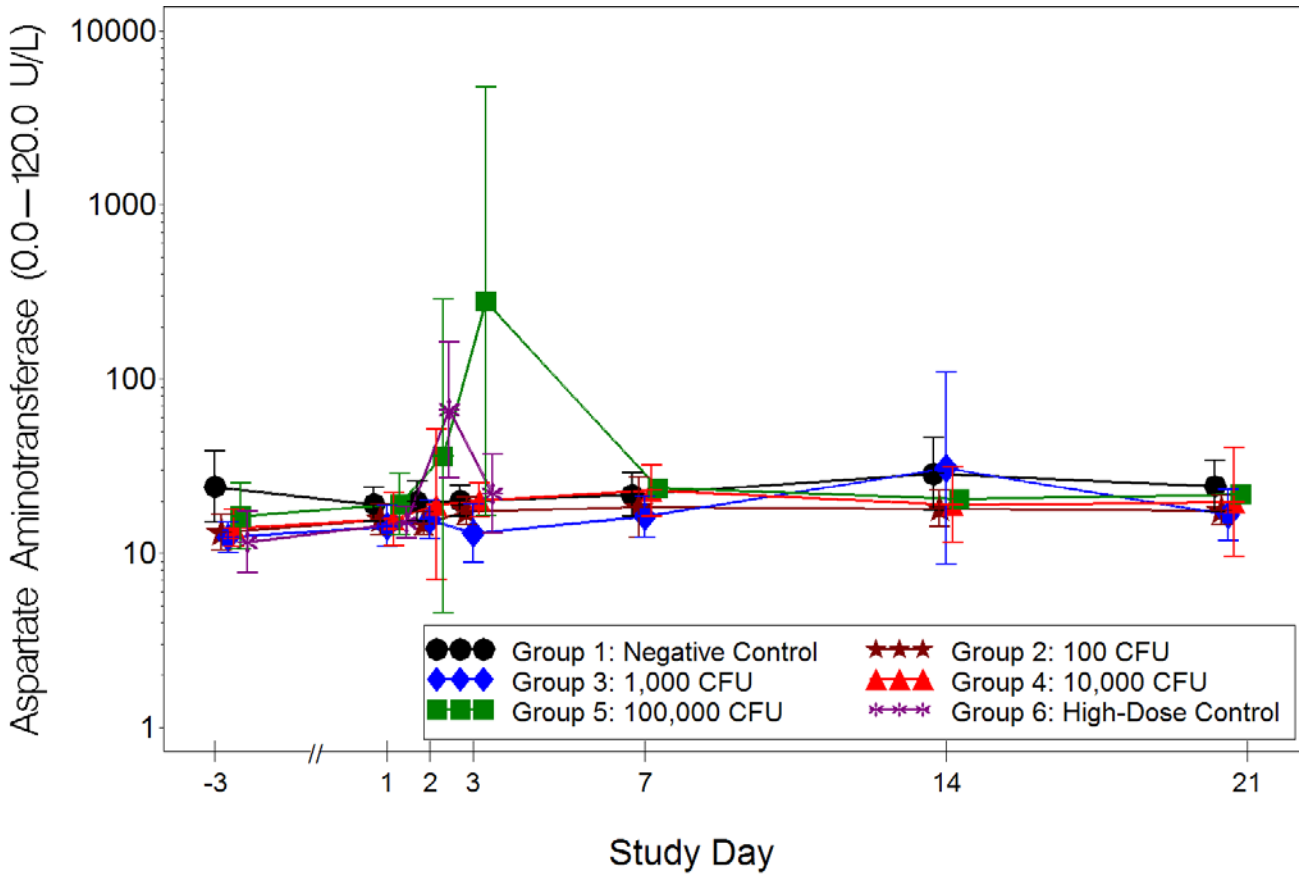

Figure 2. **Plot of Aspartate Aminotransferase over time.**

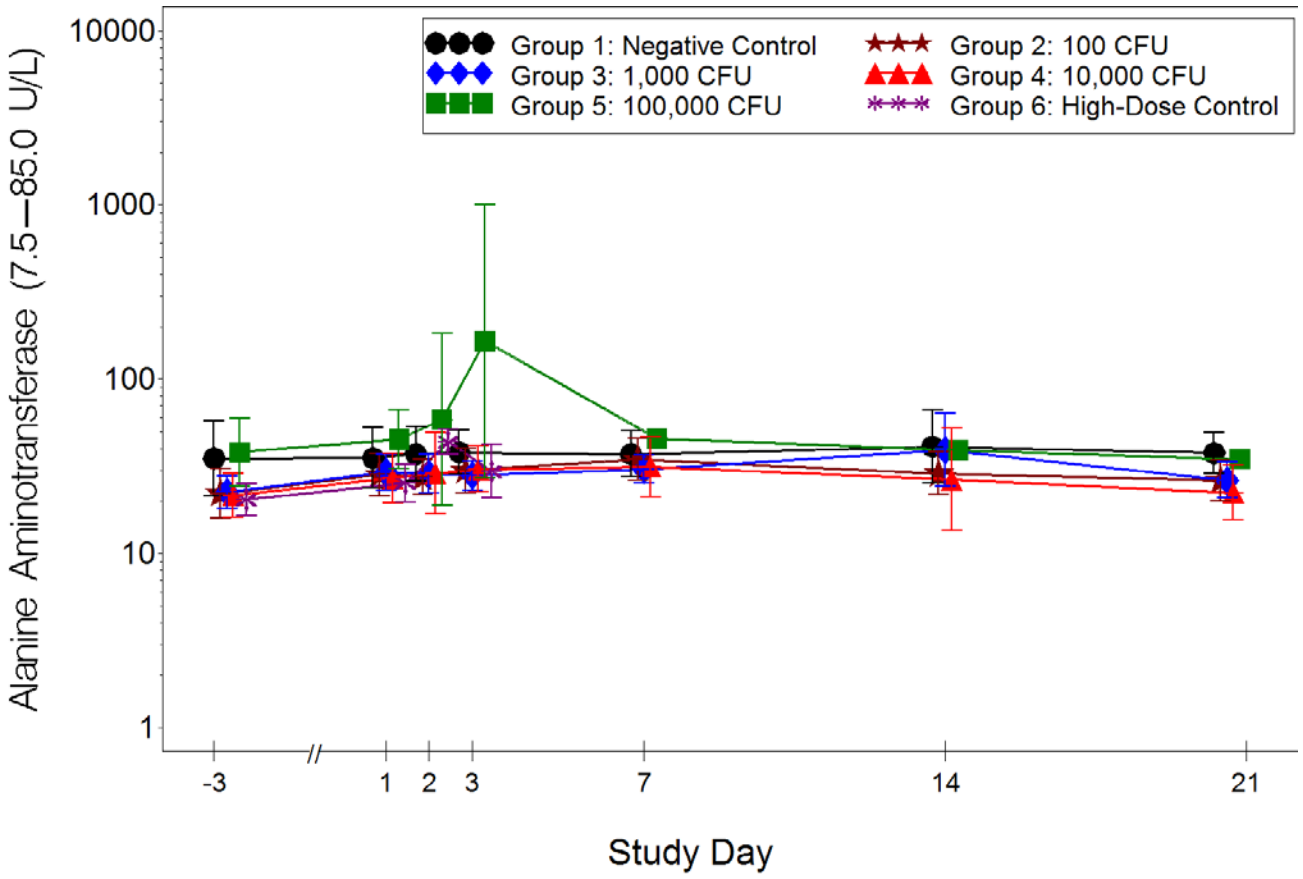

Figure 3. **Plot of Alanine Aminotransferase over time.**

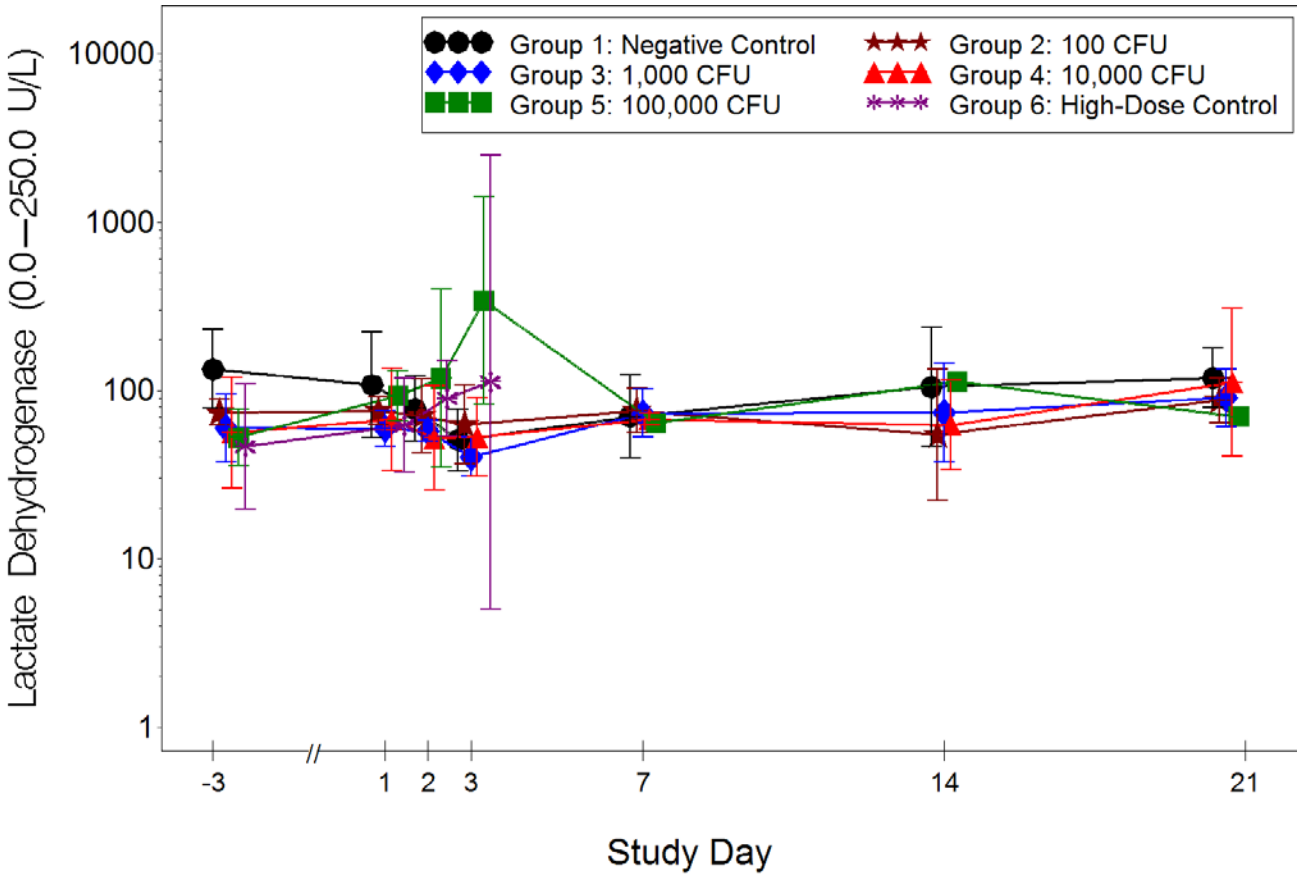

Figure 4. **Plot of Lactate Dehydrogenase over time.**

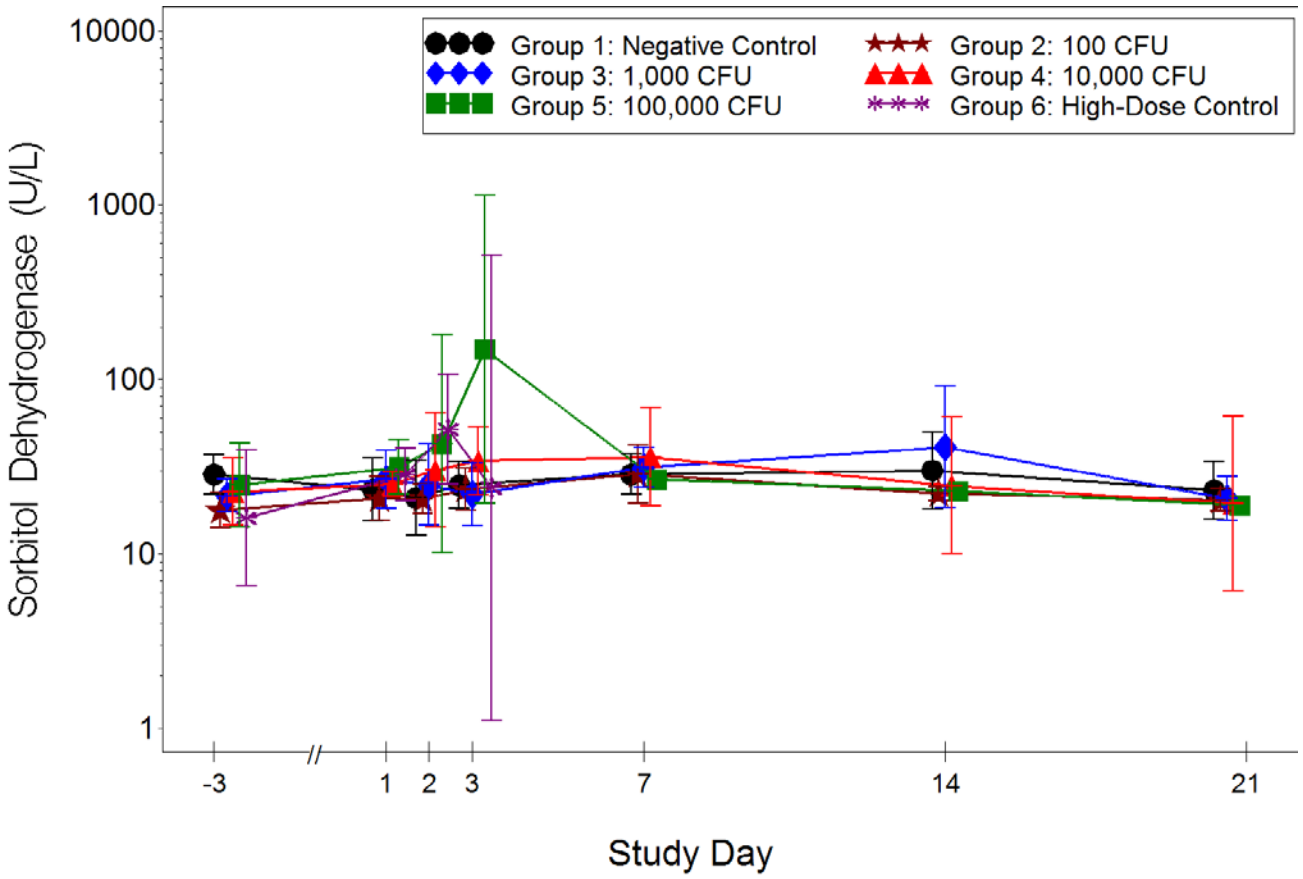

Figure 5. Plot of Sorbitol Dehydrogenase over time.

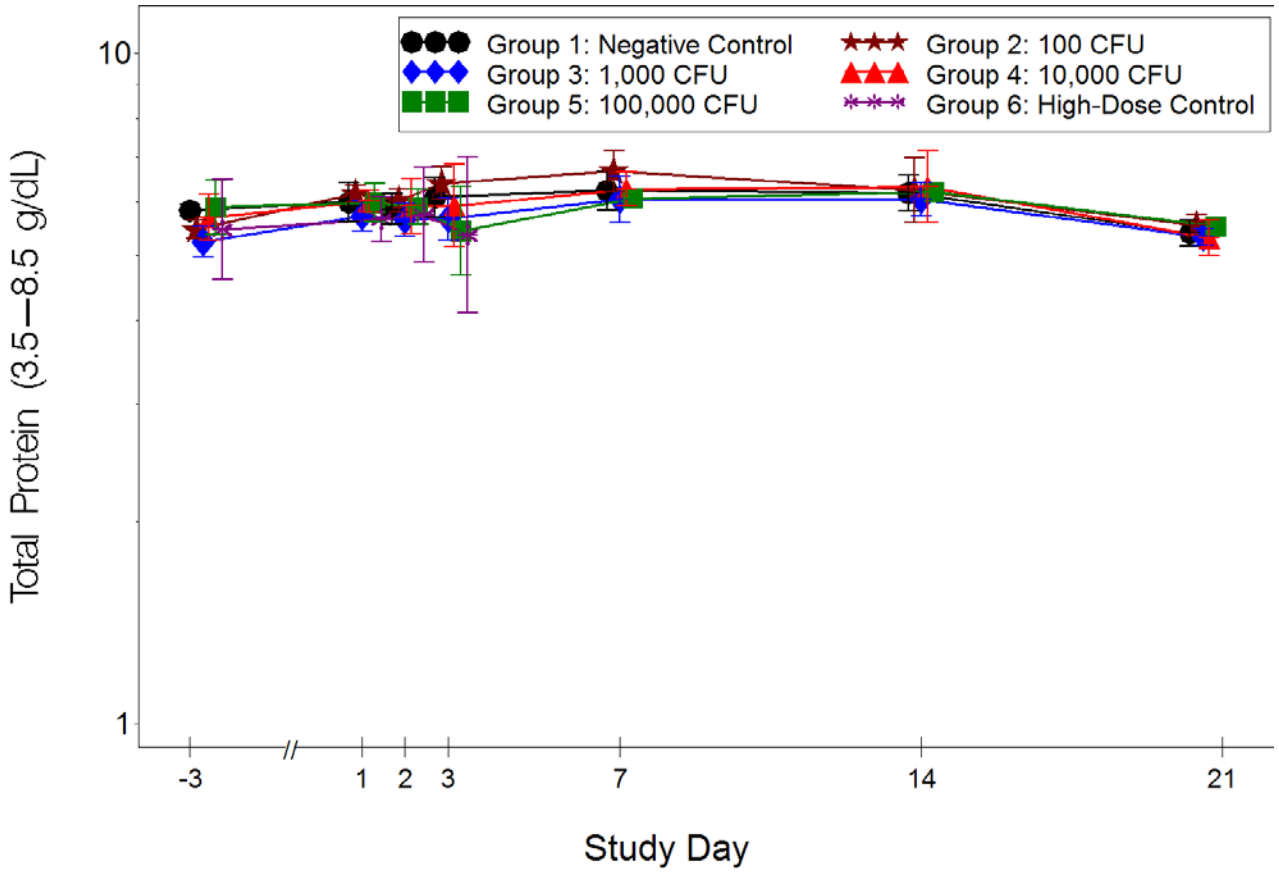

Figure 6. Plot of Total Protein over time.

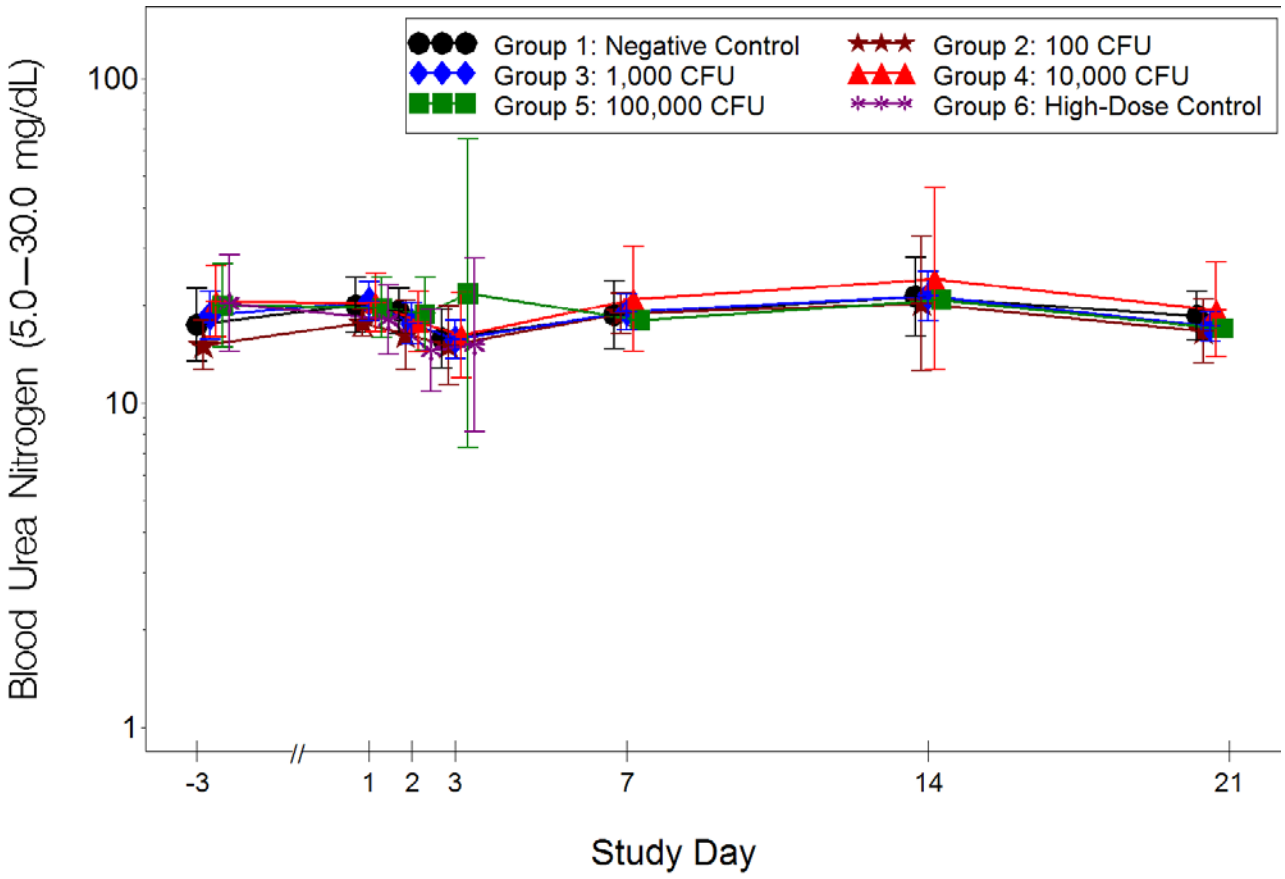

Figure 7. Plot of Blood Urea Nitrogen over time.

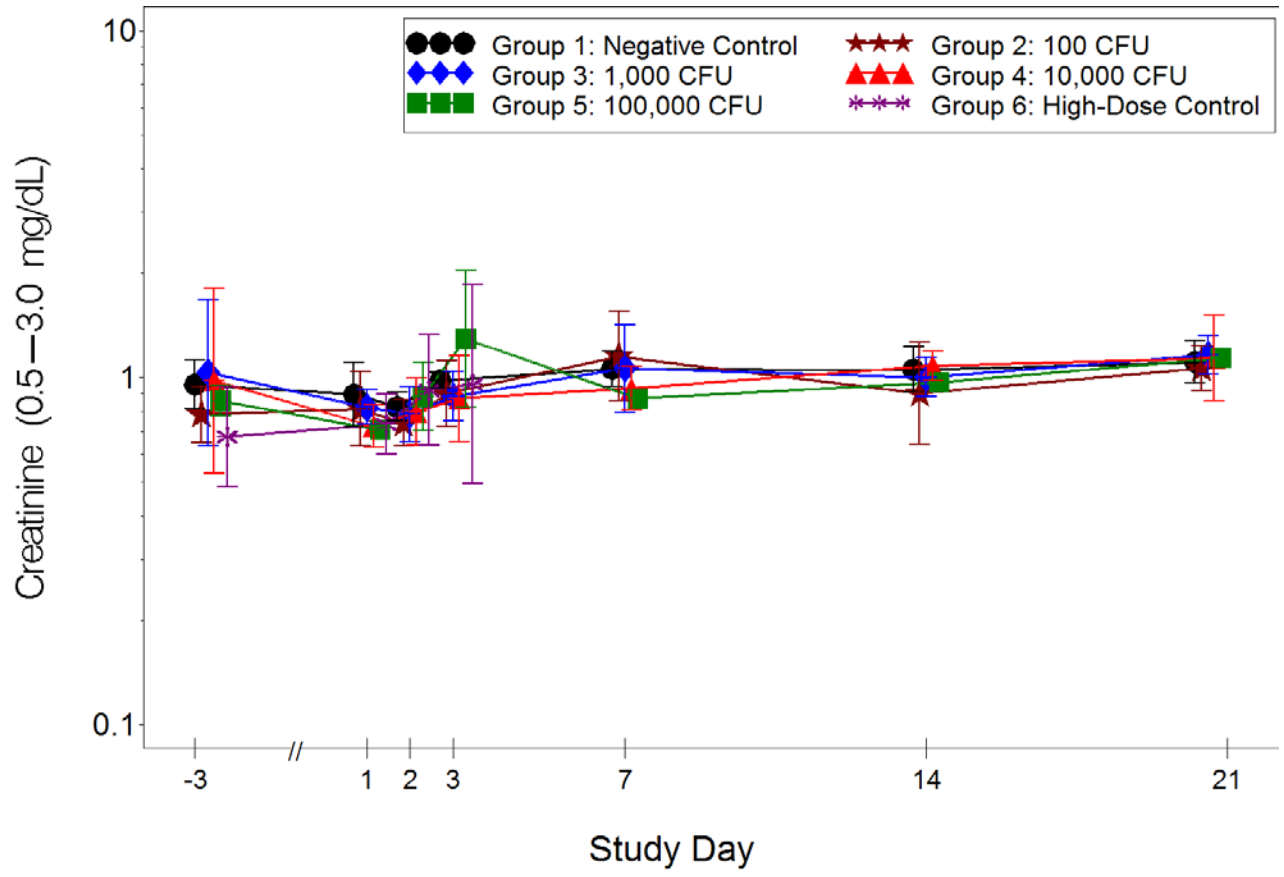

Figure 8. Plot of Creatinine over time.

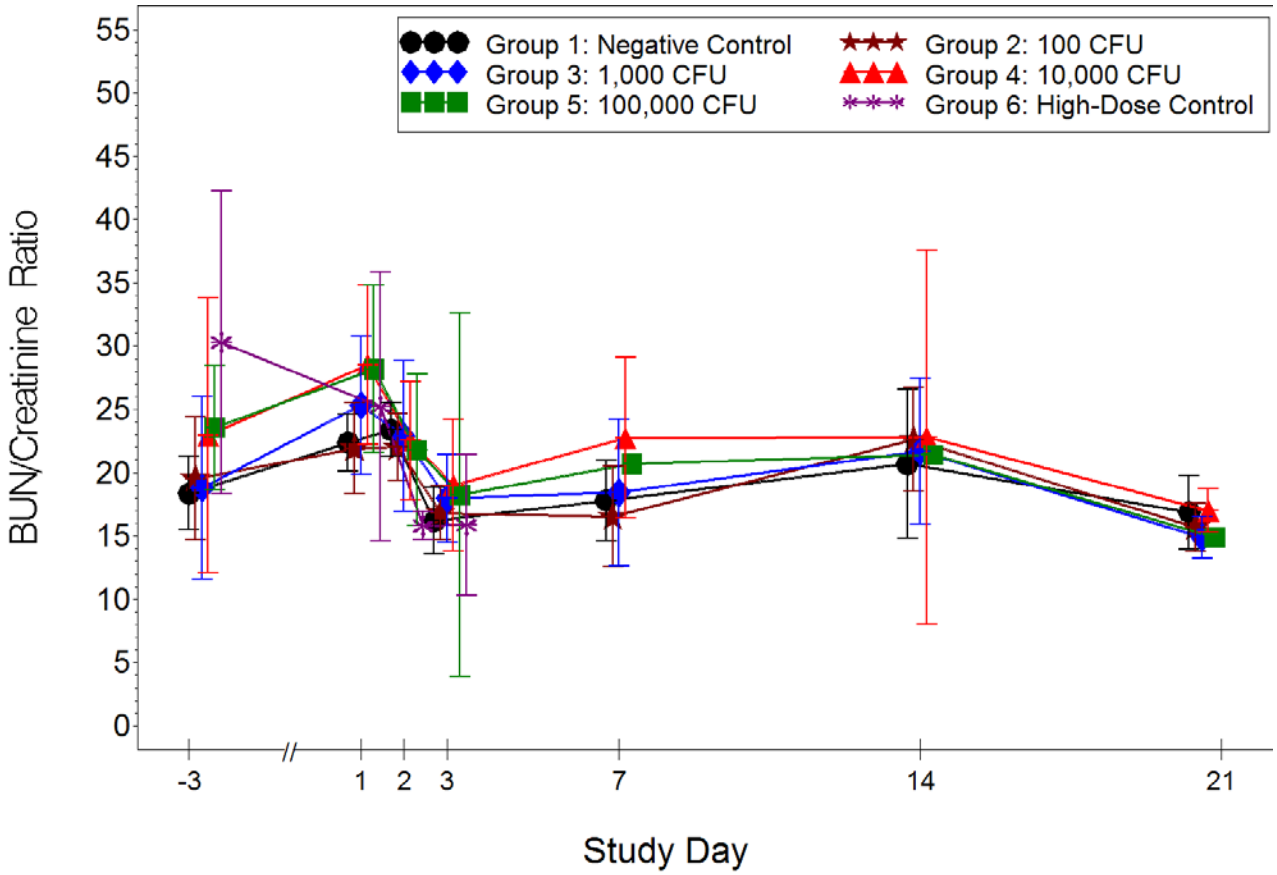

Figure 9. Plot of BUN/Creatinine Ratio over time.

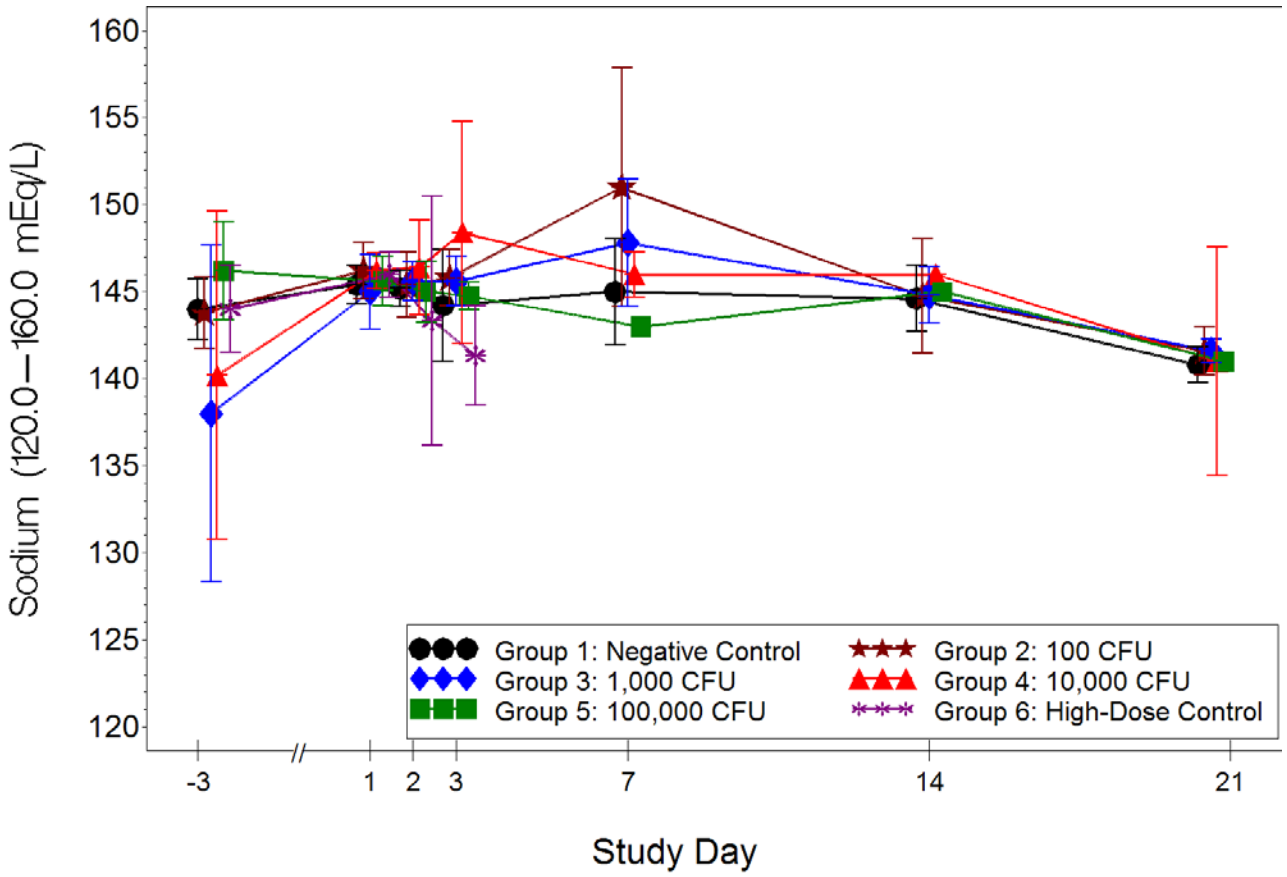

Figure 10. Plot of Sodium over time.

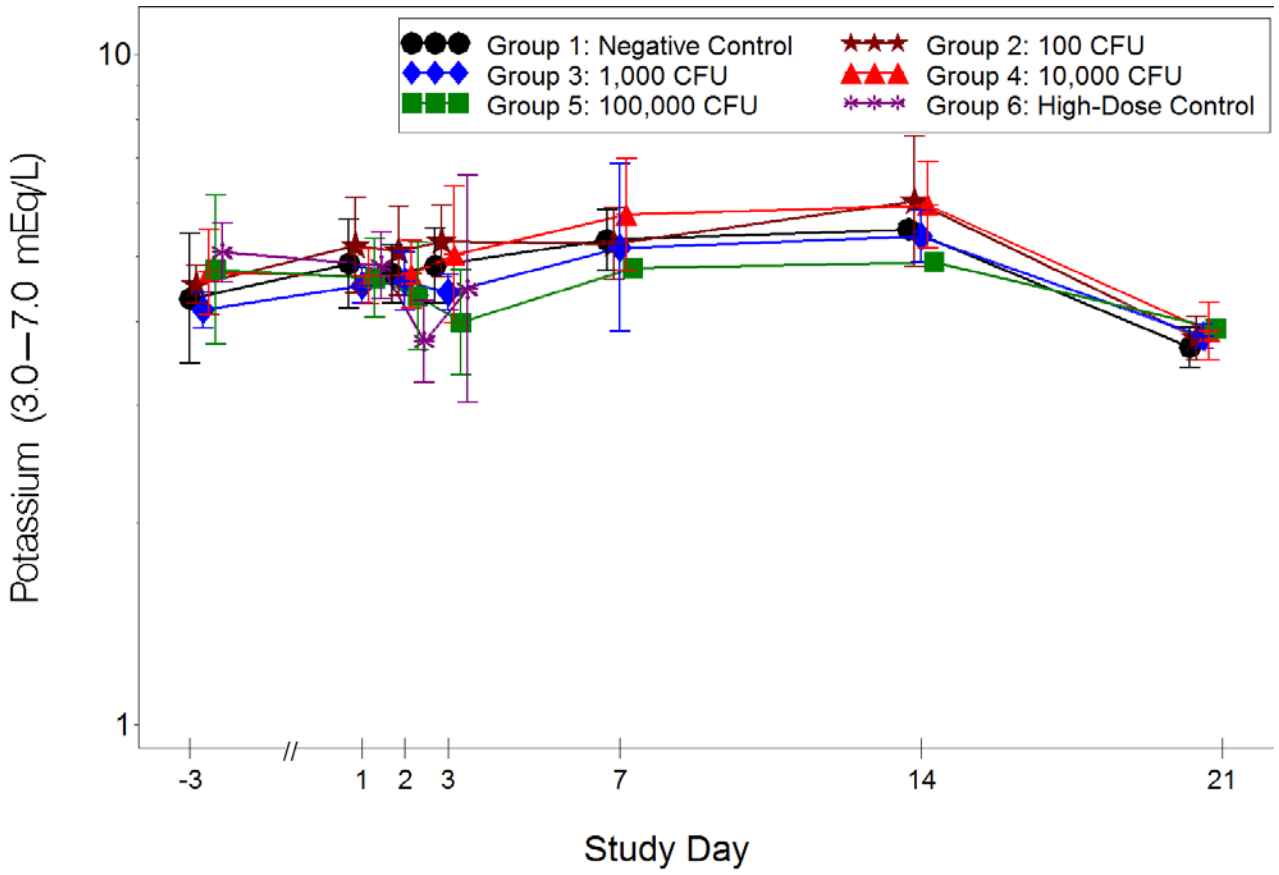

Figure 11. Plot of Potassium over time.

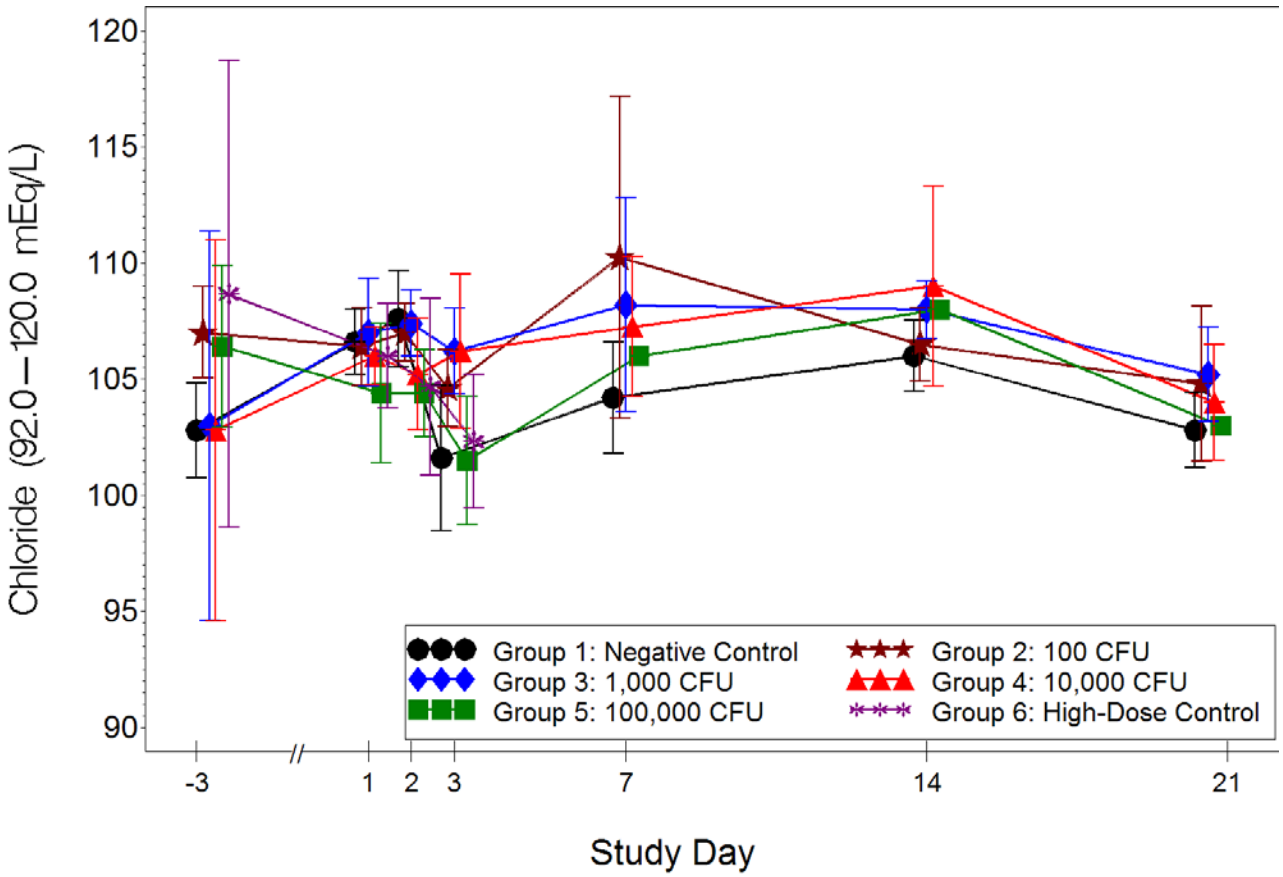

Figure 12. Plot of Chloride over time.

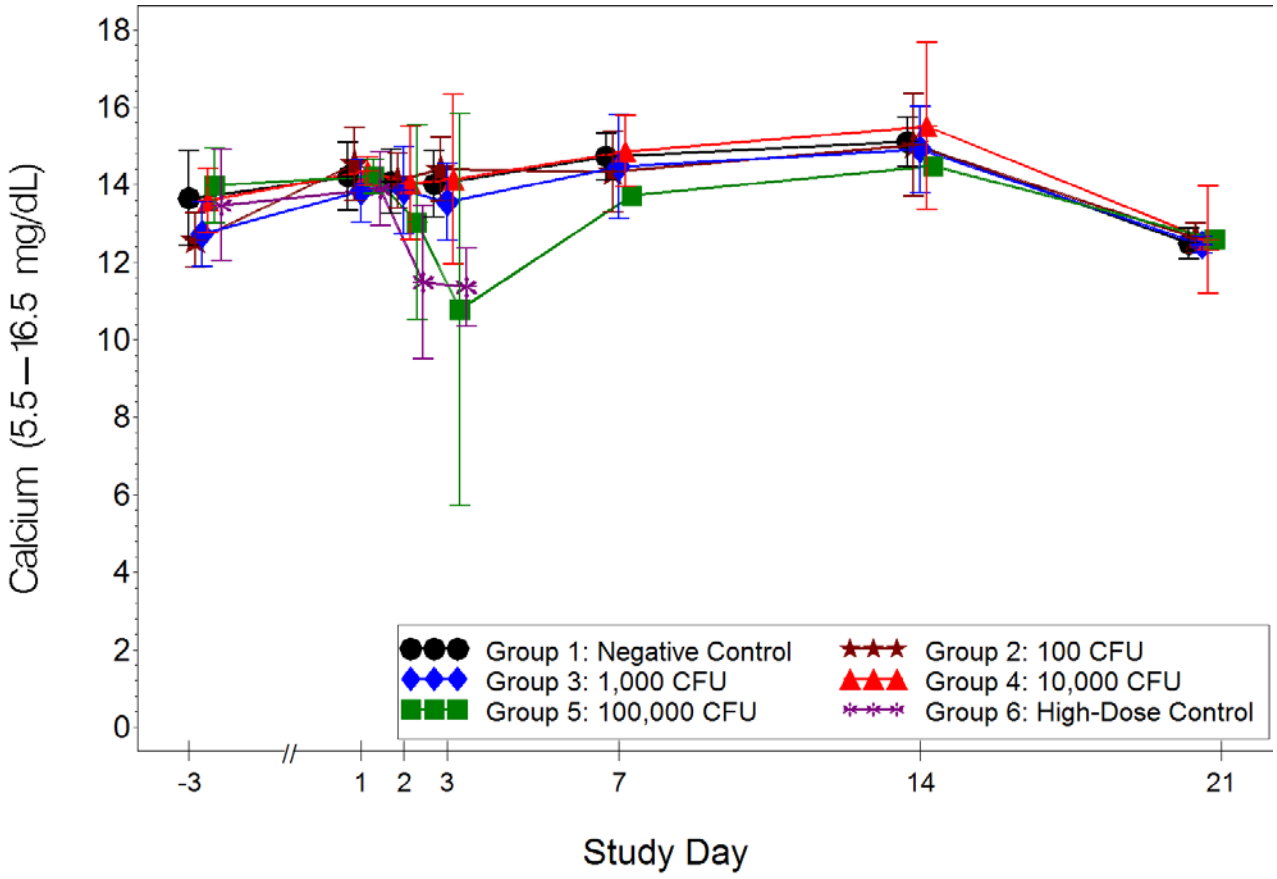

Figure 13. Plot of Calcium over time.

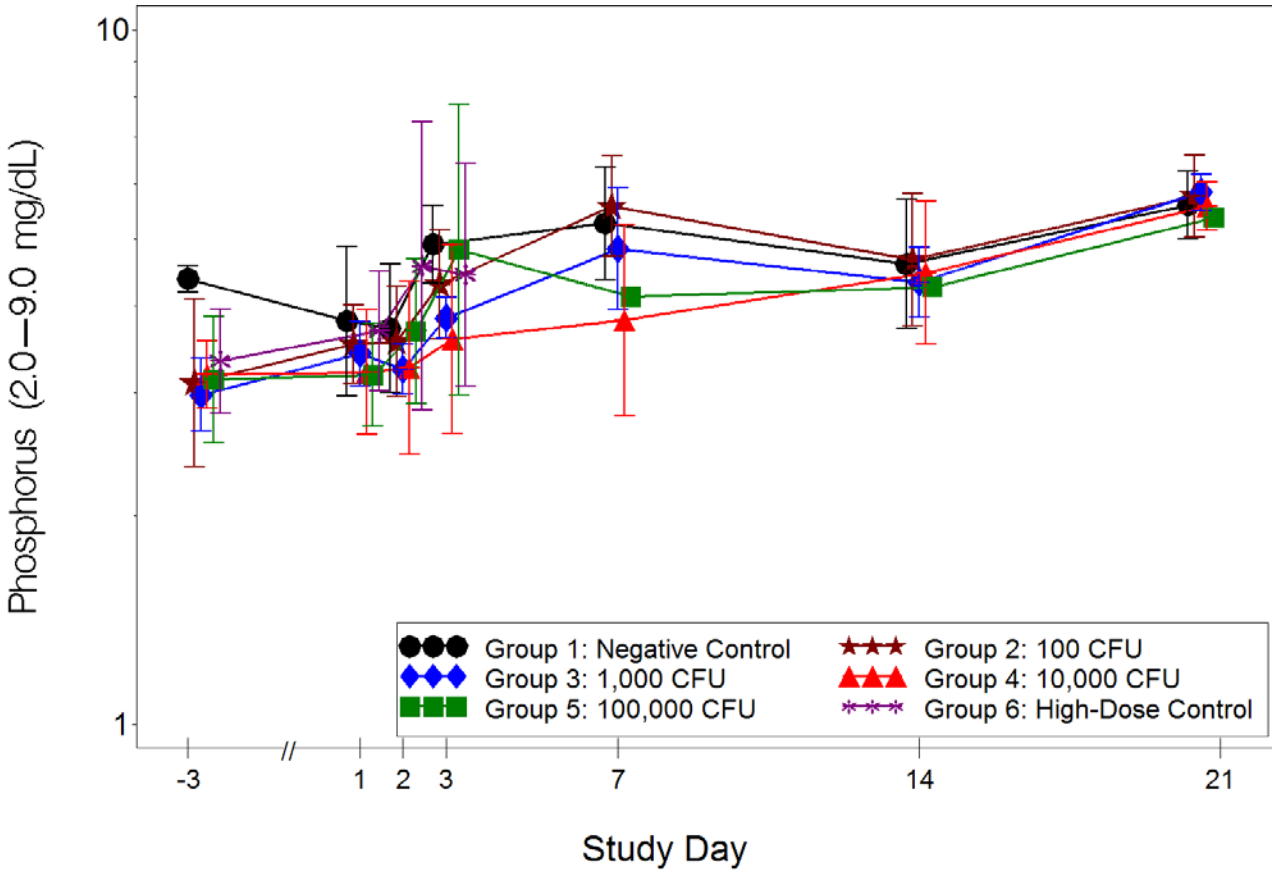

Figure 14. Plot of Phosphorus over time.

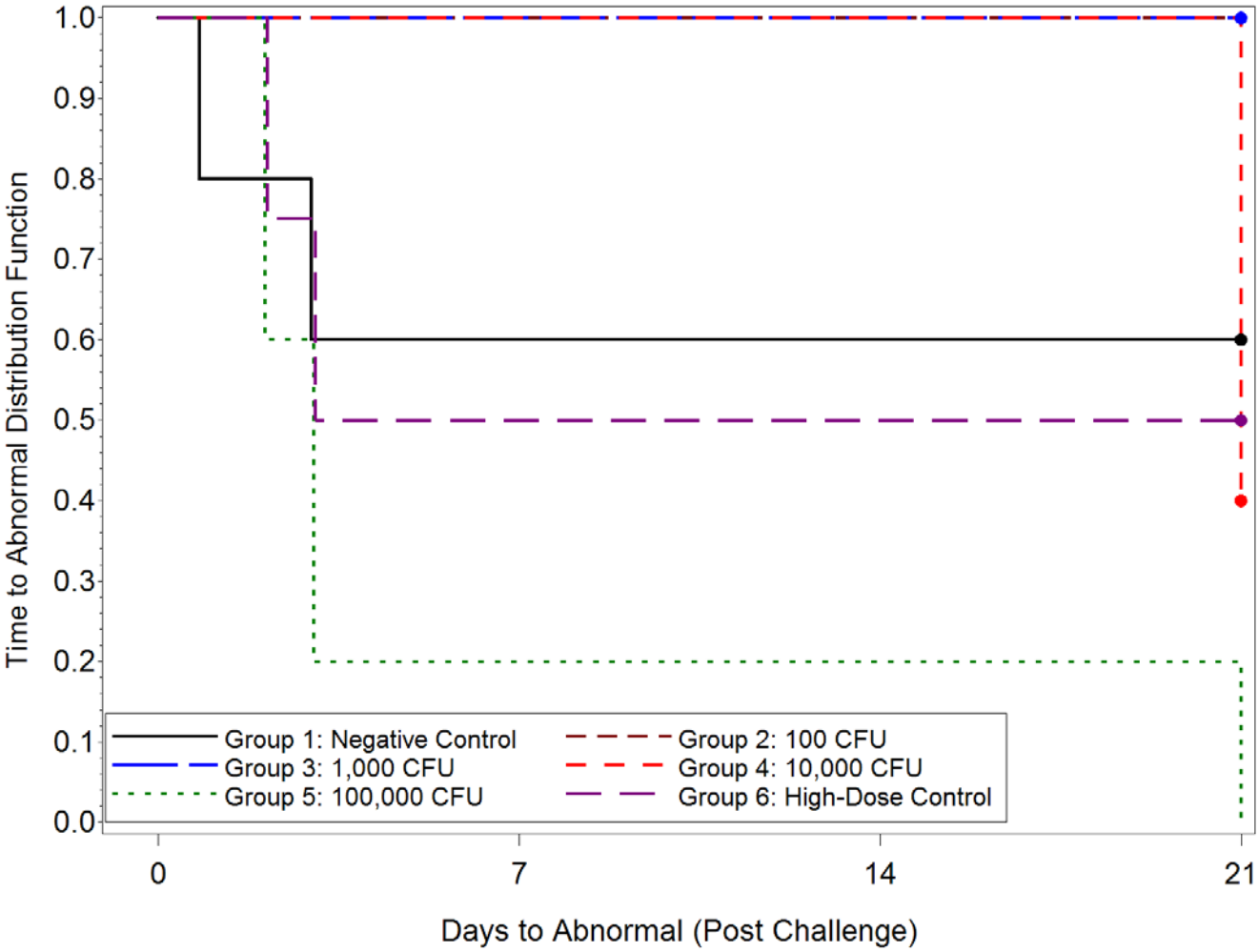

Figure 15. **Kaplan-Meier curves representing time to abnormal Lactate Dehydrogenase data for each group.**

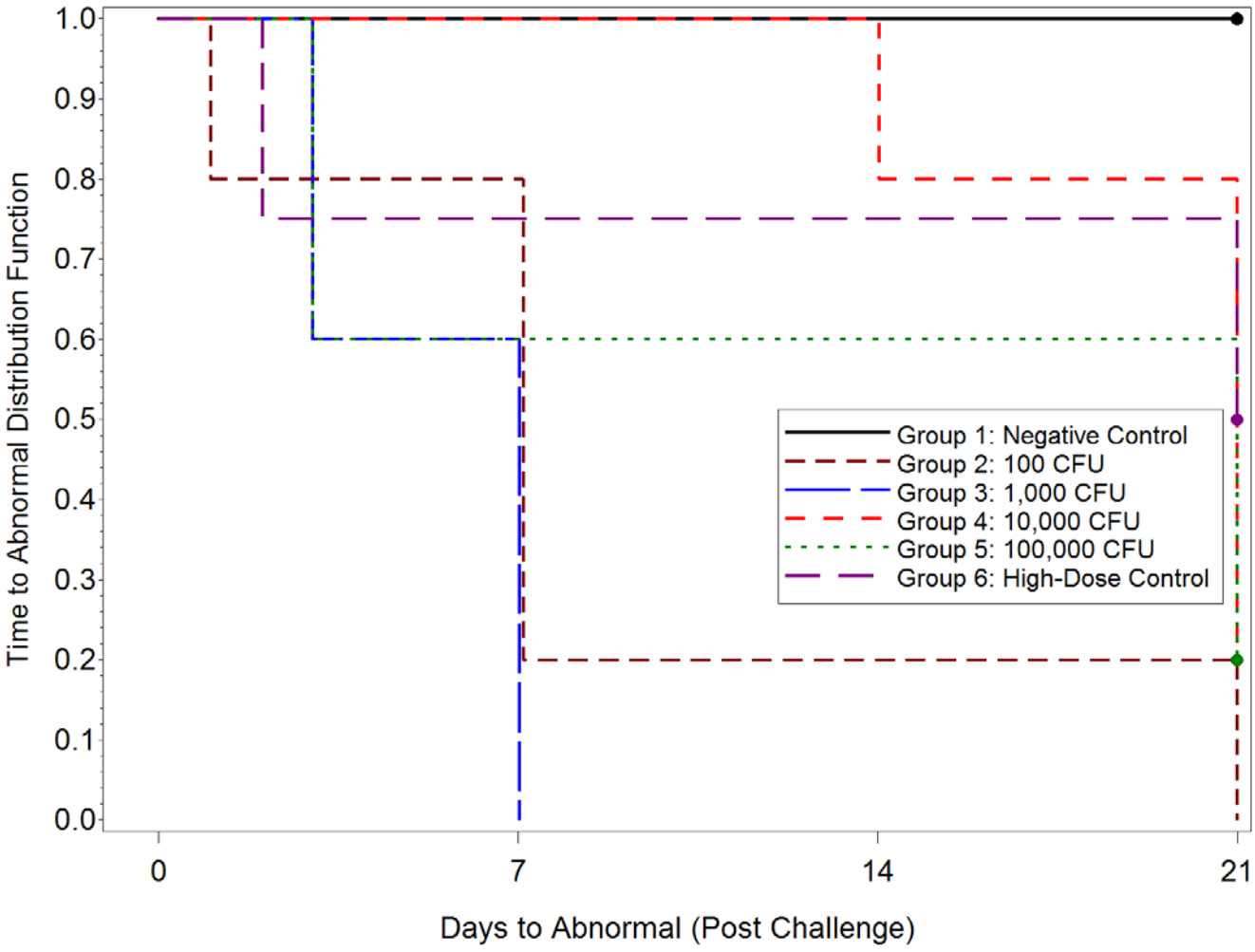

Figure 16. **Kaplan-Meier curves representing time to abnormal Phosphorus data for each group.**

Attachment 1:

Results of Analysis Repeated with Potential Outliers Excluded

Attachment 1: Results of Analysis Repeated with Potential Outliers Excluded

The clinical chemistry parameters contained a total of 18 potential outliers that are displayed in Table 2 of the report. To determine the effect of the potential outliers on the statistical analysis, the analysis was performed on the data with these observations excluded. The results that had a change in significance after excluding the potential outliers are presented below.

Tables 1-1 through 1-9 contain test results for those parameters that experienced changes in significance due to the exclusion of the potential outliers, when compared to the corresponding results shown in Tables 4b through 17b where the potential outliers were not excluded. Table entries are shown in bold if the significance changed in comparison to the corresponding results shown in Tables 4b through 17b. With the potential outliers excluded, the following changes in significance were noted:

- **AST (Table 1-1)**: There was no longer a significant increase as a proportion of baseline in Group 5 at Study Day 2. The increase in Group 6 at Study Day 2 was significantly different than the changes in Groups 2, 3, 4, and 5. There were significant increases as a proportion of baseline in Groups 2, 4, and 6 at Study Day 3. The increases in Groups 2, 4, and 6 were significantly different than the decrease in Group 1.

- **ALT (Table 1-2)**: The increase as a proportion of baseline in Group 5 at Study Day 2 was no longer significant. There were significant increases as a proportion of baseline in Groups 2, 3, and 4 at Study Day 2. The increase in Group 6 was significantly greater than that in Group 1. There were significant increases as a proportion of baseline in Groups 2, 3, and 6 at Study Day 3.

- **SDH (Table 1-3)**: There was no longer a significant increase as a proportion of baseline in Group 5 at Study Day 2. There was a significant decrease as a proportion of baseline in Group 1 at Study Day 2. The increase in Group 6 at Study Day 2 was significantly greater than those in Groups 2, 3, 4, and 5. There were significant increases as a proportion of baseline in Groups 2 and 4 at Study Day 3.

- **BUN (Table 1-4)**: At Study Day 3, there was a significant decrease as a proportion of baseline in Groups 3 and 6.

- **Creatinine (Table 1-5)**: There was a significant increase as a proportion of baseline in Groups 4 and 5 at the terminal blood draw.

- **BUN/Creatinine Ratio (Table 1-6)**: There was a significant decrease from baseline in Group 5 at Study Day 3.

- **Sodium (Table 1-7)**: There was a significant increase from baseline in Group 4, and a significant decrease from baseline in Group 5 at the terminal blood draw.

- **Chloride (Table 1-8)**: There were significant decreases from baseline in Groups 4 and 5 at the terminal blood draw.

- **Calcium (Table 1-9)**: There was a significant decrease from baseline in Group 6 at Study Day 3 that was significantly different than the changes in Groups 2, 3, and 4. The decrease from baseline in Group 5 was significantly different than the increases in Groups 1 and 4 at Study Day 3.

Table 1-1. Summary of Test Results for Aspartate Aminotransferase (AST, U/L) with Potential Outliers Excluded

Aspartate Aminotransferase†								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value#
	1	2	3	4	5	6		
2	0.83	1.13	1.26	1.36	**1.21**	5.13 ↑	<0.0001*	0.16 (1<6) <0.0001* **0.22 (2<6) 0.0003** **0.25 (3<6) 0.0008** **0.27 (4<6) 0.0014** **0.24 (5<6) 0.0009**
3	0.83	**1.33** ↑	1.06	**1.45** ↑	38.39 ↑	**1.69** ↑	<0.0001*	**0.62 (1<2) 0.0225** **0.57 (1<4) 0.0054** 0.02 (1<5) <0.0001* **0.49 (1<6) 0.0020** 0.03 (2<5) <0.0001* 0.03 (3<5) <0.0001* 0.04 (4<5) <0.0001* 0.04 (6<5) <0.0001*

† Indicates that values for this parameter were log-transformed for the analysis.

Cells contain all pairwise comparisons that are significant at the 0.05 level. The format within each cell is: (1) the ratio of shifts, (2) the relationship between the corresponding pair of Group mean shifts shown in parentheses [For example, "(1<6)" indicates that the mean shift as a proportion of baseline for Group 1 was less than that for Group 6], and (3) the Tukey-adjusted p-value.

↑, ↓ Indicate that the ratio of the geometric mean at baseline and the geometric mean at the Study Day was significantly different from one (at the 0.05 level). "↑" indicates that the geometric mean at the Study Day was greater than that at baseline**,** while "↓" indicates that the geometric mean at the Study Day was less than that at baseline.

* Group effect was significant at the 0.05 level.

Table 1-2. Summary of Test Results for Alanine Aminotransferase (ALT, U/L) with Potential Outliers Excluded

Aspartate Aminotransferase†								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value#
	1	2	3	4	5	6		
2	1.06	**1.24** ↑	**1.28** ↑	**1.34** ↑	**1.21**	1.91 ↑	**0.0443 ***	**0.56 (1<6) 0.0170**
3	1.08	**1.35** ↑	**1.25** ↑	1.41 ↑	5.33 ↑	**1.29** ↑	<0.0001*	0.20 (1<5) <0.0001* 0.25 (2<5) <0.0001* 0.23 (3<5) <0.0001* 0.26 (4<5) <0.0001* 0.24 (6<5) <0.0001*

† Indicates that values for this parameter were log-transformed for the analysis.

Cells contain all pairwise comparisons that are significant at the 0.05 level. The format within each cell is: (1) the ratio of shifts, (2) the relationship between the corresponding pair of Group mean shifts shown in parentheses [For example, "(1<6)" indicates that the mean shift as a proportion of baseline for Group 1 was less than that for Group 6], and (3) the Tukey-adjusted p-value.

↑, ↓ Indicate that the ratio of the geometric mean at baseline and the geometric mean at the Study Day was significantly different from one (at the 0.05 level). "↑" indicates that the geometric mean at the Study Day was greater than that at baseline**,** while "↓" indicates that the geometric mean at the Study Day was less than that at baseline.

* Group effect was significant at the 0.05 level.

Table 1-3. Summary of Test Results for Sorbitol Dehydrogenase (SDH, U/L) with Potential Outliers Excluded

Sorbitol Dehydrogenase†								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value#
	1	2	3	4	5	6		
2	**0.73** ↓	1.16	1.16	1.33	**1.19**	2.78 ↑	0.0006 *	0.26 (1<6) 0.0001 **0.42 (2<6) 0.0123** **0.42 (3<6) 0.0117** **0.48 (4<6) 0.0428** **0.43 (5<6) 0.0214**
3	0.87	**1.33** ↑	1.02	**1.50** ↑	10.61 ↑	1.02	<0.0001*	0.08 (1<5) <0.0001* 0.12 (2<5) <0.0001* 0.10 (3<5) <0.0001* 0.14 (4<5) <0.0001* 0.10 (6<5) <0.0001*

† Indicates that values for this parameter were log-transformed for the analysis.

Cells contain all pairwise comparisons that are significant at the 0.05 level. The format within each cell is: (1) the ratio of shifts, (2) the relationship between the corresponding pair of Group mean shifts shown in parentheses [For example, "(1<6)" indicates that the mean shift as a proportion of baseline for Group 1 was less than that for Group 6], and (3) the Tukey-adjusted p-value.

↑, ↓ Indicate that the ratio of the geometric mean at baseline and the geometric mean at the Study Day was significantly different from one (at the 0.05 level). "↑" indicates that the geometric mean at the Study Day was greater than that at baseline**,** while "↓" indicates that the geometric mean at the Study Day was less than that at baseline.

* Group effect was significant at the 0.05 level.

Table 1-4. Summary of Test Results for Blood Urea Nitrogen (mg/dL) with Potential Outliers Excluded

Blood Urea Nitrogen†								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value#
	1	2	3	4	5	6		
3	0.91	1.00	**0.84** ↓	0.79 ↓	0.90	**0.77** ↓	0.2364	

† Indicates that values for this parameter were log-transformed for the analysis.

Cells contain all pairwise comparisons that are significant at the 0.05 level. The format within each cell is: (1) the ratio of shifts, (2) the relationship between the corresponding pair of Group mean shifts shown in parentheses [For example, "(1<6)" indicates that the mean shift as a proportion of baseline for Group 1 was less than that for Group 6], and (3) the Tukey-adjusted p-value.

↑, ↓ Indicate that the ratio of the geometric mean at baseline and the geometric mean at the Study Day was significantly different from one (at the 0.05 level). "↑" indicates that the geometric mean at the Study Day was greater than that at baseline**,** while "↓" indicates that the geometric mean at the Study Day was less than that at baseline.

Table 1-5. Summary of Test Results for Creatinine (mg/dL) with Potential Outliers Excluded

Creatinine†								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value#
	1	2	3	4	5	6		
Terminal	NA	NA	NA	**2.31** ↑	**1.22** ↑	NA	NA	

† Indicates that values for this parameter were log-transformed for the analysis.

Cells contain all pairwise comparisons that are significant at the 0.05 level. The format within each cell is: (1) the ratio of shifts, (2) the relationship between the corresponding pair of Group mean shifts shown in parentheses [For example, "(3<5)" indicates that the mean shift as a proportion of baseline for Group 3 was less than that for Group 5], and (3) the Tukey-adjusted p-value.

NA Data not available for this Group at this Study Day, or not enough animals to perform analysis.

↑, ↓ Indicate that the ratio of the geometric mean at baseline and the geometric mean at the Study Day was significantly different from one (at the 0.05 level). "↑" indicates that the geometric mean at the Study Day was greater than that at baseline**,** while "↓" indicates that the geometric mean at the Study Day was less than that at baseline.

Table 1-6. Summary of Test Results for BUN/Creatinine Ratio with Potential Outliers Excluded

BUN/Creatinine Ratio								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
	1	2	3	4	5	6		
3	-2.2	-2.7	-0.8	-3.9	**-7.1** ↓	-9.9 ↓	0.1235	

There were no significant Tukey's pairwise comparisons.

↑, ↓ Indicate that the difference between the mean at baseline and the mean at the Study Day was significantly different from zero (at the 0.05 level). "↑" indicates that the mean at the Study Day was greater than that at baseline**,** while "↓" indicates that the mean at the Study Day was less than that at baseline.

Table 1-7. Summary of Test Results for Sodium (mEq/L) with Potential Outliers Excluded

Sodium								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
	1	2	3	4	5	6		
Terminal	NA	NA	NA	**12** ↑	**-2** ↓	NA	NA	

Cells contain all pairwise comparisons that are statistically significant at the 0.05 level. The format within each cell is: (1) the difference of shifts, (2) the relationship between the corresponding pair of Group mean shifts shown in parentheses [For example, "(1<4)" indicates that the mean shift from baseline for Group 1 was less than that for Group 4], and (3) the Tukey-adjusted p-value.

↑, ↓ Indicate that the difference between the mean at baseline and the mean at the Study Day was significantly different from zero (at the 0.05 level). "↑" indicates that the mean at the Study Day was greater than that at baseline**,** while "↓" indicates that the mean at the Study Day was less than that at baseline.

NA Data not available for this Group at this Study Day, or not enough animals to perform analysis.

Table 1-8. Summary of Test Results for Chloride (mEq/L) with Potential Outliers Excluded

Chloride								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value[#]
	1	2	3	4	5	6		
Terminal	NA	NA	NA	**-6 ↓**	**-10 ↓**	NA	NA	

\# Cells contain all pairwise comparisons that are statistically significant at the 0.05 level. The format within each cell is: (1) the difference of shifts, (2) the relationship between the corresponding pair of Group mean shifts shown in parentheses [For example, "(5<1)" indicates that the mean shift from baseline for Group 5 was less than that for Group 1], and (3) the Tukey-adjusted p-value.

↑, ↓ Indicate that the difference between the mean at baseline and the mean at the Study Day was significantly different from zero (at the 0.05 level). "↑" indicates that the mean at the Study Day was greater than that at baseline**,** while "↓" indicates that the mean at the Study Day was less than that at baseline.

NA Data not available for this Group at this Study Day, or not enough animals to perform analysis.

Table 1-9. Summary of Test Results for Calcium (mg/dL) with Potential Outliers Excluded

Calcium								
Study Day	Mean Shift as a Proportion of Baseline, by Group						Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value[#]
	1	2	3	4	5	6		
3	0.36	1.84 ↑	0.83	0.55	-2.36 ↓	**-1.88 ↓**	0.0003 *	**-2.72 (5<1) 0.0467** -4.19 (5<2) 0.0011 **-3.72 (6<2) 0.0009** -3.18 (5<3) 0.0148 **-2.71 (6<3) 0.0180** **-2.90 (5<4) 0.0298** **-2.43 (6<4) 0.0397**

\# Cells contain all pairwise comparisons that are statistically significant at the 0.05 level. The format within each cell is: (1) the difference of shifts, (2) the relationship between the corresponding pair of Group mean shifts shown in parentheses [For example, "(5<2)" indicates that the mean shift from baseline for Group 5 was less than that for Group 2], and (3) the Tukey-adjusted p-value.

↑, ↓ Indicate that the difference between the mean at baseline and the mean at the Study Day was significantly different from zero (at the 0.05 level). "↑" indicates that the mean at the Study Day was greater than that at baseline**,** while "↓" indicates that the mean at the Study Day was less than that at baseline.

* Group effect was significant at the 0.05 level.

APPENDIX K
HEAT SHOCK RESULTS

1020-CG920503 Heat Shock Comparison (cfu/mL)							
Sample Type	**Sample ID**	**Date of First Heat Shock**	**Enumeration Prior to First Heat Shock**	**Enumeration Post First Heat Shock**	**Date of Second Heat Shock**	**Enumeration Prior to Second Heat Shock**	**Enumeration Post Second Heat Shock**
Nebulizer	**L23204**	**9/30/2009**	9.30E+08	8.80E+08	**N/A**	N/A	N/A
Nebulizer	**L23203**	**9/30/2009**	1.48E+09	1.26E+09	**N/A**	N/A	N/A
Nebulizer	**L23213**	**9/30/2009**	1.22E+09	1.18E+09	**N/A**	N/A	N/A
Nebulizer	**L23221**	**9/30/2009**	1.33E+09	1.27E+09	**N/A**	N/A	N/A
Nebulizer	**L23232**	**9/30/2009**	1.27E+09	1.44E+09	**N/A**	N/A	N/A
Impinger	**L23204**	**9/18/2009**	7.52E+05	7.80E+05	**9/30/2009**	7.28E+05	6.16E+05
Impinger	**L23203**	**9/18/2009**	1.83E+06	1.88E+06	**9/30/2009**	1.72E+06	1.16E+06
Impinger	**L23213**	**9/18/2009**	1.59E+06	1.83E+06	**9/30/2009**	1.68E+06	1.34E+06
Impinger	**L23221**	**9/18/2009**	1.85E+06	1.73E+06	**9/30/2009**	1.60E+06	1.43E+06
Impinger	**L23232**	**9/18/2009**	1.83E+06	2.73E+06	**9/30/2009**	1.73E+06	1.35E+06

K-2

Printed By/Date: TRM 11.16.09

QA Audit Completed By/Date: [illegible] 11/16/09

QC/Technical Review By/Date: AEK 111609

APPENDIX L
CLINICAL OBSERVATIONS

Animal ID	Sex	Group	9/8/2009		9/9/2009		9/10/2009		9/11/2009		9/12/2009		9/13/2009		9/14/2009	
			AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
L23220	M	1		N	N	N	N	N	N	N	N	N	N	N	N	N
L23216	M	1		N	N	N	N	N	N	N	N	N	N	N	N	N
L23218	M	1		N	N	N	N	N	N	N	N	N	N	N	N	N
L23223	M	1		N	N	N	A[1]	N	N	N	N	N	N	N	N	N
L23222	M	1		N	N	N	N	N	N	N	N	N	N	N	N	N
L23215	M	2		N	N	N	N	N	N	N	N	N	N	N	N	N
L23206	M	2		N	N	N	N	N	N	N	N	N	N	N	N	N
L23210	M	2		N	N	N	N	N	N	N	N	N	N	N	N	N
L23219	M	2		N	N	N	N	N	N	N	N	N	N	N	N	N
L23211	M	2		N	N	N	N	N	N	N	N	N	N	N	N	N
L23217	M	3		N	N	N	N	N	N	N	N	N	N	N	N	N
L23230	M	3		N	N	N	N	N	N	N	N	N	N	N	N	N
L23228	M	3		N	N	N	N	N	N	N	N	N	N	N	N	N
L23227	M	3		N	N	N	N	N	N	N	N	N	N	N	N	N
L23229	M	3		N	N	N	N	N	N	N	N	N	N	N	N	N
L23235	M	4		N	N	N	N	N	N	N	N	N	N	N	N	N
L23205	M	4		N	N	N	N	N	N	N	N	N	N	N	N	N
L23225	M	4		N	N	N	N	N	N	N	N	N	N	N	N	N
L23231	M	4		N	N	N	N	N	N	N	N	N	N	N	N	N
L23207	M	4		N	N	N	N	N	N	N	N	N	N	N	N	N
L23201	M	5		N	N	N	N	N	N	N	N	N	N	N	N	N
L23234	M	5		N	N	N	N	N	N	N	NE	N	N	N	N	N
L23212	M	5		N	N	N	N	N	N	N	N	N	N	N	N	N
L23200	M	5		N	N	N	N	N	N	N	N	N	N	N	N	N
L23214	M	5		N	N	N	N	N	N	N	N	N	N	N	N	N
L23204	M	6		N	N	N	N	N	N	N	N	N	N	N	N	N
L23203	M	6		N	N	N	N	N	N	N	N	N	N	N	N	N
L23213	M	6		N	N	N	N	N	N	N	N	N	N	N	N	N
L23221	M	6		N	N	N	N	N	N	N	N	N	N	N	N	N
L23232	M	6		N	N	N	N	N	N	N	N	N	N	N	N	N

Animals arrived in AM
Animals challenged

COMMENTS:
[1]Animal chewed on right rear foot, sloughing skin off

KEY:
N=Normal
NE=Not Eating
A=Abnormal w/comment

L-2

QA Audit Completed
By/Date: [illegible] 1/27/10

Prepared By: JJm 1/6/10
QC/Tech Reviewed By: [illegible] 1-6-10

Animal ID	Sex	Group	9/15/2009		9/16/2009		9/17/2009		9/18/2009	
			AM	PM	AM	PM	AM	PM	AM	PM
L23220	M	1	N	N	N	N	N	N	N	
L23216	M	1	N	N	N	N	N	N	N	
L23218	M	1	N	N	N	N	N	N	N	
L23223	M	1	N	N	N	N	N	N	N	
L23222	M	1	N	N	N	N	N	N	N	
L23215	M	2	N	N	N	N	N	N	N	
L23206	M	2	N	N	N	N	N	N	N	
L23210	M	2	N	N	N	N	N	N	N	
L23219	M	2	N	N	N	N	N	N	N	
L23211	M	2	N	N	N	N	N	N	N	
L23217	M	3	N	N	N	N	N	N	N	
L23230	M	3	N	N	N	N	N	N	N	
L23228	M	3	N	N	N	N	N	N	N	
L23227	M	3	N	N	N	N	N	N	N	
L23229	M	3	N	N	N	N	N	N	N	
L23235	M	4	N	N	N	N	N	N	N	
L23205	M	4	N	N	N	N	N	N	N	
L23225	M	4	N	N	N	N	N	N	N	
L23231	M	4	N	N	N	N	N	N	N	
L23207	M	4	N	N	N	N	N	N	N	
L23201	M	5	N	N	N	N	N	N	N	
L23234	M	5	N	N	N	N	N	N	N	
L23212	M	5	N	N	N	N	N	N	N	
L23200	M	5	N	N	N	N	N	N	N	
L23214	M	5	N	N	N	N	N	N	N	
L23204	M	6	N	N	N	N	N	N	N	
L23203	M	6	N	N	N	N	N	N	N	
L23213	M	6	N	N	N	N	N	N	N	
L23221	M	6	N	N	N	N	N	N	N	
L23232	M	6	N	N	N	N	N	N	N	

Animals arrived in AM
Animals challenged

COMMENTS:
[1]Animal chewed on right rear foot, sloughing skin off

KEY:
N=Normal
NE=Not Eating
A=Abnormal w/comment

L-3

QA Audit Completed
By/Date: 1/6/10

Prepared By: 11/6/10
QC/Tech Reviewed By: 1-6-10

Animal ID	Sex	Group	9/18/2009		9/19/2009		9/20/2009		9/21/2009		9/22/2009		9/23/2009		9/24/2009		9/25/2009	
			AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
L23220	M	1		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
L23216	M	1		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
L23218	M	1		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
L23223	M	1		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
L23222	M	1		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
L23215	M	2		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
L23206	M	2		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
L23210	M	2		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
L23219	M	2		N	N	N	N	N	N	N	N	N	N	N	N	N	NE	N
L23211	M	2		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
L23217	M	3		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
L23230	M	3		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
L23228	M	3		N	N	N	N	N	N	SS	N	N	N	N	N	N	N	N
L23227	M	3		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
L23229	M	3		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
L23235	M	4		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
L23205	M	4		N	NE	N	N	N	N	N	N	N	N	N	N	N	N	N
L23225	M	4		N	N	N	N	N	NE	L	L,RA	FD						
L23231	M	4		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
L23207	M	4		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
L23201	M	5		N	N	N	N	N	NE,L	L	FD							
L23234	M	5		N	N	N	N	N	N	L	N	N	N	L	FD			
L23212	M	5		N	N	N	N	N	N	L	N	N	N	N	N	N	N	N
L23200	M	5		N	N	N	N	N	N	FD								
L23214	M	5		N	N	N	N	N	NE	L,O[1]	L	L	L,NE	L	FD			
L23204	M	6		N	N	N	N	N	NE	N	L	FD						
L23203	M	6		N	N	N	N	N	NE	L	L	L	FD					
L23213	M	6		N	N	N	N	L	L,NE	FD								
L23221	M	6		N	NE	L	FD											
L23232	M	6		N	N	N	N	N	NE,L	L	FD							

**** Observation missing please reference DR # 8477 JJM 1/6/10

α = Animals were euthanized before am observations, please reference DR # 8477 JJM 1/6/10

Prechallenge obs
Animal deceased

N = Normal
NS = No Stool
NE = Not Eating
L = Lethargic
RA = Respiratory Abnormalities
FD = Found Dead
O = Other w/ comment

COMMENTS:

[1] Little stool present
[2] Large scab on top of right hind foot- appears tender
[3] E-collar placed on animal due to chewing
[4] Scab between shoulder blades
[5] Water line discovered disconnected
[6] Ear tag fell out- multiple scabs and swollen areas on left ear- very tender
[7] Left ear tip bruised, sore and notched

L-4

QA Audit Completed
By/Date: MZ 1/27/10

Prepared By: JJM 1/6/10
QC/Tech Reviewed By: DC 1-6-10

Animal ID	Sex	Group	9/26/2009		9/27/2009		9/28/2009		9/29/2009		9/30/2009		10/1/2009		10/2/2009	
			AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
L23220	M	1	N	N	N	N	N	N	N	N	N	****	N	N	N	N
L23216	M	1	N	N	N	N	N	N	N	N	N	****	N	N	N	N
L23218	M	1	O[2]	N	N	N	N	N	N	N	O[3]	****	N	N	N	N
L23223	M	1	N	N	N	N	N	N	N	N	N	****	N	N	N	N
L23222	M	1	N	N	N	N	N	N	N	N	N	****	N	N	N	N
L23215	M	2	O[4]	N	N	N	N	N	N	N	N	****	N	N	N	N
L23206	M	2	NE,O[5]	N	N	N	N	N	N	N	N	****	N	N	N	N
L23210	M	2	NE,O[5]	N	N	N	N	N	N	N	N	****	N	N	N	N
L23219	M	2	NE,O[5,6]	N	N	N	N	N	N	N	N	****	N	N	N	N
L23211	M	2	NE,O[5]	N	N	N	N	N	N	N	N	****	N	N	N	N
L23217	M	3	NE,O[5,7]	N	N	N	N	N	N	N	N	****	N	N	N	N
L23230	M	3	NE,O[5]	N	N	N	N	N	N	N	N	****	N	N	N	N
L23228	M	3	N	N	N	N	N	N	N	N	N	****	N	N	N	N
L23227	M	3	N	N	N	N	N	N	N	N	N	****	N	N	N	N
L23229	M	3	N	N	N	N	N	N	N	N	N	****	N	N	N	N
L23235	M	4	N	N	NE,L	N	NE,L	L,O[1]	NE,NS,FD							
L23205	M	4	N	N	N	N	N	N	N	N	N	****	N	N	N	N
L23225	M	4														
L23231	M	4	N	N	N	N	N	N	N	N	N	****	N	N	N	N
L23207	M	4	N	N	N	N	N	N	N	N	N	****	N	N	N	N
L23201	M	5														
L23234	M	5														
L23212	M	5	N	N	N	N	N	N	N	N	N	****	N	N	N	N
L23200	M	5														
L23214	M	5														
L23204	M	6														
L23203	M	6														
L23213	M	6														
L23221	M	6														
L23232	M	6														

**** Observation missing please reference DR # 8477 JSM 1/26/10

α = Animals were euthanized before am observations, please reference DR # 8477 JSM 1/26/10

Prechallenge obs
Animal deceased

N = Normal
NS = No Stool
NE = Not Eating
L = Lethargic
RA = Respiratory Abnormalities
FD = Found Dead
O = Other w/ comment

COMMENTS:

[1] Little stool present
[2] Large scab on top of right hind foot- appears tender
[3] E-collar placed on animal due to chewing
[4] Scab between shoulder blades
[5] Water line discovered disconnected
[6] Ear tag fell out- multiple scabs and swollen areas on left ear- very tender
[7] Left ear tip bruised, sore and notched

L-5

QA Audit Completed By/Date: MT 1/27/10

Prepared By: JSM 1/6/10

QC/Tech Reviewed By: JC 1-10-10

Animal ID	Sex	Group	10/3/2009		10/4/2009		10/5/2009		10/6/2009		10/7/2009		10/8/2009		10/9/2009
			AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM[α]
L23220	M	1	N	N	N	N	N	N	N	N	N	N	N	N	α
L23216	M	1	N	N	N	N	N	N	N	N	N	N	N	N	α
L23218	M	1	SS	N	N	N	N	N	N	N	N	N	N	N	α
L23223	M	1	N	N	N	N	N	N	N	N	N	N	N	N	α
L23222	M	1	N	N	N	N	N	N	N	N	N	N	N	N	α
L23215	M	2	N	N	N	N	N	N	N	N	N	N	N	N	α
L23206	M	2	N	N	N	N	N	N	N	N	N	N	N	N	α
L23210	M	2	N	N	N	N	N	N	N	N	N	N	N	N	α
L23219	M	2	N	N	N	N	N	N	N	N	N	N	N	N	α
L23211	M	2	N	N	N	N	N	N	N	N	N	N	N	N	α
L23217	M	3	N	N	N	N	N	N	N	N	N	N	N	N	α
L23230	M	3	N	N	N	N	N	N	N	N	N	N	N	N	α
L23228	M	3	N	N	N	N	N	N	N	N	N	N	N	N	α
L23227	M	3	N	N	N	N	N	N	N	N	N	N	N	N	α
L23229	M	3	N	N	N	N	N	N	N	N	N	N	N	N	α
L23235	M	4													
L23205	M	4	N	N	N	N	N	N	N	N	N	N	N	N	α
L23225	M	4													
L23231	M	4	N	N	N	N	N	N	N	N	N	N	N	N	α
L23207	M	4	N	N	N	N	N	N	N	N	N	N	N	N	α
L23201	M	5													
L23234	M	5													
L23212	M	5	N	N	N	N	N	N	N	N	N	N	N	N	α
L23200	M	5													
L23214	M	5													
L23204	M	6													
L23203	M	6													
L23213	M	6													
L23221	M	6													
L23232	M	6													

**** Observation missing please reference DR # 8477 JSM 1/26/10

α = Animals were euthanized before am observations, please reference DR # 8477 JSM 1/26/10

Prechallenge obs
Animal deceased

N = Normal
NS = No Stool
NE = Not Eating
L = Lethargic
RA = Respiratory Abnormalities
FD = Found Dead
O = Other w/ comment

COMMENTS:

[1] Little stool present
[2] Large scab on top of right hind foot- appears tender
[3] E-collar placed on animal due to chewing
[4] Scab between shoulder blades
[5] Water line discovered disconnected
[6] Ear tag fell out- multiple scabs and swollen areas on left ear- very tender
[7] Left ear tip bruised, sore and notched

L-6

QA Audit Completed By/Date: MD 1/27/10

Prepared By: JSM 1/6/10

QC/Tech Reviewed By: JC 1-6-10

APPENDIX M
BODY WEIGHTS

1020-CG920503 Body Weight Summary Table

Animal ID	Group	Body Weight (kg)							
		Day 0	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Moribund/ Terminal
L23216	1	3.3	3.4	3.4	3.0	3.3	3.39	3.42	NA
L23218	1	3.3	3.3	3.4	3.3	3.3	3.29	3.29	NA
L23220	1	NA	3.0	3.0	3.0	3.0	3.13	3.10	NA
L23222	1	3.5	3.5	3.6	3.6	3.5	3.67	3.54	NA
L23223	1	3.0	3.0	3.0	3.0	3.0	3.10	3.08	NA
L23206	2	3.2	3.3	3.2	3.2	3.0	3.30	3.42	NA
L23210	2	3.0	3.5	3.1	3.1	2.9	3.24	3.29	NA
L23211	2	3.3	3.2	3.3	3.3	3.1	3.43	3.40	NA
L23215	2	3.1	3.1	3.1	3.0	3.1	3.24	3.15	NA
L23219	2	3.1	3.1	3.2	3.1	2.9	3.29	3.29	NA
L23217	3	3.2	3.2	3.2	3.3	3.1	3.30	3.29	NA
L23227	3	3.3	3.3	3.3	3.3	3.3	3.37	3.31	NA
L23228	3	3.2	3.1	3.1	3.2	3.2	3.33	3.29	NA
L23229	3	3.4	3.4	3.4	3.5	3.4	3.48	3.55	NA
L23230	3	3.0	3.0	3.1	3.1	2.8	3.11	3.06	NA
L23205	4	3.3	3.3	3.3	3.4	3.4	3.37	3.44	NA
L23207	4	3.5	3.5	3.4	3.5	3.4	3.58	3.50	NA
L23225	4	3.3	3.1	3.1	3.1				2.7
L23231	4	3.2	3.1	3.2	3.3	3.2	3.30	3.22	NA
L23235	4	3.3	3.2	3.3	3.3	3.2			3.13
L23200	5	3.4	3.2	3.2	3.3				3.3
L23201	5	3.2	3.2	3.0	3.0				2.8
L23212	5	3.2	3.2	3.2	3.3	3.3	3.33	3.31	NA
L23214	5	3.2	3.2	3.0	3.0				2.9
L23234	5	3.2	3.3	3.2	3.2				3
L23203	6	3.0	2.9	3.0	3.0				2.7
L23204	6	3.1	3.1	3.0	3.0				2.7
L23213	6	3.3	2.9	2.8	3.0				*
L23221	6	3.1	3.2						3.2
L23232	6	3.1	3.1	3.0	2.9				2.9

* = A moribund/terminal weight was not recorded for rabbit L23213

QA AUDIT COMPLETED
BY/DATE: Writz 2/22/11

Prepared By: JRC 2/22/11

QC reviewed by/date: JRC 2/22/11

APPENDIX N
INDIVIDUAL MORTALITY RESULTS

1020-CG920503
Mortality Table

Animal ID	Dose Group	Challenge Date & Time	Date & Time of Death	Time to Death (days)	Found Dead or Moribund/Euthanized/ Survived
L23220	1	9/18/09 8:19	NA	NA	Survived
L23216	1	9/18/09 8:32	NA	NA	Survived
L23218	1	9/18/09 8:44	NA	NA	Survived
L23223	1	9/18/09 8:55	NA	NA	Survived
L23222	1	9/18/09 9:05	NA	NA	Survived
L23215	2	9/18/09 9:37	NA	NA	Survived
L23206	2	9/18/09 9:47	NA	NA	Survived
L23210	2	9/18/09 9:59	NA	NA	Survived
L23219	2	9/18/09 10:07	NA	NA	Survived
L23211	2	9/18/09 10:17	NA	NA	Survived
L23217	3	9/18/09 10:27	NA	NA	Survived
L23230	3	9/18/09 10:37	NA	NA	Survived
L23228	3	9/18/09 10:48	NA	NA	Survived
L23227	3	9/18/09 10:59	NA	NA	Survived
L23229	3	9/18/09 11:11	NA	NA	Survived
L23235	4	9/18/09 11:27	9/29/2009 7:56	11	Found Dead
L23205	4	9/18/09 11:37	NA	NA	Survived
L23225	4	9/18/09 11:48	9/22/2009 15:14	4	Found Dead
L23231	4	9/18/09 11:58	NA	NA	Survived
L23207	4	9/18/09 12:08	NA	NA	Survived
L23201	5	9/18/09 12:18	9/22/2009 8:34	4	Found Dead
L23234	5	9/18/09 12:29	9/24/2009 8:32	6	Found Dead
L23212	5	9/18/09 12:39	NA	NA	Survived
L23200	5	9/18/09 12:47	9/21/2009 11:16	3	Found Dead
L23214	5	9/18/09 12:56	9/24/2009 8:32	6	Found Dead
L23204	6	9/18/09 13:07	9/22/2009 15:14	4	Found Dead
L23203	6	9/18/09 13:17	9/23/2009 8:25	5	Found Dead
L23213	6	9/18/09 13:27	9/21/2009 13:05	3	Found Dead
L23221	6	9/18/09 13:37	9/20/2009 6:33	2	Found Dead
L23232	6	9/18/09 13:48	9/22/2009 8:34	4	Found Dead

NA = Not Applicable

Prepared By: JJM 1/6/10

QA Audit Completed By/Date: MJ 1/27/10

QC/Tech Reviewed By: JC 1-6-10

APPENDIX O
FINAL SURVIVAL STATISTICAL REPORT

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List of Acronyms

BBRC ..... Battelle Biomedical Research Center
CFU ..... Colony forming unit
LD_{50} ..... Median lethal dose
N ..... Number of animals

1. Introduction

This report summarizes the statistical analysis of survival data collected under Battelle Biomedical Research Center (BBRC) Study No. 1020-CG920503. Thirty (30) male pathogen-free New Zealand White rabbits *(Oryctolagus cuniculus)* were randomized into six groups, with each group having five animals. Animals were aerosol challenged on study day zero with *Bacillus anthracis* (Ames strain) spores as indicated in Table 1. Animals were observed twice daily post-challenge and survival data was recorded.

Table 1. Study Design

Group	Number of Animals per Group	Target Spore Dose (CFU)
1[a]	5	100 x LD_{50}
2	5	100
3	5	1,000
4	5	10,000
5	5	100,000
6[b]	5	100 x LD_{50}

CFU Colony forming units
a Spores are gamma-irradiated (negative control)
b High-dose control
LD_{50} Median lethal dose

2. Statistical Methods

Estimates with exact 95% binomial confidence intervals for the proportion of surviving animals within each group were calculated using the FREQ procedure in SAS® (SAS Institute, Cary, NC, version 9.1). An overall two-sided Fisher's exact test was performed to determine if the proportions of surviving animals were significantly different between the groups. If the overall Fisher's exact test was significant, then pairwise two-sided Fisher's exact tests were performed to determine which pairs of groups were significantly different from each other. The Fisher's exact tests were performed using the SAS® FREQ procedure. The SAS® MULTTEST procedure was used to perform the Bonferroni-Holm adjustment to maintain an overall 0.05 level of significance for the multiple pairwise comparisons.

Excluding the negative control group (Group 1), a logistic regression model was fitted to the survival data as a function of the base-10 logarithm of the estimated inhaled dose to determine the effect of dose on lethality. The median lethal dose (LD_{50}) was then estimated from the predicted logistic regression curve, along with 95% Fieller's confidence intervals. The logistic regression models were fitted using the SAS® LOGISTIC procedure, while the Fieller's 95% confidence intervals were obtained by refitting the models using the SAS® PROBIT procedure as a matter of convenience.

The time-to-death data were analyzed in combination with the survival data to determine if there were significant differences between the groups in terms of susceptibility to challenge. Using the SAS® LIFETEST procedure, Kaplan-Meier curves were plotted and an overall log-rank test was performed to determine if the survival distributions within the groups were significantly different from each other. If the overall log-rank test was significant, then pairwise log-rank tests were performed to determine which groups were significantly different from each other. Again, the Bonferroni-Holm adjustment was used to maintain an overall 0.05 level of significance for the multiple pairwise comparisons.

3. Results

Table 2 contains the estimated proportion of surviving animals within each group, along with exact binomial 95% confidence intervals. All animals in the negative control group (Group 1) and the two lowest spore dose groups (Groups 2 and 3) survived. Three of the five animals in the targeted 10,000 colony forming unit (CFU) dose group (Group 4) survived, and only one of the five animals in the targeted 100,000 CFU dose group (Group 5) survived. No animals in the high dose control group (Group 6) survived.

The overall Fisher's exact test was significant (p-value<0.0001), indicating that the proportion of surviving animals in at least one of the groups was significantly different from those in the other groups. Table 3 contains the unadjusted and Bonferroni-Holm adjusted p-values from pairwise Fisher's exact tests. When all animals in both comparison groups survived, the Fisher's exact tests could not be performed; therefore, p-values of 1.0000 were substituted to indicate that the groups were not significantly different from each other. Based on the unadjusted Fisher's exact tests, the proportions of surviving animals in the negative control group (Group 1) and in the two lowest dose groups (Groups 2 and 3) were significantly greater than those in the targeted 100,000 CFU dose group (Group 5) and in the high dose control group (Group 6). However, after adjusting for the multiple comparisons, there were no significant pairwise differences between the groups.

The logistic regression model fitted to the survival data indicated a significant dose-response relationship with increased inhaled doses being associated with decreased probabilities of survival, as evidenced by the significant p-value associated with the estimated slope coefficient of -2.21 (p-value=0.0147). The estimated LD_{50} was 51,789 CFU with a 95% Fieller confidence interval ranging from 6,142 CFU to 726,639 CFU. Figure 1 displays the fitted logistic regression model overlaid on the observed survival (or mortality) data.

The overall log-rank test was significant (p-value<0.0001), indicating that the survival distribution in at least one of the groups was significantly different from those in the other groups. Table 4 contains the unadjusted and Bonferroni-Holm adjusted p-values from pairwise log-rank tests. When all animals in both comparison groups survived, the log-rank tests could not be performed; therefore, p-values of 1.0000 were substituted to indicate that the groups were not

significantly different from each other. Based on the unadjusted log-rank tests, the times to death in the negative control group (Group 1) and in the two lowest target dose groups (Groups 2 and 3) were significantly greater than those in the targeted 100,000 CFU dose group (Group 5) and in the high dose control group (Group 6). Furthermore, the targeted 10,000 CFU dose group (Group 4) also experienced significantly greater times to death than the high dose control group (Group 6). However, after adjusting for the multiple pairwise comparisons, only the times to death in the negative control group (Group 1) and in the two lowest dose groups (Groups 2 and 3) were significantly greater than that in the high dose control group (Group 6). Figure 2 displays the Kaplan-Meier curves for each of the six dose groups. Since all animals in Groups 1 through 3 survived the length of the study, Groups 2 and 3 were plotted with a slight offset so that their curves would be distinguishable. A dose-response relationship was observed, with increased target doses generally being associated with decreased times to death and greater mortality.

4. Conclusions

The proportion of surviving animals decreased for groups that received higher spore doses. All animals in the negative control and the lower dose groups (Groups 1 through 3) survived, while none of the animals in the high dose control group (Group 6) survived. Prior to adjusting for multiple pairwise comparisons, the proportions of surviving animals in the negative control and the two lower dose groups (Groups 1 through 3) were significantly greater than those in the targeted 100,000 CFU dose and high dose control groups (Groups 5 and 6). However, these differences were no longer significant after adjusting for the multiple pairwise comparisons.

The results for the logistic regression model fitted to the survival data indicated a significant dose-response relationship with increased inhaled doses being associated with decreased probabilities of survival. The estimated LD_{50} was 51,789 CFU with a 95% Fieller confidence interval ranging from 6,142 CFU to 726,639 CFU.

The overall log-rank test indicated that the survival distribution in at least one of the groups was significantly different from those in the other groups. Prior to adjusting for multiple pairwise comparisons, the times to death in the negative control and the two lowest dose groups (Groups 1 through 3) were significantly greater than those in the targeted 100,000 CFU dose and the high dose control groups (Groups 5 and 6). Furthermore, the time to death in the targeted 10,000 CFU dose group (Group 4) was significantly greater than that in the high dose control group (Group 6). After adjusting for the multiple pairwise comparisons, the times to death in the negative control and the two lowest dose groups (Groups 1 through 3) were still significantly greater than that in the high dose control group (Group 6). A dose-response relationship was observed in the Kaplan-Meier plots, with increased target doses generally being associated with decreased times to death and greater mortality.

Table 2. Proportion of Surviving Animals with Exact 95% Confidence Interval by Group

Group	Number of Surviving Animals / N	Proportion Survived (95% Confidence Interval)
1	5/5	1.00 (0.48, 1.00)
2	5/5	1.00 (0.48, 1.00)
3	5/5	1.00 (0.48, 1.00)
4	3/5	0.60 (0.15, 0.95)
5	1/5	0.20 (0.01, 0.72)
6	0/5	0.00 (0.00, 0.52)

N Number of animals.

Table 3. Results of Two-Sided Pairwise Fisher's Exact Tests

Group	Two-Sided Pairwise Fisher's Exact Test P-Values									
	Unadjusted P-Values					Bonferroni-Holm Adjusted P-Values				
	2	3	4	5	6	2	3	4	5	6
1	1.0000[a]	1.0000[a]	0.4444	0.0476*	0.0079*	1.0000[a]	1.0000[a]	1.0000	0.5714	0.1190
2		1.0000[a]	0.4444	0.0476*	0.0079*		1.0000[a]	1.0000	0.5714	0.1190
3			0.4444	0.0476*	0.0079*			1.0000	0.5714	0.1190
4				0.5238	0.1667				1.0000	1.0000
5					1.0000					1.0000

a A p-value of 1.0000 was substituted since all animals in both groups survived.
* Comparison significant at the 0.05 level.

Table 4. Results of Pairwise Log-Rank Tests

Group	Pairwise Log-rank Test P-Values									
	Unadjusted P-Values					Bonferroni-Holm Adjusted P-Values				
	2	3	4	5	6	2	3	4	5	6
1	1.0000[a]	1.0000[a]	0.1343	0.0133*	0.0018*	1.0000[a]	1.0000[a]	0.9400	0.1461	0.0276*
2		1.0000[a]	0.1343	0.0133*	0.0018*		1.0000[a]	0.9400	0.1461	0.0276*
3			0.1343	0.0133*	0.0018*			0.9400	0.1461	0.0276*
4				0.1403	0.0064*				0.9400	0.0770
5					0.0943					0.7544

a A p-value of 1.0000 was substituted since all animals in both groups survived.
* Comparison significant at the 0.05 level

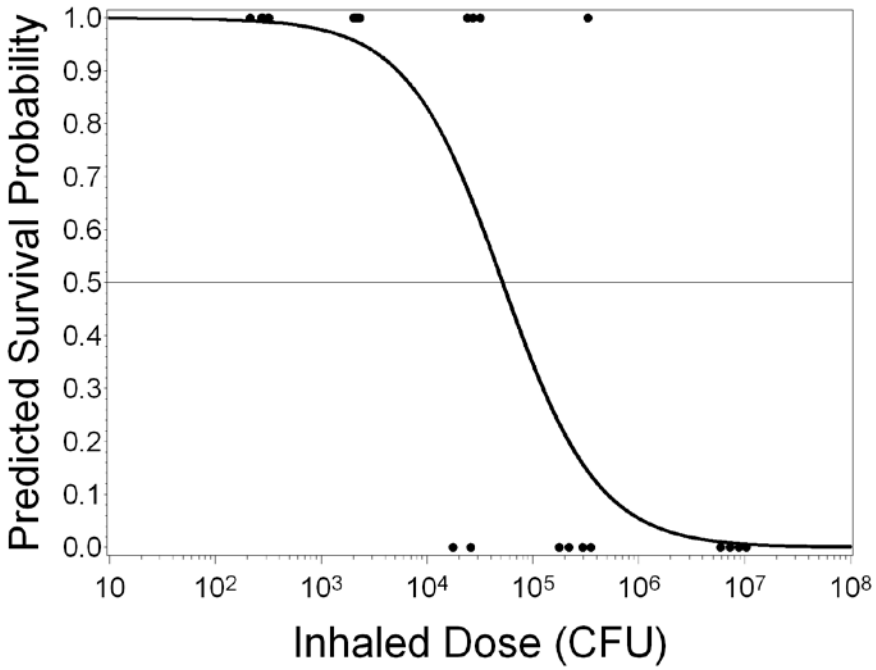

Figure 1. Estimated logistic regression curve and observed survival or mortality.

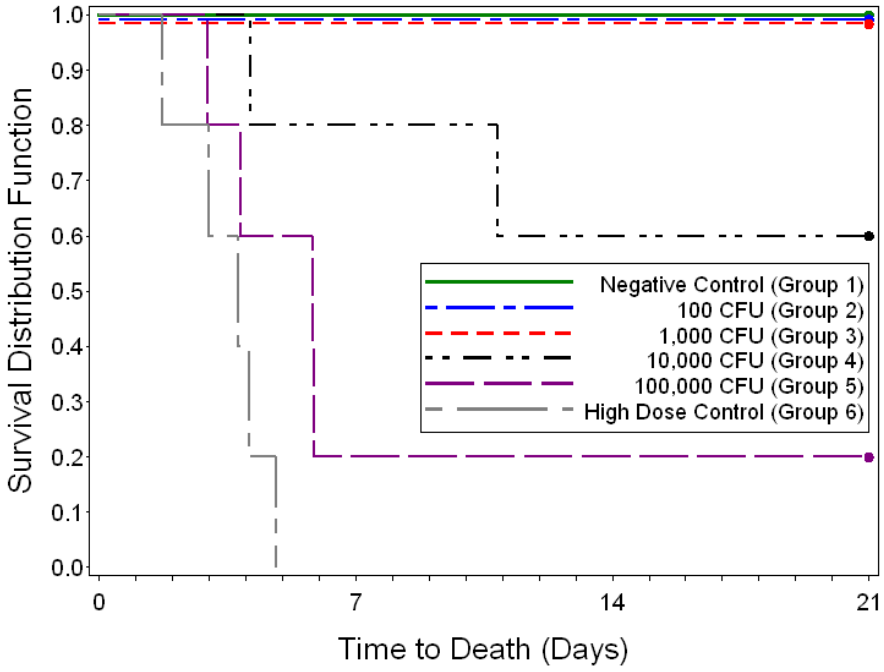

Figure 2. Kaplan-Meier curves representing time to death and survival data for each group.

APPENDIX P
INDIVIDUAL CIRCULATING PA ELISA RESULTS

1020-CG920503 PA ELISA Results

	Animal ID	Group	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
A	L23220	1	BD	BD	BD	BD	BD	BD	BD	*
	L23216	1	BD	BD	BD	BD	BD	BD	BD	*
	L23218	1	BD	BD	BD	BD	BD	BD	BD	*
	L23223	1	BD	BD	BD	BD	BD	BD	BD	*
	L23222	1	BD	BD	BD	BD	BD	BD	BD	*
	L23215	2	BD	BD	BD	BD	BD	BD	BD	*
	L23206	2	BD	BD	BD	BD	BD	BD	BD	*
	L23210	2	BD	BD	BD	BD	BD	BD	BD	*
	L23219	2	BD	BD	BD	BD	BD	BD	BD	*
	L23211	2	BD	BD	BD	BD	BD	BD	BD	*
	L23217	3	BD	BD	BD	BD	BD	BD	BD	*
	L23230	3	BD	BD	BD	BD	BD	BD	BD	*
	L23228	3	BD	BD	BD	BD	BD	BD	BD	*
	L23227	3	BD	BD	BD	BD	BD	BD	BD	*
	L23229	3	BD	BD	BD	BD	BD	BD	BD	*
	L23235	4	BD	BD	BD	BD	BD	*	*	*
	L23205	4	BD	BD	BD	BD	BD	BD	BD	*
	L23225	4	BD	BD	BD	51.199	*	*	*	32964.272
	L23231	4	BD	BD	BD	BD	BD	BD	BD	*
	L23207	4	BD	BD	BD	BD	BD	BD	BD	*
	L23201	5	BD	BD	64.857	471.046	*	*	*	*
	L23234	5	BD	BD	BD	20.082	*	*	*	*
	L23212	5	BD	BD	BD	BD	BD	BD	BD	*
	L23200	5	BD	BD	BD	*	*	*	*	BD
	L23214	5	BD	BD	28.579	*	*	*	*	*
	L23204	6	BD	BD	BD	9.637	*	*	*	25344.927
	L23203	6	BD	BD	2.833	1.631	*	*	*	*
	L23213	6	*	BD	125.095	*	*	*	*	383.515
	L23221	6	BD	BD	*	*	*	*	*	*
	L23232	6	*	BD	BD	2.107	*	*	*	*
	L23202	Extra	BD	*	*	*	*	*	*	*
	L23209	Extra	BD	*	*	*	*	*	*	*

[PA] is reported in ng/mL.
* indicates that no sample was received.
BD indicates that the result was below the detection limit of the assay.
Optical LOD is set at 0.139 per QD-186 results
LOD, LLOQ and ULOQ are set at 2.0ng/mL, 4.9ng/mL and 10000ng/mL respectively per QD-186 results

QC/Tech Review by: ALS 12-9-09

QA Audit Completed By/Date: 12/17/10

M20809

APPENDIX Q
INDIVIDUAL BACTEREMIA CULTURE RESULTS

1020-CG920503 Bacteremia (cfu/mL) [1]										
Animal ID	Group	Targeted Challenge Dose (cfu)	Day -3 [2]	Day 1 (24h PC)	Day 2 (48h PC)	Day 3 (72h PC)	Day 7	Day 14	Day 21	Terminal
L23220	1	100X LD_{50} [3]	0	0	0	0	0	0	0	
L23216	1	100X LD_{50} [3]	0	0	0	0	0	0	0	
L23218	1	100X LD_{50} [3]	0	0	0	0	0	0	0	
L23223	1	100X LD_{50} [3]	0	0	0	0	0	0	0	
L23222	1	100X LD_{50} [3]	0	0	0	0	0	0	0	
L23215	2	100	0	0	0	0	0	0	0	
L23206	2	100	0	0	0	0	0	0	0	
L23210	2	100	0	0	0	0	0	0	0	
L23219	2	100	0	0	0	0	0	0	0	
L23211	2	100	0	0	0	0	0	0	0	
L23217	3	1000	0	0	0	0	0	0	0	
L23230	3	1000	0	0	0	0	0	0	0	
L23228	3	1000	0	0	0	0	0	0	0	
L23227	3	1000	0	0	0	0	0	0	0	
L23229	3	1000	0	0	0	0	0	0	0	
L23235	4	10,000	0	0	0	0	0			1.70E+06
L23205	4	10,000	0	0	0	0	0	0	0	
L23225	4	10,000	0	0	4.53E+03	+				8.03E+05
L23231	4	10,000	0	0	0	0	0	0	0	
L23207	4	10,000	0	0	0	0	0	0	0	
L23201	5	100,000	0	0	+	9.73E+05				9.80E+05
L23234	5	100,000	0	0	0	+				5.43E+06
L23212	5	100,000	0	0	0	0	0	0	0	
L23200	5	100,000	0	+	+					+
L23214	5	100,000	0	0	8.23E+03	0 [4]				0
L23204	6	100X LD_{50}	0	+	+	2.80E+03				1.15E+05
L23203	6	100X LD_{50}	0	0	+	+				3.03E+07
L23213	6	100X LD_{50}	NS	0	+					1.31E+05
L23221	6	100X LD_{50}	0	NS						2.58E+06
L23232	6	100X LD_{50}	NS	+	0	2.77E+03				0 [5]

[1] 0 = Negative; + = Positive for *B. anthracis* but less than quantitative level of detection (2500 cfu/mL); NS = No sample received; Refer to DR-8178.

[2] Qualitative bacteremia performed.

[3] Negative control (Group 1) will be challenged with irradiated spores.

[4] Qualitative bacteremia performed; Sample was directly plated onto solid media from the cell pellet of the serum separation tube; Refer to DR-8170.

[5] Qualitative bacteremia performed; Due to nature of the sample, the sample could not be diluted for quantitative bacteremia; Refer to DR-8170.

Q-2

Printed By/Date: TRM 11.16.09

QC/Technical Review By/Date: AEK 111609

QA Audit Completed By/Date: 11/17/09

APPENDIX R
INDIVIDUAL BACTEREMIA QPCR RESULTS

1020-CG920503 Nucleic Acid Isolation and PCR Sample Summary									
Positive Control: B. anthracis Culture DNA samples located in RM 156, Freezer: 77899-5120									
DNA ID	Comments	Animal ID	Sample Collection Timepoint	Sample Type	Animal Group ID	Sample Volume Used (µL)	Final DNA Volume (µL)	PCR Plate	PCR Results (copies/µL of DNA)
1020-DNA-001	NA	Positive Control	NA	Culture	NA	100	100	1020-001	403017
1020-DNA-002	NA	L23220	Day -3	Blood	1	100	100	1020-001	0
1020-DNA-003	NA	L23216	Day -3	Blood	1	100	100	1020-001	0
1020-DNA-004	NA	L23218	Day -3	Blood	1	100	100	1020-001	0
1020-DNA-005	NA	L23223	Day -3	Blood	1	100	100	1020-001	0
1020-DNA-006	NA	L23222	Day -3	Blood	1	100	100	1020-001	0
1020-DNA-007	NA	L23215	Day -3	Blood	2	100	100	1020-001	0
1020-DNA-008	NA	L23206	Day -3	Blood	2	100	100	1020-001	0
1020-DNA-009	NA	L23210	Day -3	Blood	2	100	100	1020-001	0
1020-DNA-010	NA	L23219	Day -3	Blood	2	100	100	1020-001	0
1020-DNA-011	NA	L23211	Day -3	Blood	2	100	100	1020-001	0
1020-DNA-012	NA	L23217	Day -3	Blood	3	100	100	1020-001	0
1020-DNA-013	NA	L23230	Day -3	Blood	3	100	100	1020-001	0
1020-DNA-014	NA	L23228	Day -3	Blood	3	100	100	1020-001	0
1020-DNA-015	NA	L23227	Day -3	Blood	3	100	100	1020-001	0
1020-DNA-016	NA	L23229	Day -3	Blood	3	100	100	1020-001	0
1020-DNA-017	NA	L23235	Day -3	Blood	4	100	100	1020-001	0
1020-DNA-018	NA	L23205	Day -3	Blood	4	100	100	1020-001	0
1020-DNA-019	NA	L23225	Day -3	Blood	4	100	100	1020-001	234
1020-DNA-020	NA	L23231	Day -3	Blood	4	100	100	1020-001	0
1020-DNA-021	NA	L23207	Day -3	Blood	4	100	100	1020-001	0
1020-DNA-022	NA	L23201	Day -3	Blood	5	100	100	1020-001	0
1020-DNA-023	NA	Negative Control	NA	Water	NA	100	100	1020-001	0
1020-DNA-024	NA	Positive Control	NA	Culture	NA	100	100	1020-002	527444
1020-DNA-025	NA	L23234	Day -3	Blood	5	100	100	1020-002	0
1020-DNA-026	NA	L23212	Day -3	Blood	5	100	100	1020-002	0
1020-DNA-027	NA	L23200	Day -3	Blood	5	100	100	1020-002	0
1020-DNA-028	NA	L23214	Day -3	Blood	5	100	100	1020-002	0
1020-DNA-029	NA	L23204	Day -3	Blood	6	100	100	1020-002	0
1020-DNA-030	NA	L23203	Day -3	Blood	6	100	100	1020-002	0
1020-DNA-031	NA	L23221	Day -3	Blood	6	100	100	1020-002	0
1020-DNA-032	NA	L23220	Day 1	Blood	1	100	100	1020-002	0
1020-DNA-033	NA	L23216	Day 1	Blood	1	100	100	1020-002	0
1020-DNA-034	NA	L23218	Day 1	Blood	1	100	100	1020-002	0
1020-DNA-035	NA	L23223	Day 1	Blood	1	100	100	1020-002	0
1020-DNA-036	NA	L23222	Day 1	Blood	1	100	100	1020-002	< 2
1020-DNA-037	NA	L23215	Day 1	Blood	2	100	100	1020-002	0
1020-DNA-038	NA	L23206	Day 1	Blood	2	100	100	1020-002	0
1020-DNA-039	NA	L23210	Day 1	Blood	2	100	100	1020-002	0
1020-DNA-040	NA	L23219	Day 1	Blood	2	100	100	1020-002	0
1020-DNA-041	NA	L23211	Day 1	Blood	2	100	100	1020-002	0
1020-DNA-042	NA	L23217	Day 1	Blood	3	100	100	1020-002	0
1020-DNA-043	NA	L23230	Day 1	Blood	3	100	100	1020-002	0
1020-DNA-044	NA	L23228	Day 1	Blood	3	100	100	1020-002	0
1020-DNA-045	NA	L23227	Day 1	Blood	3	100	100	1020-002	0
1020-DNA-046	NA	Negative Control	NA	Water	NA	100	100	1020-002	0
1020-DNA-047	NA	Positive Control	NA	Culture	NA	100	100	1020-003	384408
1020-DNA-048	NA	L23229	Day 1	Blood	3	100	100	1020-003	0
1020-DNA-049	NA	L23235	Day 1	Blood	4	100	100	1020-003	0
1020-DNA-050	NA	L23205	Day 1	Blood	4	100	100	1020-003	0
1020-DNA-051	NA	L23225	Day 1	Blood	4	100	100	1020-003	21
1020-DNA-052	NA	L23231	Day 1	Blood	4	100	100	1020-003	0
1020-DNA-053	NA	L23207	Day 1	Blood	4	100	100	1020-003	0
1020-DNA-054	NA	L23201	Day 1	Blood	5	100	100	1020-003	0
1020-DNA-055	NA	L23234	Day 1	Blood	5	100	100	1020-003	0
1020-DNA-056	NA	L23212	Day 1	Blood	5	100	100	1020-003	0
1020-DNA-057	NA	L23200	Day 1	Blood	5	100	100	1020-003	9
1020-DNA-058	NA	L23214	Day 1	Blood	5	100	100	1020-003	0
1020-DNA-059	NA	L23204	Day 1	Blood	6	100	100	1020-003	< 2
1020-DNA-060	NA	L23203	Day 1	Blood	6	100	100	1020-003	0
1020-DNA-061	NA	L23213	Day 1	Blood	6	100	100	1020-003	0
1020-DNA-062	NA	L23232	Day 1	Blood	6	100	100	1020-003	0
1020-DNA-063	NA	L23220	Day 2	Blood	1	100	100	1020-003	0
1020-DNA-064	NA	L23216	Day 2	Blood	1	100	100	1020-003	0
1020-DNA-065	NA	L23218	Day 2	Blood	1	100	100	1020-003	0
1020-DNA-066	NA	L23223	Day 2	Blood	1	100	100	1020-003	0
1020-DNA-067	NA	L23222	Day 2	Blood	1	100	100	1020-003	0
1020-DNA-068	NA	L23215	Day 2	Blood	2	100	100	1020-003	0
1020-DNA-069	NA	Negative Control	NA	Water	NA	100	100	1020-003	0
1020-DNA-070	NA	Positive Control	NA	Culture	NA	100	100	1020-004	406095
1020-DNA-071	NA	L23206	Day 2	Blood	2	100	100	1020-004	0
1020-DNA-072	NA	L23210	Day 2	Blood	2	100	100	1020-004	0
1020-DNA-073	NA	L23219	Day 2	Blood	2	100	100	1020-004	0
1020-DNA-074	NA	L23211	Day 2	Blood	2	100	100	1020-004	0
1020-DNA-075	NA	L23217	Day 2	Blood	3	100	100	1020-004	0
1020-DNA-076	NA	L23230	Day 2	Blood	3	100	100	1020-004	0
1020-DNA-077	NA	L23228	Day 2	Blood	3	100	100	1020-004	0
1020-DNA-078	NA	L23227	Day 2	Blood	3	100	100	1020-004	0
1020-DNA-079	NA	L23229	Day 2	Blood	3	100	100	1020-004	0
1020-DNA-080	NA	L23235	Day 2	Blood	4	100	100	1020-004	0
1020-DNA-081	NA	L23205	Day 2	Blood	4	100	100	1020-004	0
1020-DNA-082	NA	L23225	Day 2	Blood	4	100	100	1020-004	18

Initial/Date: Bm 11/12/09

QC/Tech Review: NT 11/17/09

QA Audit Completed By/Date: MA 11/27/10

1020-CG920503 Nucleic Acid Isolation and PCR Sample Summary									
Positive Control: B. anthracis Culture DNA samples located in RM 156, Freezer: 77899-5120									
DNA ID	**Comments**	**Animal ID**	**Sample Collection Timepoint**	**Sample Type**	**Animal Group ID**	**Sample Volume Used (µL)**	**Final DNA Volume (µL)**	**PCR Plate**	**PCR Results (copies/µL of DNA)**
1020-DNA-083	NA	L23231	Day 2	Blood	4	100	100	1020-004	0
1020-DNA-084	NA	L23207	Day 2	Blood	4	100	100	1020-004	0
1020-DNA-085	NA	L23201	Day 2	Blood	5	100	100	1020-004	37
1020-DNA-086	NA	L23234	Day 2	Blood	5	100	100	1020-004	0
1020-DNA-087	NA	L23212	Day 2	Blood	5	100	100	1020-004	0
1020-DNA-088	NA	L23200	Day 2	Blood	5	100	100	1020-004	40
1020-DNA-089	NA	L23214	Day 2	Blood	5	100	100	1020-004	53
1020-DNA-090	NA	L23204	Day 2	Blood	6	100	100	1020-004	15
1020-DNA-091	NA	L23203	Day 2	Blood	6	100	100	1020-004	< 2
1020-DNA-092	NA	Negative Control	NA	Water	NA	100	100	1020-004	0
1020-DNA-093	NA	Positive Control	NA	Culture	NA	100	100	1020-005	579756
1020-DNA-094	NA	L23232	Day 2	Blood	6	100	100	1020-005	< 2
1020-DNA-095	NA	L23220	Day 3	Blood	1	100	100	1020-005	0
1020-DNA-096	NA	L23216	Day 3	Blood	1	100	100	1020-005	0
1020-DNA-097	NA	L23218	Day 3	Blood	1	100	100	1020-005	0
1020-DNA-098	NA	L23223	Day 3	Blood	1	100	100	1020-005	0
1020-DNA-099	NA	L23222	Day 3	Blood	1	100	100	1020-005	0
1020-DNA-100	NA	L23215	Day 3	Blood	2	100	100	1020-005	0
1020-DNA-101	NA	L23206	Day 3	Blood	2	100	100	1020-005	0
1020-DNA-102	NA	L23210	Day 3	Blood	2	100	100	1020-005	< 2
1020-DNA-103	NA	L23219	Day 3	Blood	2	100	100	1020-005	0
1020-DNA-104	NA	L23211	Day 3	Blood	2	100	100	1020-005	0
1020-DNA-105	NA	L23217	Day 3	Blood	3	100	100	1020-005	0
1020-DNA-106	NA	L23230	Day 3	Blood	3	100	100	1020-005	0
1020-DNA-107	NA	L23228	Day 3	Blood	3	100	100	1020-005	0
1020-DNA-108	NA	L23227	Day 3	Blood	3	100	100	1020-005	0
1020-DNA-109	NA	L23229	Day 3	Blood	3	100	100	1020-005	0
1020-DNA-110	NA	L23235	Day 3	Blood	4	100	100	1020-005	0
1020-DNA-111	NA	L23205	Day 3	Blood	4	100	100	1020-005	0
1020-DNA-112	NA	L23225	Day 3	Blood	4	100	100	1020-005	109
1020-DNA-113	NA	L23231	Day 3	Blood	4	100	100	1020-005	0
1020-DNA-114	NA	L23207	Day 3	Blood	4	100	100	1020-005	0
1020-DNA-115	NA	Negative Control	NA	Water	NA	100	100	1020-005	0
1020-DNA-116	NA	Positive Control	NA	Culture	NA	100	100	1020-006	548818
1020-DNA-117	NA	L23201	Day 3	Blood	5	100	100	1020-006	1003
1020-DNA-118	NA	L23234	Day 3	Blood	5	100	100	1020-006	4
1020-DNA-119	NA	L23212	Day 3	Blood	5	100	100	1020-006	0
1020-DNA-120	NA	L23204	Day 3	Blood	6	100	100	1020-006	23
1020-DNA-121	NA	L23203	Day 3	Blood	6	100	100	1020-006	11
1020-DNA-122	NA	L23232	Day 3	Blood	6	100	100	1020-006	33
1020-DNA-123	NA	L23220	Day 7	Blood	1	100	100	1020-006	0
1020-DNA-124	NA	L23216	Day 7	Blood	1	100	100	1020-006	0
1020-DNA-125	NA	L23218	Day 7	Blood	1	100	100	1020-006	0
1020-DNA-126	NA	L23223	Day 7	Blood	1	100	100	1020-006	0
1020-DNA-127	NA	L23222	Day 7	Blood	1	100	100	1020-006	0
1020-DNA-128	NA	L23215	Day 7	Blood	2	100	100	1020-006	0
1020-DNA-129	NA	L23206	Day 7	Blood	2	100	100	1020-006	0
1020-DNA-130	NA	L23210	Day 7	Blood	2	100	100	1020-006	0
1020-DNA-131	NA	L23219	Day 7	Blood	2	100	100	1020-006	0
1020-DNA-132	NA	L23211	Day 7	Blood	2	100	100	1020-006	0
1020-DNA-133	NA	L23217	Day 7	Blood	3	100	100	1020-006	0
1020-DNA-134	NA	L23230	Day 7	Blood	3	100	100	1020-006	0
1020-DNA-135	NA	L23228	Day 7	Blood	3	100	100	1020-006	0
1020-DNA-136	NA	L23227	Day 7	Blood	3	100	100	1020-006	0
1020-DNA-137	NA	L23229	Day 7	Blood	3	100	100	1020-006	0
1020-DNA-138	NA	Negative Control	NA	Water	NA	100	100	1020-006	0
1020-DNA-139	NA	Positive Control	NA	Culture	NA	100	100	1020-007	387793
1020-DNA-140	NA	L23235	Day 7	Blood	4	100	100	1020-007	0
1020-DNA-141	NA	L23205	Day 7	Blood	4	100	100	1020-007	0
1020-DNA-142	NA	L23231	Day 7	Blood	4	100	100	1020-007	0
1020-DNA-143	NA	L23207	Day 7	Blood	4	100	100	1020-007	0
1020-DNA-144	NA	L23212	Day 7	Blood	5	100	100	1020-007	0
1020-DNA-145	NA	L23220	Day 14	Blood	1	100	100	1020-007	0
1020-DNA-146	NA	L23216	Day 14	Blood	1	100	100	1020-007	0
1020-DNA-147	NA	L23218	Day 14	Blood	1	100	100	1020-007	0
1020-DNA-148	NA	L23223	Day 14	Blood	1	100	100	1020-007	0
1020-DNA-149	NA	L23222	Day 14	Blood	1	100	100	1020-007	0
1020-DNA-150	NA	L23215	Day 14	Blood	2	100	100	1020-007	0
1020-DNA-151	NA	L23206	Day 14	Blood	2	100	100	1020-007	0
1020-DNA-152	NA	L23210	Day 14	Blood	2	100	100	1020-007	0
1020-DNA-153	NA	L23219	Day 14	Blood	2	100	100	1020-007	0
1020-DNA-154	NA	L23211	Day 14	Blood	2	100	100	1020-007	0
1020-DNA-155	NA	L23217	Day 14	Blood	3	100	100	1020-007	0
1020-DNA-156	NA	L23230	Day 14	Blood	3	100	100	1020-007	0
1020-DNA-157	NA	L23228	Day 14	Blood	3	100	100	1020-007	0
1020-DNA-158	NA	L23227	Day 14	Blood	3	100	100	1020-007	0
1020-DNA-159	NA	L23229	Day 14	Blood	3	100	100	1020-007	0
1020-DNA-160	NA	L23205	Day 14	Blood	4	100	100	1020-007	0
1020-DNA-161	NA	Negative Control	NA	Water	NA	100	100	1020-007	0
1020-DNA-162	NA	Positive Control	NA	Culture	NA	100	100	1020-008	< 2
1020-DNA-163	NA	L23231	Day 14	Blood	4	100	100	1020-008	0
1020-DNA-164	NA	L23207	Day 14	Blood	4	100	100	1020-008	0

Initial/Date: [illegible] 11/12/09

QC/Tech Review: NT 11/17/09

QA Audit Completed
Rv/Date: [illegible] 11/17/10

1020-CG920503 Nucleic Acid Isolation and PCR Sample Summary									
Positive Control: B. anthracis Culture DNA samples located in RM 156, Freezer: 77899-5120									
DNA ID	**Comments**	**Animal ID**	**Sample Collection Timepoint**	**Sample Type**	**Animal Group ID**	**Sample Volume Used (µL)**	**Final DNA Volume (µL)**	**PCR Plate**	**PCR Results (copies/µL of DNA)**
1020-DNA-165	NA	L23212	Day 14	Blood	5	100	100	1020-008	0
1020-DNA-166	NA	L23220	Day 21	Blood	1	100	100	1020-008	0
1020-DNA-167	NA	L23216	Day 21	Blood	1	100	100	1020-008	0
1020-DNA-168	NA	L23218	Day 21	Blood	1	100	100	1020-008	0
1020-DNA-169	NA	L23223	Day 21	Blood	1	100	100	1020-008	0
1020-DNA-170	NA	L23222	Day 21	Blood	1	100	100	1020-008	0
1020-DNA-171	NA	L23215	Day 21	Blood	2	100	100	1020-008	0
1020-DNA-172	NA	L23206	Day 21	Blood	2	100	100	1020-008	0
1020-DNA-173	NA	L23210	Day 21	Blood	2	100	100	1020-008	0
1020-DNA-174	NA	L23219	Day 21	Blood	2	100	100	1020-008	0
1020-DNA-175	NA	L23211	Day 21	Blood	2	100	100	1020-008	0
1020-DNA-176	NA	L23217	Day 21	Blood	3	100	100	1020-008	0
1020-DNA-177	NA	L23230	Day 21	Blood	3	100	100	1020-008	0
1020-DNA-178	NA	L23228	Day 21	Blood	3	100	100	1020-008	0
1020-DNA-179	NA	L23227	Day 21	Blood	3	100	100	1020-008	0
1020-DNA-180	NA	L23229	Day 21	Blood	3	100	100	1020-008	0
1020-DNA-181	NA	L23205	Day 21	Blood	4	100	100	1020-008	0
1020-DNA-182	NA	L23231	Day 21	Blood	4	100	100	1020-008	0
1020-DNA-183	NA	L23207	Day 21	Blood	4	100	100	1020-008	0
1020-DNA-184	NA	Negative Control	NA	Water	NA	100	100	1020-008	0
1020-DNA-185	NA	Positive Control	NA	Culture	NA	100	100	1020-009	470808
1020-DNA-186	NA	L23212	Day 21	Blood	5	100	100	1020-009	0
1020-DNA-187	NA	L23235	Terminal	Blood	4	100	100	1020-009	7394
1020-DNA-188	NA	L23201	Terminal	Blood	5	100	100	1020-009	3820
1020-DNA-189	NA	L23234	Terminal	Blood	5	100	100	1020-009	11472
1020-DNA-190	NA	L23200	Terminal	Blood	5	100	100	1020-009	3
1020-DNA-191	NA	L23214	Terminal	Blood	5	100	100	1020-009	274027
1020-DNA-192	NA	L23203	Terminal	Blood	6	100	100	1020-009	150882
1020-DNA-193	NA	L23213	Terminal	Blood	6	100	100	1020-009	268
1020-DNA-194	NA	L23221	Terminal	Blood	6	100	100	1020-009	0
1020-DNA-195	NA	Negative Control	NA	Water	NA	100	100	1020-009	0

Initial/Date: BM 11/12/09

QC/Tech Review: NT 11/17/09

QA Audit Completed
By/Date: MD 1/27/10

APPENDIX S

INDIVIDUAL TNA RESULTS

Animal ID Information				Plate ID Information	Prescreen Test Results
Animal ID	Animal Group#	Bleed Day	TNA Sample ID#	Plate ID	Positive or Negative
L23223	1	Day -3	L23223 Day -3	111809-376	Negative
L23222	1	Day -3	L23222 Day -3	111809-376	Negative
L23230	3	Day -3	L23230 Day -3	111809-376	Negative
L23227	3	Day -3	L23227 Day -3	111809-376	Negative
L23205	4	Day -3	L23205 Day -3	111809-376	Negative
L23225	4	Day -3	L23225 Day -3	111809-376	Negative
L23234	5	Day -3	L23234 Day -3	111809-376	Negative
L23212	5	Day -3	L23212 Day -3	111809-376	Negative
L23220	1	Day 1	L23220 Day 1	111809-376	Negative
L23216	1	Day 1	L23216 Day 1	111809-376	Negative
L23218	1	Day 1	L23218 Day 1	111809-376	Negative
L23223	1	Day 1	L23223 Day 1	111809-376	Negative
L23222	1	Day 1	L23222 Day 1	111809-376	Negative
L23215	2	Day 1	L23215 Day 1	111809-376	Negative
L23206	2	Day 1	L23206 Day 1	111809-376	Negative
L23210	2	Day 1	L23210 Day 1	111809-376	Negative
L23211	2	Day 1	L23211 Day 1	111809-376	Negative
L23217	3	Day 1	L23217 Day 1	111809-376	Negative
L23230	3	Day 1	L23230 Day 1	111809-376	Negative
L23228	3	Day 1	L23228 Day 1	111809-376	Negative
L23227	3	Day 1	L23227 Day 1	111809-376	Negative
L23229	3	Day 1	L23229 Day 1	111809-376	Negative
L23235	4	Day 1	L23235 Day 1	111809-376	Negative

Animal ID Information				Plate ID Information	Prescreen Test Results
Animal ID	Animal Group#	Bleed Day	TNA Sample ID#	Plate ID	Positive or Negative
L23205	4	Day 1	L23205 Day 1	111809-377	Negative
L23225	4	Day 1	L23225 Day 1	111809-377	Negative
L23231	4	Day 1	L23231 Day 1	111809-377	Negative
L23207	4	Day 1	L23207 Day 1	111809-377	Negative
L23201	5	Day 1	L23201 Day 1	111809-377	Negative
L23234	5	Day 1	L23234 Day 1	111809-377	Negative
L23212	5	Day 1	L23212 Day 1	111809-377	Negative
L23200	5	Day 1	L23200 Day 1	111809-377	Negative
L23214	5	Day 1	L23214 Day 1	111809-377	Negative
L23204	6	Day 1	L23204 Day 1	111809-377	Negative
L23203	6	Day 1	L23203 Day 1	111809-377	Negative
L23213	6	Day 1	L23213 Day 1	111809-377	Negative
L23232	6	Day 1	L23232 Day 1	111809-377	Negative
L23220	1	Day 2	L23220 Day2	111809-377	Negative
L23216	1	Day 2	L23216 Day2	111809-377	Negative
L23223	1	Day 2	L23223 Day2	111809-377	Negative
L23210	2	Day 2	L23210 Day2	111809-377	Negative
L23211	2	Day 2	L23211 Day2	111809-377	Negative
L23230	3	Day 2	L23230 Day2	111809-377	Negative
L23235	4	Day 2	L23235 Day2	111809-377	Negative
L23225	4	Day 2	L23225 Day2	111809-377	Negative
L23201	5	Day 2	L23201 Day2	111809-377	Negative
L23214	5	Day 2	L23214 Day2	111809-377	Negative

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Animal ID Information				Plate ID Information	Prescreen Test Results
Animal ID	Animal Group#	Bleed Day	TNA Sample ID#	Plate ID	Positive or Negative
L23203	6	Day 2	L23203 Day2	111809-378	Negative
L23232	6	Day 2	L23232 Day2	111809-378	Negative
L23220	1	Day 3	L23220 Day 3	111809-378	Negative
L23218	1	Day 3	L23218 Day 3	111809-378	Negative
L23223	1	Day 3	L23223 Day 3	111809-378	Negative
L23222	1	Day 3	L23222 Day 3	111809-378	Negative
L23215	2	Day 3	L23215 Day 3	111809-378	Negative
L23206	2	Day 3	L23206 Day 3	111809-378	Negative
L23210	2	Day 3	L23210 Day 3	111809-378	Negative
L23217	3	Day 3	L23217 Day 3	111809-378	Negative
L23230	3	Day 3	L23230 Day 3	111809-378	Negative
L23228	3	Day 3	L23228 Day 3	111809-378	Negative
L23227	3	Day 3	L23227 Day 3	111809-378	Negative
L23235	4	Day 3	L23235 Day 3	111809-378	Negative
L23205	4	Day 3	L23205 Day 3	111809-378	Negative
L23225	4	Day 3	L23225 Day 3	111809-378	Negative
L23231	4	Day 3	L23231 Day 3	111809-378	Negative
L23201	5	Day 3	L23201 Day 3	111809-378	Negative
L23234	5	Day 3	L23234 Day 3	111809-378	Negative
L23200	5	Terminal	L23200 Terminal	111809-378	Negative
L23214	5	Day 3	L23214 Day 3	111809-378	Negative
L23203	6	Day 3	L23203 Day 3	111809-378	Negative
L23213	6	Terminal	L23213 Terminal	111809-378	Negative

Animal ID Information				Plate ID Information	Prescreen Test Results
Animal ID	Animal Group#	Bleed Day	TNA Sample ID#	Plate ID	Positive or Negative
L23220	1	Day 7	L23220 Day 7	111809-379	Negative
L23216	1	Day 7	L23216 Day 7	111809-379	Negative
L23218	1	Day 7	L23218 Day 7	111809-379	Negative
L23223	1	Day 7	L23223 Day 7	111809-379	Negative
L23222	1	Day 7	L23222 Day 7	111809-379	Negative
L23206	2	Day 7	L23206 Day 7	111809-379	Negative
L23211	2	Day 7	L23211 Day 7	111809-379	Negative
L23217	3	Day 7	L23217 Day 7	111809-379	Negative
L23228	3	Day 7	L23228 Day 7	111809-379	Negative
L23227	3	Day 7	L23227 Day 7	111809-379	Negative
L23229	3	Day 7	L23229 Day 7	111809-379	Negative
L23235	4	Day 7	L23235 Day 7	111809-379	Negative
L23205	4	Day 7	L23205 Day 7	111809-379	Negative
L23231	4	Day 7	L23231 Day 7	111809-379	Negative
L23207	4	Day 7	L23207 Day 7	111809-379	Negative
L23212	5	Day 7	L23212 Day 7	111809-379	Negative
L23220	1	Day 14	L23220 Day 14	111809-379	Negative
L23206	2	Day 14	L23206 Day 14	111809-379	Negative
L23219	2	Day 14	L23219 Day 14	111809-379	Negative
L23230	3	Day 14	L23230 Day 14	111809-379	Negative
L23228	3	Day 14	L23228 Day 14	111809-379	Negative
L23229	3	Day 14	L23229 Day 14	111809-379	Negative
L23231	4	Day 14	L23231 Day 14	111809-379	Negative

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Animal ID Information			Plate ID Information	Prescreen Test Results
Animal ID	Animal Group#	TNA Sample ID#	Plate ID	Positive or Negative
L23207	4	L23207 Day 14	111809-380	Negative
L23212	5	L23212 Day 14	111809-380	Negative
L23220	1	L23220 Day 21	111809-380	Negative
L23216	1	L23216 Day 21	111809-380	Negative
L23218	1	L23218 Day 21	111809-380	Negative
L23223	1	L23223 Day 21	111809-380	Negative
L23222	1	L23222 Day 21	111809-380	Negative
L23215	2	L23215 Day 21	111809-380	Negative
L23206	2	L23206 Day 21	111809-380	Negative
L23210	2	L23210 Day 21	111809-380	Negative
L23219	2	L23219 Day 21	111809-380	Negative
L23211	2	L23211 Day 21	111809-380	Negative
L23217	3	L23217 Day 21	111809-380	Negative
L23230	3	L23230 Day 21	111809-380	Negative
L23228	3	L23228 Day 21	111809-380	Negative
L23227	3	L23227 Day 21	111809-380	Negative
L23229	3	L23229 Day 21	111809-380	Negative
L23205	4	L23205 Day 21	111809-380	Negative
L23231	4	L23231 Day 21	111809-380	Negative
L23207	4	L23207 Day 21	111809-380	Negative
L23212	5	L23212 Day 21	111809-380	Negative
L23204	6	L23204 Terminal	111809-380	Negative

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Animal ID Information				Plate ID Information				ED50			
Animal ID	Animal Group #	Time Point	TNA Sample ID#	Test 1		Test 2 [1]		Test 1	Test 2	Mean ED50	Median ED50
				Analyst	Plate ID	Analyst	Plate ID	ED50	ED50		
L23200	5	Day 2	L23200 Day 2	PKIII	111809-382	KN	111809-381	0	0	<LOD	<LOD
L23204	6	Day 2	L23204 Day 2	PKIII	111809-382	KN	111809-381	0	0	<LOD	<LOD
L23212	5	Day 2	L23212 Day 2	PKIII	111809-382	KN	111809-381	0	0	<LOD	<LOD
L23214	5	Day -3	L23214 Day -3	PKIII	111809-382	KN	111809-381	0	0	<LOD	<LOD
L23215	2	Day 7	L23215 Day 7	PKIII	111809-382	KN	111809-381	0	0	<LOD	<LOD
L23222	1	Day 2	L23222 Day 2	PKIII	111809-382	KN	111809-381	0	0	<LOD	<LOD
L23225	4	Terminal	[2] L23225 Terminal	PKIII	111809-382	KN	111809-381	0	8	<LOD	<LOD
L23230	3	Day 7	L23230 Day 7	PKIII	111809-382	KN	111809-381	0	0	<LOD	<LOD

[1] Added by hand, data accepted

[2] Sample exhibits cytotoxic effect. ED50 values inaccurate. See memo dated 12/21/09

Animal ID Information				NF50				
Animal ID	Animal Group #	Time Point	TNA Sample ID#	Test 1	Test 2	Mean NF50	Median NF50	Reactivity
				NF50	NF50			
L23200	5	Day 2	L23200 Day 2	NA	NA	<LOD	<LOD	Non-Reactive
L23204	6	Day 2	L23204 Day 2	NA	NA	<LOD	<LOD	Non-Reactive
L23212	5	Day 2	L23212 Day 2	NA	NA	<LOD	<LOD	Non-Reactive
L23214	5	Day -3	L23214 Day -3	NA	NA	<LOD	<LOD	Non-Reactive
L23215	2	Day 7	L23215 Day 7	NA	NA	<LOD	<LOD	Non-Reactive
L23222	1	Day 2	L23222 Day 2	NA	NA	<LOD	<LOD	Non-Reactive
L23225	4	Terminal	[2] L23225 Terminal	NA	0.025	<LOD	<LOD	Non-Reactive
L23230	3	Day 7	L23230 Day 7	NA	NA	<LOD	<LOD	Non-Reactive

QC/Tech Rev By/Date: DN 4/5/10

1020 CG920503

QA AUDIT COMPLETED

BY/DATE: DN 6/2/10

APPENDIX T
INDIVIDUAL ANTI-PA IGG ELISA RESULTS

QC/TR by: MFF 2/15/10

1020-CG920503 ELISA Results

Animal ID	Group	ELISA Code (Day)	Date Taken	Test Operator 1 Plate Number	Test Operator 2 Plate Number	Test Operator 3 Plate Number	Test Operator 4 Plate Number	Results of test operator 1	Results of test operator 2	Results of test operator 3	Results of test operator 4	Mean ELISA anti-PA IgG (µg/mL)	Std Dev of the Test Operators	%CV Between Test Operators	Median ELISA anti-PA IgG (µg/mL)	ELISA Reportable Values
L23220	1	-3														
		1		101209-105 Pre-Screen GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		3		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		7		101209-108 AMB	102109-159 KAS			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		14		101209-112 AMB	102109-163 KAS			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		21		102109-165 DP	102609-191 JNG			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		Terminal														
L23216	1	-3														
		1		101209-105 Pre-Screen GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		3														
		7		101209-108 AMB	102109-159 KAS			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		14														
		21		102109-165 DP	102609-191 JNG			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		Terminal														
L23218	1	-3														
		1		101209-105 Pre-Screen GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2														
		3		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		7		101209-108 AMB	102109-159 KAS			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		14														
		21		102109-165 DP	102609-191 JNG			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		Terminal														
L23223	1	-3		101209-105 Pre-Screen - GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		1		101209-105 Pre-Screen GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		3		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		7		101209-108 AMB	102109-159 KAS			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		14														
		21		102109-166 DP	102609-191 JNG			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		Terminal														
L23222	1	-3		101209-105 Pre-Screen - GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		1		101209-105 Pre-Screen GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN						--	--	--	--	--
		3		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		7		101209-109 AMB	102109-160 KAS			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		14														
		21		102109-166 DP	102609-192 JNG			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		Terminal														
L23215	2	-3														
		1		101209-105 Pre-Screen GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2														
		3		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		7		111909-284 LAN	111909-285 ALS			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		14														
		21		102109-166 DP	102609-192 JNG			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		Terminal														

QA AUDIT COMPLETED
BY/DATE: JM 6/9/10

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MFF 2/15/10

QC/TR by: Mff 2/15/10

1020-CG920503 ELISA Results

Animal ID	Group	ELISA Code (Day)	Date Taken	Test Operator 1 Plate Number	Test Operator 2 Plate Number	Test Operator 3 Plate Number	Test Operator 4 Plate Number	Results of test operator 1	Results of test operator 2	Results of test operator 3	Results of test operator 4	Mean ELISA anti-PA IgG (µg/mL)	Std Dev of the Test Operators	%CV Between Test Operators	Median ELISA anti-PA IgG (µg/mL)	ELISA Reportable Values
L23206	2	-3														
		1		101209-105 Pre-Screen GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2														
		3		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		7		101209-109 AMB	102109-160 KAS			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		14		101209-112 AMB	102109-163 KAS			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		21		102109-166 DP	102609-192 JNG			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		Terminal														
L23210	2	-3														
		1		101209-105 Pre-Screen GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		3		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		7														
		14														
		21		102109-167 DP	102609-192 JNG			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		Terminal														
L23219	2	-3														
		1														
		2														
		3														
		7														
		14		101209-113 AMB	102109-163 KAS			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		21		102109-167 DP	102109-171 JNG			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		Terminal														
L23211	2	-3														
		1		101209-105 Pre-Screen GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		3														
		7		101209-109 AMB	102109-160 KAS			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		14														
		21		102109-167 DP	102109-171 JNG			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		Terminal														
L23217	3	-3														
		1		101209-105 Pre-Screen GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2														
		3		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		7		101209-109 AMB	102109-160 KAS			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		14														
		21		102109-167 DP	102109-171 JNG			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		Terminal														
L23230	3	-3		101209-105 Pre-Screen - GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		1		101209-105 Pre-Screen GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		3		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		7		111909-284 LAN	111909-285 ALS			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		14		101209-113 AMB	102109-163 KAS			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		21		102109-168 DP	102109-171 JNG			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		Terminal														

QA AUDIT COMPLETED
BY/DATE: Jm 6/9/10

Mff 2/15/10

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QC/TR by: MFF 2/15/10

1020-CG920503 ELISA Results

Animal ID	Group	ELISA Code (Day)	Date Taken	Test Operator 1 Plate Number	Test Operator 2 Plate Number	Test Operator 3 Plate Number	Test Operator 4 Plate Number	Results of test operator 1	Results of test operator 2	Results of test operator 3	Results of test operator 4	Mean ELISA anti-PA IgG (µg/mL)	Std Dev of the Test Operators	%CV Between Test Operators	Median ELISA anti-PA IgG (µg/mL)	ELISA Reportable Values
L23228	3	-3														
		1		101209-105 Pre-Screen GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2														
		3		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		7		101209-110 AMB	102109-161 KAS			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		14		101209-113 AMB	102109-164 KAS			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		21		102109-168 DP	102109-172 JNG			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		Terminal														
L23227	3	-3		101209-105 Pre-Screen - GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		1		101209-105 Pre-Screen GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2														
		3		101209-107 Pre-Screen GEM		Pre-Screen - 102109-178 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		7		101209-110 AMB	102109-161 KAS			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		14														
		21		102109-168 DP	102109-172 JNG			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		Terminal														
L23229	3	-3														
		1		101209-105 Pre-Screen GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2														
		3														
		7		101209-110 AMB	102109-161 KAS			0.000	0.772			0.386	0.546	141.42%	0.386	<LOQ
		14		101209-113 AMB	102109-164 KAS			0.000	1.003			0.502	0.709	141.42%	0.502	<LOQ
		21		102109-168 DP	102109-172 JNG			0.807	0.000			0.404	0.571	141.42%	0.404	<LOQ
		Terminal														
L23235	4	-3														
		1		101209-105 Pre-Screen GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		3		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		7		101209-110 AMB	102109-161 KAS			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		14														
		21														
		Terminal														
L23205	4	-3		101209-105 Pre-Screen - GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		1		101209-105 Pre-Screen GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2														
		3		101209-107 Pre-Screen GEM		Pre-Screen - 102109-178 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		7		101209-111 AMB	102109-162 KAS			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		14														
		21		102109-169 DP	102109-172 JNG			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		Terminal														
L23225	4	-3		101209-105 Pre-Screen - GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		1		101209-105 Pre-Screen GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		3		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		7														
		14														
		21														
		Terminal		101209-114 AMB	102109-170 DP			0.000	0.000			0.000	0.000	--	0.000	<LOQ

QA AUDIT COMPLETED
BY/DATE: Jm 6/9/10

MFF 2/15/10

QC/TR by: Mff 2/15/10

1020-CG920503 ELISA Results

Animal ID	Group	ELISA Code (Day)	Date Taken	Test Operator 1 Plate Number	Test Operator 2 Plate Number	Test Operator 3 Plate Number	Test Operator 4 Plate Number	Results of test operator 1	Results of test operator 2	Results of test operator 3	Results of test operator 4	Mean ELISA anti-PA IgG (µg/mL)	Std Dev of the Test Operators	%CV Between Test Operators	Median ELISA anti-PA IgG (µg/mL)	ELISA Reportable Values
L23291	4	-3														
		1		101209-105 Pre-Screen GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2														
		3		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		7		101209-111 AMB	102109-162 KAS			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		14		101209-114 AMB	102109-164 KAS			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		21		102109-169 DP	102109-173 JNG			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		Terminal														
L23207	4	-3														
		1		101209-105 Pre-Screen GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2														
		3														
		7		101209-111 AMB	102109-162 KAS			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		14		101209-114 AMB	102109-164 KAS			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		21		102109-169 DP	102109-173 JNG			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		Terminal														
L23201	5	-3														
		1		101209-105 Pre-Screen GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		3		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		7														
		14														
		21														
		Terminal														
L23234	5	-3		101209-105 Pre-Screen - GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		1		101209-105 Pre-Screen GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2														
		3		101209-107 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		7														
		14														
		21														
		Terminal														
L23212	5	-3		101209-105 Pre-Screen - GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		1		101209-105 Pre-Screen GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		3														
		7		101209-111 AMB	102109-162 KAS			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		14		101209-114 AMB	102109-165 DP			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		21		102109-169 DP	102109-173 JNG			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		Terminal														
L23200	5	-3														
		1		101209-105 Pre-Screen GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2		111909-283 LAN	111909-285 ALS			0.000	0.000			0.000	0.000	--	0.000	<LOQ
		3														
		7														
		14														
		21														
		Terminal		101209-115 AMB	102109-170 DP			0.000	0.000			0.000	0.000	--	0.000	<LOQ

QA AUDIT COMPLETED
BY/DATE: Jm 6/9/10

Mff 2/15/10

QC/TR by: MKF 2/15/10

1020-CG920503 ELISA Results

Animal ID	Group	ELISA Code (Day)	Date Taken	Test Operator 1 Plate Number	Test Operator 2 Plate Number	Test Operator 3 Plate Number	Test Operator 4 Plate Number	Results of test operator 1	Results of test operator 2	Results of test operator 3	Results of test operator 4	Mean ELISA anti-PA IgG (µg/mL)	Std Dev of the Test Operators	%CV Between Test Operators	Median ELISA anti-PA IgG (µg/mL)	ELISA Reportable Values
L23214	5	-3		111909-283 LAN	111909-285 ALS											
		1		101209-105 Pre-Screen GEM		Pre-Screen - 102109-176 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		3		101209-107 Pre-Screen GEM		Pre-Screen - 102109-178 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		7										0.000	0.000	--	0.000	<LOQ
		14														
		21														
		Terminal														
L23204	6	-3														
		1		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2		111909-283 LAN	NA			0.000	NA			0.000	--	--	0.000	<LOQ
		3														
		7														
		14														
		21														
		Terminal		101209-115 AMB	102109-170 DP			0.000	0.000			0.000	0.000	--	0.000	<LOQ
L23203	6	-3														
		1		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		3		101209-107 Pre-Screen GEM		Pre-Screen - 102109-178 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		7														
		14														
		21														
		Terminal														
L23213	6	-3														
		1		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2														
		3														
		7														
		14														
		21														
		Terminal		101209-115 AMB	102109-170 DP			0.000	0.000			0.000	0.000	--	0.000	<LOQ
L23221	6	-3														
		1														
		2														
		3														
		7														
		14														
		21														
		Terminal														
L23232	6	-3														
		1		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		2		101209-106 Pre-Screen GEM		Pre-Screen - 102109-177 LAN		0.000	0.000			0.000	0.000	--	0.000	<LOQ
		3														
		7														
		14														
		21														
		Terminal														

%CV >30% but sample is <LOQ
Not enough sample remaining to process

QA AUDIT COMPLETED
BY/DATE: JM 6/9/10

MKF 2/15/10
WD MKF 2/15/10

APPENDIX U
INDIVIDUAL HEMATOLOGY RESULTS

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
White Blood Cell Count (2.90-8.10 10^3/uL)	1	L23216	M	7.61	4.61	4.56	7.40	**8.16**	6.51	4.36	--
	1	L23218	M	5.86	5.51	3.49	6.35	6.03	5.44 *	3.71	--
	1	L23220	M	7.74	7.83	5.81	6.23	2.76 *	2.47 *	4.05	--
	1	L23222	M	**12.47**	**8.98**	7.94 *	**9.59**	7.71	7.87	5.25	--
	1	L23223	M	6.58	4.99	5.95	2.75	5.05	2.84 *	3.32	--
		Average		8.05	6.38	5.55	6.46	5.94	5.03	4.14	--
		Std Dev		2.59	1.92	1.67	2.48	2.18	2.33	0.73	--
	2	L23206	M	7.12	7.94	7.16 *	5.99	4.12 *	7.39	4.56	--
	2	L23210	M	7.06	6.73	7.34	--	--	5.02 *	--	--
	2	L23211	M	4.97	5.61	5.09	5.83	4.62	6.40 *	2.21	--
	2	L23215	M	6.66	5.11	6.01	6.41	4.93	4.86 *	3.89	--
	2	L23219	M	8.02	6.84	7.74	8.03 *	6.20	4.82 *	4.94	--
		Average		6.77	6.45	6.67	6.57	4.97	5.70	3.90	--
		Std Dev		1.12	1.11	1.09	1.01	0.89	1.15	1.21	--
	3	L23217	M	**9.44**	**10.18**	**10.87**	**11.21**	**8.28**	**8.49**	6.64	--
	3	L23227	M	7.97	**8.27**	7.67	7.31	7.09	**8.19**	4.39	--
	3	L23228	M	6.72	7.38	7.49	6.80	6.36	6.58	3.58	--
	3	L23229	M	5.90	6.53	6.15	7.27	6.03	6.52	4.09	--
	3	L23230	M	5.98	6.68	6.49	5.73	4.65	3.18 *	3.32	--
		Average		7.20	7.81	7.73	7.66	6.48	6.59	4.40	--
		Std Dev		1.50	1.49	1.87	2.08	1.34	2.11	1.32	--
	4	L23205	M	7.59	7.63	**8.14**	7.53	6.83	**8.47**	5.09	--
	4	L23207	M	5.93	6.93	5.80	6.05	5.39	5.20	2.79	--
	4	L23225	M	5.80	6.96	7.20	3.67	--	--	--	--
	4	L23231	M	7.70	**8.30**	8.09	7.74	7.76	6.44	2.94	--
	4	L23235	M	6.89	6.90	**8.72**	7.82	**11.99**	--	--	--
		Average		6.78	7.34	7.59	6.56	7.99	6.70	3.61	--
		Std Dev		0.89	0.61	1.14	1.77	2.84	1.65	1.29	--
	5	L23200	M	6.20	3.43	4.33	--	--	--	--	**8.91**
	5	L23201	M	**9.73**	**10.02**	**12.64**	**13.47**	--	--	--	--
	5	L23212	M	7.75	8.03	**8.33**	**8.33**	6.88 *	5.25	4.25	--
	5	L23214	M	**8.64**	**8.23**	3.47	--	--	--	--	--
	5	L23234	M	**9.22**	**8.72**	**9.23**	**8.51**	--	--	--	--
		Average		8.31	7.69	7.60	10.10	6.88	5.25	4.25	8.91
		Std Dev		1.39	2.50	3.75	2.92	--	--	--	--
	6	L23203	M	**9.13**	**8.80**	6.71	4.68	--	--	--	--
	6	L23204	M	5.37	6.68	7.05	5.43	--	--	--	--
	6	L23213	M	--	**10.32**	--	--	--	--	--	**12.85**
	6	L23221	M	6.02	--	--	--	--	--	--	--
	6	L23232	M	--	5.69	**8.78**	1.55	--	--	--	--
		Average		6.84	7.87	7.51	3.89	--	--	--	12.85
		Std Dev		2.01	2.08	1.11	2.06	--	--	--	--

U-2

Bold > Normal Range
Underscore < Normal Range
* Clot Removed
-- No Value

QA Audit Completed By/Date: ML 1.7.10

AC 12-21-09

QC/Tech Rev. By/Date: AC 12-21-09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
Red Blood Cell Count (4.20-6.70 10^6/uL)	1	L23216	M	6.01	6.03	5.52	5.08	5.80	6.45	6.10	--
	1	L23218	M	5.17	5.62	3.67	5.89	6.00	6.05 *	5.66	--
	1	L23220	M	5.64	5.79	5.28	5.38	5.53 *	5.46 *	5.60	--
	1	L23222	M	6.51	5.92	6.04 *	5.90	6.14	6.27	5.98	--
	1	L23223	M	5.66	5.99	5.67	5.02	5.66	5.48 *	5.63	--
		Average		5.80	5.87	5.24	5.45	5.83	5.94	5.79	--
		Std Dev		0.50	0.17	0.92	0.43	0.25	0.45	0.23	--
	2	L23206	M	5.83	5.56	5.76 *	5.53	6.02 *	6.04	5.29	--
	2	L23210	M	5.85	5.97	5.67	--	--	5.85 *	--	--
	2	L23211	M	6.47	6.45	5.75	5.84	5.65	**6.79 ***	5.66	--
	2	L23215	M	6.42	6.29	5.87	5.69	6.26	6.42 *	6.04	--
	2	L23219	M	**6.76**	6.04	6.22	6.35 *	**6.98**	6.35 *	6.08	--
		Average		6.27	6.06	5.85	5.85	6.23	6.29	5.77	--
		Std Dev		0.41	0.34	0.22	0.36	0.56	0.36	0.37	--
	3	L23217	M	6.14	5.76	5.56	5.70	6.63	6.41	5.83	--
	3	L23227	M	6.29	6.42	6.02	5.88	6.24	6.20	6.11	--
	3	L23228	M	5.70	5.72	5.56	5.67	5.71	6.01	4.94	--
	3	L23229	M	5.82	5.68	5.55	5.93	6.17	6.52	5.97	--
	3	L23230	M	6.13	6.06	5.72	5.55	6.35	5.59 *	5.73	--
		Average		6.02	5.93	5.68	5.75	6.22	6.15	5.72	--
		Std Dev		0.25	0.31	0.20	0.16	0.33	0.37	0.46	--
	4	L23205	M	5.00	5.10	4.90	4.98	5.42	5.57	5.21	--
	4	L23207	M	6.28	5.95	6.13	6.19	5.81	6.44	5.60	--
	4	L23225	M	6.47	6.14	5.26	4.93	--	--	--	--
	4	L23231	M	5.45	5.66	5.34	5.27	5.77	5.94	5.58	--
	4	L23235	M	6.15	5.59	5.69	5.53	5.76	--	--	--
		Average		5.87	5.69	5.46	5.38	5.69	5.98	5.46	--
		Std Dev		0.62	0.40	0.47	0.51	0.18	0.44	0.22	--
	5	L23200	M	6.06	5.70	5.66	--	--	--	--	5.60
	5	L23201	M	6.31	5.63	5.21	5.29	--	--	--	--
	5	L23212	M	5.96	5.80	5.76	5.61	5.86 *	6.07	5.90	--
	5	L23214	M	5.99	6.07	5.38	--	--	--	--	--
	5	L23234	M	5.92	5.65	5.45	5.12	--	--	--	--
		Average		6.05	5.77	5.49	5.34	5.86	6.07	5.90	5.60
		Std Dev		0.16	0.18	0.22	0.25	--	--	--	--
	6	L23203	M	5.97	6.06	5.19	4.78	--	--	--	--
	6	L23204	M	5.77	5.55	5.17	5.08	--	--	--	--
	6	L23213	M	--	5.65	--	--	--	--	--	6.07
	6	L23221	M	5.23	--	--	--	--	--	--	--
	6	L23232	M	--	6.03	5.29	5.33	--	--	--	--
		Average		5.66	5.82	5.22	5.06	--	--	--	6.07
		Std Dev		0.38	0.26	0.06	0.28	--	--	--	--

Bold > Normal Range
Underscore < Normal Range
* Clot Removed
-- No Value

ATC 12-21-09

QA Audit Completed By/Date: MD 12/7/10

QC/Tech Rev. By/Date: ATC 12-21-09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
Hemoglobin (9.5-14.5 g/dL)	1	L23216	M	12.8	12.3	11.4	10.4	11.9	13.2	12.3	--
	1	L23218	M	11.3	11.5	7.6	12.2	12.4	12.4 *	11.5	--
	1	L23220	M	12.0	11.6	10.6	10.8	11.4 *	11.2 *	11.4	--
	1	L23222	M	13.0	11.4	11.6 *	11.2	11.8	12.1	11.5	--
	1	L23223	M	11.9	12.2	11.4	10.3	11.5	11.2 *	11.4	--
		Average		12.2	11.8	10.5	11.0	11.8	12.0	11.6	--
		Std Dev		0.7	0.4	1.7	0.8	0.4	0.8	0.4	--
	2	L23206	M	12.7	11.5	12.0 *	11.4	12.6 *	12.6	10.9	--
	2	L23210	M	12.5	12.4	11.6	--	--	12.2 *	--	--
	2	L23211	M	13.2	12.7	11.3	11.6	11.3	13.7 *	11.3	--
	2	L23215	M	13.2	12.3	11.6	11.4	12.4	12.8 *	11.8	--
	2	L23219	M	13.5	12.0	12.4	12.5 *	14.4	12.7 *	12.3	--
		Average		13.0	12.2	11.8	11.7	12.7	12.8	11.6	--
		Std Dev		0.4	0.5	0.4	0.5	1.3	0.6	0.6	--
	3	L23217	M	12.0	11.1	10.8	11.2	13.0	12.4	11.3	--
	3	L23227	M	12.7	12.5	11.8	11.5	12.3	12.3	11.9	--
	3	L23228	M	12.2	11.9	11.6	11.8	12.0	12.6	10.4	--
	3	L23229	M	12.8	12.1	11.6	12.4	12.9	13.7	12.2	--
	3	L23230	M	13.0	12.3	11.6	11.3	13.1	11.5 *	11.7	--
		Average		12.5	12.0	11.5	11.6	12.7	12.5	11.5	--
		Std Dev		0.4	0.5	0.4	0.5	0.5	0.8	0.7	--
	4	L23205	M	11.4	11.3	10.7	11.0	12.0	12.4	11.4	--
	4	L23207	M	12.7	11.4	11.8	12.1	11.2	12.5	10.9	--
	4	L23225	M	13.4	12.1	10.4	9.8	--	--	--	--
	4	L23231	M	11.9	11.9	11.3	11.1	12.1	12.7	10.9	--
	4	L23235	M	13.8	12.0	12.1	12.0	12.6	--	--	--
		Average		12.6	11.7	11.3	11.2	12.0	12.5	11.1	--
		Std Dev		1.0	0.4	0.7	0.9	0.6	0.2	0.3	--
	5	L23200	M	12.4	11.4	11.1	--	--	--	--	11.0
	5	L23201	M	13.6	11.6	10.6	11.0	--	--	--	--
	5	L23212	M	12.9	12.1	11.9	11.7	12.4 *	12.7	12.3	--
	5	L23214	M	12.5	12.1	10.9	--	--	--	--	--
	5	L23234	M	12.9	11.7	11.3	10.6	--	--	--	--
		Average		12.9	11.8	11.2	11.1	12.4	12.7	12.3	11.0
		Std Dev		0.5	0.3	0.5	0.6	--	--	--	--
	6	L23203	M	12.6	12.3	10.5	9.8	--	--	--	--
	6	L23204	M	11.7	10.6	9.9	9.7	--	--	--	--
	6	L23213	M	--	11.5	--	--	--	--	--	12.3
	6	L23221	M	11.6	--	--	--	--	--	--	--
	6	L23232	M	--	12.5	11.0	11.3	--	--	--	--
		Average		12.0	11.7	10.5	10.3	--	--	--	12.3
		Std Dev		0.6	0.9	0.6	0.9	--	--	--	--

Bold > Normal Range
Underscore < Normal Range
* Clot Removed
-- No Value

QA Audit Completed By/Date: 12/27/10

Page 3

PHC 12·21·09

QC/Tech Rev. By/Date: PHC 12·21·09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
Hematocrit (27.2-45.9 %)	1	L23216	M	38.4	38.7	35.1	32.2	38.3	41.3	38.4	--
	1	L23218	M	34.1	36.2	23.8	38.9	40.3	39.1 *	35.3	--
	1	L23220	M	36.0	37.0	34.3	35.0	35.6 *	33.8 *	34.6	--
	1	L23222	M	38.7	34.8	36.6 *	34.6	36.3	37.5	35.2	--
	1	L23223	M	36.8	39.1	36.0	31.4	36.0	34.6 *	34.9	--
		Average		36.8	37.2	33.2	34.4	37.3	37.3	35.7	--
		Std Dev		1.9	1.8	5.3	2.9	2.0	3.1	1.5	--
	2	L23206	M	37.9	36.2	37.8 *	36.9	39.9 *	39.4	33.7	--
	2	L23210	M	38.3	39.4	36.1	--	--	37.9 *	--	--
	2	L23211	M	39.9	38.9	34.1	35.5	35.8	41.6 *	33.3	--
	2	L23215	M	38.6	38.0	35.6	34.5	38.9	40.3 *	37.5	--
	2	L23219	M	42.4	38.3	38.9	39.8 *	**46.5**	38.7 *	37.4	--
		Average		39.4	38.2	36.5	36.7	40.3	39.6	35.5	--
		Std Dev		1.8	1.2	1.9	2.3	4.5	1.4	2.3	--
	3	L23217	M	36.5	34.4	33.7	35.0	41.0	38.9	34.9	--
	3	L23227	M	39.0	41.2	37.1	36.1	41.3	38.7	37.2	--
	3	L23228	M	37.1	37.6	36.3	36.6	38.5	39.1	31.3	--
	3	L23229	M	37.9	37.1	35.8	38.7	40.0	41.8	37.8	--
	3	L23230	M	38.1	37.1	35.0	33.8	41.8	33.9 *	34.8	--
		Average		37.7	37.5	35.6	36.0	40.5	38.5	35.2	--
		Std Dev		1.0	2.4	1.3	1.8	1.3	2.9	2.6	--
	4	L23205	M	33.9	34.5	32.8	34.7	37.6	38.3	35.1	--
	4	L23207	M	38.8	36.2	37.6	39.9	36.6	39.6	33.6	--
	4	L23225	M	40.3	37.2	31.5	29.4	--	--	--	--
	4	L23231	M	35.6	36.3	34.5	34.6	39.8	38.9	33.5	--
	4	L23235	M	41.8	36.9	37.5	37.2	38.7	--	--	--
		Average		38.1	36.2	34.8	35.2	38.2	38.9	34.1	--
		Std Dev		3.3	1.0	2.7	3.9	1.4	0.7	0.9	--
	5	L23200	M	38.9	35.4	35.2	--	--	--	--	36.9
	5	L23201	M	41.7	35.7	32.0	32.5	--	--	--	--
	5	L23212	M	38.9	37.3	36.8	35.5	37.9 *	38.5	37.3	--
	5	L23214	M	38.9	39.7	33.5	--	--	--	--	--
	5	L23234	M	38.8	36.6	34.7	32.7	--	--	--	--
		Average		39.4	36.9	34.4	33.6	37.9	38.5	37.3	36.9
		Std Dev		1.3	1.7	1.8	1.7	--	--	--	--
	6	L23203	M	37.9	38.3	32.0	29.3	--	--	--	--
	6	L23204	M	35.7	33.3	30.2	30.0	--	--	--	--
	6	L23213	M	--	35.2	--	--	--	--	--	44.7
	6	L23221	M	34.7	--	--	--	--	--	--	--
	6	L23232	M	--	39.0	33.9	33.5	--	--	--	--
		Average		36.1	36.5	32.0	30.9	--	--	--	44.7
		Std Dev		1.6	2.7	1.9	2.3	--	--	--	--

U-5

Bold > Normal Range
Underscore < Normal Range
* Clot Removed
-- No Value

QA Audit Completed By/Date: [illegible] 1/27/10

PTC 12-21-09

QC/Tech Rev. By/Date: PTC 12-21-09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
MCV (59.1-75.4 fL)	1	L23216	M	64.0	64.1	63.5	63.3	66.1	64.0	62.9	--
	1	L23218	M	65.9	64.4	64.7	65.9	67.2	64.7 *	62.5	--
	1	L23220	M	63.9	64.0	64.9	65.0	64.4 *	62.0 *	61.8	--
	1	L23222	M	59.5	58.7	60.7 *	58.7	59.0	59.8	58.9	--
	1	L23223	M	65.1	65.2	63.4	62.5	63.7	63.1 *	62.0	--
		Average		63.7	63.3	63.4	63.1	64.1	62.7	61.6	--
		Std Dev		2.5	2.6	1.7	2.8	3.2	1.9	1.6	--
	2	L23206	M	64.9	65.1	65.6 *	66.7	66.3 *	65.1	63.7	--
	2	L23210	M	65.4	66.1	63.6	--	--	64.8 *	--	--
	2	L23211	M	61.6	60.4	59.3	60.7	63.4	61.2 *	58.9	--
	2	L23215	M	60.1	60.5	60.7	60.5	62.2	62.8 *	62.0	--
	2	L23219	M	62.8	63.5	62.5	62.6 *	66.6	61.0 *	61.5	--
		Average		63.0	63.1	62.3	62.6	64.6	63.0	61.5	--
		Std Dev		2.2	2.6	2.5	2.9	2.2	1.9	2.0	--
	3	L23217	M	59.5	59.8	60.6	61.4	61.8	60.7	59.8	--
	3	L23227	M	62.0	64.3	61.6	61.4	66.2	62.4	60.9	--
	3	L23228	M	65.2	65.8	65.4	64.4	67.5	65.0	63.4	--
	3	L23229	M	65.2	65.4	64.5	65.3	64.9	64.1	63.3	--
	3	L23230	M	62.1	61.2	61.2	60.9	65.7	60.6 *	60.9	--
		Average		62.8	63.3	62.7	62.7	65.2	62.6	61.7	--
		Std Dev		2.4	2.7	2.1	2.0	2.1	2.0	1.6	--
	4	L23205	M	67.6	67.7	67.0	69.7	69.3	68.7	67.3	--
	4	L23207	M	61.8	60.9	61.3	64.5	63.0	61.5	59.9	--
	4	L23225	M	62.3	60.5	59.8	59.6	--	--	--	--
	4	L23231	M	65.3	64.2	64.6	65.7	69.1	65.4	60.1	--
	4	L23235	M	67.9	66.0	65.9	67.3	67.1	--	--	--
		Average		65.0	63.9	63.7	65.4	67.1	65.2	62.4	--
		Std Dev		2.9	3.1	3.1	3.8	2.9	3.6	4.2	--
	5	L23200	M	64.2	62.1	62.1	--	--	--	--	65.9
	5	L23201	M	66.0	63.5	61.4	61.5	--	--	--	--
	5	L23212	M	65.4	64.2	64.0	63.2	64.7 *	63.5	63.2	--
	5	L23214	M	64.9	65.4	62.3	--	--	--	--	--
	5	L23234	M	65.5	64.8	63.7	63.9	--	--	--	--
		Average		65.2	64.0	62.7	62.9	64.7	63.5	63.2	65.9
		Std Dev		0.7	1.3	1.1	1.2	--	--	--	--
	6	L23203	M	63.5	63.2	61.7	61.2	--	--	--	--
	6	L23204	M	61.9	60.1	58.4	59.1	--	--	--	--
	6	L23213	M	--	62.3	--	--	--	--	--	73.7
	6	L23221	M	66.3	--	--	--	--	--	--	--
	6	L23232	M	--	64.6	64.1	62.9	--	--	--	--
		Average		63.9	62.6	61.4	61.1	--	--	--	73.7
		Std Dev		2.2	1.9	2.9	1.9	--	--	--	--

U-6

Bold > Normal Range
Underscore < Normal Range
* Clot Removed
-- No Value

AC 12-21-09

QA Audit Completed By/Date: AL 12/1/09

QC/Tech Rev. By/Date: AC 12-21-09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
MCH (19.7-24.6 pg)	1	L23216	M	21.2	20.4	20.6	20.5	20.5	20.5	20.2	--
	1	L23218	M	21.9	20.5	20.7	20.7	20.8	20.5 *	20.3	--
	1	L23220	M	21.3	20.0	20.1	20.1	20.7 *	20.5 *	20.4	--
	1	L23222	M	20.0	19.2	19.2 *	19.0	19.1	19.3	19.3	--
	1	L23223	M	21.0	20.3	20.1	20.5	20.3	20.5 *	20.2	--
		Average		21.1	20.1	20.1	20.2	20.3	20.3	20.1	--
		Std Dev		0.7	0.5	0.6	0.7	0.7	0.5	0.4	--
	2	L23206	M	21.7	20.6	20.9 *	20.6	21.0 *	20.8	20.7	--
	2	L23210	M	21.3	20.8	20.4	--	--	20.8 *	--	--
	2	L23211	M	20.4	19.7	19.7	19.9	20.0	20.2 *	19.9	--
	2	L23215	M	20.6	19.6	19.7	20.0	19.9	19.9 *	19.6	--
	2	L23219	M	19.9	19.9	19.9	19.8 *	20.7	20.0 *	20.3	--
		Average		20.8	20.1	20.1	20.1	20.4	20.3	20.1	--
		Std Dev		0.7	0.5	0.5	0.4	0.5	0.4	0.5	--
	3	L23217	M	19.6	19.2	19.5	19.7	19.6	19.3	19.5	--
	3	L23227	M	20.3	19.4	19.6	19.6	19.7	19.8	19.4	--
	3	L23228	M	21.5	20.8	20.9	20.8	21.0	20.9	21.0	--
	3	L23229	M	22.0	21.3	20.9	21.0	20.9	21.0	20.5	--
	3	L23230	M	21.3	20.2	20.2	20.4	20.6	20.6 *	20.5	--
		Average		20.9	20.2	20.2	20.3	20.4	20.3	20.2	--
		Std Dev		1.0	0.9	0.7	0.6	0.7	0.7	0.7	--
	4	L23205	M	22.7	22.1	21.9	22.2	22.2	22.2	21.9	--
	4	L23207	M	20.2	19.2	19.3	19.5	19.3	19.4	19.5	--
	4	L23225	M	20.8	19.6	19.8	20.0	--	--	--	--
	4	L23231	M	21.9	21.1	21.1	21.2	21.1	21.3	19.5	--
	4	L23235	M	22.4	21.5	21.3	21.6	21.9	--	--	--
		Average		21.6	20.7	20.7	20.9	21.1	21.0	20.3	--
		Std Dev		1.1	1.2	1.1	1.1	1.3	1.4	1.4	--
	5	L23200	M	20.5	20.1	19.6	--	--	--	--	19.6
	5	L23201	M	21.5	20.6	20.4	20.7	--	--	--	--
	5	L23212	M	21.7	20.8	20.6	20.9	21.2 *	20.9	20.9	--
	5	L23214	M	20.9	19.9	20.2	--	--	--	--	--
	5	L23234	M	21.7	20.8	20.7	20.7	--	--	--	--
		Average		21.3	20.4	20.3	20.8	21.2	20.9	20.9	19.6
		Std Dev		0.5	0.4	0.4	0.1	--	--	--	--
	6	L23203	M	21.2	20.3	20.2	20.5	--	--	--	--
	6	L23204	M	20.2	19.1	19.2	19.1	--	--	--	--
	6	L23213	M	--	20.3	--	--	--	--	--	20.3
	6	L23221	M	22.2	--	--	--	--	--	--	--
	6	L23232	M	--	20.7	20.9	21.2	--	--	--	--
		Average		21.2	20.1	20.1	20.3	--	--	--	20.3
		Std Dev		1.0	0.7	0.9	1.1	--	--	--	--

U-7

Bold > Normal Range
Underscore < Normal Range
* Clot Removed
-- No Value

ATC 12.21.09

QA Audit Completed/
By/Date: AMZ 12/7/10

QC/Tech Rev. By/Date: ATC 12.21.09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
MCHC (31.0-34.9 g/dL)	1	L23216	M	33.2	31.9	32.4	32.4	31.0	32.1	32.1	--
	1	L23218	M	33.2	31.9	32.0	31.3	30.9	31.7 *	32.4	--
	1	L23220	M	33.4	31.2	31.0	31.0	32.1 *	33.1 *	33.0	--
	1	L23222	M	33.6	32.7	31.6 *	32.5	32.4	32.2	32.8	--
	1	L23223	M	32.3	31.1	31.7	32.8	31.9	32.5 *	32.6	--
		Average		33.1	31.8	31.7	32.0	31.7	32.3	32.6	--
		Std Dev		0.5	0.6	0.5	0.8	0.7	0.5	0.3	--
	2	L23206	M	33.5	31.7	31.9 *	31.0	31.6 *	31.9	32.4	--
	2	L23210	M	32.6	31.5	32.1	--	--	32.1 *	--	--
	2	L23211	M	33.1	32.7	33.2	32.8	31.5	32.9 *	33.9	--
	2	L23215	M	34.3	32.4	32.5	33.0	32.0	31.8 *	31.6	--
	2	L23219	M	31.7	31.3	31.8	31.6 *	31.1	32.8 *	32.9	--
		Average		33.0	31.9	32.3	32.1	31.6	32.3	32.7	--
		Std Dev		1.0	0.6	0.6	1.0	0.4	0.5	1.0	--
	3	L23217	M	33.0	32.1	32.1	32.0	31.7	31.8	32.5	--
	3	L23227	M	32.7	30.3	31.9	31.9	29.8	31.7	31.9	--
	3	L23228	M	32.9	31.7	31.9	32.4	31.1	32.2	33.1	--
	3	L23229	M	33.7	32.6	32.5	32.1	32.2	32.7	32.4	--
	3	L23230	M	34.2	33.1	33.1	33.5	31.4	33.9 *	33.7	--
		Average		33.3	32.0	32.3	32.4	31.2	32.5	32.7	--
		Std Dev		0.6	1.1	0.5	0.7	0.9	0.9	0.7	--
	4	L23205	M	33.6	32.6	32.7	31.8	32.0	32.3	32.5	--
	4	L23207	M	32.7	31.6	31.4	30.2	30.7	31.5	32.5	--
	4	L23225	M	33.4	32.5	33.2	33.5	--	--	--	--
	4	L23231	M	33.5	32.8	32.8	32.2	30.5	32.6	32.5	--
	4	L23235	M	33.0	32.6	32.3	32.2	32.6	--	--	--
		Average		33.2	32.4	32.5	32.0	31.5	32.1	32.5	--
		Std Dev		0.4	0.5	0.7	1.2	1.0	0.6	0.0	--
	5	L23200	M	31.9	32.3	31.6	--	--	--	--	29.7
	5	L23201	M	32.6	32.4	33.2	33.7	--	--	--	--
	5	L23212	M	33.2	32.4	32.2	33.0	32.7 *	32.9	33.0	--
	5	L23214	M	32.3	30.4	32.4	--	--	--	--	--
	5	L23234	M	33.2	32.0	32.5	32.4	--	--	--	--
		Average		32.6	31.9	32.4	33.0	32.7	32.9	33.0	29.7
		Std Dev		0.6	0.9	0.6	0.7	--	--	--	--
	6	L23203	M	33.3	32.1	32.7	33.5	--	--	--	--
	6	L23204	M	32.7	31.8	32.9	32.4	--	--	--	--
	6	L23213	M	--	32.6	--	--	--	--	--	27.5
	6	L23221	M	33.5	--	--	--	--	--	--	--
	6	L23232	M	--	32.1	32.6	33.7	--	--	--	--
		Average		33.2	32.2	32.7	33.2	--	--	--	27.5
		Std Dev		0.4	0.3	0.2	0.7	--	--	--	--

Bold > Normal Range
Underscore < Normal Range
* Clot Removed
-- No Value

ATC 12-21-09

QA Audit Complete; By/Date: [illegible] 1/27/10

QC/Tech Rev. By/Date: ATC 12-21-09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
RDW (10.3-14.4 %)	1	L23216	M	11.7	11.7	11.4	11.4	13.0	11.5	11.3	--
	1	L23218	M	12.2	12.4	12.4	12.4	12.4	11.5 *	11.1	--
	1	L23220	M	13.4	13.4	13.1	13.5	12.9 *	11.7 *	11.6	--
	1	L23222	M	11.5	11.7	11.8 *	12.1	12.6	12.0	11.2	--
	1	L23223	M	12.3	12.0	12.0	12.2	12.8	12.2 *	12.0	--
		Average		12.2	12.2	12.1	12.3	12.7	11.8	11.4	--
		Std Dev		0.7	0.7	0.6	0.8	0.2	0.3	0.4	--
	2	L23206	M	13.2	14.2	14.1 *	14.2	13.4 *	12.6	11.8	--
	2	L23210	M	12.7	12.3	12.7	--	--	12.7 *	--	--
	2	L23211	M	12.8	13.1	13.1	13.4	14.2	12.3 *	11.7	--
	2	L23215	M	12.7	13.1	13.3	14.0	**14.5**	12.8 *	12.0	--
	2	L23219	M	12.1	12.1	12.2	12.7 *	12.8	11.7 *	11.0	--
		Average		12.7	13.0	13.1	13.6	13.7	12.4	11.6	--
		Std Dev		0.4	0.8	0.7	0.7	0.8	0.4	0.4	--
	3	L23217	M	13.5	13.4	13.7	14.1	**14.5**	13.2	12.5	--
	3	L23227	M	12.7	13.1	13.4	13.9	14.0	12.7	11.8	--
	3	L23228	M	12.3	12.3	12.5	13.0	13.5	12.5	12.0	--
	3	L23229	M	12.9	12.7	12.7	13.0	13.5	12.0	11.9	--
	3	L23230	M	12.8	13.0	13.0	13.5	**14.5**	12.8 *	12.2	--
		Average		12.8	12.9	13.1	13.5	14.0	12.6	12.1	--
		Std Dev		0.4	0.4	0.5	0.5	0.5	0.4	0.3	--
	4	L23205	M	12.9	13.0	13.1	13.3	14.3	12.7	12.4	--
	4	L23207	M	12.7	12.7	12.9	12.8	13.3	12.4	12.0	--
	4	L23225	M	11.8	12.6	12.1	12.4	--	--	--	--
	4	L23231	M	12.3	12.3	12.4	13.1	13.5	12.2	12.0	--
	4	L23235	M	12.9	12.3	12.6	12.9	12.8	--	--	--
		Average		12.5	12.6	12.6	12.9	13.5	12.4	12.1	--
		Std Dev		0.5	0.3	0.4	0.3	0.6	0.3	0.2	--
	5	L23200	M	12.1	12.2	12.3	--	--	--	--	12.2
	5	L23201	M	13.3	13.6	13.6	12.7	--	--	--	--
	5	L23212	M	12.6	12.4	12.4	13.1	13.8 *	12.3	12.0	--
	5	L23214	M	12.8	12.8	12.8	--	--	--	--	--
	5	L23234	M	13.3	13.4	13.8	14.1	--	--	--	--
		Average		12.8	12.9	13.0	13.3	13.8	12.3	12.0	12.2
		Std Dev		0.5	0.6	0.7	0.7	--	--	--	--
	6	L23203	M	13.5	13.7	13.5	13.0	--	--	--	--
	6	L23204	M	12.6	12.8	12.6	12.6	--	--	--	--
	6	L23213	M	--	12.4	--	--	--	--	--	12.4
	6	L23221	M	12.4	--	--	--	--	--	--	--
	6	L23232	M	--	12.8	12.6	12.5	--	--	--	--
		Average		12.8	12.9	12.9	12.7	--	--	--	12.4
		Std Dev		0.6	0.6	0.5	0.3	--	--	--	--

U-9

Bold > Normal Range
Underscore < Normal Range
* Clot Removed
-- No Value

QA Audit Complete
By/Date: [illegible] 1/27/10

ATC 12-21-09

QC/Tech Rev. By/Date: ATC 12-21-09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
Platelet Count (137-558 10^3/uL)	1	L23216	M	426	339	257	270	**733**	451	248	--
	1	L23218	M	350	355	224	364	408	73 *	451	--
	1	L23220	M	**741**	**1137**	**1087**	**806**	120 *	26 *	**685**	--
	1	L23222	M	501	415	306 *	410	509	373	352	--
	1	L23223	M	**707**	**896**	**826**	10	**652**	31 *	**631**	--
		Average		545	628	540	372	484	191	473	--
		Std Dev		172	365	392	288	239	205	184	--
	2	L23206	M	**616**	**693**	435 *	**623**	62 *	520	**591**	--
	2	L23210	M	**611**	**569**	**580**	--	--	258 *	--	--
	2	L23211	M	381	328	266	325	211	240 *	114	--
	2	L23215	M	413	355	267	362	430	208 *	558	--
	2	L23219	M	346	417	435	377 *	358	91 *	340	--
		Average		473	472	397	422	265	263	401	--
		Std Dev		130	155	133	136	163	157	221	--
	3	L23217	M	440	363	326	370	233	345	296	--
	3	L23227	M	474	416	342	416	390	324	353	--
	3	L23228	M	307	352	351	399	343	257	113	--
	3	L23229	M	510	446	366	461	393	470	275	--
	3	L23230	M	456	405	388	428	404	26 *	381	--
		Average		437	396	355	415	353	284	284	--
		Std Dev		77	39	24	34	71	164	104	--
	4	L23205	M	486	476	454	513	414	506	454	--
	4	L23207	M	486	425	479	549	512	503	456	--
	4	L23225	M	380	**563**	299	408	--	--	--	--
	4	L23231	M	**588**	**588**	517	**575**	443	**564**	448	--
	4	L23235	M	345	203	264	322	166	--	--	--
		Average		457	451	403	473	384	524	453	--
		Std Dev		97	153	113	106	151	34	4	--
	5	L23200	M	**653**	457	417	--	--	--	--	408
	5	L23201	M	**634**	555	220	173	--	--	--	--
	5	L23212	M	426	349	359	396	251 *	325	170	--
	5	L23214	M	**600**	**626**	270	--	--	--	--	--
	5	L23234	M	**588**	531	534	353	--	--	--	--
		Average		580	504	360	307	251	325	170	408
		Std Dev		90	105	124	118	--	--	--	--
	6	L23203	M	421	426	198	288	--	--	--	--
	6	L23204	M	539	486	250	476	--	--	--	--
	6	L23213	M	--	**568**	--	--	--	--	--	193
	6	L23221	M	518	--	--	--	--	--	--	--
	6	L23232	M	--	555	298	107	--	--	--	--
		Average		493	509	249	290	--	--	--	193
		Std Dev		63	66	50	185	--	--	--	--

Bold > Normal Range
Underscore < Normal Range
* Clot Removed
-- No Value

AC 12-21-09

QA Audit Complete
By/Date: [illegible] 12/7/10

QC/Tech Rev. By/Date: AC 12-21-09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
MPV (5.2-7.7 fL)	1	L23216	M	**8.2**	7.1	7.0	7.0	6.1	6.7	7.5	--
	1	L23218	M	**8.4**	6.6	6.7	7.5	7.0	**8.5** *	6.1	--
	1	L23220	M	**8.7**	7.7	6.9	6.7	**9.0** *	**10.6** *	6.6	--
	1	L23222	M	7.1	6.6	6.5 *	6.1	5.7	6.6	6.3	--
	1	L23223	M	**9.8**	**8.4**	6.8	**13.1**	6.6	**11.4** *	6.2	--
		Average		8.4	7.3	6.8	8.1	6.9	8.8	6.5	--
		Std Dev		1.0	0.8	0.2	2.9	1.3	2.2	0.6	--
	2	L23206	M	**8.6**	7.5	7.5 *	**8.1**	**9.0** *	6.7	6.6	--
	2	L23210	M	**8.7**	7.6	6.0	--	--	7.3 *	--	--
	2	L23211	M	7.3	6.5	6.1	6.1	7.6	7.4 *	7.3	--
	2	L23215	M	7.7	7.0	7.1	6.4	7.3	**7.9** *	6.3	--
	2	L23219	M	**8.3**	7.1	7.2	7.1 *	7.4	6.9 *	6.1	--
		Average		8.1	7.1	6.8	6.9	7.8	7.2	6.6	--
		Std Dev		0.6	0.4	0.7	0.9	0.8	0.5	0.5	--
	3	L23217	M	**9.0**	6.9	6.4	6.3	**8.6**	6.9	6.2	--
	3	L23227	M	**8.3**	7.5	6.3	7.7	**8.5**	6.7	6.5	--
	3	L23228	M	**9.0**	7.2	6.2	**7.8**	**8.3**	7.4	7.5	--
	3	L23229	M	7.1	6.4	6.1	6.7	7.7	6.2	7.0	--
	3	L23230	M	**7.9**	7.0	6.4	7.4	**7.8**	**13.1** *	6.0	--
		Average		8.3	7.0	6.3	7.2	8.2	8.1	6.6	--
		Std Dev		0.8	0.4	0.1	0.7	0.4	2.9	0.6	--
	4	L23205	M	**8.4**	6.0	6.0	**8.1**	**8.1**	6.5	5.9	--
	4	L23207	M	7.0	6.0	5.7	7.0	6.2	5.7	5.6	--
	4	L23225	M	**8.7**	6.1	**8.3**	**15.2**	--	--	--	--
	4	L23231	M	**8.2**	6.3	6.3	**9.1**	**9.2**	6.7	5.5	--
	4	L23235	M	7.6	6.8	7.2	7.3	**10.0**	--	--	--
		Average		8.0	6.2	6.7	9.3	8.4	6.3	5.7	--
		Std Dev		0.7	0.3	1.1	3.4	1.6	0.5	0.2	--
	5	L23200	M	**8.5**	6.5	6.6	--	--	--	--	**9.2**
	5	L23201	M	7.1	5.8	6.7	**11.8**	--	--	--	--
	5	L23212	M	7.6	7.3	7.6	**8.7**	**8.4** *	7.6	**7.9**	--
	5	L23214	M	6.9	6.0	6.3	--	--	--	--	--
	5	L23234	M	7.6	6.4	6.1	7.5	--	--	--	--
		Average		7.5	6.4	6.7	9.3	8.4	7.6	7.9	9.2
		Std Dev		0.6	0.6	0.6	2.2	--	--	--	--
	6	L23203	M	**7.9**	6.8	6.7	**14.4**	--	--	--	--
	6	L23204	M	**8.0**	6.1	7.4	**13.1**	--	--	--	--
	6	L23213	M	--	5.9	--	--	--	--	--	**14.4**
	6	L23221	M	**8.8**	--	--	--	--	--	--	--
	6	L23232	M	--	5.8	6.5	**21.7**	--	--	--	--
		Average		8.2	6.2	6.9	16.4	--	--	--	14.4
		Std Dev		0.5	0.5	0.5	4.6	--	--	--	--

U-11

Bold > Normal Range
Underscore < Normal Range
* Clot Removed
-- No Value

AC 12-21-09

QA Audit Complete. Rv/Date: 12/21/09

QC/Tech Rev. By/Date: AC 12-21-09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
%NEUT (17.2-40.0 %)	1	L23216	M	23.2	7.2	17.1	17.7	31.6	24.5	20.3	--
	1	L23218	M	27.4	27.5	26.0	23.5	29.9	30.3 *	28.1	--
	1	L23220	M	**60.9**	30.4	36.0	29.9	26.4 *	15.7 *	24.6	--
	1	L23222	M	23.6	17.8	17.2 *	17.6	16.6	24.1	19.5	--
	1	L23223	M	22.6	17.7	22.6	6.3	22.4	10.3 *	17.9	--
		Average		31.5	20.1	23.8	19.0	25.4	21.0	22.1	--
		Std Dev		16.5	9.2	7.8	8.7	6.0	7.9	4.2	--
	2	L23206	M	24.0	20.2	16.4 *	14.9	20.5 *	26.0	15.5	--
	2	L23210	M	31.7	25.3	25.9	--	--	23.2 *	--	--
	2	L23211	M	26.3	27.6	26.4	20.8	24.7	27.9 *	18.1	--
	2	L23215	M	24.8	25.6	17.4	19.9	22.2	19.9 *	17.5	--
	2	L23219	M	21.9	17.7	19.5	11.5 *	20.3	18.3 *	12.6	--
		Average		25.7	23.3	21.1	16.8	21.9	23.1	15.9	--
		Std Dev		3.7	4.1	4.7	4.4	2.0	4.0	2.5	--
	3	L23217	M	18.6	14.0	12.0	11.4	20.1	18.6	14.6	--
	3	L23227	M	23.1	15.8	16.9	15.9	18.4	19.4	17.8	--
	3	L23228	M	30.8	27.4	28.2	30.1	30.2	34.2	23.5	--
	3	L23229	M	20.2	16.8	19.6	15.2	21.8	19.4	15.1	--
	3	L23230	M	22.5	26.3	21.3	24.6	21.5	18.0 *	16.3	--
		Average		23.0	20.1	19.6	19.4	22.4	21.9	17.5	--
		Std Dev		4.7	6.3	6.0	7.7	4.6	6.9	3.6	--
	4	L23205	M	21.8	16.9	14.4	14.0	20.9	17.8	15.4	--
	4	L23207	M	26.5	24.0	26.7	21.6	24.0	24.6	15.9	--
	4	L23225	M	36.9	39.9	**51.1**	9.5	--	--	--	--
	4	L23231	M	28.6	28.0	29.6	30.2	33.6	22.0	16.8	--
	4	L23235	M	20.6	18.6	13.2	13.6	33.1	--	--	--
		Average		26.9	25.5	27.0	17.8	27.9	21.5	16.0	--
		Std Dev		6.5	9.2	15.3	8.2	6.4	3.4	0.7	--
	5	L23200	M	30.0	2.7	6.5	--	--	--	--	14.2
	5	L23201	M	27.5	26.5	34.3	36.1	--	--	--	--
	5	L23212	M	16.2	15.6	13.4	12.7	19.2 *	15.6	13.2	--
	5	L23214	M	39.4	33.2	**69.3**	--	--	--	--	--
	5	L23234	M	**40.9**	34.0	29.6	**53.3**	--	--	--	--
		Average		30.8	22.4	30.6	34.0	19.2	15.6	13.2	14.2
		Std Dev		10.0	13.3	24.4	20.4	--	--	--	--
	6	L23203	M	15.6	23.0	**49.1**	25.1	--	--	--	--
	6	L23204	M	30.9	31.3	**52.3**	37.2	--	--	--	--
	6	L23213	M	--	23.0	--	--	--	--	--	29.3
	6	L23221	M	32.5	--	--	--	--	--	--	--
	6	L23232	M	--	31.0	**69.8**	**42.3**	--	--	--	--
		Average		26.3	27.1	57.1	34.9	--	--	--	29.3
		Std Dev		9.3	4.7	11.1	8.8	--	--	--	--

Bold > Normal Range
Underscore < Normal Range
* Clot Removed
-- No Value

MTC 12-21-09

QA Audit Complete: By/Date: 1/12 12/21/10

QC/Tech Rev. By/Date: MTC 12-21-09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
%LYMPH (57.5-81.1 %)	1	L23216	M	65.5	78.7	72.7	72.9	56.8	64.2	68.5	--
	1	L23218	M	65.2	63.2	65.3	69.1	61.2	60.1 *	62.3	--
	1	L23220	M	30.6	58.5	52.1	60.1	64.6 *	76.7 *	64.2	--
	1	L23222	M	69.1	73.7	75.6 *	74.5	75.5	68.8	71.5	--
	1	L23223	M	65.9	74.2	70.8	**89.1**	70.6	**82.5** *	74.1	--
		Average		59.3	69.7	67.3	73.1	65.7	70.5	68.1	--
		Std Dev		16.1	8.4	9.3	10.5	7.4	9.1	4.9	--
	2	L23206	M	66.9	71.9	76.0 *	78.6	71.0 *	65.5	76.3	--
	2	L23210	M	59.1	67.1	66.7	--	--	67.5 *	--	--
	2	L23211	M	64.3	64.6	63.9	70.7	66.4	59.7 *	69.7	--
	2	L23215	M	69.8	67.6	77.1	74.6	71.1	73.4 *	76.1	--
	2	L23219	M	70.2	75.3	71.5	**82.8** *	67.3	72.1 *	77.2	--
		Average		66.1	69.3	71.0	76.7	69.0	67.6	74.8	--
		Std Dev		4.6	4.3	5.7	5.2	2.5	5.5	3.5	--
	3	L23217	M	71.7	75.3	74.7	76.8	63.4	67.9	70.9	--
	3	L23227	M	69.1	74.6	74.0	75.4	73.5	73.1	72.8	--
	3	L23228	M	55.3	60.7	60.4	57.7	53.9	53.6	64.6	--
	3	L23229	M	73.3	76.6	72.9	77.6	70.4	73.8	77.6	--
	3	L23230	M	71.4	67.8	73.4	70.1	69.1	75.2 *	79.1	--
		Average		68.2	71.0	71.1	71.5	66.1	68.7	73.0	--
		Std Dev		7.3	6.7	6.0	8.3	7.7	8.9	5.8	--
	4	L23205	M	70.0	73.9	75.6	79.5	67.9	72.9	75.8	--
	4	L23207	M	64.9	68.7	65.5	68.8	64.7	64.9	74.1	--
	4	L23225	M	51.1	54.0	45.1	75.8	--	--	--	--
	4	L23231	M	64.9	65.3	63.3	62.3	57.7	71.0	72.8	--
	4	L23235	M	70.2	73.7	78.7	76.6	59.2	--	--	--
		Average		64.2	67.1	65.6	72.6	62.4	69.6	74.2	--
		Std Dev		7.8	8.2	13.2	7.0	4.8	4.2	1.5	--
	5	L23200	M	60.6	**87.5**	**85.6**	--	--	--	--	73.6
	5	L23201	M	64.3	67.3	61.3	60.5	--	--	--	--
	5	L23212	M	77.1	76.9	80.7	81.1	67.9 *	74.8	76.0	--
	5	L23214	M	46.4	54.7	23.0	--	--	--	--	--
	5	L23234	M	51.2	60.2	64.2	44.1	--	--	--	--
		Average		59.9	69.3	63.0	61.9	67.9	74.8	76.0	73.6
		Std Dev		12.0	13.1	24.6	18.5	--	--	--	--
	6	L23203	M	78.7	68.8	46.3	68.5	--	--	--	--
	6	L23204	M	56.8	58.7	44.7	56.0	--	--	--	--
	6	L23213	M	--	68.6	--	--	--	--	--	59.6
	6	L23221	M	59.5	--	--	--	--	--	--	--
	6	L23232	M	--	60.9	27.5	48.4	--	--	--	--
		Average		65.0	64.3	39.5	57.6	--	--	--	59.6
		Std Dev		11.9	5.2	10.4	10.1	--	--	--	--

Bold > Normal Range
Underscore < Normal Range
* Clot Removed
-- No Value

QA Audit Complete: By/Date: 12/11/09

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ATC 12-21-09

QC/Tech Rev. By/Date: ATC 12-21-09

U-13

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
%MONO (0.0-6.1 %)	1	L23216	M	1.3	0.4	0.5	3.2	4.3	1.1	0.9	--
	1	L23218	M	1.9	2.3	0.8	1.3	1.8	4.1 *	1.8	--
	1	L23220	M	2.2	3.2	3.8	2.1	1.4 *	0.9 *	1.1	--
	1	L23222	M	1.0	0.9	1.1 *	1.6	0.7	1.2	0.8	--
	1	L23223	M	5.4	0.8	1.0	0.5	0.9	1.1 *	0.8	--
		Average		2.4	1.5	1.4	1.7	1.8	1.7	1.1	--
		Std Dev		1.8	1.2	1.3	1.0	1.5	1.4	0.4	--
	2	L23206	M	1.3	1.3	2.4 *	2.2	1.1 *	0.8	0.6	--
	2	L23210	M	1.8	1.3	1.3	--	--	1.0 *	--	--
	2	L23211	M	1.8	1.5	2.3	1.8	1.8	3.6 *	1.2	--
	2	L23215	M	1.2	1.3	1.0	1.1	1.0	0.8 *	0.9	--
	2	L23219	M	1.2	0.8	2.7	1.0 *	3.0	0.8 *	1.0	--
		Average		1.5	1.2	1.9	1.5	1.7	1.4	0.9	--
		Std Dev		0.3	0.3	0.7	0.6	0.9	1.2	0.3	--
	3	L23217	M	1.4	1.0	1.9	1.7	2.2	3.1	1.5	--
	3	L23227	M	1.3	2.8	2.4	1.0	1.5	2.0	1.0	--
	3	L23228	M	3.8	1.3	1.6	1.7	3.8	1.2	1.6	--
	3	L23229	M	1.4	2.0	2.3	2.3	0.9	0.8	1.3	--
	3	L23230	M	1.1	0.7	1.0	1.1	2.3	1.1 *	0.7	--
		Average		1.8	1.6	1.8	1.6	2.1	1.6	1.2	--
		Std Dev		1.1	0.8	0.6	0.5	1.1	0.9	0.4	--
	4	L23205	M	1.8	2.3	3.1	1.2	1.5	3.3	1.7	--
	4	L23207	M	3.5	2.6	1.4	3.6	3.9	3.8	1.2	--
	4	L23225	M	4.8	1.5	0.4	**8.3**	--	--	--	--
	4	L23231	M	2.2	1.5	2.3	2.5	2.8	0.8	1.7	--
	4	L23235	M	1.7	1.2	1.7	3.7	1.2	--	--	--
		Average		2.8	1.8	1.8	3.9	2.4	2.6	1.5	--
		Std Dev		1.3	0.6	1.0	2.7	1.2	1.6	0.3	--
	5	L23200	M	1.6	0.5	0.6	--	--	--	--	6.0
	5	L23201	M	4.0	1.6	1.5	1.0	--	--	--	--
	5	L23212	M	0.9	1.2	1.5	0.9	1.1 *	1.4	0.6	--
	5	L23214	M	2.5	2.7	2.5	--	--	--	--	--
	5	L23234	M	3.0	1.4	2.6	0.1	--	--	--	--
		Average		2.4	1.5	1.7	0.7	1.1	1.4	0.6	6.0
		Std Dev		1.2	0.8	0.8	0.5	--	--	--	--
	6	L23203	M	1.3	2.0	0.4	0.6	--	--	--	--
	6	L23204	M	4.5	2.0	0.6	1.6	--	--	--	--
	6	L23213	M	--	1.2	--	--	--	--	--	**7.4**
	6	L23221	M	1.7	--	--	--	--	--	--	--
	6	L23232	M	--	2.3	0.4	1.4	--	--	--	--
		Average		2.5	1.9	0.5	1.2	--	--	--	7.4
		Std Dev		1.7	0.5	0.1	0.5	--	--	--	--

Bold > Normal Range
Underscore < Normal Range
* Clot Removed
-- No Value

ATC 12·21·09

QA Audit Complete
By/Date: 12 11/7/10

QC/Tech Rev. By/Date: ATC 12·21·09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
%EOS (0.1-7.0 %)	1	L23216	M	2.4	3.9	2.7	1.2	2.2	3.0	2.7	--
	1	L23218	M	2.3	3.2	3.4	2.7	3.7	2.5 *	4.3	--
	1	L23220	M	2.1	2.1	2.8	3.0	3.1 *	3.1 *	3.2	--
	1	L23222	M	1.3	2.4	2.2 *	1.9	2.3	1.8	2.3	--
	1	L23223	M	2.2	3.1	1.6	2.0	2.4	2.8 *	3.0	--
		Average		2.1	2.9	2.5	2.2	2.7	2.6	3.1	--
		Std Dev		0.4	0.7	0.7	0.7	0.6	0.5	0.8	--
	2	L23206	M	2.3	2.7	2.0 *	1.5	2.6 *	2.6	3.1	--
	2	L23210	M	1.4	2.6	1.6	--	--	3.0 *	--	--
	2	L23211	M	2.8	2.2	2.0	2.1	2.9	2.4 *	3.9	--
	2	L23215	M	1.6	1.9	1.4	1.4	2.4	2.3 *	2.2	--
	2	L23219	M	1.8	2.0	1.5	1.5 *	2.5	3.6 *	2.8	--
		Average		2.0	2.3	1.7	1.6	2.6	2.8	3.0	--
		Std Dev		0.6	0.4	0.3	0.3	0.2	0.5	0.7	--
	3	L23217	M	1.9	2.4	2.9	1.8	3.7	2.3	2.8	--
	3	L23227	M	0.9	1.4	1.2	1.7	1.5	1.5	1.4	--
	3	L23228	M	2.3	2.4	2.1	2.9	3.5	3.5	3.3	--
	3	L23229	M	1.1	1.3	1.6	1.3	2.2	1.4	1.7	--
	3	L23230	M	2.1	2.4	1.9	1.6	2.6	2.9 *	1.7	--
		Average		1.7	2.0	1.9	1.9	2.7	2.3	2.2	--
		Std Dev		0.6	0.6	0.6	0.6	0.9	0.9	0.8	--
	4	L23205	M	1.6	1.2	1.9	1.2	2.9	1.5	1.7	--
	4	L23207	M	1.7	1.7	1.9	2.2	2.5	1.6	2.8	--
	4	L23225	M	2.3	1.7	1.3	1.9	--	--	--	--
	4	L23231	M	1.3	1.6	1.9	1.4	2.4	1.9	2.5	--
	4	L23235	M	1.2	1.3	1.7	1.5	1.4	--	--	--
		Average		1.6	1.5	1.7	1.6	2.3	1.7	2.3	--
		Std Dev		0.4	0.2	0.3	0.4	0.6	0.2	0.6	--
	5	L23200	M	1.8	3.6	3.3	--	--	--	--	1.5
	5	L23201	M	1.3	1.6	0.9	0.4	--	--	--	--
	5	L23212	M	2.0	1.8	1.1	1.7	4.0 *	2.7	3.5	--
	5	L23214	M	2.8	3.6	1.3	--	--	--	--	--
	5	L23234	M	1.9	1.7	1.0	0.8	--	--	--	--
		Average		2.0	2.5	1.5	1.0	4.0	2.7	3.5	1.5
		Std Dev		0.5	1.0	1.0	0.7	--	--	--	--
	6	L23203	M	1.4	2.1	1.3	2.8	--	--	--	--
	6	L23204	M	1.0	1.6	0.7	1.2	--	--	--	--
	6	L23213	M	--	1.6	--	--	--	--	--	0.9
	6	L23221	M	2.0	--	--	--	--	--	--	--
	6	L23232	M	--	1.8	0.5	3.2	--	--	--	--
		Average		1.5	1.8	0.8	2.4	--	--	--	0.9
		Std Dev		0.5	0.2	0.4	1.1	--	--	--	--

U-15

Bold > Normal Range
Underscore < Normal Range
* Clot Removed
-- No Value

QA Audit Completed By/Date: [illegible] 12/17/10

AC 12-21-09

QC/Tech Rev. By/Date: AC 12-21-09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
%BASO (2.2-9.7 %)	1	L23216	M	7.2	9.6	6.9	4.9	5.0	7.0	7.4	--
	1	L23218	M	3.0	3.8	4.5	3.2	3.2	2.8 *	3.4	--
	1	L23220	M	3.6	5.7	5.1	4.5	4.5 *	3.4 *	6.7	--
	1	L23222	M	3.5	5.1	3.8 *	4.3	4.8	4.2	5.6	--
	1	L23223	M	3.3	4.1	3.9	2.0	3.7	3.1 *	4.2	--
		Average		4.1	5.7	4.8	3.8	4.2	4.1	5.5	--
		Std Dev		1.7	2.3	1.3	1.2	0.8	1.7	1.7	--
	2	L23206	M	4.8	3.7	3.2 *	2.8	4.5 *	5.0	4.4	--
	2	L23210	M	3.9	3.6	3.4	--	--	5.2 *	--	--
	2	L23211	M	4.6	4.1	5.5	4.4	4.0	6.2 *	7.2	--
	2	L23215	M	2.4	3.5	3.1	3.0	3.2	3.4 *	3.2	--
	2	L23219	M	4.9	4.0	4.7	3.0 *	5.8	5.2 *	6.0	--
		Average		4.1	3.8	4.0	3.3	4.4	5.0	5.2	--
		Std Dev		1.0	0.3	1.1	0.7	1.1	1.0	1.8	--
	3	L23217	M	5.7	7.0	8.1	7.8	**10.3**	7.9	**10.1**	--
	3	L23227	M	5.4	5.1	5.5	5.9	4.9	3.4	6.5	--
	3	L23228	M	7.5	8.0	7.6	7.4	8.3	7.4	6.7	--
	3	L23229	M	3.3	3.2	3.5	3.5	4.6	4.5	4.1	--
	3	L23230	M	2.8	2.7	2.2	2.4	4.3	2.6 *	2.0	--
		Average		4.9	5.2	5.4	5.4	6.5	5.2	5.9	--
		Std Dev		1.9	2.3	2.5	2.4	2.7	2.4	3.0	--
	4	L23205	M	4.3	5.2	4.9	3.9	6.5	4.4	4.9	--
	4	L23207	M	3.1	3.1	4.4	3.7	4.8	5.1	5.7	--
	4	L23225	M	4.3	2.5	1.8	3.7	--	--	--	--
	4	L23231	M	2.7	3.1	2.5	3.4	3.3	4.2	6.1	--
	4	L23235	M	4.2	5.2	4.3	4.5	3.6	--	--	--
		Average		3.7	3.8	3.6	3.8	4.6	4.6	5.6	--
		Std Dev		0.8	1.3	1.3	0.4	1.5	0.5	0.6	--
	5	L23200	M	3.8	5.5	3.8	--	--	--	--	4.1
	5	L23201	M	2.7	2.9	1.9	1.1	--	--	--	--
	5	L23212	M	3.7	4.3	3.1	3.4	7.6 *	5.4	6.7	--
	5	L23214	M	6.0	5.7	3.8	--	--	--	--	--
	5	L23234	M	2.4	2.5	2.4	1.7	--	--	--	--
		Average		3.7	4.2	3.0	2.1	7.6	5.4	6.7	4.1
		Std Dev		1.4	1.5	0.8	1.2	--	--	--	--
	6	L23203	M	2.9	4.0	2.7	2.8	--	--	--	--
	6	L23204	M	6.1	6.3	1.4	3.2	--	--	--	--
	6	L23213	M	--	5.5	--	--	--	--	--	1.8
	6	L23221	M	4.0	--	--	--	--	--	--	--
	6	L23232	M	--	3.8	1.7	3.9	--	--	--	--
		Average		4.3	4.9	1.9	3.3	--	--	--	1.8
		Std Dev		1.6	1.2	0.7	0.6	--	--	--	--

U-16

Bold > Normal Range
Underscore < Normal Range
* Clot Removed
-- No Value

QA Audit Completed By/Date: [illegible] 12/27/10

ATC 12-21-09

QC/Tech Rev. By/Date: ATC 12-21-09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
#NEUT (0.80-2.90 10^3/uL)	1	L23216	M	1.77	0.33	0.78	1.31	2.58	1.60	0.89	--
	1	L23218	M	1.61	1.52	0.91	1.49	1.80	1.65 *	1.05	--
	1	L23220	M	**4.71**	2.38	2.09	1.86	0.73 *	0.39 *	1.00	--
	1	L23222	M	**2.94**	1.59	1.36 *	1.68	1.28	1.90	1.02	--
	1	L23223	M	1.49	0.88	1.34	0.17	1.13	0.29 *	0.59	--
		Average		2.50	1.34	1.30	1.30	1.50	1.17	0.91	--
		Std Dev		1.36	0.78	0.51	0.67	0.71	0.76	0.19	--
	2	L23206	M	1.71	1.60	1.18 *	0.89	0.84 *	1.92	0.71	--
	2	L23210	M	2.24	1.71	1.90	--	--	1.16 *	--	--
	2	L23211	M	1.30	1.55	1.34	1.21	1.14	1.79 *	0.40	--
	2	L23215	M	1.65	1.31	1.05	1.28	1.10	0.97 *	0.68	--
	2	L23219	M	1.75	1.21	1.51	0.93 *	1.26	0.88 *	0.62	--
		Average		1.73	1.48	1.40	1.08	1.09	1.34	0.60	--
		Std Dev		0.34	0.21	0.33	0.20	0.18	0.48	0.14	--
	3	L23217	M	1.76	1.42	1.30	1.28	1.67	1.58	0.97	--
	3	L23227	M	1.84	1.31	1.29	1.16	1.31	1.59	0.78	--
	3	L23228	M	2.07	2.02	2.11	2.05	1.92	2.25	0.84	--
	3	L23229	M	1.19	1.10	1.20	1.10	1.31	1.26	0.62	--
	3	L23230	M	1.34	1.76	1.38	1.41	1.00	0.57 *	0.54	--
		Average		1.64	1.52	1.46	1.40	1.44	1.45	0.75	--
		Std Dev		0.36	0.37	0.37	0.38	0.36	0.61	0.17	--
	4	L23205	M	1.65	1.29	1.17	1.05	1.43	1.51	0.78	--
	4	L23207	M	1.57	1.66	1.55	1.30	1.29	1.28	0.44	--
	4	L23225	M	2.14	2.78	**3.68**	0.35	--	--	--	--
	4	L23231	M	2.20	2.32	2.40	2.34	2.60	1.42	0.49	--
	4	L23235	M	1.42	1.28	1.15	1.06	**3.97**	--	--	--
		Average		1.80	1.87	1.99	1.22	2.32	1.40	0.57	--
		Std Dev		0.35	0.66	1.07	0.72	1.25	0.12	0.18	--
	5	L23200	M	1.86	0.09	0.28	--	--	--	--	1.27
	5	L23201	M	2.68	2.66	**4.33**	**4.86**	--	--	--	--
	5	L23212	M	1.26	1.25	1.11	1.06	1.32 *	0.82	0.56	--
	5	L23214	M	**3.40**	2.73	2.40	--	--	--	--	--
	5	L23234	M	**3.77**	**2.96**	2.73	**4.53**	--	--	--	--
		Average		2.59	1.94	2.17	3.48	1.32	0.82	0.56	1.27
		Std Dev		1.04	1.23	1.56	2.11	--	--	--	--
	6	L23203	M	1.42	2.02	**3.30**	1.18	--	--	--	--
	6	L23204	M	1.66	2.09	**3.69**	2.02	--	--	--	--
	6	L23213	M	--	2.38	--	--	--	--	--	**3.77**
	6	L23221	M	1.96	--	--	--	--	--	--	--
	6	L23232	M	--	1.77	**6.13**	0.65	--	--	--	--
		Average		1.68	2.07	4.37	1.28	--	--	--	3.77
		Std Dev		0.27	0.25	1.53	0.69	--	--	--	--

U-17

Bold > Normal Range
Underscore < Normal Range
* Clot Removed
-- No Value

Atc 12-21-09

QA Audit Completed By/Date: [illegible] 12/21/10

QC/Tech Rev. By/Date: Atc 12-21-09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
#LYMPH (2.20-5.30 10^3/uL)	1	L23216	M	4.98	3.63	3.31	**5.40**	4.64	4.18	2.99	--
	1	L23218	M	3.82	3.48	2.28	4.39	3.69	3.27 *	2.32	--
	1	L23220	M	2.37	4.58	3.03	3.75	1.79 *	1.89 *	2.60	--
	1	L23222	M	**8.62**	**6.62**	**6.00** *	**7.15**	**5.83**	**5.41**	3.75	--
	1	L23223	M	4.33	3.70	4.22	2.45	3.57	2.35 *	2.46	--
		Average		4.82	4.40	3.77	4.63	3.90	3.42	2.82	--
		Std Dev		2.33	1.31	1.43	1.77	1.49	1.42	0.57	--
	2	L23206	M	4.76	**5.71**	**5.44** *	4.71	2.92 *	4.84	3.48	--
	2	L23210	M	4.17	4.51	4.90	--	--	3.39 *	--	--
	2	L23211	M	3.19	3.62	3.25	4.12	3.07	3.82 *	1.54	--
	2	L23215	M	4.65	3.46	4.63	4.78	3.51	3.57 *	2.96	--
	2	L23219	M	**5.63**	5.15	**5.53**	**6.65** *	4.18	3.48 *	3.82	--
		Average		4.48	4.49	4.75	5.07	3.42	3.82	2.95	--
		Std Dev		0.89	0.97	0.92	1.10	0.57	0.59	1.00	--
	3	L23217	M	**6.76**	**7.66**	**8.12**	**8.61**	5.25	**5.76**	4.71	--
	3	L23227	M	**5.50**	**6.17**	**5.67**	**5.52**	5.21	**5.99**	3.19	--
	3	L23228	M	3.72	4.48	4.52	3.93	3.43	3.53	2.31	--
	3	L23229	M	4.32	5.00	4.48	**5.64**	4.25	4.81	3.18	--
	3	L23230	M	4.27	4.53	4.77	4.01	3.21	2.40 *	2.62	--
		Average		4.91	5.57	5.51	5.54	4.27	4.50	3.20	--
		Std Dev		1.22	1.35	1.54	1.90	0.96	1.52	0.92	--
	4	L23205	M	**5.31**	**5.64**	**6.16**	**5.99**	4.64	**6.17**	3.86	--
	4	L23207	M	3.85	4.76	3.80	4.16	3.49	3.37	2.07	--
	4	L23225	M	2.97	3.76	3.24	2.78	--	--	--	--
	4	L23231	M	4.99	**5.42**	5.12	4.82	4.48	4.57	2.14	--
	4	L23235	M	4.84	5.09	**6.86**	**5.99**	**7.10**	--	--	--
		Average		4.39	4.93	5.04	4.75	4.93	4.70	2.69	--
		Std Dev		0.96	0.74	1.53	1.35	1.54	1.40	1.01	--
	5	L23200	M	3.76	3.00	3.71	--	--	--	--	**6.56**
	5	L23201	M	**6.26**	**6.74**	**7.75**	**8.14**	--	--	--	--
	5	L23212	M	**5.97**	**6.18**	**6.72**	**6.75**	4.67 *	3.93	3.23	--
	5	L23214	M	4.00	4.50	0.80	--	--	--	--	--
	5	L23234	M	4.72	5.25	**5.93**	3.75	--	--	--	--
		Average		4.94	5.13	4.98	6.21	4.67	3.93	3.23	6.56
		Std Dev		1.13	1.47	2.77	2.24	--	--	--	--
	6	L23203	M	**7.19**	**6.05**	3.11	3.21	--	--	--	--
	6	L23204	M	3.05	3.92	3.15	3.04	--	--	--	--
	6	L23213	M	--	**7.08**	--	--	--	--	--	**7.65**
	6	L23221	M	3.58	--	--	--	--	--	--	--
	6	L23232	M	--	3.47	2.41	0.75	--	--	--	--
		Average		4.61	5.13	2.89	2.33	--	--	--	7.65
		Std Dev		2.25	1.72	0.42	1.37	--	--	--	--

U-18

Bold > Normal Range
Underscore < Normal Range
* Clot Removed
-- No Value

Ptc 12.21.09

QA Audit Completed
By/Date: [illegible] 12/21/10

QC/Tech Rev. By/Date: Ptc 12.21.09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
#MONO (0.00-0.40 10^3/uL)	1	L23216	M	0.10	0.02	0.02	0.23	0.35	0.07	0.04	--
	1	L23218	M	0.11	0.13	0.03	0.08	0.11	0.22 *	0.07	--
	1	L23220	M	0.17	0.25	0.22	0.13	0.04 *	0.02 *	0.05	--
	1	L23222	M	0.13	0.08	0.09 *	0.15	0.05	0.09	0.04	--
	1	L23223	M	0.35	0.04	0.06	0.01	0.04	0.03 *	0.03	--
		Average		0.17	0.10	0.08	0.12	0.12	0.09	0.05	--
		Std Dev		0.10	0.09	0.08	0.08	0.13	0.08	0.02	--
	2	L23206	M	0.09	0.10	0.17 *	0.13	0.05 *	0.06	0.03	--
	2	L23210	M	0.12	0.09	0.09	--	--	0.05 *	--	--
	2	L23211	M	0.09	0.08	0.12	0.11	0.08	0.23 *	0.03	--
	2	L23215	M	0.08	0.07	0.06	0.07	0.05	0.04 *	0.03	--
	2	L23219	M	0.09	0.06	0.21	0.08 *	0.19	0.04 *	0.05	--
		Average		0.09	0.08	0.13	0.10	0.09	0.08	0.04	--
		Std Dev		0.02	0.02	0.06	0.03	0.07	0.08	0.01	--
	3	L23217	M	0.13	0.11	0.20	0.19	0.19	0.26	0.10	--
	3	L23227	M	0.11	0.23	0.19	0.08	0.11	0.17	0.05	--
	3	L23228	M	0.26	0.10	0.12	0.11	0.24	0.08	0.06	--
	3	L23229	M	0.08	0.13	0.14	0.17	0.06	0.05	0.05	--
	3	L23230	M	0.06	0.05	0.06	0.06	0.11	0.03 *	0.02	--
		Average		0.13	0.12	0.14	0.12	0.14	0.12	0.06	--
		Std Dev		0.08	0.07	0.06	0.06	0.07	0.10	0.03	--
	4	L23205	M	0.14	0.18	0.25	0.09	0.10	0.28	0.08	--
	4	L23207	M	0.21	0.18	0.08	0.22	0.21	0.20	0.03	--
	4	L23225	M	0.28	0.10	0.03	0.30	--	--	--	--
	4	L23231	M	0.17	0.13	0.18	0.19	0.22	0.05	0.05	--
	4	L23235	M	0.12	0.08	0.15	0.29	0.14	--	--	--
		Average		0.18	0.13	0.14	0.22	0.17	0.18	0.05	--
		Std Dev		0.06	0.05	0.09	0.09	0.06	0.12	0.03	--
	5	L23200	M	0.10	0.02	0.03	--	--	--	--	**0.54**
	5	L23201	M	0.39	0.16	0.19	0.14	--	--	--	--
	5	L23212	M	0.07	0.09	0.12	0.08	0.08 *	0.07	0.03	--
	5	L23214	M	0.22	0.22	0.09	--	--	--	--	--
	5	L23234	M	0.28	0.12	0.24	0.01	--	--	--	--
		Average		0.21	0.12	0.13	0.08	0.08	0.07	0.03	0.54
		Std Dev		0.13	0.07	0.08	0.07	--	--	--	--
	6	L23203	M	0.11	0.18	0.02	0.03	--	--	--	--
	6	L23204	M	0.24	0.13	0.04	0.08	--	--	--	--
	6	L23213	M	--	0.12	--	--	--	--	--	**0.95**
	6	L23221	M	0.10	--	--	--	--	--	--	--
	6	L23232	M	--	0.13	0.04	0.02	--	--	--	--
		Average		0.15	0.14	0.03	0.04	--	--	--	0.95
		Std Dev		0.08	0.03	0.01	0.03	--	--	--	--

U-19

Bold > Normal Range
Underscore < Normal Range
* Clot Removed
-- No Value

ATC 12-21-09

QA Audit Completed
By/Date: 1/2 1/17/10

QC/Tech Rev By/Date: ATC 12-21-09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
#EOS (0.00-0.40 10^3/uL)	1	L23216	M	0.18	0.18	0.12	0.09	0.18	0.20	0.12	--
	1	L23218	M	0.14	0.17	0.12	0.17	0.22	0.14 *	0.16	--
	1	L23220	M	0.16	0.17	0.16	0.19	0.09 *	0.08 *	0.13	--
	1	L23222	M	0.16	0.22	0.17 *	0.18	0.18	0.14	0.12	--
	1	L23223	M	0.15	0.15	0.10	0.05	0.12	0.08 *	0.10	--
		Average		0.16	0.18	0.13	0.14	0.16	0.13	0.13	--
		Std Dev		0.01	0.03	0.03	0.06	0.05	0.05	0.02	--
	2	L23206	M	0.16	0.21	0.14 *	0.09	0.11 *	0.20	0.14	--
	2	L23210	M	0.10	0.17	0.12	--	--	0.15 *	--	--
	2	L23211	M	0.14	0.12	0.10	0.12	0.13	0.15 *	0.09	--
	2	L23215	M	0.11	0.10	0.09	0.09	0.12	0.11 *	0.09	--
	2	L23219	M	0.14	0.13	0.12	0.12 *	0.16	0.17 *	0.14	--
		Average		0.13	0.15	0.11	0.11	0.13	0.16	0.12	--
		Std Dev		0.02	0.04	0.02	0.02	0.02	0.03	0.03	--
	3	L23217	M	0.18	0.24	0.31	0.21	0.30	0.20	0.19	--
	3	L23227	M	0.07	0.12	0.09	0.12	0.11	0.12	0.06	--
	3	L23228	M	0.15	0.18	0.15	0.20	0.23	0.23	0.12	--
	3	L23229	M	0.06	0.09	0.10	0.10	0.13	0.09	0.07	--
	3	L23230	M	0.12	0.16	0.12	0.09	0.12	0.09 *	0.06	--
		Average		0.12	0.16	0.15	0.14	0.18	0.15	0.10	--
		Std Dev		0.05	0.06	0.09	0.06	0.08	0.07	0.06	--
	4	L23205	M	0.12	0.09	0.15	0.09	0.20	0.12	0.09	--
	4	L23207	M	0.10	0.12	0.11	0.13	0.14	0.08	0.08	--
	4	L23225	M	0.13	0.12	0.09	0.07	--	--	--	--
	4	L23231	M	0.10	0.13	0.15	0.11	0.19	0.12	0.07	--
	4	L23235	M	0.08	0.09	0.15	0.12	0.17	--	--	--
		Average		0.11	0.11	0.13	0.10	0.18	0.11	0.08	--
		Std Dev		0.02	0.02	0.03	0.02	0.03	0.02	0.01	--
	5	L23200	M	0.11	0.12	0.14	--	--	--	--	0.14
	5	L23201	M	0.12	0.16	0.11	0.06	--	--	--	--
	5	L23212	M	0.15	0.14	0.09	0.15	0.27 *	0.14	0.15	--
	5	L23214	M	0.24	0.29	0.04	--	--	--	--	--
	5	L23234	M	0.18	0.14	0.09	0.07	--	--	--	--
		Average		0.16	0.17	0.09	0.09	0.27	0.14	0.15	0.14
		Std Dev		0.05	0.07	0.04	0.05	--	--	--	--
	6	L23203	M	0.13	0.18	0.08	0.13	--	--	--	--
	6	L23204	M	0.06	0.11	0.05	0.06	--	--	--	--
	6	L23213	M	--	0.16	--	--	--	--	--	0.11
	6	L23221	M	0.12	--	--	--	--	--	--	--
	6	L23232	M	--	0.10	0.05	0.05	--	--	--	--
		Average		0.10	0.14	0.06	0.08	--	--	--	0.11
		Std Dev		0.04	0.04	0.02	0.04	--	--	--	--

Bold > Normal Range
Underscore < Normal Range
* Clot Removed
-- No Value

AHC 12-21-09

QA Audit Complete/ By/Date: [illegible]

QC/Tech Rev. By/Date: AHC 12-21-09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
#BASO (0.10-0.60 10^3/uL)	1	L23216	M	0.55	0.44	0.32	0.36	0.41	0.46	0.32	--
	1	L23218	M	0.17	0.21	0.16	0.20	0.19	0.15 *	0.13	--
	1	L23220	M	0.28	0.45	0.30	0.28	0.12 *	0.08 *	0.27	--
	1	L23222	M	0.44	0.46	0.30 *	0.41	0.37	0.33	0.29	--
	1	L23223	M	0.22	0.20	0.23	0.05	0.19	0.09 *	0.14	--
		Average		0.33	0.35	0.26	0.26	0.26	0.22	0.23	--
		Std Dev		0.16	0.13	0.07	0.14	0.13	0.17	0.09	--
	2	L23206	M	0.34	0.29	0.23 *	0.17	0.19 *	0.37	0.20	--
	2	L23210	M	0.28	0.24	0.25	--	--	0.26 *	--	--
	2	L23211	M	0.23	0.23	0.28	0.26	0.18	0.40 *	0.16	--
	2	L23215	M	0.16	0.18	0.19	0.19	0.16	0.17 *	0.12	--
	2	L23219	M	0.39	0.27	0.36	0.24 *	0.36	0.25 *	0.30	--
		Average		0.28	0.24	0.26	0.22	0.22	0.29	0.20	--
		Std Dev		0.09	0.04	0.06	0.04	0.09	0.09	0.08	--
	3	L23217	M	0.54	**0.71**	**0.88**	**0.88**	**0.85**	**0.67**	**0.67**	--
	3	L23227	M	0.43	0.42	0.42	0.43	0.35	0.27	0.29	--
	3	L23228	M	0.50	0.59	0.57	0.50	0.53	0.49	0.24	--
	3	L23229	M	0.19	0.21	0.22	0.25	0.28	0.29	0.17	--
	3	L23230	M	0.17	0.18	0.14	0.13	0.20	0.08 *	0.07	--
		Average		0.37	0.42	0.45	0.44	0.44	0.36	0.29	--
		Std Dev		0.17	0.23	0.30	0.29	0.26	0.23	0.23	--
	4	L23205	M	0.32	0.40	0.40	0.30	0.45	0.37	0.25	--
	4	L23207	M	0.18	0.21	0.25	0.23	0.26	0.27	0.16	--
	4	L23225	M	0.25	0.18	0.13	0.14	--	--	--	--
	4	L23231	M	0.21	0.26	0.20	0.26	0.25	0.27	0.18	--
	4	L23235	M	0.29	0.36	0.38	0.35	0.43	--	--	--
		Average		0.25	0.28	0.27	0.26	0.35	0.30	0.20	--
		Std Dev		0.06	0.09	0.12	0.08	0.11	0.06	0.05	--
	5	L23200	M	0.23	0.19	0.16	--	--	--	--	0.37
	5	L23201	M	0.26	0.29	0.24	0.15	--	--	--	--
	5	L23212	M	0.28	0.34	0.26	0.29	0.52 *	0.28	0.28	--
	5	L23214	M	0.52	0.47	0.13	--	--	--	--	--
	5	L23234	M	0.22	0.22	0.22	0.14	--	--	--	--
		Average		0.30	0.30	0.20	0.19	0.52	0.28	0.28	0.37
		Std Dev		0.12	0.11	0.05	0.08	--	--	--	--
	6	L23203	M	0.26	0.35	0.18	0.13	--	--	--	--
	6	L23204	M	0.33	0.42	0.10	0.17	--	--	--	--
	6	L23213	M	--	0.56	--	--	--	--	--	0.24
	6	L23221	M	0.24	--	--	--	--	--	--	--
	6	L23232	M	--	0.22	0.15	0.06	--	--	--	--
		Average		0.28	0.39	0.14	0.12	--	--	--	0.24
		Std Dev		0.05	0.14	0.04	0.06	--	--	--	--

U-21

Bold > Normal Range
Underscore < Normal Range
* Clot Removed
-- No Value

ATC 12-21-09

QA Audit Completed By/Date: [illegible] 12/21/10

QC/Tech Rev. By/Date: ATC 12-21-09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
Neut#/Lymph# Ratio	1	L23216	M	0.355	0.091	0.236	0.243	0.556	0.383	0.298	--
	1	L23218	M	0.421	0.437	0.399	0.339	0.488	0.505 *	0.453	--
	1	L23220	M	1.987	0.520	0.690	0.496	0.408 *	0.206 *	0.385	--
	1	L23222	M	0.341	0.240	0.227 *	0.235	0.220	0.351	0.272	--
	1	L23223	M	0.344	0.238	0.318	0.069	0.317	0.123 *	0.240	--
		Average		0.690	0.305	0.374	0.276	0.398	0.314	0.330	--
		Std Dev		0.726	0.172	0.190	0.157	0.134	0.151	0.088	--
	2	L23206	M	0.359	0.280	0.217 *	0.189	0.288 *	0.397	0.204	--
	2	L23210	M	0.537	0.379	0.388	--	--	0.342 *	--	--
	2	L23211	M	0.408	0.428	0.412	0.294	0.371	0.469 *	0.260	--
	2	L23215	M	0.355	0.379	0.227	0.268	0.313	0.272 *	0.230	--
	2	L23219	M	0.311	0.235	0.273	0.140 *	0.301	0.253 *	0.162	--
		Average		0.394	0.340	0.303	0.223	0.318	0.347	0.214	--
		Std Dev		0.087	0.080	0.091	0.071	0.037	0.089	0.042	--
	3	L23217	M	0.260	0.185	0.160	0.149	0.318	0.274	0.206	--
	3	L23227	M	0.335	0.212	0.228	0.210	0.251	0.265	0.245	--
	3	L23228	M	0.556	0.451	0.467	0.522	0.560	0.637	0.364	--
	3	L23229	M	0.275	0.220	0.268	0.195	0.308	0.262	0.195	--
	3	L23230	M	0.314	0.389	0.289	0.352	0.312	0.238 *	0.206	--
		Average		0.348	0.291	0.282	0.286	0.350	0.335	0.243	--
		Std Dev		0.120	0.120	0.114	0.152	0.121	0.169	0.070	--
	4	L23205	M	0.311	0.229	0.190	0.175	0.308	0.245	0.202	--
	4	L23207	M	0.408	0.349	0.408	0.313	0.370	0.380	0.213	--
	4	L23225	M	0.721	0.739	1.136	0.126	--	--	--	--
	4	L23231	M	0.441	0.428	0.469	0.485	0.580	0.311	0.229	--
	4	L23235	M	0.293	0.251	0.168	0.177	0.559	--	--	--
		Average		0.435	0.399	0.474	0.255	0.454	0.312	0.215	--
		Std Dev		0.172	0.206	0.393	0.146	0.136	0.068	0.014	--
	5	L23200	M	0.495	0.030	0.075	--	--	--	--	0.194
	5	L23201	M	0.428	0.395	0.559	0.597	--	--	--	--
	5	L23212	M	0.211	0.202	0.165	0.157	0.283 *	0.209	0.173	--
	5	L23214	M	0.850	0.607	3.000	--	--	--	--	--
	5	L23234	M	0.799	0.564	0.460	1.208	--	--	--	--
		Average		0.557	0.360	0.852	0.654	0.283	0.209	0.173	0.194
		Std Dev		0.267	0.244	1.217	0.528	--	--	--	--
	6	L23203	M	0.197	0.334	1.061	0.368	--	--	--	--
	6	L23204	M	0.544	0.533	1.171	0.664	--	--	--	--
	6	L23213	M	--	0.336	--	--	--	--	--	0.493
	6	L23221	M	0.547	--	--	--	--	--	--	--
	6	L23232	M	--	0.510	2.544	0.867	--	--	--	--
		Average		0.429	0.428	1.592	0.633	--	--	--	0.493
		Std Dev		0.201	0.108	0.826	0.251	--	--	--	--

U-22

Bold > Normal Range
Underscore < Normal Range
* Clot Removed
-- No Value

ATC 12·21·09

QA Audit Completed By/Date: 1/L 1/17/10

QC/Tech Rev. By/Date: ATC 12·21·09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
Neut%/Lymph% Ratio	1	L23216	M	0.354	0.091	0.235	0.243	0.556	0.382	0.296	--
	1	L23218	M	0.420	0.435	0.398	0.340	0.489	0.504 *	0.451	--
	1	L23220	M	1.990	0.520	0.691	0.498	0.409 *	0.205 *	0.383	--
	1	L23222	M	0.342	0.242	0.228 *	0.236	0.220	0.350	0.273	--
	1	L23223	M	0.343	0.239	0.319	0.071	0.317	0.125 *	0.242	--
		Average		0.690	0.305	0.374	0.278	0.398	0.313	0.329	--
		Std Dev		0.728	0.171	0.190	0.157	0.134	0.150	0.086	--
	2	L23206	M	0.359	0.281	0.216 *	0.190	0.289 *	0.397	0.203	--
	2	L23210	M	0.536	0.377	0.388	--	--	0.344 *	--	--
	2	L23211	M	0.409	0.427	0.413	0.294	0.372	0.467 *	0.260	--
	2	L23215	M	0.355	0.379	0.226	0.267	0.312	0.271 *	0.230	--
	2	L23219	M	0.312	0.235	0.273	0.139 *	0.302	0.254 *	0.163	--
		Average		0.394	0.340	0.303	0.223	0.319	0.347	0.214	--
		Std Dev		0.086	0.079	0.092	0.071	0.037	0.089	0.041	--
	3	L23217	M	0.259	0.186	0.161	0.148	0.317	0.274	0.206	--
	3	L23227	M	0.334	0.212	0.228	0.211	0.250	0.265	0.245	--
	3	L23228	M	0.557	0.451	0.467	0.522	0.560	0.638	0.364	--
	3	L23229	M	0.276	0.219	0.269	0.196	0.310	0.263	0.195	--
	3	L23230	M	0.315	0.388	0.290	0.351	0.311	0.239 *	0.206	--
		Average		0.348	0.291	0.283	0.286	0.350	0.336	0.243	--
		Std Dev		0.120	0.120	0.114	0.152	0.121	0.169	0.070	--
	4	L23205	M	0.311	0.229	0.190	0.176	0.308	0.244	0.203	--
	4	L23207	M	0.408	0.349	0.408	0.314	0.371	0.379	0.215	--
	4	L23225	M	0.722	0.739	1.133	0.125	--	--	--	--
	4	L23231	M	0.441	0.429	0.468	0.485	0.582	0.310	0.231	--
	4	L23235	M	0.293	0.252	0.168	0.178	0.559	--	--	--
		Average		0.435	0.400	0.473	0.256	0.455	0.311	0.216	--
		Std Dev		0.172	0.206	0.391	0.146	0.136	0.068	0.014	--
	5	L23200	M	0.495	0.031	0.076	--	--	--	--	0.193
	5	L23201	M	0.428	0.394	0.560	0.597	--	--	--	--
	5	L23212	M	0.210	0.203	0.166	0.157	0.283 *	0.209	0.174	--
	5	L23214	M	0.849	0.607	3.013	--	--	--	--	--
	5	L23234	M	0.799	0.565	0.461	1.209	--	--	--	--
		Average		0.556	0.360	0.855	0.654	0.283	0.209	0.174	0.193
		Std Dev		0.267	0.243	1.223	0.528	--	--	--	--
	6	L23203	M	0.198	0.334	1.060	0.366	--	--	--	--
	6	L23204	M	0.544	0.533	1.170	0.664	--	--	--	--
	6	L23213	M	--	0.335	--	--	--	--	--	0.492
	6	L23221	M	0.546	--	--	--	--	--	--	--
	6	L23232	M	--	0.509	2.538	0.874	--	--	--	--
		Average		0.429	0.428	1.589	0.635	--	--	--	0.492
		Std Dev		0.200	0.108	0.823	0.255	--	--	--	--

Bold > Normal Range
Underscore < Normal Range
* Clot Removed
-- No Value

PTC 12-21-09

QA Audit Complete
By/Date: MP 1/27/10

QC/Tech Rev. By/Date: PTC 12-21-09

APPENDIX V
INDIVIDUAL CLINICAL CHEMISTRY RESULTS

Clinical Chemistry

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
Blood Urea Nitrogen (5.0-30.0 mg/dL)	1	L23216	M	15.3	20.3	17.1	15.2	20.1	23.0	16.7	--
	1	L23218	M	15.6	16.8	17.2	14.7	14.0	14.3	15.5	--
	1	L23220	M	15.6	17.9	19.0	12.8	17.4	22.6	19.7	--
	1	L23222	M	25.1	25.4	23.4 *	19.8	23.4	24.1	21.6	--
	1	L23223	M	17.3	20.6	20.1	17.4	19.9	24.4	20.0	--
		Average		17.8	20.2	19.4	16.0	19.0	21.7	18.7	--
		Std Dev		4.2	3.3	2.6	2.7	3.5	4.2	2.5	--
	2	L23206	M	18.0	18.1	19.4	19.8	21.1	20.8	17.4	--
	2	L23210	M	13.8	19.1	19.8 *	17.5	--	24.3	20.8	--
	2	L23211	M	16.2	18.7	16.7	14.9	19.1	+	17.9	--
	2	L23215	M	12.5	16.0	12.7	11.1	17.0	+	12.8	--
	2	L23219	M	15.4	16.7	13.8	13.5	18.6	16.6	15.4	--
		Average		15.2	17.7	16.5	15.4	19.0	20.6	16.9	--
		Std Dev		2.1	1.3	3.2	3.4	1.7	3.9	3.0	--
	3	L23217	M	16.5	19.1	15.4	14.0	19.3	18.4	19.4	--
	3	L23227	M	22.1	22.9	17.9	13.9	22.0	22.1	17.1	--
	3	L23228	M	19.5	19.9	21.0	17.2	19.9	25.9	17.4	--
	3	L23229	M	15.9	18.1	16.4	16.3	17.2	18.8	15.3	--
	3	L23230	M	19.7 *	23.3	17.9	17.4	17.3	22.5	17.1	--
		Average		18.7	20.7	17.7	15.8	19.1	21.5	17.3	--
		Std Dev		2.5	2.3	2.1	1.7	2.0	3.1	1.5	--
	4	L23205	M	18.5	20.5	15.6	18.4	20.8	18.5	16.6	--
	4	L23207	M	29.5	26.4	24.0	22.8	28.2	**31.1**	21.4	--
	4	L23225	M	18.0	18.9	16.9	12.8	--	--	--	**66.9** ****
	4	L23231	M	18.8	20.5	17.1	15.9	20.5	24.3	20.4	--
	4	L23235	M	19.9	16.8	16.4	13.0	15.9	--	--	--
		Average		20.9	20.6	18.0	16.6	21.4	24.6	19.5	66.9
		Std Dev		4.8	3.6	3.4	4.2	5.1	6.3	2.5	--
	5	L23200	M	22.6	21.0	22.7	--	--	--	--	20.5
	5	L23201	M	27.2	25.0	21.9 *, ^^	**60.3** *, ^^	--	--	--	--
	5	L23212	M	17.5	18.4	17.6	16.6	18.0	20.8	17.0	--
	5	L23214	M	20.1	19.5	20.0 *	17.0 ^^	--	--	--	--
	5	L23234	M	14.7	15.6	13.5	13.2	--	--	--	--
		Average		20.4	19.9	19.1	26.8	18.0	20.8	17.0	20.5
		Std Dev		4.8	3.5	3.7	22.4	--	--	--	--
	6	L23203	M	17.5	17.1	12.9	12.3	--	--	--	--
	6	L23204	M	23.0	22.7	16.3 *	14.1 ****	--	--	--	**65.9** ****
	6	L23213	M	--	16.2	--	--	--	--	--	28.8 *
	6	L23221	M	20.8	--	--	--	--	--	--	--
	6	L23232	M	--	16.9	14.6	19.9	--	--	--	--
		Average		20.4	18.2	14.6	15.4	--	--	--	47.4
		Std Dev		2.8	3.0	1.7	4.0	--	--	--	26.2

V-2

Bold > Normal Range; Underscore < Normal Range
* Slight Hemolysis; **** Severe Hemolysis
^^ Moderate Lipemia
~~ Instrument unable to calculate result
+ Sample quantity insufficient
-- No Value

AMC 10-22-09

QA Audit Completed By/Date: Jm 5/10/10

QC/Tech Rev. By/Date: AMC 10-22-09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
Creatinine (0.5-3.0 mg/dL)	1	L23216	M	0.92	0.89	0.82	0.93	1.07	1.13	1.21	--
	1	L23218	M	0.93	0.76	0.75	0.97	1.03	1.07	0.99	--
	1	L23220	M	0.94	0.75	0.82	0.96	1.00	0.85	0.98	--
	1	L23222	M	1.19	1.07	0.93 *	1.04	1.24	1.10	1.25	--
	1	L23223	M	0.83	1.06	0.81	1.01	0.98	1.15	1.14	--
		Average		0.96	0.91	0.83	0.98	1.06	1.06	1.11	--
		Std Dev		0.13	0.16	0.07	0.04	0.10	0.12	0.12	--
	2	L23206	M	0.75	0.74	0.78	1.00	1.18	0.96	1.13	--
	2	L23210	M	0.92	1.11	0.88 *	1.09	--	0.99	1.13	--
	2	L23211	M	0.91	0.86	0.74	0.90	1.27	+	1.17	--
	2	L23215	M	0.71	0.66	0.66	0.68	0.88	+	0.87	--
	2	L23219	M	0.66	0.76	0.66	0.88	1.34	0.77	1.05	--
		Average		0.79	0.83	0.74	0.91	1.17	0.91	1.07	--
		Std Dev		0.12	0.17	0.09	0.15	0.20	0.12	0.12	--
	3	L23217	M	0.92	0.94	0.94	1.03	1.57	1.18	1.37	--
	3	L23227	M	1.24	0.73	0.72	0.73	0.89	0.89	1.05	--
	3	L23228	M	0.73	0.80	0.82	0.88	0.99	0.96	1.14	--
	3	L23229	M	0.76	0.81	0.84	0.96	0.89	1.01	1.19	--
	3	L23230	M	1.86 *	0.84	0.64	0.84	1.10	1.00	1.09	--
		Average		1.10	0.82	0.79	0.89	1.09	1.01	1.17	--
		Std Dev		0.47	0.08	0.12	0.11	0.28	0.11	0.12	--
	4	L23205	M	0.58	0.65	0.70	0.78	0.86	1.05	1.00	--
	4	L23207	M	1.13	0.74	0.90	1.17	1.02	1.06	1.20	--
	4	L23225	M	2.13	0.83	1.01	1.05	--	--	--	**4.92 ******
	4	L23231	M	0.83	0.78	0.77	0.77	0.99	1.13	1.23	--
	4	L23235	M	0.78	0.64	0.66	0.68	0.86	--	--	--
		Average		1.09	0.73	0.81	0.89	0.93	1.08	1.14	4.92
		Std Dev		0.61	0.08	0.15	0.21	0.08	0.04	0.13	--
	5	L23200	M	0.85	0.69	0.77	--	--	--	--	1.04
	5	L23201	M	0.96	0.70	1.18 *, ^^	1.92 *, ^^	--	--	--	--
	5	L23212	M	0.79	0.71	0.78	0.99	0.87	0.97	1.14	--
	5	L23214	M	0.90	0.71	0.95 *	1.29 ^^	--	--	--	--
	5	L23234	M	0.80	0.73	0.79	1.14	--	--	--	--
		Average		0.86	0.71	0.89	1.34	0.87	0.97	1.14	1.04
		Std Dev		0.07	0.01	0.18	0.41	--	--	--	--
	6	L23203	M	0.68	0.76	0.79	0.71	--	--	--	--
	6	L23204	M	0.77	0.65	1.06 *	1.06 ****	--	--	--	**5.01 ******
	6	L23213	M	--	0.68	--	--	--	--	--	1.65 *
	6	L23221	M	0.59	--	--	--	--	--	--	--
	6	L23232	M	--	0.86	0.93	1.17	--	--	--	--
		Average		0.68	0.74	0.93	0.98	--	--	--	3.33
		Std Dev		0.09	0.09	0.14	0.24	--	--	--	2.38

Bold > Normal Range; Underscore < Normal Range
* Slight Hemolysis; **** Severe Hemolysis
^^ Moderate Lipemia
~~ Instrument unable to calculate result
+ Sample quantity insufficient
-- No Value

PAC 10-22-09

QA Audit Completed
By/Date: JM 5/10/10

QC/Tech Rev. By/Date: PAC 10-22-09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
BUN/Creatinine Ratio	1	L23216	M	16.6	22.8	20.9	16.3	18.8	20.4	13.8	--
	1	L23218	M	16.8	22.1	22.9	15.2	13.6	13.4	15.7	--
	1	L23220	M	16.6	23.9	23.2	13.3	17.4	26.6	20.1	--
	1	L23222	M	21.1	23.7	25.2 *	19.0	18.9	21.9	17.3	--
	1	L23223	M	20.8	19.4	24.8	17.2	20.3	21.2	17.5	--
		Average		18.4	22.4	23.4	16.2	17.8	20.7	16.9	--
		Std Dev		2.3	1.8	1.7	2.1	2.6	4.7	2.3	--
	2	L23206	M	24.0	24.5	24.9	19.8	17.9	21.7	15.4	--
	2	L23210	M	15.0	17.2	22.5 *	16.1	--	24.5	18.4	--
	2	L23211	M	17.8	21.7	22.6	16.6	15.0	+	15.3	--
	2	L23215	M	17.6	24.2	19.2	16.3	19.3	+	14.7	--
	2	L23219	M	23.3	22.0	20.9	15.3	13.9	21.6	14.7	--
		Average		19.5	21.9	22.0	16.8	16.5	22.6	15.7	--
		Std Dev		3.9	2.9	2.1	1.7	2.5	1.6	1.5	--
	3	L23217	M	17.9	20.3	16.4	13.6	12.3	15.6	14.2	--
	3	L23227	M	17.8	31.4	24.9	19.0	24.7	24.8	16.3	--
	3	L23228	M	26.7	24.9	25.6	19.5	20.1	27.0	15.3	--
	3	L23229	M	20.9	22.3	19.5	17.0	19.3	18.6	12.9	--
	3	L23230	M	10.6 *	27.7	28.0	20.7	15.7	22.5	15.7	--
		Average		18.8	25.3	22.9	18.0	18.4	21.7	14.9	--
		Std Dev		5.8	4.4	4.8	2.8	4.7	4.6	1.3	--
	4	L23205	M	31.9	31.5	22.3	23.6	24.2	17.6	16.6	--
	4	L23207	M	26.1	35.7	26.7	19.5	27.6	29.3	17.8	--
	4	L23225	M	8.5	22.8	16.7	12.2	--	--	--	13.6 ****
	4	L23231	M	22.7	26.3	22.2	20.6	20.7	21.5	16.6	--
	4	L23235	M	25.5	26.3	24.8	19.1	18.5	--	--	--
		Average		22.9	28.5	22.5	19.0	22.8	22.8	17.0	13.6
		Std Dev		8.7	5.1	3.8	4.2	4.0	6.0	0.7	--
	5	L23200	M	26.6	30.4	29.5	--	--	--	--	19.7
	5	L23201	M	28.3	35.7	18.6 *, ^^	31.4 *, ^^	--	--	--	--
	5	L23212	M	22.2	25.9	22.6	16.8	20.7	21.4	14.9	--
	5	L23214	M	22.3	27.5	21.1 *	13.2 ^^	--	--	--	--
	5	L23234	M	18.4	21.4	17.1	11.6	--	--	--	--
		Average		23.6	28.2	21.8	18.3	20.7	21.4	14.9	19.7
		Std Dev		3.9	5.3	4.8	9.0	--	--	--	--
	6	L23203	M	25.7	22.5	16.3	17.3	--	--	--	--
	6	L23204	M	29.9	34.9	15.4 *	13.3 ****	--	--	--	13.2 ****
	6	L23213	M	--	23.8	--	--	--	--	--	17.5 *
	6	L23221	M	35.3	--	--	--	--	--	--	--
	6	L23232	M	--	19.7	15.7	17.0	--	--	--	--
		Average		30.3	25.2	15.8	15.9	--	--	--	15.4
		Std Dev		4.8	6.7	0.5	2.2	--	--	--	3.0

Bold > Normal Range; Underscore < Normal Range
* Slight Hemolysis; **** Severe Hemolysis
^^ Moderate Lipemia
-- Instrument unable to calculate result
+ Sample quantity insufficient
-- No Value

AHC 10-22-09

QA Audit Completed By/Date: QM 5/10/10

QC/Tech Rev. By/Date: AHC 10-22-09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
Sodium (125-160 mEq/L)	1	L23216	M	143	145	146	141	147	145	141	--
	1	L23218	M	143	145	146	148	148	146	142	--
	1	L23220	M	146	145	145	145	144	143	140	--
	1	L23222	M	143	145	144 *	143	142	143	140	--
	1	L23223	M	145	147	145	144	144	146	141	--
		Average		144	145	145	144	145	145	141	--
		Std Dev		1	1	1	3	2	2	1	--
	2	L23206	M	143	145	145	147	153	146	143	--
	2	L23210	M	142	147	144 *	145	--	143	141	--
	2	L23211	M	146	148	147	146	151	147	142	--
	2	L23215	M	143	145	144	144	145	+	142	--
	2	L23219	M	145	146	147	147	155	143	140	--
		Average		144	146	145	146	151	145	142	--
		Std Dev		2	1	2	1	4	2	1	--
	3	L23217	M	141	144	145	145	148	143	142	--
	3	L23227	M	135	148	146	146	149	145	141	--
	3	L23228	M	146	145	145	146	148	146	142	--
	3	L23229	M	142	144	145	147	143	146	142	--
	3	L23230	M	126 *	144	147	144	151	144	141	--
		Average		138	145	146	146	148	145	142	--
		Std Dev		8	2	1	1	3	1	1	--
	4	L23205	M	143	147	146	150	146	146	143	--
	4	L23207	M	146	147	150	156	147	146	138	--
	4	L23225	M	127	146	144	142	--	--	--	139 ****
	4	L23231	M	141	145	146	147	146	146	142	--
	4	L23235	M	144	146	146	147	145	--	--	--
		Average		140	146	146	148	146	146	141	139
		Std Dev		8	1	2	5	1	0	3	--
	5	L23200	M	145	144	146	--	--	--	--	143
	5	L23201	M	150	146	146 *, ^^	145 *, ^^	--	--	--	--
	5	L23212	M	144	145	144	145	143	145	141	--
	5	L23214	M	146	147	143 *	145 ^^	--	--	--	--
	5	L23234	M	146	146	146	144	--	--	--	--
		Average		146	146	145	145	143	145	141	143
		Std Dev		2	1	1	1	--	--	--	--
	6	L23203	M	144	147	145	140	--	--	--	--
	6	L23204	M	145	146	140 *	142 ****	--	--	--	121 ****
	6	L23213	M	--	145	--	--	--	--	--	150 *
	6	L23221	M	143	--	--	--	--	--	--	--
	6	L23232	M	--	146	145	142	--	--	--	--
		Average		144	146	143	141	--	--	--	136
		Std Dev		1	1	3	1	--	--	--	21

Bold > Normal Range; Underscore < Normal Range
* Slight Hemolysis; **** Severe Hemolysis
^^ Moderate Lipemia
~~ Instrument unable to calculate result
+ Sample quantity insufficient
-- No Value

ATC 10-22-09

QA Audit Completed
By/Date: JM 5/10/10

QC/Tech Rev. By/Date: ATC 10-22-09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
Potassium (3.0-7.0 mEq/L)	1	L23216	M	3.7	4.8	4.3	4.3	5.6	5.5	3.7	--
	1	L23218	M	4.0	4.2	4.4	4.8	5.9	5.4	3.5	--
	1	L23220	M	3.9	4.7	4.7	4.6	5.2	5.5	3.5	--
	1	L23222	M	5.8	4.9	5.0 *	5.7	4.8	5.4	3.6	--
	1	L23223	M	4.5	5.9	5.2	4.9	5.0	5.6	4.0	--
		Average		4.4	4.9	4.7	4.9	5.3	5.5	3.7	--
		Std Dev		0.8	0.6	0.4	0.5	0.4	0.1	0.2	--
	2	L23206	M	4.7	5.2	5.7	6.0	4.8	6.3	3.8	--
	2	L23210	M	4.7	6.3	5.2 *	5.3	--	6.0	3.9	--
	2	L23211	M	4.7	5.4	4.2	5.0	5.4	7.0	3.9	--
	2	L23215	M	4.2	4.7	4.9	4.6	5.7	+	3.4	--
	2	L23219	M	4.4	4.5	5.6	5.5	5.0	5.0	3.9	--
		Average		4.5	5.2	5.1	5.3	5.2	6.1	3.8	--
		Std Dev		0.2	0.7	0.6	0.5	0.4	0.8	0.2	--
	3	L23217	M	4.2	4.6	4.5	4.3	4.1	5.0	3.8	--
	3	L23227	M	4.4	4.5	4.4	4.2	6.6	5.6	3.6	--
	3	L23228	M	4.3	4.8	5.2	4.3	6.6	5.7	3.9	--
	3	L23229	M	4.0	4.5	4.7	4.6	4.7	4.9	3.8	--
	3	L23230	M	3.9 *	4.2	4.2	4.7	4.3	5.6	3.9	--
		Average		4.2	4.5	4.6	4.4	5.3	5.4	3.8	--
		Std Dev		0.2	0.2	0.4	0.2	1.2	0.4	0.1	--
	4	L23205	M	4.3	4.8	4.6	4.9	5.2	5.6	3.7	--
	4	L23207	M	5.6	5.0	5.5	6.9	6.3	6.3	3.9	--
	4	L23225	M	5.0	4.8	4.3	5.0	--	--	--	**21.2 ******
	4	L23231	M	4.2	4.7	4.7	4.4	6.5	6.0	4.0	--
	4	L23235	M	4.7	4.1	4.5	4.3	5.2	--	--	--
		Average		4.8	4.7	4.7	5.1	5.8	6.0	3.9	21.2
		Std Dev		0.6	0.3	0.5	1.1	0.7	0.4	0.2	--
	5	L23200	M	5.1	4.8	5.1	--	--	--	--	**11.4**
	5	L23201	M	6.5	4.5	3.7 *, ^^	3.7 *, ^^	--	--	--	--
	5	L23212	M	4.2	4.3	5.0	4.7	4.8	4.9	3.9	--
	5	L23214	M	4.7	5.5	3.8 *	3.7 ^^	--	--	--	--
	5	L23234	M	3.8	4.2	4.4	3.9	--	--	--	--
		Average		4.9	4.7	4.4	4.0	4.8	4.9	3.9	11.4
		Std Dev		1.0	0.5	0.7	0.5	--	--	--	--
	6	L23203	M	5.0	5.2	3.5	3.8	--	--	--	--
	6	L23204	M	4.9	4.5	3.8 *	5.2 ****	--	--	--	**29.3 ******
	6	L23213	M	--	4.6	--	--	--	--	--	**17.3 ***
	6	L23221	M	5.3	--	--	--	--	--	--	--
	6	L23232	M	--	5.1	3.9	4.5	--	--	--	--
		Average		5.1	4.9	3.7	4.5	--	--	--	23.3
		Std Dev		0.2	0.4	0.2	0.7	--	--	--	8.5

V-6

Bold > Normal Range; Underscore < Normal Range
* Slight Hemolysis; **** Severe Hemolysis
^^ Moderate Lipemia
~~ Instrument unable to calculate result
+ Sample quantity insufficient
-- No Value

QA Audit Completed By/Date: JM 5/10/10

AC 10-22-09

QC/Tech Rev. By/Date: AC 10-22-09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
Chloride (92-120 mEq/L)	1	L23216	M	104	106	108	98	107	106	103	--
	1	L23218	M	101	108	109	101	105	106	102	--
	1	L23220	M	102	107	105	102	102	105	105	--
	1	L23222	M	105	107	109 *	105	103	108	102	--
	1	L23223	M	102	105	107	102	104	105	102	--
		Average		103	107	108	102	104	106	103	--
		Std Dev		2	1	2	3	2	1	1	--
	2	L23206	M	106	108	108	106	114	108	105	--
	2	L23210	M	105	105	106 *	103	--	106	102	--
	2	L23211	M	109	107	107	104	111	106	105	--
	2	L23215	M	107	107	106	106	104	+	109	--
	2	L23219	M	108	105	108	104	112	106	103	--
		Average		107	106	107	105	110	107	105	--
		Std Dev		2	1	1	1	4	1	3	--
	3	L23217	M	108	107	108	107	104	109	106	--
	3	L23227	M	101	109	106	108	109	109	104	--
	3	L23228	M	107	107	107	104	107	108	103	--
	3	L23229	M	107	104	107	106	107	107	106	--
	3	L23230	M	92 *	108	109	106	114	107	107	--
		Average		103	107	107	106	108	108	105	--
		Std Dev		7	2	1	1	4	1	2	--
	4	L23205	M	109	107	108	108	107	108	105	--
	4	L23207	M	104	107	105	106	110	111	104	--
	4	L23225	M	92	105	103	102	--	--	--	86 ****
	4	L23231	M	102	105	106	106	106	108	103	--
	4	L23235	M	107	106	104	109	106	--	--	--
		Average		103	106	105	106	107	109	104	86
		Std Dev		7	1	2	3	2	2	1	--
	5	L23200	M	105	101	102	--	--	--	--	95
	5	L23201	M	111	107	105 *, ^^	102 *, ^^	--	--	--	--
	5	L23212	M	104	103	105	102	106	108	103	--
	5	L23214	M	105	106	104 *	99 ^^	--	--	--	--
	5	L23234	M	107	105	106	103	--	--	--	--
		Average		106	104	104	102	106	108	103	95
		Std Dev		3	2	2	2	--	--	--	--
	6	L23203	M	111	107	106	103	--	--	--	--
	6	L23204	M	111	106	103 *	101 ****	--	--	--	77 ****
	6	L23213	M	--	107	--	--	--	--	--	94 *
	6	L23221	M	104	--	--	--	--	--	--	--
	6	L23232	M	--	104	105	103	--	--	--	--
		Average		109	106	105	102	--	--	--	86
		Std Dev		4	1	2	1	--	--	--	12

Bold > Normal Range; Underscore < Normal Range
* Slight Hemolysis; **** Severe Hemolysis
^^ Moderate Lipemia
~~ Instrument unable to calculate result
+ Sample quantity insufficient
-- No Value

V-7

QA Audit Complete
Init/Date: Jm 5/10/10

AC 10.22.09

QC/Tech Rev. By/Date: AC 10.22.09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
Calcium (5.50-16.50 mg/dL)	1	L23216	M	12.44	14.43	14.25	13.33	15.13	15.78	12.71	--
	1	L23218	M	13.26	13.26	13.08	14.61	15.21	14.52	12.39	--
	1	L23220	M	13.44	13.68	13.73	13.20	14.23	14.67	11.95	--
	1	L23222	M	15.12	14.74	14.64 *	14.58	14.20	15.19	12.69	--
	1	L23223	M	13.98	14.91	14.66	14.32	14.84	15.37	12.68	--
		Average		13.65	14.20	14.07	14.01	14.72	15.11	12.48	--
		Std Dev		0.99	0.71	0.67	0.69	0.48	0.52	0.33	--
	2	L23206	M	13.08	14.03	14.48	15.20	14.06	15.38	12.67	--
	2	L23210	M	11.97	15.84	14.93 *	14.65	--	15.37	13.05	--
	2	L23211	M	12.62	14.39	13.67	13.98	13.84	15.54	12.78	--
	2	L23215	M	13.14	14.46	13.64	13.51	15.30	+	12.26	--
	2	L23219	M	12.01	13.97	13.81	14.67	14.13	13.78	12.39	--
		Average		12.56	14.54	14.11	14.40	14.33	15.02	12.63	--
		Std Dev		0.56	0.76	0.57	0.66	0.66	0.83	0.31	--
	3	L23217	M	12.02	13.37	13.19	12.59	13.06	13.99	12.39	--
	3	L23227	M	13.61	13.46	13.92	12.82	14.98	14.24	12.65	--
	3	L23228	M	12.30	14.34	14.41	14.14	15.95	16.27	12.47	--
	3	L23229	M	13.25	14.75	15.01	14.35	14.27	14.88	12.56	--
	3	L23230	M	12.43 *	13.29	12.77	13.85	14.07	15.19	12.20	--
		Average		12.72	13.84	13.86	13.55	14.47	14.91	12.45	--
		Std Dev		0.68	0.66	0.90	0.80	1.08	0.90	0.17	--
	4	L23205	M	13.06	14.19	14.08	14.44	14.63	14.51	12.60	--
	4	L23207	M	14.57	14.31	15.57	**16.93**	15.22	15.97	12.01	--
	4	L23225	M	13.05	14.23	12.27	12.17	--	--	--	16.09 ****
	4	L23231	M	13.27	14.86	14.23	13.68	15.43	16.05	13.13	--
	4	L23235	M	14.01	14.16	14.03	13.47	14.16	--	--	--
		Average		13.59	14.35	14.04	14.14	14.86	15.51	12.58	16.09
		Std Dev		0.67	0.29	1.17	1.76	0.58	0.87	0.56	--
	5	L23200	M	14.30	13.98	14.70	--	--	--	--	15.59
	5	L23201	M	15.06	14.39	9.90 *, ^^	6.45 *, ^^	--	--	--	--
	5	L23212	M	13.12	14.01	14.19	13.99	13.72	14.48	12.59	--
	5	L23214	M	14.07	14.77	12.09 *	10.79 ^^	--	--	--	--
	5	L23234	M	13.32	13.95	14.26	11.89	--	--	--	--
		Average		13.97	14.22	13.03	10.78	13.72	14.48	12.59	15.59
		Std Dev		0.78	0.36	2.02	3.18	--	--	--	--
	6	L23203	M	12.86	13.09	10.56	11.00	--	--	--	--
	6	L23204	M	13.54	14.22	11.97 *	11.27 ****	--	--	--	13.09 ****
	6	L23213	M	--	13.82	--	--	--	--	--	12.32 *
	6	L23221	M	14.01	--	--	--	--	--	--	--
	6	L23232	M	--	14.45	11.91	11.80	--	--	--	--
		Average		13.47	13.90	11.48	11.36	--	--	--	12.71
		Std Dev		0.58	0.60	0.80	0.41	--	--	--	0.54

V-8

Bold > Normal Range; Underscore < Normal Range
* Slight Hemolysis; **** Severe Hemolysis
^^ Moderate Lipemia
~~ Instrument unable to calculate result
+ Sample quantity insufficient
-- No Value

QA Audit Completed By/Date: Jm 5/10/10

Atc 10-22-09

QC/Tech Rev. By/Date: Atc 10-22-09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
Phosphorus (2.0-9.0 mg/dL)	1	L23216	M	4.34	3.73	3.09	4.75	5.90	4.93	5.74	--
	1	L23218	M	4.41	3.38	3.45	4.93	5.12	3.91	4.94	--
	1	L23220	M	4.59	3.20	4.34	5.46	6.11	4.94	5.31	--
	1	L23222	M	4.16	3.71	3.34 *	4.19	4.17	3.79	5.79	--
	1	L23223	M	4.41	5.33	4.57	5.29	5.24	5.68	6.26	--
		Average		4.38	3.87	3.76	4.92	5.31	4.65	5.61	--
		Std Dev		0.15	0.85	0.65	0.50	0.76	0.79	0.50	--
	2	L23206	M	4.01	4.12	4.45	5.34	5.57	5.14	6.83	--
	2	L23210	M	2.16	3.65	3.43 *	4.51	--	4.56	5.43	--
	2	L23211	M	3.16	3.14	2.98	3.67	5.91	+	5.76	--
	2	L23215	M	3.18	3.27	3.69	3.89	4.81	+	5.09	--
	2	L23219	M	3.29	3.53	3.38	4.26	6.08	4.33	5.84	--
		Average		3.16	3.54	3.59	4.33	5.59	4.68	5.79	--
		Std Dev		0.66	0.38	0.55	0.65	0.56	0.42	0.65	--
	3	L23217	M	3.13	3.43	3.60	3.71	5.38	4.71	5.43	--
	3	L23227	M	3.05	3.76	3.33	3.58	4.84	4.64	5.88	--
	3	L23228	M	2.73	3.14	3.09	4.11	4.62	4.26	6.12	--
	3	L23229	M	2.67	3.13	3.15	3.88	3.78	3.72	5.70	--
	3	L23230	M	3.37 *	3.68	3.07	3.97	5.79	4.38	6.05	--
		Average		2.99	3.43	3.25	3.85	4.88	4.34	5.84	--
		Std Dev		0.29	0.29	0.22	0.21	0.77	0.39	0.28	--
	4	L23205	M	3.17	3.21	2.96	3.38	4.01	4.95	5.74	--
	4	L23207	M	3.37	3.33	3.95	4.54	4.10	4.39	5.59	--
	4	L23225	M	3.44	3.86	4.32	4.77	--	--	--	**16.39 ******
	4	L23231	M	3.28	3.37	2.95	3.03	4.52	4.10	5.38	--
	4	L23235	M	2.74	2.45	2.47	2.67	2.86	--	--	--
		Average		3.20	3.24	3.33	3.68	3.87	4.48	5.57	16.39
		Std Dev		0.28	0.51	0.77	0.93	0.71	0.43	0.18	--
	5	L23200	M	3.34	3.63	3.46	--	--	--	--	6.38
	5	L23201	M	3.48	2.97	4.40 *, ^^	7.35 *, ^^	--	--	--	--
	5	L23212	M	3.17	3.29	3.51	3.69	4.13	4.26	5.36	--
	5	L23214	M	3.51	3.54	4.48 *	4.87 ^^	--	--	--	--
	5	L23234	M	2.34	2.60	2.82	4.10	--	--	--	--
		Average		3.17	3.21	3.73	5.00	4.13	4.26	5.36	6.38
		Std Dev		0.48	0.42	0.70	1.64	--	--	--	--
	6	L23203	M	3.61	3.95	5.28	3.99	--	--	--	--
	6	L23204	M	3.17	3.32	3.67 *	4.17 ****	--	--	--	**17.55 ******
	6	L23213	M	--	3.31	--	--	--	--	--	**19.69 ***
	6	L23221	M	3.24	--	--	--	--	--	--	--
	6	L23232	M	--	4.23	4.91	5.26	--	--	--	--
		Average		3.34	3.70	4.62	4.47	--	--	--	18.62
		Std Dev		0.24	0.46	0.84	0.69	--	--	--	1.51

V-9

Bold > Normal Range; Underscore < Normal Range
* Slight Hemolysis; **** Severe Hemolysis
^^ Moderate Lipemia
~~ Instrument unable to calculate result
+ Sample quantity insufficient
-- No Value

MC 10.22.09

QA Audit Completed
By/Date: Dm 5/10/10

QC/Tech Rev. By/Date: MC 10.22.09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
Total Protein (3.5-8.5 g/dL)	1	L23216	M	5.90	6.29	6.09	6.20	6.77	6.62	5.68	--
	1	L23218	M	5.84	5.96	5.75	6.58	6.44	6.21	5.45	--
	1	L23220	M	5.69	5.54	5.52	5.76	5.97	5.82	5.23	--
	1	L23222	M	5.94	5.90	5.83 *	5.82	5.99	5.98	5.21	--
	1	L23223	M	5.79	6.34	6.12	6.20	6.10	6.34	5.40	--
		Average		5.83	6.01	5.86	6.11	6.25	6.19	5.39	--
		Std Dev		0.10	0.33	0.25	0.33	0.34	0.31	0.19	--
	2	L23206	M	5.59	6.14	6.19	6.75	6.75	6.57	5.72	--
	2	L23210	M	5.31	6.14	6.10 *	6.27	--	6.03	5.50	--
	2	L23211	M	5.47	6.42	5.89	6.23	6.68	+	5.39	--
	2	L23215	M	5.61	6.02	5.78	6.09	6.28	+	5.70	--
	2	L23219	M	5.34	6.18	6.20	6.67	6.98	6.15	5.37	--
		Average		5.46	6.18	6.03	6.40	6.67	6.25	5.54	--
		Std Dev		0.14	0.15	0.19	0.29	0.29	0.28	0.17	--
	3	L23217	M	5.00	5.68	5.59	5.63	5.89	5.94	5.24	--
	3	L23227	M	5.39	5.58	5.51	5.13	5.91	5.65	5.22	--
	3	L23228	M	5.00	5.83	5.89	5.84	6.37	6.29	5.26	--
	3	L23229	M	5.40	6.08	5.93	5.91	5.59	6.30	5.40	--
	3	L23230	M	5.42 *	5.45	5.33	5.86	6.55	6.13	5.50	--
		Average		5.24	5.72	5.65	5.67	6.06	6.06	5.32	--
		Std Dev		0.22	0.24	0.26	0.32	0.39	0.27	0.12	--
	4	L23205	M	5.35	5.73	5.48	5.91	5.98	5.98	5.25	--
	4	L23207	M	6.26	6.22	6.71	7.14	6.23	6.45	5.45	--
	4	L23225	M	5.66	6.04	5.90	5.23	--	--	--	4.30 ****
	4	L23231	M	5.42	6.10	5.66	5.80	6.53	6.56	5.21	--
	4	L23235	M	5.82	6.03	5.92	5.69	6.35	--	--	--
		Average		5.70	6.02	5.93	5.95	6.27	6.33	5.30	4.30
		Std Dev		0.36	0.18	0.47	0.71	0.23	0.31	0.13	--
	5	L23200	M	5.83	5.98	6.20	--	--	--	--	5.96
	5	L23201	M	6.69	6.18	5.97 *, ^^	5.09 *, ^^	--	--	--	--
	5	L23212	M	5.57	5.90	6.14	5.87	6.08	6.21	5.52	--
	5	L23214	M	5.90	6.37	5.66 *	5.91 ^^	--	--	--	--
	5	L23234	M	5.55	5.53	5.55	4.92	--	--	--	--
		Average		5.91	5.99	5.90	5.45	6.08	6.21	5.52	5.96
		Std Dev		0.46	0.32	0.29	0.52	--	--	--	--
	6	L23203	M	5.07	5.28	5.34	4.84	--	--	--	--
	6	L23204	M	5.50	5.87	5.84 *	5.29 ****	--	--	--	4.42 ****
	6	L23213	M	--	5.66	--	--	--	--	--	5.79 *
	6	L23221	M	5.82	--	--	--	--	--	--	--
	6	L23232	M	--	5.82	6.07	6.00	--	--	--	--
		Average		5.46	5.66	5.75	5.38	--	--	--	5.11
		Std Dev		0.38	0.27	0.37	0.58	--	--	--	0.97

V-10

Bold > Normal Range; Underscore < Normal Range
* Slight Hemolysis; **** Severe Hemolysis
^^ Moderate Lipemia
-- Instrument unable to calculate result
+ Sample quantity insufficient
-- No Value

QA Audit Completed By/Date: Jm 5/10/10

AtC 10-22-09

QC/Tech Rev. By/Date: AtC 10-22-09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
Total Bilirubin (0.0-1.0 mg/dL)	1	L23216	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	--
	1	L23218	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	--
	1	L23220	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	--
	1	L23222	M	0.0	0.0	0.0 *	0.0	0.0	0.0	0.0	--
	1	L23223	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	--
		Average		0.0	0.0	0.0	0.0	0.0	0.0	0.0	--
		Std Dev		0.0	0.0	0.0	0.0	0.0	0.0	0.0	--
	2	L23206	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	--
	2	L23210	M	0.0	0.0	0.0 *	0.0	--	0.0	0.0	--
	2	L23211	M	0.0	0.0	0.0	0.0	0.0	+	0.0	--
	2	L23215	M	0.0	0.0	0.0	0.0	0.0	+	0.0	--
	2	L23219	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	--
		Average		0.0	0.0	0.0	0.0	0.0	0.0	0.0	--
		Std Dev		0.0	0.0	0.0	0.0	0.0	0.0	0.0	--
	3	L23217	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	--
	3	L23227	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	--
	3	L23228	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	--
	3	L23229	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	--
	3	L23230	M	0.0 *	0.0	0.0	0.0	0.0	0.0	0.0	--
		Average		0.0	0.0	0.0	0.0	0.0	0.0	0.0	--
		Std Dev		0.0	0.0	0.0	0.0	0.0	0.0	0.0	--
	4	L23205	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	--
	4	L23207	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	--
	4	L23225	M	0.0	0.0	0.0	0.0	--	--	--	. ****, ~~
	4	L23231	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	--
	4	L23235	M	0.0	0.0	0.0	0.0	0.0	--	--	--
		Average		0.0	0.0	0.0	0.0	0.0	0.0	0.0	--
		Std Dev		0.0	0.0	0.0	0.0	0.0	0.0	0.0	--
	5	L23200	M	0.0	0.0	0.0	--	--	--	--	0.0
	5	L23201	M	0.0	0.0	0.0 *, ^^	0.0 *, ^^	--	--	--	--
	5	L23212	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	--
	5	L23214	M	0.0	0.0	0.0 *	0.0 ^^	--	--	--	--
	5	L23234	M	0.0	0.0	0.0	0.0	--	--	--	--
		Average		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Std Dev		0.0	0.0	0.0	0.0	--	--	--	--
	6	L23203	M	0.0	0.0	0.0	0.0	--	--	--	--
	6	L23204	M	0.0	0.0	0.0 *	0.0 ****	--	--	--	. ****, ~~
	6	L23213	M	--	0.0	--	--	--	--	--	0.0 *
	6	L23221	M	0.0	--	--	--	--	--	--	--
	6	L23232	M	--	0.0	0.0	0.0	--	--	--	--
		Average		0.0	0.0	0.0	0.0	--	--	--	0.0
		Std Dev		0.0	0.0	0.0	0.0	--	--	--	--

Bold > Normal Range; Underscore < Normal Range
* Slight Hemolysis; **** Severe Hemolysis
^^ Moderate Lipemia
~~ Instrument unable to calculate result
+ Sample quantity insufficient
-- No Value

ATC 12-10-09

QA Audit Completed
By/Date: Jm 5/10/10

QC/Tech Rev. By/Date: ATC 12-10-09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
Aspartate Aminotransferase (0.0-120.0 U/L)	1	L23216	M	18.5	20.0	20.4	18.7	15.2	53.2	27.2	--
	1	L23218	M	18.1	16.7	15.0	16.2	24.0	21.1	30.2	--
	1	L23220	M	42.5	15.6	27.0	25.1	25.6	23.2	28.9	--
	1	L23222	M	19.0	19.4	17.8 *	19.2	26.4	22.4	15.3	--
	1	L23223	M	29.8	25.1	21.1	21.9	19.4	32.5	23.3	--
		Average		25.6	19.4	20.3	20.2	22.1	30.5	25.0	--
		Std Dev		10.6	3.7	4.5	3.4	4.7	13.5	6.0	--
	2	L23206	M	15.8	17.2	16.8	18.9	18.3	16.4	19.7	--
	2	L23210	M	12.0	15.9	14.1 *	17.9	--	19.8	13.9	--
	2	L23211	M	10.8	13.7	13.7	15.3	15.5	+	18.8	--
	2	L23215	M	11.7	13.0	13.0	14.9	15.3	+	16.5	--
	2	L23219	M	16.3	19.3	16.9	21.1	26.2	18.2	20.2	--
		Average		13.3	15.8	14.9	17.6	18.8	18.1	17.8	--
		Std Dev		2.5	2.6	1.8	2.6	5.1	1.7	2.6	--
	3	L23217	M	11.1	16.2	14.2	13.2	17.3	16.6	14.0	--
	3	L23227	M	13.1	16.7	13.8	18.1	18.0	50.1	21.9	--
	3	L23228	M	12.7	13.2	22.1	12.2	13.2	**146.8**	13.7	--
	3	L23229	M	15.3	16.7	15.8	16.3	21.4	18.8	22.9	--
	3	L23230	M	10.2 *	10.1	13.4	8.1	12.8	12.1	13.2	--
		Average		12.5	14.6	15.9	13.6	16.5	48.9	17.1	--
		Std Dev		2.0	2.9	3.6	3.9	3.6	56.8	4.8	--
	4	L23205	M	12.1	13.7	10.7	21.6	19.5	16.7	23.9	--
	4	L23207	M	13.5	19.2	22.3	20.6	25.1	17.1	14.1	--
	4	L23225	M	15.7	23.1	72.4	20.7	--	--	--	**944.0 ******
	4	L23231	M	18.3	13.0	12.2	24.4	29.7	23.9	22.5	--
	4	L23235	M	11.2	12.0	11.6	15.0	19.0	--	--	--
		Average		14.2	16.2	25.8	20.5	23.3	19.2	20.2	944.0
		Std Dev		2.9	4.8	26.5	3.4	5.1	4.0	5.3	--
	5	L23200	M	16.8	15.8	15.0	--	--	--	--	**121.2**
	5	L23201	M	28.8	25.4	**701.8 *, ^^**	**1292.8 *, ^^**	--	--	--	--
	5	L23212	M	13.9	15.9	15.1	21.5	23.7	20.5	21.8	--
	5	L23214	M	11.2	14.0	22.6 *	**394.6 ^^**	--	--	--	--
	5	L23234	M	15.4	28.8	17.1	**551**	--	--	--	--
		Average		17.2	20.0	154.3	565.0	23.7	20.5	21.8	121.2
		Std Dev		6.8	6.7	306.1	533.6	--	--	--	--
	6	L23203	M	13.8	12.6	83.9	18.3	--	--	--	--
	6	L23204	M	10.0	14.8	44.0 *	27.7 ****	--	--	--	**416.8 ******
	6	L23213	M	--	17.1	--	--	--	--	--	**218.0 ***
	6	L23221	M	11.3	--	--	--	--	--	--	--
	6	L23232	M	--	16.1	80.7	21.1	--	--	--	--
		Average		11.7	15.2	69.5	22.4	--	--	--	317.4
		Std Dev		1.9	1.9	22.2	4.8	--	--	--	140.6

Bold > Normal Range; Underscore < Normal Range
* Slight Hemolysis; **** Severe Hemolysis
^^ Moderate Lipemia
-- Instrument unable to calculate result
+ Sample quantity insufficient
-- No Value

Atc 10-22-09

QA Audit Complete
ew/Date: Jkn 5/10/10

QC/Tech Rev. By/Date: Atc 10-22-09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
Alanine Aminotransferase (7.5-85.0 U/L)	1	L23216	M	36.2	44.9	44.7	44.1	42.6	64.3	49.2	--
	1	L23218	M	19.4	20.3	22.5	25.2	25.5	24.1	30.7	--
	1	L23220	M	37.8	37.8	41.2	40.6	33.1	33.8	33.9	--
	1	L23222	M	33.6	36.9	38.8 *	39.0	43.3	40.1	32.1	--
	1	L23223	M	59.6	43.5	44.9	45.1	45.6	54.5	45.8	--
		Average		37.3	36.7	38.4	38.8	38.0	43.4	38.3	--
		Std Dev		14.4	9.8	9.3	8.0	8.5	16.1	8.5	--
	2	L23206	M	24.5	28.4	28.8	30.8	35.5	32.6	26.9	--
	2	L23210	M	16.1	19.8	20.6 *	20.8	--	26.0	21.1	--
	2	L23211	M	19.2	28.5	25.6	27.8	26.8	+	22.3	--
	2	L23215	M	32.3	37.5	33.6	37.8	39.8	+	36.9	--
	2	L23219	M	21.4	30.0	30.6	35.4	37.2	28.1	26.2	--
		Average		22.7	28.8	27.8	30.5	34.8	28.9	26.7	--
		Std Dev		6.2	6.3	5.0	6.7	5.6	3.4	6.2	--
	3	L23217	M	26.9	34.1	30.5	32.0	34.8	43.1	33.8	--
	3	L23227	M	26.9	29.0	30.3	31.1	31.2	58.3	30.0	--
	3	L23228	M	19.6	36.4	38.0	30.7	35.0	53.8	25.7	--
	3	L23229	M	19.2	23.7	23.0	22.7	26.0	25.1	21.9	--
	3	L23230	M	20.5 *	24.7	24.1	24.6	26.3	27.4	22.2	--
		Average		22.6	29.6	29.2	28.2	30.7	41.5	26.7	--
		Std Dev		3.9	5.6	6.0	4.3	4.4	15.0	5.1	--
	4	L23205	M	15.5	19.0	18.3	24.4	22.1	19.4	20.6	--
	4	L23207	M	29.9	34.0	40.2	41.1	38.8	32.0	26.4	--
	4	L23225	M	21.1	36.2	51.0	37.3	--	--	--	**143.8 ******
	4	L23231	M	22.0	25.0	23.1	28.5	36.1	30.0	20.5	--
	4	L23235	M	21.6	24.9	22.8	24.0	31.6	--	--	--
		Average		22.0	27.8	31.1	31.1	32.2	27.1	22.5	143.8
		Std Dev		5.1	7.1	13.9	7.8	7.3	6.8	3.4	--
	5	L23200	M	28.0	30.9	31.6	--	--	--	--	56.4
	5	L23201	M	71.5	67.2	**290.6 *, ^^**	**588.1 *, ^^**	--	--	--	--
	5	L23212	M	33.1	36.5	35.8	37.0	45.7	39.1	34.7	--
	5	L23214	M	34.6	44.8	41.4 *	**171.8 ^^**	--	--	--	--
	5	L23234	M	34.7	55.5	50.6	**198.4**	--	--	--	--
		Average		40.4	47.0	90.0	248.8	45.7	39.1	34.7	56.4
		Std Dev		17.6	14.6	112.4	237.0	--	--	--	--
	6	L23203	M	22.4	21.0	46.3	31.2	--	--	--	--
	6	L23204	M	19.0	26.3	40.7 *	25.1 ****	--	--	--	**142.3 ******
	6	L23213	M	--	30.6	--	--	--	--	--	**124.3 ***
	6	L23221	M	19.7	--	--	--	--	--	--	--
	6	L23232	M	--	24.3	44.1	32.9	--	--	--	--
		Average		20.4	25.6	43.7	29.7	--	--	--	133.3
		Std Dev		1.8	4.0	2.8	4.1	--	--	--	12.7

Bold > Normal Range; Underscore < Normal Range
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^^ Moderate Lipemia
-- Instrument unable to calculate result
+ Sample quantity insufficient
-- No Value

ATC 10-22-09

QA Audit Complete
Init/Date: Jm 5/10/10

QC/Tech Rev. By/Date: ATC 10-22-09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
Lactate Dehydrogenase (0.0-250.0 U/L)	1	L23216	M	93	138	60	39	38	58	86	--
	1	L23218	M	121	97	59	59	76	123	156	--
	1	L23220	M	**285**	50	61	86	128	76	86	--
	1	L23222	M	120	89	118 *	41	54	77	115	--
	1	L23223	M	112	245	114	41	83	**305**	175	--
		Average		146	124	82	53	76	128	124	--
		Std Dev		78	75	31	20	34	102	41	--
	2	L23206	M	89	95	111	99	63	39	64	--
	2	L23210	M	69	74	88 *	91	--	52	70	--
	2	L23211	M	64	71	39	35	99	+	106	--
	2	L23215	M	68	63	80	65	71	+	104	--
	2	L23219	M	84	80	58	48	75	80	103	--
		Average		75	77	75	68	77	57	89	--
		Std Dev		11	12	28	27	15	21	21	--
	3	L23217	M	59	54	52	36	83	35	53	--
	3	L23227	M	67	65	58	51	68	116	113	--
	3	L23228	M	55	77	70	40	64	136	117	--
	3	L23229	M	35	46	54	30	54	58	90	--
	3	L23230	M	100 *	59	55	48	107	68	96	--
		Average		63	60	58	41	75	83	94	--
		Std Dev		24	12	7	9	21	42	25	--
	4	L23205	M	32	48	31	41	67	58	127	--
	4	L23207	M	80	90	67	46	63	51	71	--
	4	L23225	M	138	158	121	114	--	--	--	**5189 ******
	4	L23231	M	37	45	50	47	69	82	155	--
	4	L23235	M	43	45	31	41	71	--	--	--
		Average		66	77	60	58	68	64	118	5189
		Std Dev		44	49	37	32	3	16	43	--
	5	L23200	M	56	116	85	--	--	--	--	**447**
	5	L23201	M	75	128	**574 *, ^^**	**602 *, ^^**	--	--	--	--
	5	L23212	M	32	65	49	90	64	113	70	--
	5	L23214	M	53	78	155 *	**493 ^^**	--	--	--	--
	5	L23234	M	57	93	62	**509**	--	--	--	--
		Average		55	96	185	424	64	113	70	447
		Std Dev		15	26	221	227	--	--	--	--
	6	L23203	M	35	55	106	43	--	--	--	--
	6	L23204	M	42	105	71 *	**462 ********	--	--	--	**4782 ********
	6	L23213	M	--	40	--	--	--	--	--	**846 ***
	6	L23221	M	68	--	--	--	--	--	--	--
	6	L23232	M	--	64	96	72	--	--	--	--
		Average		48	66	91	192	--	--	--	2814
		Std Dev		17	28	18	234	--	--	--	2783

Bold > Normal Range; Underscore < Normal Range
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~~ Instrument unable to calculate result
+ Sample quantity insufficient
-- No Value

Atc 10-22-09

QA Audit Complete
By/Date: Jm 5/10/10

QC/Tech Rev. By/Date: Atc 10-22-09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
Sorbitol Dehydrogenase (U/L)	1	L23216	M	24.4	27.7	29.0	27.2	28.4	40.6	23.2	--
	1	L23218	M	25.3	17.7	12.2	20.5	21.8	18.3	28.9	--
	1	L23220	M	36.3	16.5	17.8	22.4	26.2	24.6	17.4	--
	1	L23222	M	23.9	22.8	19.7 *	20.3	29.9	26.0	16.8	--
	1	L23223	M	35.5	37.2	32.8	36.9	39.2	51.1	33.8	--
		Average		29.1	24.4	22.3	25.5	29.1	32.1	24.0	--
		Std Dev		6.3	8.4	8.4	7.0	6.4	13.4	7.3	--
	2	L23206	M	20.0	20.0	20.4	27.1	27.0	22.8	22.9	--
	2	L23210	M	13.3	14.9	18.2 *	23.7	--	22.5	19.2	--
	2	L23211	M	18.0	23.2	20.6	20.4	24.5	+	18.0	--
	2	L23215	M	17.0	20.3	18.3	17.7	24.8	+	19.1	--
	2	L23219	M	21.6	27.7	26.9	31.1	41.2	21.2	23.4	--
		Average		18.0	21.2	20.9	24.0	29.4	22.2	20.5	--
		Std Dev		3.2	4.7	3.5	5.3	8.0	0.9	2.5	--
	3	L23217	M	21.7	28.0	29.1	25.9	34.4	33.9	23.7	--
	3	L23227	M	23.3	28.3	24.7	22.3	31.8	87.1	22.7	--
	3	L23228	M	24.5	42.1	47.1	33.7	41.8	75.4	27.1	--
	3	L23229	M	15.9	20.9	18.5	18.1	23.9	24.5	15.9	--
	3	L23230	M	23.4 *	19.5	15.4	14.2	27.9	21.3	16.7	--
		Average		21.8	27.8	27.0	22.8	32.0	48.4	21.2	--
		Std Dev		3.4	9.0	12.5	7.5	6.8	30.6	4.8	--
	4	L23205	M	17.4	22.3	20.0	49.7	30.2	25.2	26.8	--
	4	L23207	M	15.0	21.6	23.2	19.7	22.0	16.9	11.4	--
	4	L23225	M	35.5	26.2	87.2	40.7	--	--	--	210.5 ****
	4	L23231	M	22.3	26.7	21.5	40.0	46.8	34.9	23.9	--
	4	L23235	M	29.4	29.7	29.0	29.0	53.5	--	--	--
		Average		23.9	25.3	36.2	35.8	38.1	25.7	20.7	210.5
		Std Dev		8.5	3.3	28.7	11.6	14.5	9.0	8.2	--
	5	L23200	M	35.0	36.7	33.2	--	--	--	--	135.9
	5	L23201	M	44.9	42.7	331.1 *, ^^	497.2 *, ^^	--	--	--	--
	5	L23212	M	16.4	21.9	20.6	24.5	26.7	22.9	19.0	--
	5	L23214	M	21.0	24.3	24.5 *	178.9 ^^	--	--	--	--
	5	L23234	M	17.6	37.0	25.3	223.1	--	--	--	--
		Average		27.0	32.5	86.9	230.9	26.7	22.9	19.0	135.9
		Std Dev		12.5	9.0	136.6	196.9	--	--	--	--
	6	L23203	M	23.8	24.2	69.9	30.5	--	--	--	--
	6	L23204	M	11.7	25.7	38.9 *	. ****, ~~	--	--	--	54.8 ****
	6	L23213	M	--	39.5	--	--	--	--	--	266.3 *
	6	L23221	M	14.9	--	--	--	--	--	--	--
	6	L23232	M	--	26.8	51.1	18.8	--	--	--	--
		Average		16.8	29.1	53.3	24.7	--	--	--	160.6
		Std Dev		6.3	7.0	15.6	8.3	--	--	--	149.6

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^^ Moderate Lipemia
~~ Instrument unable to calculate result
+ Sample quantity insufficient
-- No Value

Atc 10-22-09

QA Audit Completed
5/10/10

QC/Tech Rev. By/Date: Atc 10-22-09

Parameter	Group ID	Animal ID	Sex	Day -3	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Terminal
C-Reactive Protein (mg/dL)	1	L23216	M	<0.5	<0.5	1.89	0.73	<0.5	<0.5	<0.5	--
	1	L23218	M	<0.5	0.56	<0.5	<0.5	2.94	2.62	<0.5	--
	1	L23220	M	2.98	2.57	3.75	1.77	<0.5	<0.5	<0.5	--
	1	L23222	M	<0.5	0.87	0.56 *	<0.5	<0.5	<0.5	<0.5	--
	1	L23223	M	5.26	<0.5	0.56	<0.5	<0.5	<0.5	<0.5	--
		Average		1.80	0.90	1.40	0.65	0.79	0.72	0.25	--
		Std Dev		2.27	0.97	1.46	0.66	1.20	1.06	0.00	--
	2	L23206	M	<0.5	2.37	1.00	1.03	<0.5	<0.5	<0.5	--
	2	L23210	M	<0.5	1.53	0.59 *	<0.5	--	<0.5	<0.5	--
	2	L23211	M	<0.5	1.10	<0.5	<0.5	0.68	+	<0.5	--
	2	L23215	M	<0.5	0.89	0.51	<0.5	<0.5	+	<0.5	--
	2	L23219	M	<0.5	1.00	<0.5	<0.5	<0.5	<0.5	<0.5	--
		Average		0.25	1.38	0.52	0.41	0.36	0.25	0.25	--
		Std Dev		0.00	0.61	0.31	0.35	0.22	0.00	0.00	--
	3	L23217	M	<0.5	0.57	<0.5	<0.5	<0.5	<0.5	<0.5	--
	3	L23227	M	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	--
	3	L23228	M	0.82	2.19	1.02	0.53	<0.5	0.86	0.55	--
	3	L23229	M	<0.5	0.64	<0.5	<0.5	<0.5	<0.5	<0.5	--
	3	L23230	M	<0.5 *	1.02	<0.5	<0.5	<0.5	<0.5	<0.5	--
		Average		0.36	0.93	0.40	0.31	0.25	0.37	0.31	--
		Std Dev		0.25	0.75	0.34	0.13	0.00	0.27	0.13	--
	4	L23205	M	<0.5	0.50	0.57	<0.5	<0.5	<0.5	<0.5	--
	4	L23207	M	<0.5	1.20	0.51	<0.5	<0.5	<0.5	<0.5	--
	4	L23225	M	1.47	2.74	7.96	9.59	--	--	--	6.28 ****
	4	L23231	M	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	--
	4	L23235	M	<0.5	0.61	<0.5	<0.5	7.48	--	--	--
		Average		0.49	1.06	1.91	2.12	2.06	0.25	0.25	6.28
		Std Dev		0.55	1.00	3.39	4.18	3.62	0.00	0.00	--
	5	L23200	M	<0.5	1.87	0.88	--	--	--	--	3.27
	5	L23201	M	<0.5	1.76	3.59 *, ^^	3.83 *, ^^	--	--	--	--
	5	L23212	M	<0.5	2.58	0.95	<0.5	<0.5	<0.5	<0.5	--
	5	L23214	M	<0.5	0.74	1.27 *	3.78 ^^	--	--	--	--
	5	L23234	M	1.00	1.97	0.53	3.74	--	--	--	--
		Average		0.40	1.78	1.44	2.90	0.25	0.25	0.25	3.27
		Std Dev		0.34	0.66	1.23	1.77	--	--	--	--
	6	L23203	M	<0.5	2.66	4.28	9.62	--	--	--	--
	6	L23204	M	<0.5	1.12	6.79 *	11.17 ****	--	--	--	7.30 ****
	6	L23213	M	--	0.87	--	--	--	--	--	8.46 *
	6	L23221	M	0.50	--	--	--	--	--	--	--
	6	L23232	M	--	2.68	8.29	12.88	--	--	--	--
		Average		0.33	1.83	6.45	11.22	--	--	--	7.88
		Std Dev		0.14	0.97	2.03	1.63	--	--	--	0.82

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* Slight Hemolysis; **** Severe Hemolysis
^^ Moderate Lipemia
-- Instrument unable to calculate result
+ Sample quantity insufficient
-- No Value

AtC 10·22·09

QA Audit Completed By/Date: [illegible] 5/6/10

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APPENDIX W
ANATOMIC PATHOLOGY

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Figure 5. Animal 605 (L23213): Lymph node, sinuses: Anthrax bacilli (arrow head). HE. 4X 1-6

Figure 6. Animal 605 (L23213): Lymph node, sinuses: Hemorrhage and Lymphocyte Depletion HE. 4X 1-7

Figure 7. Animal 605 (L23213): Lymph node, sinuses: Anthrax bacilli (arrow head). HE. 40X 1-8

Figure 8.. Animal 502 (L23234): Thymus: Lymphocyte depletion (arrow heads). HE. 4X 1-9

List of Appendices

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List of Acronyms

CFU ..... colony forming units
LD_{50} ..... median lethal dose
min ..... minute
mL ..... milliliter
mm ..... millimeter
NZW ..... New Zealand White

1.0 Introduction

The objective of this study was to determine physiological markers of disease following a single exposure to *Bacillus anthracis* Ames strain spores. Thirty (30) male pathogen-free New Zealand White (NZW) rabbits were aerosol challenged with targeted doses of 100,000, 10,000, 1,000, or 100 colony forming units (CFUs) of *B. anthracis* (Ames strain). Additionally, a high dose control group was challenged with 100 LD_{50} and a negative control group was exposed to an equivalent of 100 LD_{50} of gamma irradiated spores as outlined in Table 1.

Table 1. Study Design and Challenge Doses

Group	Spore Dose (CFU)	Number of Spore Challenges	# of Rabbits
1 (negative) control*	100 x LD_{50}	1	5
2	100	1	5
3	1000	1	5
4	10,000	1	5
5	100,000	1	5
6 (high dose control)	100 x LD_{50}	1	5

*Negative controls were challenged with irradiated spores

Complete necropsies were performed on all rabbits following spontaneous death or euthanasia, including rabbits surviving to study termination on Day 21. The lungs and gross lesions from each animal were collected. The meninges, ependyma, ventricles, frontal cortex, hippocampus, thalamus, cerebellum, and brain stem were included when gross lesions were present in the brain. The brain and standard sections of all other collected tissues were placed in 10% neutral buffered formalin, processed to approximately 5 micron slides, stained with hematoxylin and eosin, and examined histologically by a board-certified pathologist. All microscopic findings were graded semi-quantitatively according to the following scale, with the associated numerical score used to calculate average severity grades for each lesion by group. Minimal (Grade 1) represented the least detectible lesion; mild (Grade 2) represented an easily discernible lesion; moderate (Grade 3) represented a change affecting a large area of the represented tissue; and marked (Grade 4) represented a lesion that approached maximal. Gross and microscopic diagnoses were entered into the PATH/TOX SYSTEM

(Xybion Medical Systems Corporation, Cedar Knolls New Jersey) for data tabulation and analysis.

2.0 Pathology

2.1 Necropsy

Two (40%) rabbits in Group 4, four (80%) rabbits in Group 5, and five (100%) rabbits in Group 6 died following challenge with *B. anthracis* spores. Mortality rates are summarized in Table 2.

Table 2. Mortality (Found Dead) Due to *B. anthracis* Infection

Group	Mortality Rate per Group (Males)	Mortality Rate per Group (Total) (%)
1[a] (100 x LD_{50})	0/5	0
2 (100 CFU)	0/5	0
3 (1,000 CFU)	0/5	0
4 (10,000 CFU)	2/5	40
5 (100,000 CFU)	4/5	80
6 (100 x LD_{50})	5/5	100

[a]Group 1 animals were challenged with irradiated spores

Gross lesions in unscheduled death rabbits (rabbits that succumbed to challenge with *B. anthracis*) included: discoloration of the brain (meninges), crusting of the skin, abdominal and thoracic cavity fluid, bronchial and mediastinal lymph node enlargement, small intestinal fluid, and thymic fluid. These gross lesions were typical of anthrax in rabbits (Zaucha et al., 1998) and correlated histologically with hemorrhage, necrosis, edema, and/or suppurative inflammation. A gross lesion was present in only one scheduled death (Day 21) rabbit (L23231) consisting of a skin crust of the left hind limb correlating microscopically to dermal and epidermal necrosis. No intralesional anthrax bacilli were found; hence this lesion was not attributed to anthrax. Gross lesions are detailed in the Individual Gross and Microscopic Observation tables (Attachment 1 to Appendix W).

2.2 Histopathology

The lungs and all gross lesions were examined microscopically for evidence of anthrax. Microscopic lesions typical of anthrax (Zaucha, et al., 1998) were present in all unscheduled death rabbits, and included minimal to moderate suppurative inflammation (predominately degenerate and viable heterophils [polymorphonuclear cells]), necrosis, hemorrhage, fibrin and/or large rod-shaped bacteria in the lungs, bronchial and mediastinal lymph nodes, skin, small intestine, brain (meninges), and thymus. Lung lesions consisted of suppurative inflammation and bacteria generally found in the pulmonary interstitium and associated with alveolar capillaries or larger pulmonary blood vessels. Scheduled death rabbits (Day 21) had little or no inflammation in the lungs (inflammation when present was generally suppurative); additional anthrax-related lesions were found in lymph nodes, meninges, skin, thymus, and small intestine. Lymph node findings included hemorrhage, lymphoid necrosis, fibrin, and bacteria. There was minimal to mild intravascular bacteria, suppurative inflammation, and hemorrhage (with vascular necrosis) primarily in the meninges of the brain of one rabbit (L23232). The skin of rabbit L23234 had areas of suppurative inflammation/edema, hemorrhage, necrosis, and intra-/extravascular bacteria. The thymus had mild to moderate atrophy of lymphoid tissue, intravascular bacteria, and evidence of edema. Additionally, there was suppurative inflammation and intra- and extravascular bacteria in the small intestine (jejunum) of one rabbit (L23221). Where suppurative inflammation occurred in tissues (other than the lung), it was generally associated with bacteria (bacilli).

Multinucleated giant cells (occasionally organizing towards granuloma formation) were present in the lungs of some rabbits in all groups, including control rabbits. These multinucleated giant cells were randomly distributed throughout the lung in ruptured or intact airways or less frequently in or near pulmonary blood vessels or lymphatics. The multinucleated giant cells often surrounded or contained birefringent foreign debris. These cells and debris were also seen in the lungs of two control animals; but occurred with greater frequency and severity in anthrax challenged rabbits. Cell aggregates of this type were not described in a previous acute anthrax rabbit study (Zaucha et al., 1998); hence, it is uncertain

if anthrax is contributory to the development of this lesion. While many pulmonary macrophages in challenged rabbits contained foamy or granular cytoplasm and cellular debris, bacilli were not identifiable in most cases. However, it is possible that these lesions were caused by an indirect effect of prolonged anthrax septicemia on macrophage function. Macrophage dysfunction has been described as occurring late in sepsis (Pahuja et al., 2008). Inhaled debris or emboli from indwelling vascular access ports may have also contributed to the development of multinucleated giant cell infiltrate/granuloma formation. Pulmonary granulomas/multinucleated giant cells, thromboemboli, and perivascular eosinophils have been described in animals fitted with indwelling vascular access ports (Taketoh et al., 2009). Microscopically the skin of rabbit (L23231) had superficial and dermal necrosis with underlying granulation tissue. Coccoid bacteria were present but there were no bacteria characteristic of anthrax in this lesion, so it was not attributed to anthrax. The incidence summary of microscopic observations is presented in Table 3 for survivors humanely terminated on Day 21 and in Table 4 for unscheduled-death rabbits. In both tables, average severity for a given lesion was calculated as the sum of severity scores in a study group divided by the total number of animals examined in the group (unweighted). Photomicrographs representative of anthrax are included in Attachment 1.

Table 3. Incidence Summary of Microscopic Nonneoplastic Graded Observations with Average Severity - Day 21

Tissue/Observation	Group:	Number Observed Per Group					
		1*	2	3	4	5	6
Brain	Number Examined:	0	0	0	0	0	0
Bacteria		-	-	-	-	-	-
	Average Severity:	-	-	-	-	-	-
Hemorrhage		-	-	-	-	-	-
	Average Severity:	-	-	-	-	-	-
Inflammation, Suppurative		-	-	-	-	-	-
	Average Severity:	-	-	-	-	-	-
Intestine, Small	Number Examined:	0	0	0	0	0	0
Bacteria (bacilli)		-	-	-	-	-	-
	Average Severity:	-	-	-	-	-	-
Inflammation, Suppurative		-	-	-	-	-	-
	Average Severity:	-	-	-	-	-	-
Lung	Number Examined:	5	5	5	3	1	0
Bacteria (bacilli)		0	0	1	1	0	-
	Average Severity:	0.0	0.0	0.2	0.3	0.0	-
Inflammation, Nonsuppurative		0	0	0	3	0	-
	Average Severity:	0.0	0.0	0.0	1.0	0.0	-
Inflammation, Suppurative		0	3	4	0	1	-
	Average Severity:	0.0	0.6	1.2	0.0	1.0	-
Multi-Nucleated Giant Cells		2	2	2	1	0	-
	Average Severity:	0.4	0.4	0.8	0.7	0.0	-
Perivascular Eosinophils		0	0	1	1	0	-
	Average Severity:	0.0	0.0	0.6	0.3	0.0	-

* Irradiated spores

Group Legend: 1=100 X LD_{50}; 2=100 CFU; 3=1,000 CFU; 4=10,000 CFU; 5=100,000 CFU; 6=100 x LD_{50}

Table 3. Incidence Summary of Microscopic Nonneoplastic Graded Observations with Average Severity - Day 21 (Continued)

Tissue/Observation	Group:	Number Observed Per Group					
		1*	2	3	4	5	6
Lymph Node, Bronchial	Number Examined:	0	0	0	0	0	0
Bacteria (bacilli)		-	-	-	-	-	-
	Average Severity:	-	-	-	-	-	-
Fibrin		-	-	-	-	-	-
	Average Severity:	-	-	-	-	-	-
Hemorrhage		-	-	-	-	-	-
	Average Severity:	-	-	-	-	-	-
Necrosis, Lymphoid		-	-	-	-	-	-
	Average Severity:	-	-	-	-	-	-
Lymph Node, Mediastinal	Number Examined:	0	0	0	0	0	0
Bacteria (bacilli)		-	-	-	-	-	-
	Average Severity:	-	-	-	-	-	-
Fibrin		-	-	-	-	-	-
	Average Severity:	-	-	-	-	-	-
Hemorrhage		-	-	-	-	-	-
	Average Severity:	-	-	-	-	-	-
Histiocytosis		-	-	-	-	-	-
	Average Severity:	-	-	-	-	-	-
Necrosis, Lymphoid		-	-	-	-	-	-
	Average Severity:	-	-	-	-	-	-
Necrosis, Vascular		-	-	-	-	-	-
	Average Severity:	-	-	-	-	-	-

* Irradiated spores

Group Legend: 1=100 X LD_{50}; 2=100 CFU; 3=1,000 CFU; 4=10,000 CFU; 5=100,000 CFU; 6=100 x LD_{50}

Table 3. Incidence Summary of Microscopic Nonneoplastic Graded Observations with Average Severity -, Day 21 (Continued)

Tissue/Observation	Group:	Number Observed Per Group					
		1*	2	3	4	5	6
Skin	Number Examined:	0	0	0	1	0	0
Bacteria (bacilli)		-	-	-	0	-	-
	Average Severity:	-	-	-	0.0	-	-
Hemorrhage		-	-	-	0	-	-
	Average Severity:	-	-	-	0.0	-	-
Inflammation, Suppurative		-	-	-	0	-	-
	Average Severity:	-	-	-	0.0	-	-
Necrosis		-	-	-	1	-	-
	Average Severity:	-	-	-	3.0	-	-
Thymus	Number Examined:	0	0	0	0	0	0
Atrophy, Lymphoid		-	-	-	-	-	-
	Average Severity:	-	-	-	-	-	-
Bacteria		-	-	-	-	-	-
	Average Severity:	-	-	-	-	-	-
Edema		-	-	-	-	-	-
	Average Severity:	-	-	-	-	-	-

* Irradiated spores

Group Legend: 1=100 X LD_{50}; 2=100 CFU; 3=1,000 CFU; 4=10,000 CFU; 5=100,000 CFU; 6=100 x LD_{50}

Table 4. Incidence Summary of Microscopic Nonneoplastic Graded Observations with Average Severity - Unscheduled Death

Tissue/Observation	Group:	Number Observed Per Group					
		1*	2	3	4	5	6
Brain	Number Examined:	0	0	0	0	0	1
Bacteria		-	-	-	-	-	1
	Average Severity:	-	-	-	-	-	1.0
Hemorrhage		-	-	-	-	-	1
	Average Severity:	-	-	-	-	-	2.0
Inflammation, Suppurative		-	-	-	-	-	1
	Average Severity:	-	-	-	-	-	3.0
Intestine, Small	Number Examined:	0	0	0	0	0	1
Bacteria (bacilli)		-	-	-	-	-	1
	Average Severity:	-	-	-	-	-	2.0
Inflammation, Suppurative		-	-	-	-	-	1
	Average Severity:	-	-	-	-	-	2.0
Lung	Number Examined:	0	0	0	2	4	5
Bacteria (bacilli)		-	-	-	1	3	5
	Average Severity:	-	-	-	1.5	1.8	1.8
Inflammation, Nonsuppurative		-	-	-	0	0	0
	Average Severity:	-	-	-	0.0	0.0	0.0
Inflammation, Suppurative		-	-	-	2	4	5
	Average Severity:	-	-	-	1.5	2.0	2.4
Multi-Nucleated Giant Cells		-	-	-	0	4	2
	Average Severity:	-	-	-	0.0	1.8	0.6
Perivascular Eosinophils		-	-	-	0	0	0
	Average Severity:	-	-	-	0.0	0.0	0.0

* Irradiated spores

Group Legend: 1=100 X LD_{50}; 2=100 CFU; 3=1,000 CFU; 4=10,000 CFU; 5=100,000 CFU; 6=100 x LD_{50}

Table 4. Incidence Summary of Microscopic Nonneoplastic Graded Observations with Average Severity - Unscheduled Death (Continued)

Tissue/Observation	Group:	Number Observed Per Group					
		1*	2	3	4	5	6
Lymph Node, Bronchial	Number Examined:	0	0	0	0	0	1
Bacteria (bacilli)		-	-	-	-	-	1
	Average Severity:	-	-	-	-	-	1.0
Fibrin		-	-	-	-	-	1
	Average Severity:	-	-	-	-	-	1.0
Hemorrhage		-	-	-	-	-	1
	Average Severity:	-	-	-	-	-	2.0
Necrosis, Lymphoid		-	-	-	-	-	1
	Average Severity:	-	-	-	-	-	2.0
Lymph Node, Mediastinal	Number Examined:	0	0	0	0	0	2
Bacteria (bacilli)		-	-	-	-	-	2
	Average Severity:	-	-	-	-	-	2.5
Fibrin		-	-	-	-	-	2
	Average Severity:	-	-	-	-	-	2.0
Hemorrhage		-	-	-	-	-	1
	Average Severity:	-	-	-	-	-	1.5
Histiocytosis		-	-	-	-	-	1
	Average Severity:	-	-	-	-	-	1.0
Necrosis, Lymphoid		-	-	-	-	-	2
	Average Severity:	-	-	-	-	-	3.0
Necrosis, Vascular		-	-	-	-	-	1
	Average Severity:	-	-	-	-	-	1.0

*Irradiated spores

Group Legend: 1=100 X LD_{50}; 2=100 CFU; 3=1,000 CFU; 4=10,000 CFU; 5=100,000 CFU; 6=100 x LD_{50}

Table 4. Incidence Summary of Microscopic Nonneoplastic Graded Observations with Average Severity - Unscheduled Death (Continued)

Tissue/Observation	Group:	Number Observed Per Group					
		1*	2	3	4	5	6
Skin	Number Examined:	0	0	0	0	1	0
Bacteria (bacilli)		-	-	-	-	1	-
	Average Severity:	-	-	-	-	3.0	-
Hemorrhage		-	-	-	-	1	-
	Average Severity:	-	-	-	-	2.0	-
Inflammation, Suppurative		-	-	-	-	1	-
	Average Severity:	-	-	-	-	2.0	-
Necrosis		-	-	-	-	1	-
	Average Severity:	-	-	-	-	2.0	-
Thymus	Number Examined:	0	0	0	0	1	1
Atrophy, Lymphoid		-	-	-	-	1	1
	Average Severity:	-	-	-	-	3.0	2.0
Bacteria		-	-	-	-	1	0
	Average Severity:	-	-	-	-	2.0	0.0
Edema		-	-	-	-	1	1
	Average Severity:	-	-	-	-	2.0	2.0

*Irradiated spores
Group Legend: 1=100 x LD50; 2=100 CFU; 3=1,000 CFU; 4=10,000 CFU; 5=100,000 CFU; 6=100 xLD50

Table 5. Summary of Individual Gross and Microscopic Observations

Group Number	Animal Number/ Death Status	Gross Findings	Microscopic Findings
1 100 LD_{50} (Irradiated)	101 (L23220)/FS		Lung: Unremarkable
	102 (L23216)/FS		Lung: Unremarkable
	103 (L23218)/FS		Lung: Multi-nucleated giant cells, minimal
	104 (L23223)/FS		Lung: Unremarkable
	105 (L23222)/FS		Lung: Multi-nucleated giant cells, minimal
2 100 CFU	201 (L23215)/FS		Lung: Inflammation, suppurative, minimal Lung: Multi-nucleated giant cells, minimal
	202 (L23206)/FS		Lung: Inflammation, suppurative, minimal
	203 (L23210)/FS		Lung: Unremarkable
	204 (L23219)/FS		Lung: Inflammation, suppurative, minimal Lung: Multi-nucleated giant cells, minimal
	205 (L23211)/FS		Lung: Unremarkable
3 1000 CFU	301 (L23217)/FS		Lung: Inflammation, suppurative, moderate Lung: Multi-nucleated giant cells, moderate Lung: Perivascular eosinophils, moderate
	302 (L23230)/FS		Lung: Inflammation, suppurative, minimal
	303 (L23228)/FS		Lung: Inflammation, suppurative, minimal Lung: Multi-nucleated giant cells, minimal
	304 (L23227)/FS		Lung: Bacteria (bacilli), minimal
	305 (L23229)/FS		Lung: Inflammation, suppurative, minimal

FD = Found Dead, FS = Final Phase Sacrifice

Table 5. Summary of Individual Gross and Microscopic Observations (Continued)

Group Number	Animal Number/ Death Status	Gross Findings	Microscopic Findings
4 10,000 CFU	401 L23235)/FD		Lung: Inflammation, suppurative, minimal
	402 (L23205)/FS		Lung: Inflammation, nonsuppurative, minimal Lung: Perivascular eosinophils, minimal
	403 (L23225)/FD	Cavity, Abdominal: Fluid, red, ~30 mL. Cavity, Thoracic: Fluid, red, ~15 mL.	Lung: Inflammation, suppurative, mild Lung: Bacteria (bacilli), moderate
	404 (L23231)/FS	Skin: Crust(s), hindlimb, red, left hind limb, 20 mm x 20 mm.	Lung: Inflammation, nonsuppurative, minimal Skin: Necrosis, moderate
	405 (L23207)/FS		Lung: Multi-nucleated giant cells, mild Lung: Bacteria (bacilli), minimal Lung: Inflammation, nonsuppurative, minimal
5 100,000 CFU	501 (L23201)/FD	Cavity, Thoracic: Fluid, red, ~15 mL.	Lung: Inflammation, suppurative, mild Lung: Multi-nucleated giant cells, mild Lung: Bacteria (bacilli), mild
	502 (L23234)/FD	Cavity, Thoracic: Fluid, red, ~10 mL. Skin: Crust(s), hindlimb, dark, left hindlimb, 30 mm 5 mm. Skin: Crust(s), dark, back, 5 mm 5 mm. Skin: Fluid, abdominal, clear, ventral abdomen, ~8 mL. Thymus: Fluid, clear, ~8 mL.	Lung: Inflammation, suppurative, moderate Lung: Multi-nucleated giant cells, mild. Lung: Bacteria (bacilli), moderate Skin: Necrosis, mild Skin: Bacteria (bacilli), moderate Skin: Inflammation, suppurative, mild Skin: Hemorrhage, mild Thymus: Atrophy, lymphoid, moderate Thymus: Edema, mild Thymus: Bacteria, mild
	503 (L23212)/FS		Lung: Inflammation, suppurative, minimal

FD = Found Dead, FS = Final Phase Sacrifice

Table 5. Summary of Individual Gross and Microscopic Observations (Continued)

Group Number	Animal Number/ Death Status	Gross Findings	Microscopic Findings
	504 (L23200)/FD	Cavity, Thoracic: Fluid, red, ~20 mL.	Lung: Inflammation, suppurative, minimal Lung: Multi-nucleated giant cells, minimal
	505 (L23214)/FD		Lung: Inflammation, suppurative, mild Lung: Multi-nucleated giant cells, mild Lung: Bacteria (bacilli), mild
6 100 LD_{50}	601 (L23204)/FD	Cavity, Thoracic: Fluid, red, ~15 mL.	Lung: Inflammation, suppurative, mild Lung: Multi-nucleated giant cells, mild Lung: Bacteria (bacilli), mild
	602 (L23203)/FD		Lung: Inflammation, suppurative, mild Lung: Multi-nucleated giant cells, mild Lung: Bacteria (bacilli), mild
	603 (L23232)/FD	Brain: Discoloration(s), meninges, diffuse, red, affects all lobes. Lymph Node, Bronchial: Enlarged, dark, 3x. Lymph Node, Mediastinal: Enlarged, dark, 3x.	Brain: Inflammation, suppurative, moderate Brain: Hemorrhage, mild Brain: Bacteria, minimal Lung: Inflammation, suppurative, moderate Lung: Bacteria (bacilli), minimal Lymph Node, Bronchial: Fibrin, minimal Hemorrhage, mild Necrosis, lymphoid, mild Bacteria (bacilli), minimal Lymph Node, Mediastinal: Fibrin, minimal Histiocytosis, mild Necrosis, lymphoid, mild Necrosis, vascular, mild Bacteria (bacilli), minimal Thymus: Atrophy, lymphoid, mild Thymus: Edema, mild

FD = Found Dead, FS = Final Phase Sacrifice

Table 5. Summary of Individual Gross and Microscopic Observations (Continued)

Group Number	Animal Number/ Death Status	Gross Findings	Microscopic Findings
	604 (L23221)/FD	Cavity, Abdominal: Fluid, red, ~60 mL. Intestine, Small: Fluid, jejunum, green, ~50 mL. Abdomen was distended.	Intestine, Small: Inflammation, suppurative, mild Bacteria (bacilli), mild Lung: Inflammation, suppurative, mild Lung: Bacteria (bacilli), moderate
	605 (L23213)/FD	Lymph Node, Mediastinal: Enlarged, dark, 3x.	Lung: Inflammation, suppurative, mild Lung: Bacteria (bacilli), minimal Lymph Node Mediastinal: Fibrin, moderate Hemorrhage, moderate Necrosis, lymphoid, marked Bacteria (bacilli), marked

FD = Found Dead, FS = Final Phase Sacrifice
Blank Space = No gross lesions observed on tissue

Table 6. Individual Gross and Microscopic Observations

Animal ID: 101 (L23220)		Group: 100 x LD_{50}*
Day of Death: 21 (Final Phase Sacrifice)		
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Lung	No gross lesions observed on tissue.	Unremarkable.

Animal ID: 102 (L23216)		Group: 100 x LD_{50}*
Day of Death: 21 (Final Phase Sacrifice)		
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Lung	No gross lesions observed on tissue.	Unremarkable.

Animal ID: 103 (L23218)		Group: 100 x LD_{50}*
Day of Death: 21 (Final Phase Sacrifice)		
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Lung	No gross lesions observed on tissue.	Multi-nucleated giant cells, minimal.

Animal ID: 104 (L23223)		Group: 100 x LD_{50}*
Day of Death: 21 (Final Phase Sacrifice)		
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Lung	No gross lesions observed on tissue.	Unremarkable.

Animal ID: 105 (L23222)	Group: 100 x LD_{50}*	
Day of Death: 21 (Final Phase Sacrifice)		
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Lung	No gross lesions observed on tissue.	Multi-nucleated giant cells, minimal.

Animal ID: 201 (L23215)		Group: 100 CFU
Day of Death: 21 (Final Phase Sacrifice)		
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Lung	No gross lesions observed on tissue.	Inflammation, suppurative, minimal. Multi-nucleated giant cells, minimal.

* Irradiated spores.

Table 6. Individual Gross and Microscopic Observations (Continued)

Animal ID: 202 (L23206)		Group: 100 CFU
Day of Death: 21 (Final Phase Sacrifice)		
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Lung	No gross lesions observed on tissue.	Inflammation, suppurative, minimal.

Animal ID: 203 (L23210)		Group: 100 CFU
Day of Death: 21 (Final Phase Sacrifice)		
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Lung	No gross lesions observed on tissue.	Unremarkable.

Animal ID: 205 (L23211)		Group: 100 CFU
Day of Death: 21 (Final Phase Sacrifice)		
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Lung	No gross lesions observed on tissue.	Inflammation, suppurative, minimal. Multi-nucleated giant cells, minimal.

Animal ID: 301 (L23217)		Group: 1,000 CFU
Day of Death: 21 (Final Phase Sacrifice)		
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Lung	No gross lesions observed on tissue.	Inflammation, suppurative, moderate. Multi-nucleated giant cells, moderate. Perivascular eosinophils, moderate.

Animal ID: 302 (L23230)		Group: 1,000 CFU
Day of Death: 21 (Final Phase Sacrifice)		
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Lung	No gross lesions observed on tissue.	Inflammation, suppurative, minimal.

Table 6. Individual Gross and Microscopic Observations (Continued)

Animal ID: 303 (L23228) Day of Death: 21 (Final Phase Sacrifice)		Group: 1,000 CFU
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Lung	No gross lesions observed on tissue.	Inflammation, suppurative, minimal. Multi-nucleated giant cells, minimal.

Animal ID: 304 (L23227) Day of Death: 21 (Final Phase Sacrifice)		Group: 1,000 CFU
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Lung	No gross lesions observed on tissue.	Bacteria (bacilli), minimal.

Animal ID: 305 (L23229) Day of Death: 21 (Final Phase Sacrifice)		Group: 1,000 CFU
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Lung	No gross lesions observed on tissue.	Inflammation, suppurative, minimal.

Animal ID: 402 (L23205) Day of Death: 21(Final Phase Sacrifice)		Group: 10,000 CFU
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Lung	No gross lesions observed on tissue.	Inflammation, nonsuppurative, minimal. Perivascular eosinophils, minimal.

Animal ID: 403 (L23225) Day of Death: 4 (Found Dead)		Group: 10,000 CFU
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Cavity, Abdominal	Fluid, red, G1/ G1= ~30 mL; no section taken.	
Cavity, Thoracic	Fluid, red, G2/ G2= ~15 mL; no section taken.	
Lung	No gross lesions observed on tissue.	Inflammation, suppurative, mild. Bacteria (bacilli), moderate.

Table 6. Individual Gross and Microscopic Observations (Continued)

Animal ID: 404 (L23231)		Group: 10,000 CFU
Day of Death: 21 (Final Phase Sacrifice)		
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Lung	No gross lesions observed on tissue.	Inflammation, nonsuppurative, minimal.
Skin	Crust(s), hindlimb, red, G1/ G1=left hind limb; 20mm x 20mm.	Necrosis, moderate. Note: G1 = necrosis.

Animal ID: 405 (L23207)		Group: 10,000 CFU
Day of Death: 21 (Final Phase Sacrifice)		
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Lung	No gross lesions observed on tissue.	Multi-nucleated giant cells, mild. Bacteria (bacilli), minimal. Inflammation, nonsuppurative, minimal.

Animal ID: 501 (L23201)		Group: 100,000 CFU
Day of Death: 4 (Found Dead)		
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Cavity, Thoracic	Fluid, red, G1/ G1= ~15mL; no sample taken.	
Lung	No gross lesions observed on tissue.	Inflammation, suppurative, mild. Multi-nucleated giant cells, mild. Bacteria (bacilli), mild.

"G" numbers are internal tracking numbers used to correlate gross and microscopic lesions (e.g., G1, G2, etc.).

Table 6. Individual Gross and Microscopic Observations (Continued)

Animal ID: 502 (L23234) Day of Death: 6 (Found Dead)		Group: 100,000 CFU
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Cavity, Thoracic	Fluid, red, G5/ G5= ~10 mL; no sample taken.	
Lung	No gross lesions observed on tissue.	Inflammation, suppurative, moderate. Multi-nucleated giant cells, mild. Bacteria (bacilli), moderate.
Skin	Crust(s), hindlimb, dark, G1/ G1=left hindlimb; 30mmx5mm. Crust(s), dark, G2/ G2=back; 5mmx5mm. Fluid, abdominal, clear, G3/ G3=ventral abdomen; ~8 mL.	Necrosis, mild. Bacteria (bacilli), moderate. Inflammation, suppurative, mild. Hemorrhage, mild. Note: G1-G3 = necrosis (escar formation).
Thymus	Fluid, clear, G4/ G4= ~8 mL.	Atrophy, lymphoid, moderate. Edema, mild. Bacteria, mild. Note: G4 = edema.

Animal ID: 503 (L23212) Day of Death: 21 (Final Phase Sacrifice)		Group: 100,000 CFU
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Lung	No gross lesions observed on tissue.	Inflammation, suppurative, minimal.

Animal ID: 504 (L23200) Day of Death: 3 (Found Dead)		Group: 100,000 CFU
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Cavity, Thoracic	Fluid, red, G1/ G1= ~20 mL; no section taken.	
Lung	No gross lesions observed on tissue.	Inflammation, suppurative, minimal. Multi-nucleated giant cells, minimal.

Table 6. Individual Gross and Microscopic Observations (Continued)

Animal ID: 505 (L23214) Day of Death: 6 (Found Dead)		Group: 100,000 CFU
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Lung	No gross lesions observed on tissue.	Inflammation, suppurative, mild. Multi-nucleated giant cells, mild. Bacteria (bacilli), mild.

Animal ID: 601 (L23204) Day of Death: 4 (Found Dead)		Group: 100 x LD_{50}
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Cavity, Thoracic	Fluid, red, G1/ G1= ~15mL; no sample taken.	
Lung	No gross lesions observed on tissue.	Inflammation, suppurative, mild. Multi-nucleated giant cells, mild. Bacteria (bacilli), mild.

Animal ID: 602 (L23203) Day of Death: 5 (Found Dead)		Group: 100 x LD_{50}
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Lung	No gross lesions observed on tissue.	Inflammation, suppurative, moderate. Multi-nucleated giant cells, minimal. Bacteria (bacilli), mild.

"G" numbers are internal tracking numbers used to correlate gross and microscopic lesions (e.g., G1, G2, etc.).

Table 6. Individual Gross and Microscopic Observations (Continued)

Animal ID: 603 (L23232)		Group: 100 x LD_{50}
Day of Death: 4 (Found Dead)		
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Brain	Discoloration(s), meninges, diffuse, red, G3/ G3=affects all lobes.	Inflammation, suppurative, moderate. Hemorrhage, mild. Bacteria, minimal. Note: G3 = hemorrhage.
Lung	No gross lesions observed on tissue.	Inflammation, suppurative, moderate. Bacteria (bacilli), minimal.
Lymph Node, Bronchial	Enlarged, dark, G1/ G1=3x.	Fibrin, minimal. Hemorrhage, mild. Necrosis, lymphoid, mild. Bacteria (bacilli), minimal. Note: G1 = necrosis and hemorrhage.
Lymph Node, Mediastinal	Enlarged, dark, G2/ G2=3x.	Fibrin, minimal. Histiocytosis, mild. Necrosis, lymphoid, mild. Necrosis, vascular, mild. Bacteria (bacilli), minimal. Note: G2 = necrosis and hemorrhage.
Thymus	No gross lesions observed on tissue.	Atrophy, lymphoid, mild. Edema, mild.

Table 6. Individual Gross and Microscopic Observations (Continued)

Animal ID: 604 (L23221) Day of Death: 2 (Found Dead)		Group: 100 x LD_{50}
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Cavity, Abdominal	Fluid, red, G1/ G1= ~60 mL; no section taken.	
Intestine, Small	Fluid, jejunum, green, G2/ G2= ~50 mL; abdomen was distended due to G2.	Inflammation, suppurative, mild. Bacteria (bacilli), mild. Note: G2 = inflammation, suppurative.
Lung	No gross lesions observed on tissue.	Inflammation, suppurative, mild. Bacteria (bacilli), moderate.

Animal ID: 605 (L23213) Day of Death: 3 (Found Dead)		Group: 100 x LD_{50}
Tissue	**Gross Observation(s)**	**Microscopic Observation(s)**
Lung	No gross lesions observed on tissue.	Inflammation, suppurative, mild. Bacteria (bacilli), minimal.
Lymph Node, Mediastinal	Enlarged, dark, G1/ G1=3x.	Fibrin, moderate. Hemorrhage, moderate. Necrosis, lymphoid, marked. Bacteria (bacilli), marked. Note: G1 = necrosis and hemorrhage.

"G" numbers are internal tracking numbers used to correlate gross and microscopic lesions (e.g., G1, G2, etc.).

4.0 CONCLUSIONS

Exposure to a single dose of *B. anthracis* resulted in 0, 0, 0, 40, 80, and 100 % mortality in the negative control, 100 CFU, 1,000 CFU, 10,000 CFU, 100,000 CFU, and 100 x LD_{50} treatment groups, respectively. Death in these rabbits was attributed to *B. anthracis*. Gross and microscopic lesions were consistent with inhalation anthrax and generally increased in severity (see Individual Gross and Microscopic Observations in Table 5 and Table 6) as the dose increased. The most severe microscopic lesions occurred in the 100 × LD_{50} treatment group. Inflammation was generally more suppurative (versus nonsuppurative) in tissues where bacteria were present. Multinucleated giant cells were present in all groups, but occurred with greater frequency and severity in treated rabbits (when compared to controls). The relationship of these cellular aggregates to anthrax is unclear.

5.0 REFERENCES

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Taketoh, J., Komatsu, S., Adachi, K., and Asanuma, K. 2009. Application of an indwelling vascular access port for intravenous administration in a repeated and intermittent dose toxicity study in rats. J of Toxicol Sci 34(1)39-52.

Zaucha, G.M., Pitt. L.M., Estep, J., Ivins, B.E., and Friedlander, A.M. 1998. The pathology of experimental anthrax in rabbits exposed by inhalation and subcutaneous inoculation. Arch Pathol Lab Med 122(11)982-992.

Attachment 1

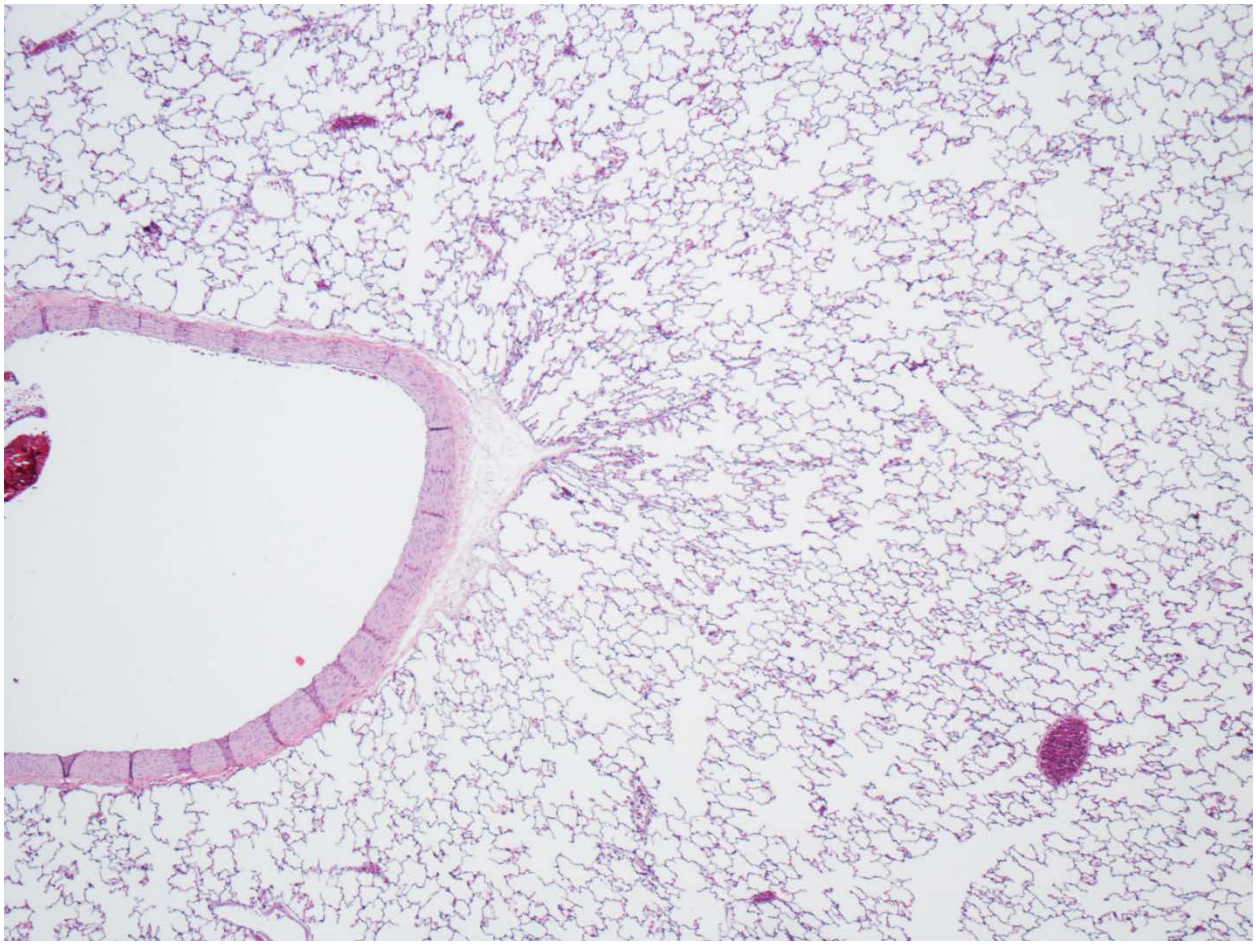

Figure 1. Animal 103 (L23218): Lung, Normal. HE. 4X

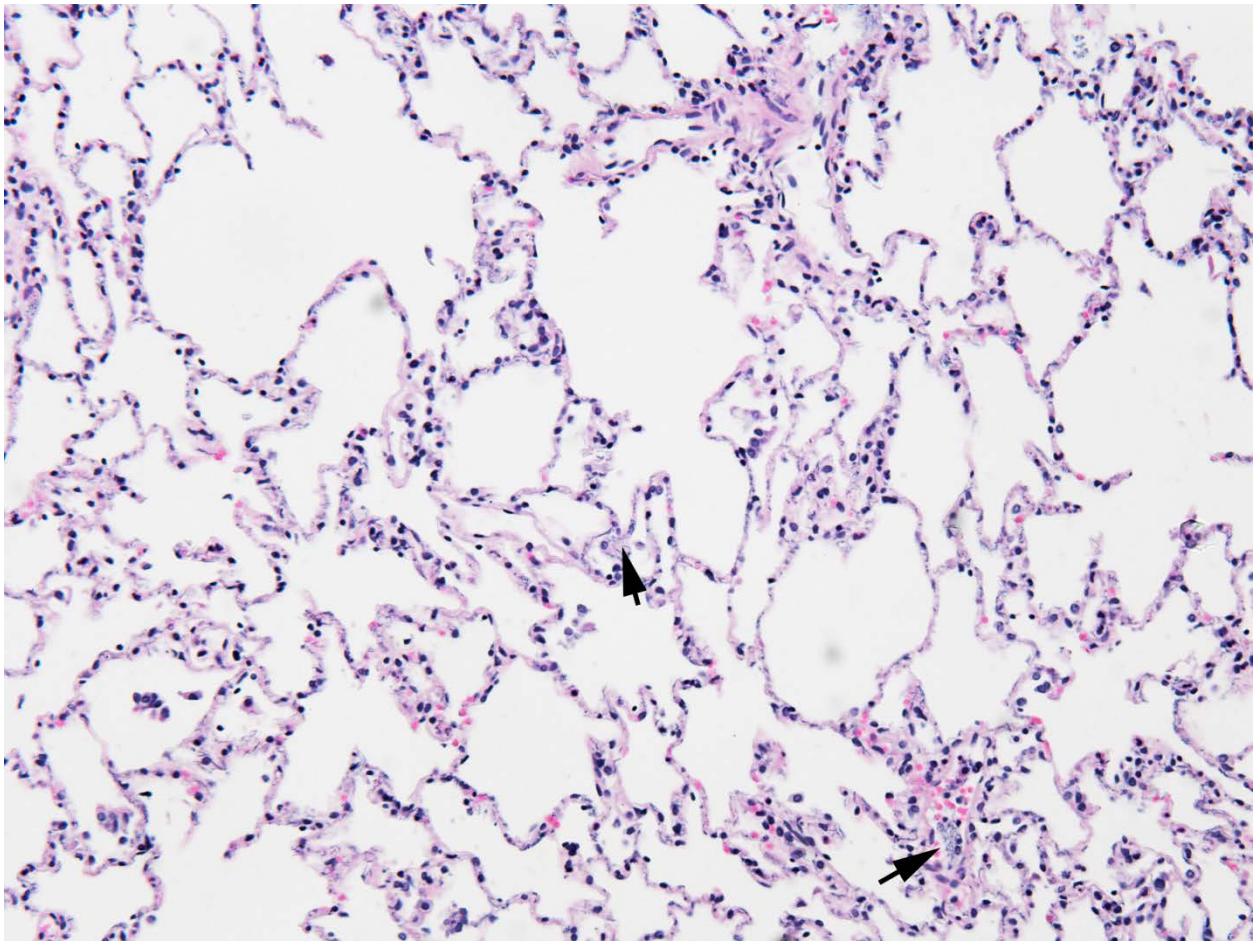

Figure 2. Animal 402 (L23225): Lung, alveoli. Interstitial inflammation and intravascular and interstitial anthrax bacilli (arrow). HE. 20X

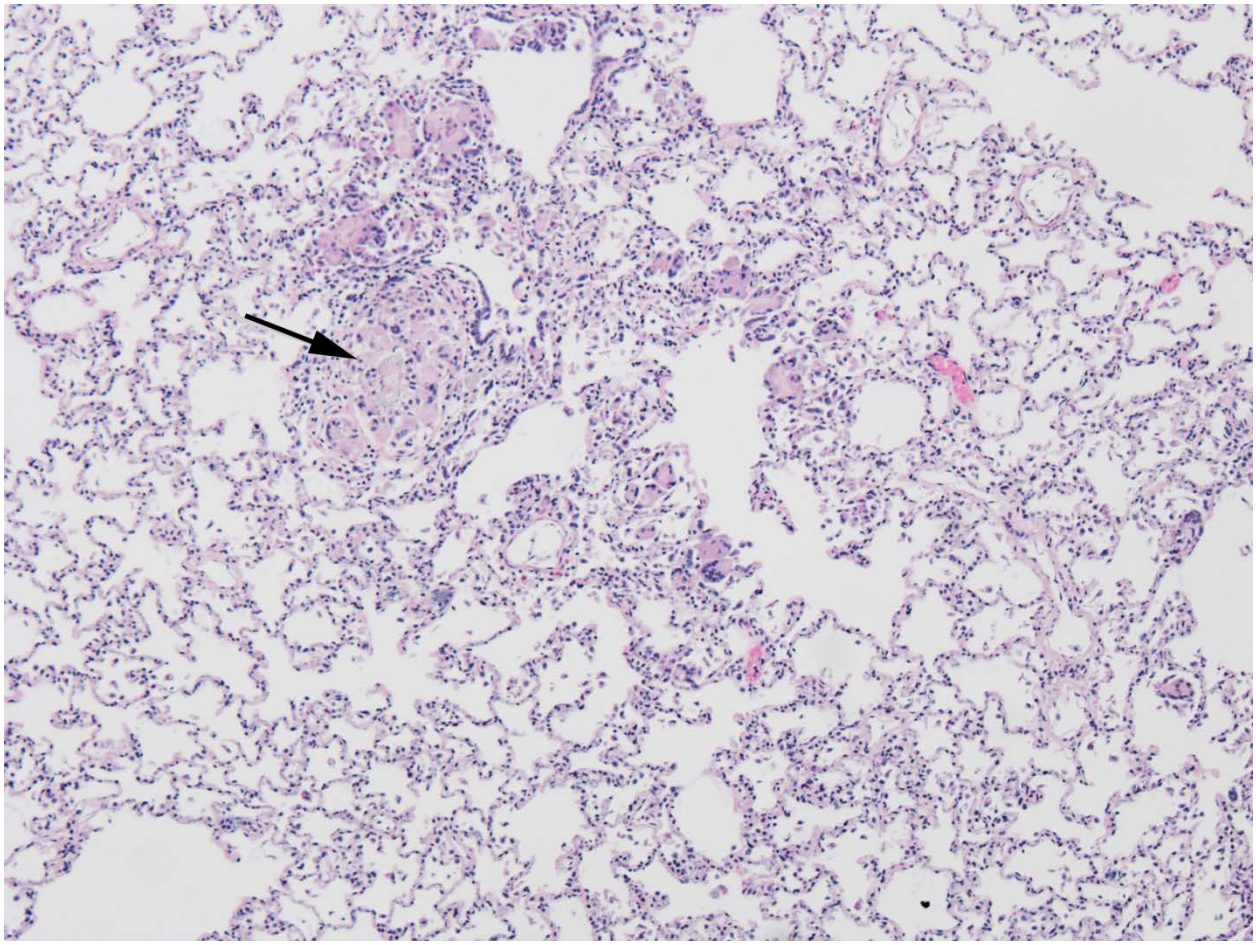

Figure 3. Animal 601(L23204) -Lung, alveolus. Multinucleated Giant Cells (arrow). HE. 10X

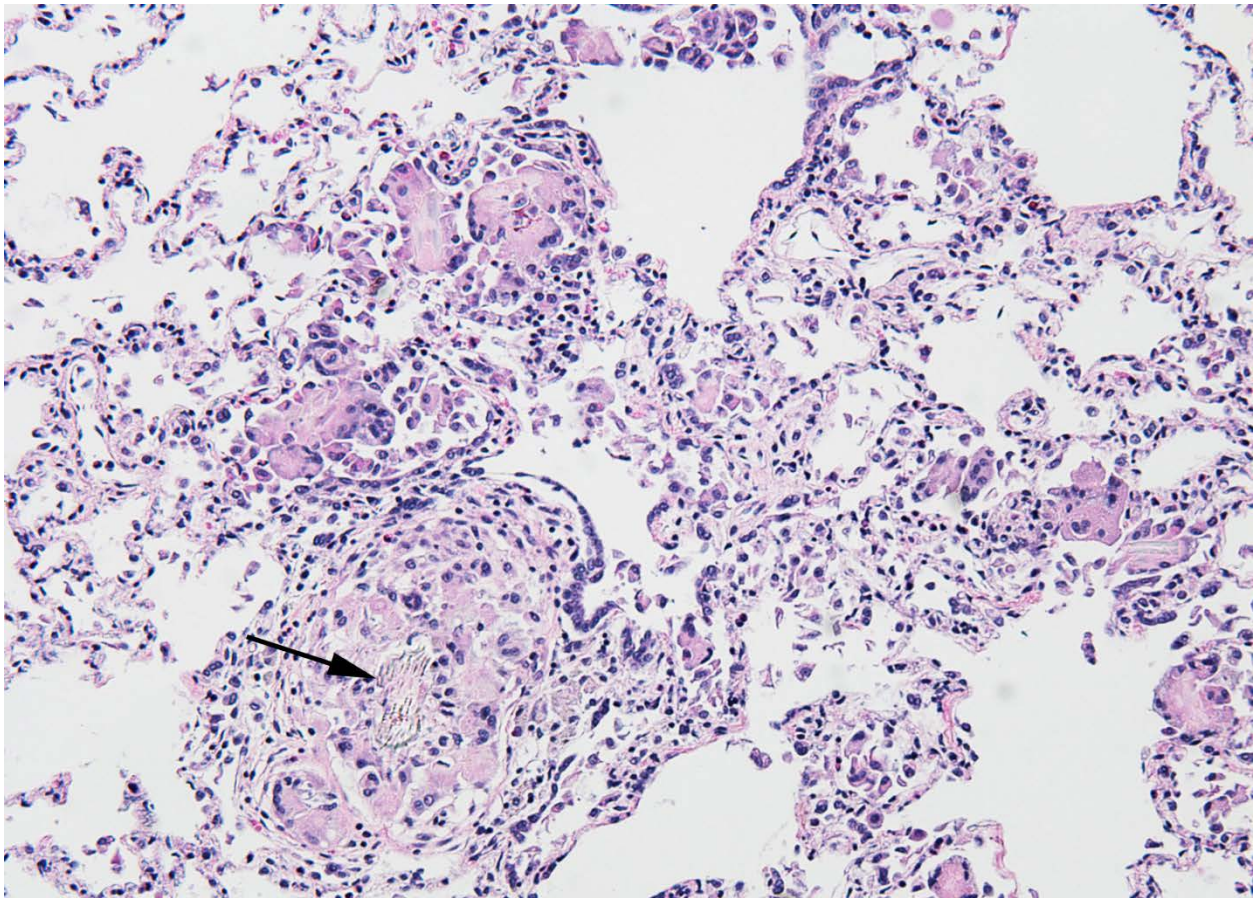

Figure 4. Animal 601 (L23204): Lung, alveolus: Multinucleated Giant Cell Foreign Body (arrow). HE. 20X

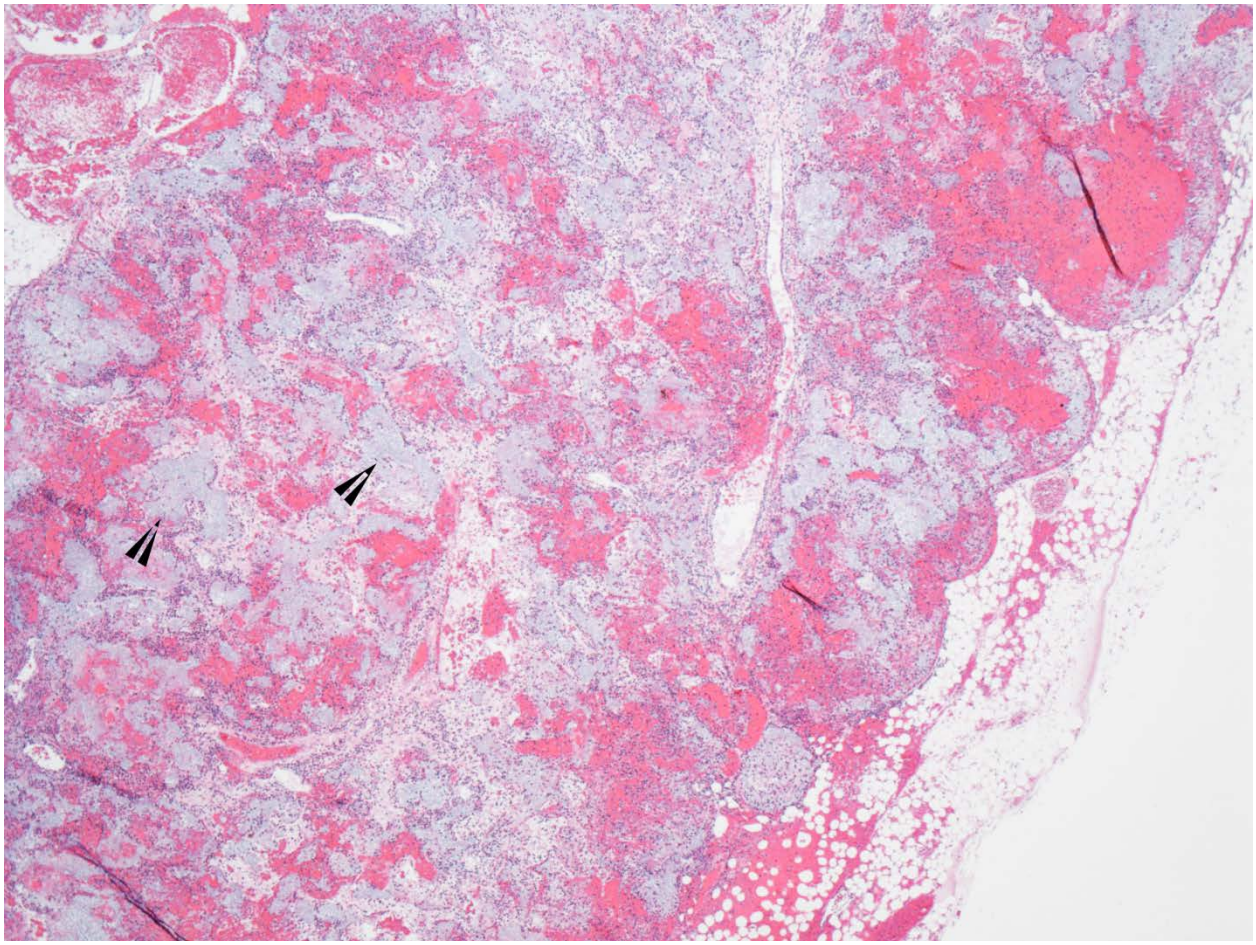

Figure 5. Animal 605 (L23213): Lymph node, sinuses: Anthrax bacilli (arrow head). HE. 4X

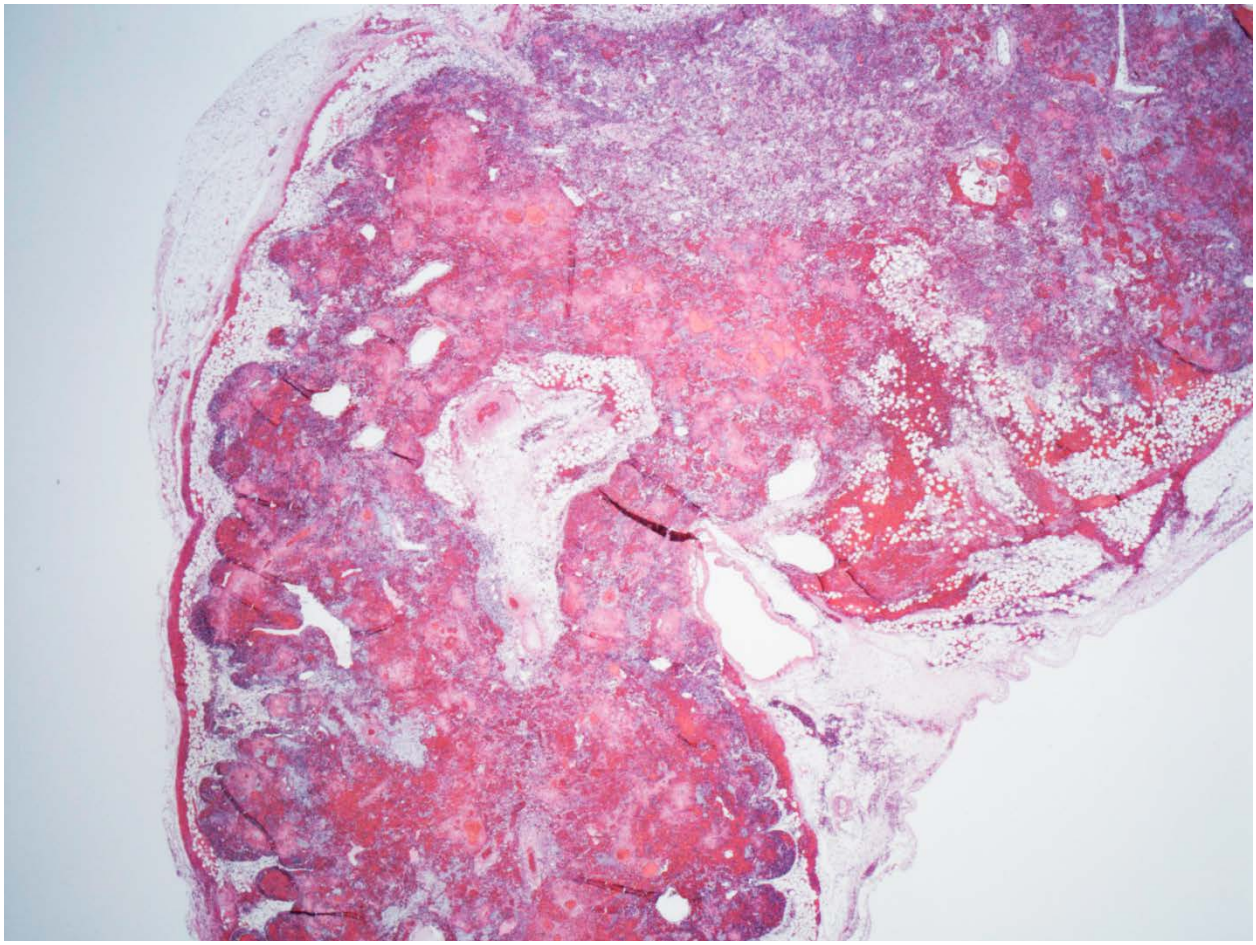

Figure 6. Animal 605 (L23213): Lymph node, sinuses: Hemorrhage and Lymphocyte Depletion HE. 4X

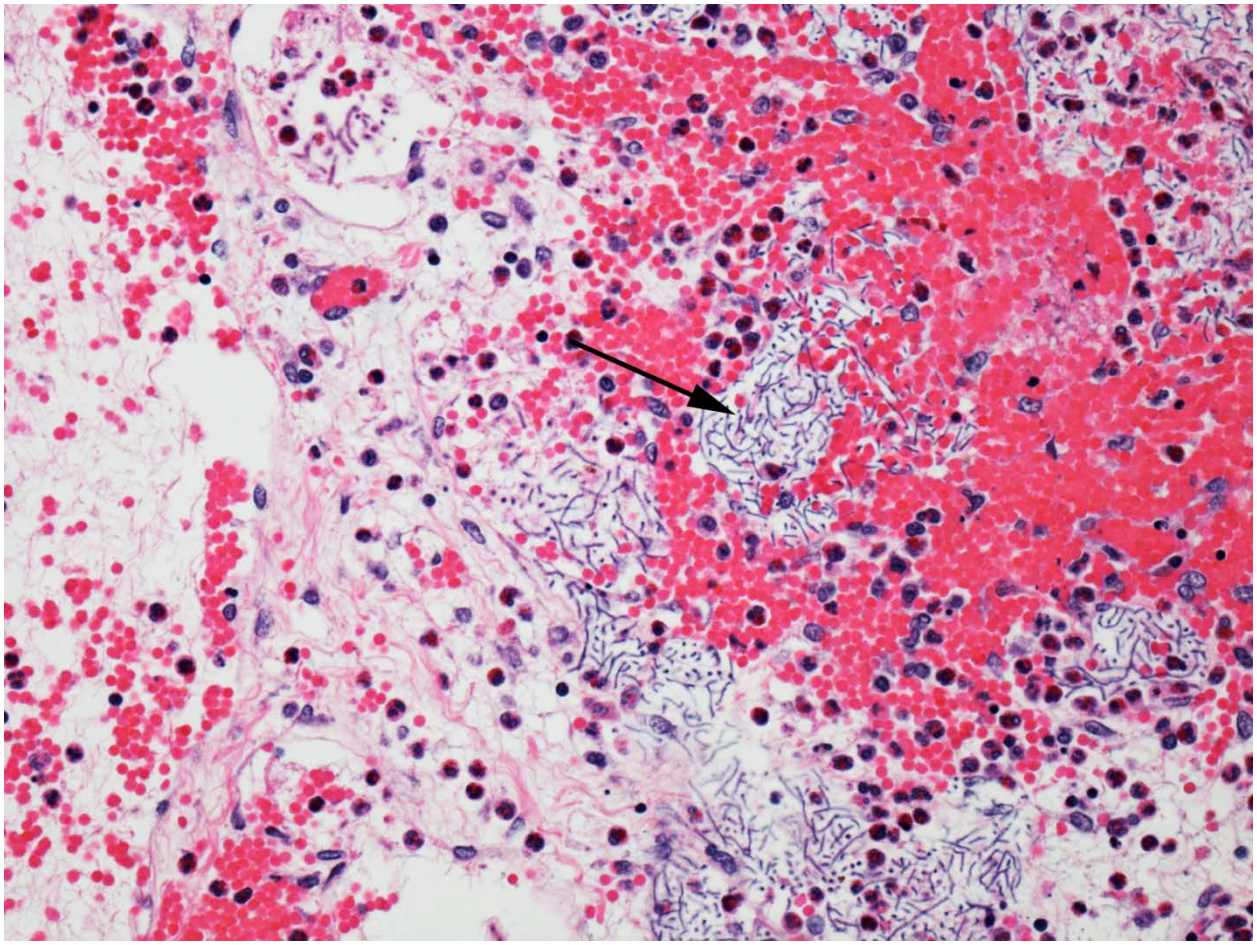

Figure 7. Animal 605 (L23213): Lymph node, sinuses: Anthrax bacilli (arrow head). HE. 40X

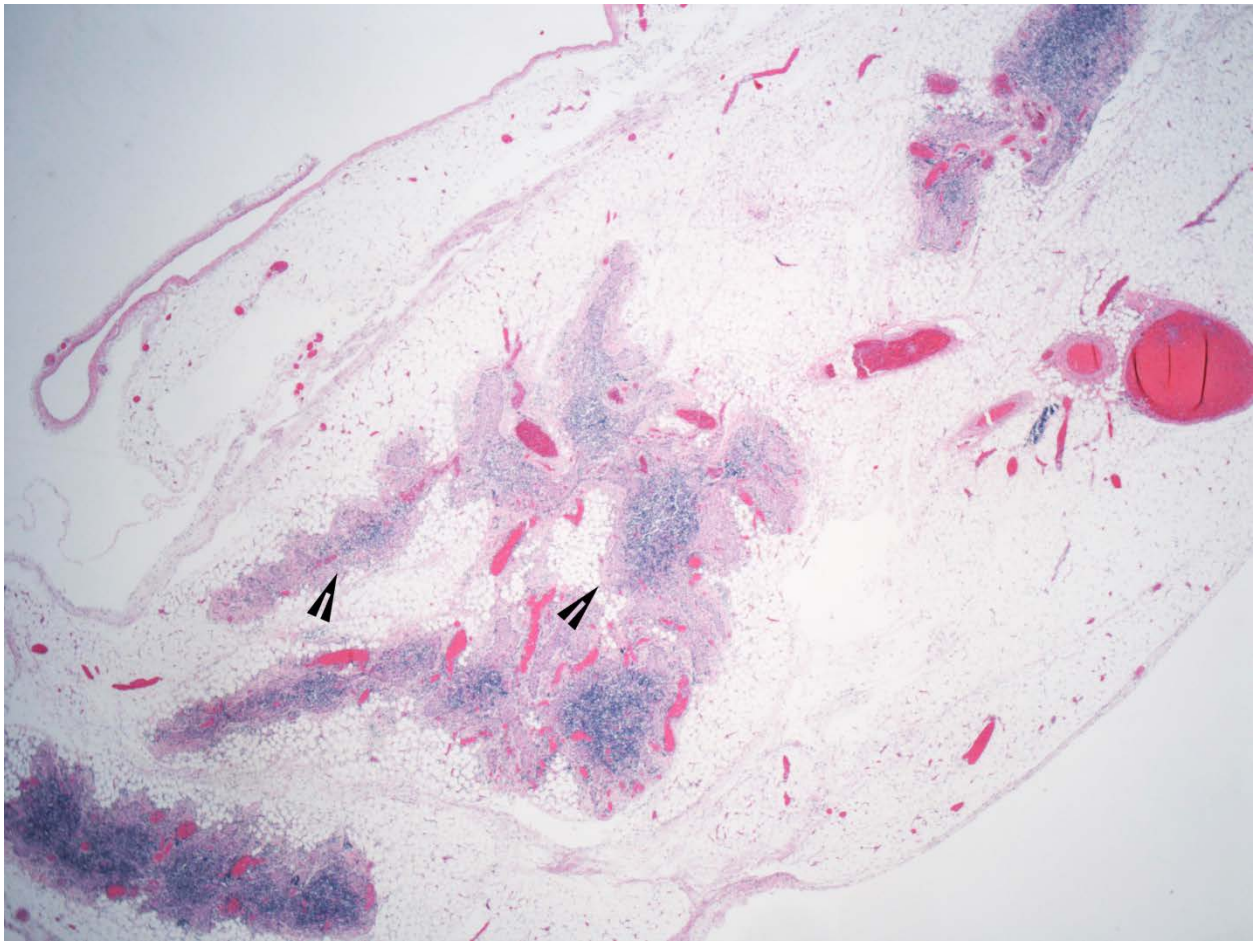

Figure 8. Animal 502 (L23234): Thymus: Lymphocyte depletion (arrow heads). HE. 4X

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