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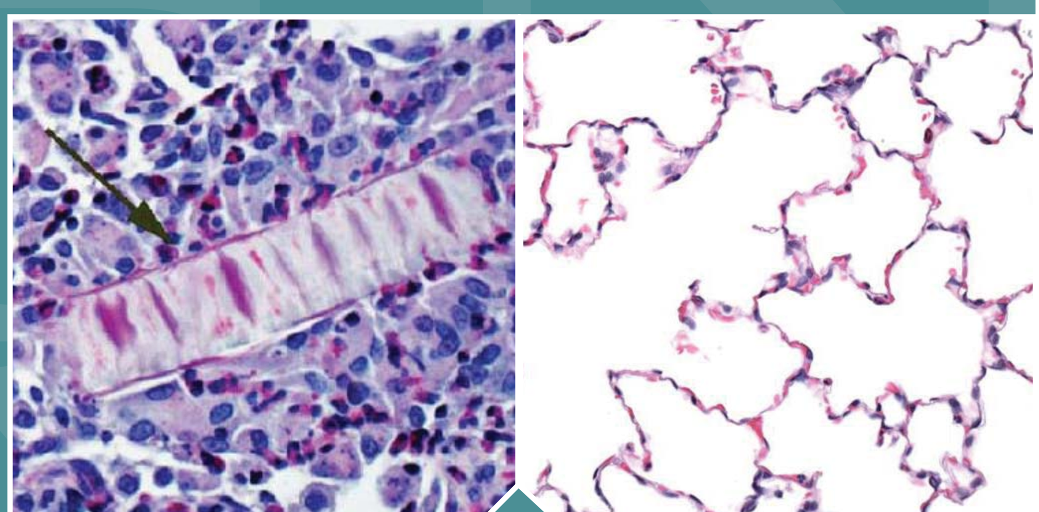
Multiple Daily Low-Dose *Bacillus anthracis* Ames Inhalation Exposures in the Rabbit



Office of Research and Development
National Homeland Security Research Center

EPA/600/R-11/145

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United States Environmental Protection Agency
Cincinnati, Ohio 45268

Disclaimer

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No official endorsement should be inferred. EPA does not endorse the purchase or sale of any commercial products or services.

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Acronyms and Abbreviations

ADD .... average daily dose per animal
ANOVA .... analysis of variance
APS .... Aerodynamic Particle Sizer
BBRC .... Battelle Biomedical Research Center
BMD .... benchmark dose
BMD_x .... benchmark dose at an x level of BMR
$BMDL_x$ .... lower confidence limit of the BMD at an x level of BMR
BMDS .... benchmark dose software
BMI .... Battelle Memorial Institute
BMR .... benchmark response
BMR_x .... benchmark response at an *x* level
BPM .... beats per minute
BL3 .... Biosafety Level 3
CBRNIAC ... Chemical, Biological, Radiological, and Nuclear Defense Information Analysis Center
CFU .... colony forming unit
cm .... centimeter
CRP .... C-reactive protein
C_t .... cycle threshold
dL .... deciliter
DNA .... deoxyribonucleic acid
DQA .... data quality audit
DR .... deviation report
ED50 .... effective dose 50%
EDTA .... ethylenediaminetetraacetic 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
F/T	freeze/thaw
g	gram
GSD	geometric standard deviation
HCT	hematocrit
HEC	human equivalent concentration
HED	human equivalent dose
Hg	mercury
HGB	hemoglobin
HRP	horseradish peroxidase
IgG	immunoglobulin G
InD	inhaled dose
IR	investigation report
kg	kilogram
L	liter
LD50	median lethal dose
LF	lethal factor
LOD	limit of detection
m3	cubic meter
µg	microgram
µL	microliter
µm	micrometer
mg	milligram
min	minute
mL	milliliter
mm	millimeter
MMAD	mass median aerodynamic diameter
MTT	3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide
NF50	neutralization factor 50%
ng	nanogram
nM	nanomolar

NHSRC	National Homeland Security Research Center
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NTC	no template control
NZW	New Zealand White
OD	optical density
PA	protective antigen
PBBK	physiologically based biokinetic model
PCR	polymerase chain reaction
PLT	platelet count
QAP	quality assurance plan
qPCR	quantitative real-time polymerase chain reaction
RBC	red blood cell
RCPM	respiratory cycles per minute
RDW	red cell distribution width
RNA	ribonucleic acid
RP	respiratory period
rPA	recombinant protective antigen
rpoB	DNA-directed ribonucleic acid polymerase subunit beta
SD	standard deviation
SST	serum separator tube
TAD	total aggregate dose
TATV	total accumulated tidal volume
TCAD	Threat and Consequence Assessment Division
TNA	toxin neutralization assay
TNTC	too numerous to count
TSA	tryptic soy agar
TSAT	technical systems audit
VAP	vascular access port
WBC	white blood cell
4PL	four-parameter logistic log

Acknowledgments

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Foreword

Following the events of September 11, 2001, 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 to carry 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 in preparing for and recovering 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

The U.S. Environmental Protection Agency (EPA), as one of the lead federal agencies supporting decontamination activities after a biological incident (U.S. DHS, 2008), has been systematically evaluating microbial dose-response data and its application for decision making to support decontamination activities. Site-specific risk-based decision making following a biological threat agent release poses extremely difficult and unique challenges, especially for a persistent agent such as *Bacillus anthracis*. Inhalation exposure risk from *B. anthracis* spores can result from aerosolized spores during a terrorist attack, subsequent re-aerosolized spores before cleanup takes place, or re-aerosolized residual spores after remediation is complete. The objective of this study was to evaluate physiological responses following 15 inhalation exposures (5 days a week for 3 weeks) to low doses of *B. anthracis* Ames spores representative of potential exposures that might be encountered in a reoccupancy/reuse scenario.

Three groups of seven New Zealand White (NZW) rabbits were implanted with telemetry transmitters and subsequently aerosol challenged with average daily inhaled doses of 2.91×10^2 to 1.18×10^4 colony forming units (CFU) of *B. anthracis* Ames spores. Five control rabbits also implanted with the telemetry transmitters were challenged with irradiated (nonviable) spores as sham challenge controls. The rabbits were then monitored for changes in nonspecific parameters: activity levels, body temperature, heart and respiration rates, hematology, and C-reactive protein. *Bacillus anthracis*-specific parameters were also measured and included bacteremia and toxemia as evidenced by the presence of protective antigen (PA, a polypeptide produced by *B. anthracis*) in the serum. All rabbits underwent necropsy, with the lungs and any gross lesions examined microscopically to identify anthrax-specific lesions. The challenge doses and mortality data were then used to identify a benchmark dose value for rabbits.

All seven rabbits exposed to a mean daily dose of 2.91×10^2 CFU survived to the end of the study and showed no physiological changes that could be attributed to the exposures. One of the seven rabbits exposed to a mean daily dose of 1.29×10^3 CFU died 17.9 days after the first

challenge. Four of the seven rabbits that received a mean daily dose of 1.18×10^4 CFU died during the study with a mean time to death of 14.8 days.

The rabbits that died on study presented with increased respiration rate, heart rate, body temperature, toxemia, and bacteremia. One animal in the highest dose group responded in the same physiological manner as those that died, but subsequently initiated a robust neutrophilic response, seroconverted (developed a humoral response to PA), and survived to the end of the study.

The calculated benchmark dose lower 95% confidence limit for 50% mortality ($BMDL_{50}$) for the average daily dose was 2.60×10^3 inhaled CFU, and the calculated BMDL for 10% mortality ($BMDL_{10}$) was 2.90×10^2 inhaled CFU. The calculated $BMDL_{50}$ and $BMDL_{10}$ for the total accumulated doses were, respectively, 4.40×10^4 and 4.90×10^3 total inhaled CFU.

These data represent the first characterization of multiple low-dose inhalation exposures of *B. anthracis* in an animal model.

1 Introduction

The U.S. Environmental Protection Agency (EPA), as one of the lead federal agencies supporting decontamination activities after a biological incident (U.S. DHS, 2008), has been systematically evaluating microbial dose-response data and its application for decision making to support decontamination activities. As part of the response to a biological incident, risk-based approaches to decontamination are desirable as they provide a formalized process to evaluate the hazard posed by the released material, assist in the identification of clearance goals, and facilitate assessment of the residual risk posed by selected management approaches. There is significant interest in the development of a risk-based management approach for *Bacillus anthracis* incidents because of its high lethality and prior use in a domestic terrorism event in 2001.

B. anthracis poses unique challenges in the site-specific risk assessment process because of the combination of high persistence and documented lethality at low inhalation doses. Inhalation exposure to *B. anthracis* spores can result from spores that aerosolize upon initial contact with the air as well as subsequent reaerosolization after settling on surfaces. Given the high lethality of *B. anthracis* spores from the inhalation route of exposure, the evaluation of clearance goals requires the ability to model the inhalation hazard posed by the low levels of spores that may remain on surfaces subsequent to decontamination.

It has been over 10 years since the anthrax letter attacks of 2001 and the lack of an acceptable dose-response relationship continues to challenge the development of risk-based management approaches. Although *B. anthracis* is the most highly studied of the currently known biothreat agents (Wilkening, 2006), there are significant data gaps in the dose-response assessment of low dose exposures (Gutting et al., 2008). The primary data gap is the lack of dose-response data suitable for modeling multiple dose exposures in the low dose-region. For use in risk-based decision making, studies must assess multiple doses in a manner that is consistent with the recurring exposure pattern of receptors who will reoccupy the locations of the biological incident.

In view of the lack of historical low-dose exposure studies and the critical need for credible science to support risk-based cleanup decisions, U.S. EPA (2011) conducted an acute low-exposure study (hereafter referred to as acute study). This study aimed 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. In the acute low-exposure study, four groups of five NZW rabbits were implanted with D70-PCT telemetry transmitters and subsequently aerosol challenged with a single average inhaled dose of 2.86×10^2, 2.06×10^3, 2.45×10^4, or 2.75×10^5 CFU. The rabbits were then monitored for 21 days post-challenge for changes in nonspecific 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.

In the acute study, four of the five rabbits that received an average single 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 in the acute study were bacteremic and 73% were positive for PA in serum. Increases in respiration, heart rate, and body temperature were also observed in rabbits that succumbed to anthrax. In addition, neutrophilia and increased liver enzymes in the sera were associated with disease. Animals that survived to the end of the study never became bacteremic or toxemic.

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

While the acute study determined the physiological effects of a single low dose exposure, the effects of repeated exposures, such as would be encountered in a reoccupancy/reuse scenario, remained unknown. To fill this knowledge gap, using the acute study as a guide, the follow-on study described in this report was performed to determine physiological changes arising during and after 3 weeks of exposure to sublethal doses of *B. anthracis* spores.

2 Materials and Methods

2.1 Test System

The protocol for the study, along with the methods referred to herein, are provided in Appendix A. All study deviations are documented in Appendix B. Thirty male specific pathogen-free NZW rabbits (*Oryctolagus cuniculus*) weighing approximately 2.7 kilograms (kg) were purchased from Covance (Denver, PA) (Appendix C identifies the pathogen list for testing). Twenty-six rabbits were placed on study and the remaining four served as replacements. Rabbits were quarantined for 5 days prior to exposure. The study was performed at the Battelle Biomedical Research Center (BBRC) located in West Jefferson, OH. A veterinarian implanted a Data Sciences International (St. Paul, MN) model D70-PCT telemetric device and vascular access ports (VAPs) in each of the rabbits prior to 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 any potential correlation with active *B. bronchiseptica* infection and response in this study. All animals were negative for *B. bronchiseptica* infection; the results of the testing are presented in Appendix C. *B bronchiseptica* status was not a criterion for rabbit placement on the study.

2.2 Randomization of Animals

Prior to challenge, the animals were randomized by weight into one group of five and three groups of seven rabbits (Table 1). The rabbits within each group were randomized for challenge order based on ear tag numbers. 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 4 were challenged last. The randomization report is located in Appendix D.

Table 1. Study Design and Challenge Doses

Group	Targeted Inhaled Spore Dose (CFU)	Number of Spore Challenges†	Number of Rabbits
1 (Sham challenge*)	10,000*	15	5
2	100	15	7
3	1,000	15	7
4	10,000	15	7

*Spores were inactivated/killed by irradiation.
†Rabbits were challenged once each day for five straight days (Monday through Friday) each week for three consecutive weeks.

2.3 *Bacillus anthracis* Ames Strain Spores

B. anthracis Ames strain spores (spore lot Ames B36) 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%	0.34%
% Spore refractility	≤ 5% nonrefractile spore	0.72% nonrefractile spore
Viable spore count	≥ 1 $x10^9$ CFU/mL	1.82 x 10^{10} CFU/mL
Guinea pig LD_{50}	< 10 spores/dose intradermal	2.49 spores/dose intradermal
Endotoxin content	< 1.0 EU/mL	0.14 EU/mL
Phenol content	0.8–1.2%	0.83%

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

On each of the 15 challenge days, 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 or 1.0×10^4 CFU of *B. anthracis* spores (Table 1). The challenge dose was controlled by the concentration of spores in the nebulizer and the length or exposure time. The sham challenge group was exposed to 1.0×10^4 gamma-irradiated spores as described below.

The volume of material loaded onto the nebulizer was the same for each concentration, 8 mL loaded. The dose was controlled by varying the concentration per mL in the nebulizer which produced the resulting aerosol concentration. For example, log increases in the nebulizer concentration will result in a log increase in the aerosol concentration (CFU/L) which results in the log difference in the dose when the same volume (TATV) of atmosphere is inhaled.

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. This nebulizer was designed to generate aerosols with an approximate aerodynamic mean diameter of 1 to 2 micrometers (µm). The nebulizer was characterized for a pressure that results in approximately 7.5 liters/minute (L/min) flow, which normally is approximately 28.0 pounds per square inch, Collison nebulizer dependent.

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 using an impinger (model 7541; Ace Glass Inc., Vineland, 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. The impinger flow rates were recorded throughout the exposure and the mean rate was used in the dose calculation. 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., Shoreview, 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 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 a sealed plethysmographic chamber. The TATV and the aerosol concentration were 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., Shawnee, KS) that was attached to a differential pressure transducer (model DP-45; Validyne Engineering Corp., Northridge, CA). Pressure differential measurements from inhalations and exhalations were transmitted to BioSystem XA version 1.5.7 software (BioSystem 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 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 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 1.05×10^5 inhaled CFU/animal (Zaucha et al., 1998).

Impinger samples were enumerated by the serial dilution (10^{-1} to 10^{-3}) and plating on tryptic soy agar [TSA] plates in triplicate. 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 no. 145-0045; Fisher Scientific, Pittsburgh, PA). The filter then 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 without diluting (0.1 mL) 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 1.

(1)

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 time (Equation 2). This equation assumes near 100% impinger sampling efficiency. The total number of viable CFU captured during each exposure was the product of the impinger concentration (C) and the impinger sampler volume (V). The total number of viable CFU 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 as the product of the aerosol concentration and the TATV.

(2)

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).

Additional details of the aerosol exposure system and a detailed schematic are found in Appendix E.

2.5 Telemetric Monitoring

The rabbits were surgically implanted with telemetry units (model D70-PCT transmitters) 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 7 days prechallenge (baseline) and for 39 days post-first challenge.

Each animal's cage was equipped with a Data Sciences International telemetry receiver. The transmitters, receivers, consolidation matrices, 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.

2.6 Clinical Observations and Body Weights

Throughout the study, the rabbits were observed twice daily for survivability and clinical signs of illness that could be attributable to anthrax infection (e.g., moribund, respiratory distress, appetite, activity, and seizures). Animals were weighed on Study Days 2, 9, 16, 23, 30, and 37. The statistical methods used to analyze the survival data and body weights are described in Appendices G and H, respectively. Individual clinical observations and body weights are presented in Appendices K and L, respectively.

2.7 Blood Collection

On Study Days -3, 2, 4, 9, 11, 16, 18, 23, 25, 30, 32, and 37, blood was collected into ethylene- diaminetetraacetic acid (EDTA; ~1.0 mL) tubes and serum separator tubes (SSTs; ~2.0 to 2.5 mL) (Table 3). Blood samples also were taken from animals found dead or prior to euthanasia. On Study Day 39, all surviving rabbits were terminally bled via cardiac puncture according to Table 3.

Table 3. Blood Collection Schedule

	Study Day												
Tube Type	**Day -3**	**Day 2**	**Day 4**	**Day 9**	**Day 11**	**Day 16**	**Day 18**	**Day 23**	**Day 25**	**Day 30**	**Day 32**	**Day 37**	**Day 39**
EDTA (~mL)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	2.0
SST (~mL)	2.5	2.0	2.5	2.0	2.5	2.0	2.5	2.0	2.5	2.0	2.5	2.0	10.0
Total per day (~mL)	3.5	3.0	3.5	3.0	3.5	3.0	3.5	3.0	3.5	3.0	3.5	3.0	12.0

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 4 hours of collection; blood was stored at 2°C through 8°C if not analyzed within 4 hours. Blood was collected from the VAP throughout the study. If a VAP failed, the medial auricular artery or the marginal ear vein was used for blood collection regardless of the sample time point. If a blood sample was not collected from either the VAP or other appropriate vessel, it was documented in the study file. Appendix I contains the exact blood collection times.

2.8 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 using 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 (Catalog 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 Biotechnology, Inc., 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 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.

2.9 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). 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 1.0 x 10^1 to 1.0 x 10^9 and spread plating 100 microliters (µL) of each dilution onto 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 morphology consistent 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 nonfluorescent 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. 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.

2.10 TNA/ELISA

To determine if the rabbits elicited an immune response following challenge, serum samples were analyzed by an anti-PA IgG ELISA and high-throughput toxin neutralization assay (TNA) as described below. The ELISA was designed to quantify IgG antibodies against anthrax 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 were 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 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 serummediated neutralization of anthrax lethal toxin manifested as a suppression of cytotoxicity, and hence preservation of cell viability.

Microtiter cell plates were seeded with a murine monocyte-macrophage cell line (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 (lethal factor [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 the ELx800 microplate reader at a wavelength of 570 nanometers using a 690 nanometer reference wavelength. The TNA Statistical Analysis System (SAS®; SAS Institute Inc., Cary, NC) 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}]). The ED_{50} was the reciprocal of the dilution of a serum sample that results in 50% neutralization of the lethal toxin. The NF_{50} is the quotient of the ED_{50} of the test sample and the ED_{50} of the reference serum standard. The NF_{50} was calculated to determine the neutralization capacity of the test sample relative to the reference serum standard on that plate, thus normalizing day-to-day assay variability.

2.11 Hematology and C-Reactive Protein

Complete hematological analysis was performed on blood samples collected in EDTA tubes using the Advia® 120 hematology analyzer (Siemens Healthcare Diagnostics, Deerfield, IL) according to the 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 (RBC) 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 J.

After hematological analysis was complete, plasma was harvested from the residual sample by centrifugation. The plasma sample was then assayed for C-reactive protein (CRP) levels using the Advia® 1200 chemistry analyzer (Siemens Healthcare Diagnostics, Deerfield, IL) according to the manufacturer's recommendations. The statistical methods used to analyze the CRP data are described in Appendix J.

2.12 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 39. The lungs and gross lesions from each animal were collected. The tissues collected for microscopic evaluation varied from animal to animal and included skin, cecum, appendix, and mediastinal lymph node. The 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.

2.13 Benchmark Dose Analysis and Dosimetric Adjustment

A benchmark dose (BMD) analysis was conducted using the survival data collected in this study (challenge doses and mortality). The outputs of the BMD analysis were then used as the inputs for a dosimetric adjustment to derive human equivalent dose (HED) and human equivalent concentration (HEC) values.

Two dose metrics of inhaled dose were evaluated in the benchmark dose analysis: the average daily dose per animal (ADD) and the total aggregate dose per animal (TAD). For the ADD, daily inhaled doses were averaged across all exposure and non-dosing days until the death of the animal or the exposure duration for those animals that survived the length of the study. The exposure duration of the study was 19 days, which captures the total number of study days including days to allow for calculation of an ADD consistent with EPA guidance for discontinuous exposures (U.S. EPA, 2002). For the TAD, daily inhaled doses were summed across all exposure days until the death of the animal or the exposure duration for those animals that survived the length of the study.

For the BMD evaluation, the current version of EPA's benchmark dose software (BMDS 2.1.2 Version 2.1.2.60, Build 06/11/10) (U.S. EPA, 2010a) was used to fit models to the dose-response data. Models from the BMDS dichotomous and dichotomous-alternative model suites were evaluated in the analysis: Weibull model, Weibull model run as exponential (with the power coefficient fixed as one), probit, $\log_e$ probit, logistic, $\log_e$ logistic, Gamma model, dichotomous Hill, probit-background response, $\log_e$ probit-background response, logistic-background response, and $\log_e$ logistic-background response. Mortality data were modeled on an individual basis using each estimated dose (i.e., with n=1 at each dose).

Benchmark dose analysis estimates the BMD for a specified level of benchmark response (BMR) observed. The BMR is defined as the level of change in the response rate (in this case mortality). For example, a BMR of 10% would be equivalent to a 10% response rate of the endpoint of interest. For this assessment, BMRs of 0.50, 0.10, and 0.01 were reported to allow for comparison of different model estimates at various points in the dose-response relationship. When used as inputs in the calculation of BMDs, these BMR values correspond to estimates of 50% lethality (i.e., LD_{50}), 10% lethality, and 1% lethality; the resulting BMDs would be written BMD_{50}, BMD_{10}, and BMD_{01}, respectively. The 95% lower confidence limit of the calculated BMD is the benchmark dose limit (BMDL).

A dosimetric adjustment was conducted using the assumptions identified in Table 5 and the ADD $BMDL_{10}$ value calculated using the best fitting mathematical model identified during the benchmark dose analysis. As part of this adjustment, assumptions were identified for the human inhalation rate, the rabbit pulmonary deposition rate, and human pulmonary deposition rate. With the exception of generating a particle size distribution-specific pulmonary deposition rate using the Regionally Deposited Dose Ratio (RDDR) Model (U.S. EPA, 1994), the approach to calculate the HED and HEC followed that presented in U.S. EPA (2010b).

Table 5. Assumptions Used to Generate Human Equivalent Dose and Human Equivalent Concentration

Parameter	Value	Units	Source
Rabbit Pulmonary Deposition Rate	0.056	Unitless	Value calculated with RDDR Model v. 2.3 (U.S. EPA, 1994) with Inputs of: • MMAD = 0.82 µm and GSD = 1.53 (Data Source: Figure 3, Appendix E, Aerosol Report), • Body Weight of 2,850 g (Data Source: Appendix L, Individual Body Weights, Arithmetic Average of Body Weight on Days 2, 9, and 16), and • Minute Volume of 1.3 L (Data Source: Arithmetic Average of Calculated Minute Volume, Product of Tidal Volume Inhaled and Sampling Time from Table 17, Table 24, and Table 30 [i.e., Days 2, 9, and 15]).
Human Inhalation Rate	16	m^3/day	31 to <51 Years of Age, Mean Value (Table 6-1 in U.S. EPA 2009).
Human Deposition Rate	0.2	Unitless	Higher End of the Range of Human Depositional Values for 1 to 2 µm particles (Figure 6-6, U.S. EPA 2004).

g - gram
GSD - geometric standard deviation
L - liter
MMAD – median aerodynamic diameter
µm - micron

The complete methodology used in the benchmark analysis and dosimetric adjustment is provided in Appendix V.

3 Results

3.1 Aerosol Challenges

To determine the rabbits' physiological responses to multiple, daily, low-dose aerosol exposures to *B. anthracis* spores, three groups of seven rabbits were exposed to targeted inhaled doses of 1.0×10^2 to 1.0×10^4 CFU. The individual mean challenge doses for the 15 challenge days as well as the group means are listed in Table 6. Figure 1 illustrates the group mean challenge daily doses over the 15 challenge days. All challenge days had consistent dosing except Challenge Day 3 where the challenge dose of Group 2 was higher than expected. This was most likely caused by an error in the dilution of the challenge material. Plate counts of the impinger samples revealed that individual mean actual inhaled doses for the 15 days of challenge ranged from 2.32×10^2 ($\pm 1.28 \times 10^1$) CFU to 1.44×10^4 ($\pm 5.99 \times 10^3$) CFU. The mass median aerodynamic diameter (MMAD) for challenge material for each group as determined by an APS is presented in Table 6 and Appendix E, Figure 3. Details of the aerosol challenge data are contained in Appendix E.

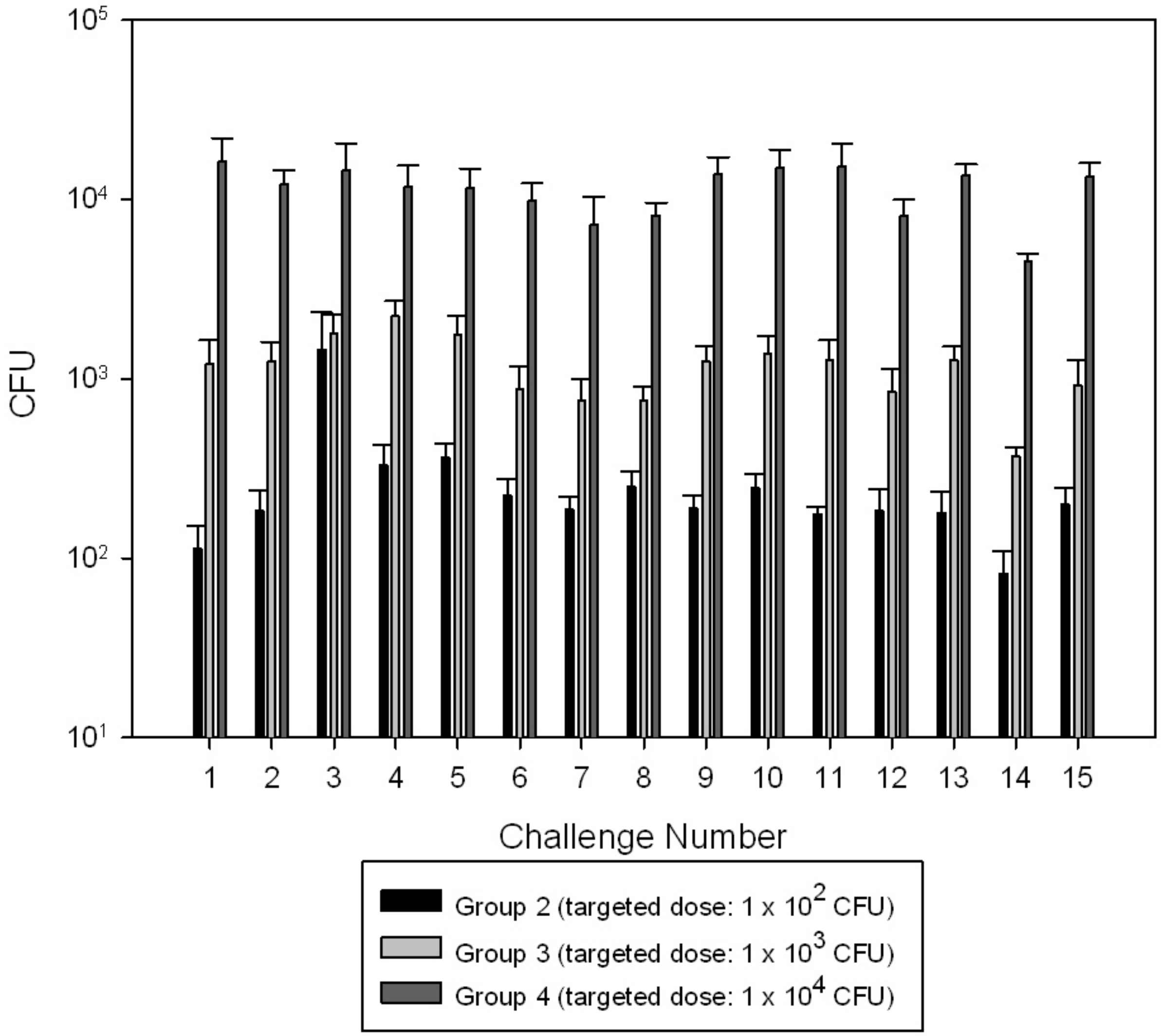

Figure 1. Mean challenge doses in CFU for each of the 15 exposure days.

Table 6. Individual and Group Mean Challenge Doses over the 15 Exposure Days

Group	Animal ID	Daily Mean Inhaled Dose (CFU/Animal)		Group Mean Inhaled Dose (CFU/Animal SD)	Challenge Dose (LD_{50})*		Group Mean Challenge Dose (LD_{50})	MMAD in µm (GSD)	Time to Death (day)
		Mean	SD		Mean	SD			
Group 1	40	0	0	0	0	0	0	0.81 (1.53)	Survived
	7	0	0		0	0			Survived
	5	0	0		0	0			Survived
	9	0	0		0	0			Survived
	37	0	0		0	0			Survived
Group 2	13	3.85×10^2	7.57×10^2	2.91×10^2 (3.88×10^2)	8.16×10^{-1}	7.22×10^{-3}	2.77×10^{-3} (3.70×10^{-3})	0.79 (1.52)	Survived
	34	3.17×10^2	4.48×10^2		2.13	4.27×10^{-3}			Survived
	25	2.79×10^2	3.54×10^2		1.56	3.38×10^{-3}			Survived
	15	3.17×10^2	3.27×10^2		9.40×10^{-1}	3.11×10^{-3}			Survived
	30	2.72×10^2	2.33×10^2		1.88	2.22×10^{-3}			Survived
	28	2.34×10^2	1.49×10^2		1.75	1.42×10^{-3}			Survived
	19	2.32×10^2	1.28×10^2		1.19	1.22×10^{-3}			Survived
Group 3	14	7.38×10^2	2.99×10^2	1.22×10^3 (5.59×10^2)	8.82×10^{-1}	2.85×10^{-3}	1.16×10^{-2} (5.33×10^{-3})	0.82 (1.53)	Survived
	11	1.12×10^3	5.01×10^2		6.98×10^{-1}	4.77×10^{-3}			Survived
	2	1.33×10^3	5.95×10^2		1.37×10^{-1}	5.50×10^{-3}			17.9
	8	1.41×10^3	6.06×10^2		5.12×10^{-1}	5.76×10^{-3}			Survived
	12	1.30×10^3	4.90×10^2		7.62×10^{-1}	4.67×10^{-3}			Survived
	18	1.21×10^3	5.47×10^2		1.14	5.30×10^{-3}			Survived
	32	1.44×10^3	5.92×10^2		2.02	1.78×10^{-2}			Survived
Group 4	6	6.41×10^3	2.57×10^3	1.17×10^4 4.64×10^3	6.55×10^{-1}	2.44×10^{-2}	1.12×10^{-1} (4.43×10^{-2})	0.86 (1.49)	10.9
	33	9.75×10^3	2.58×10^3		3.08E+00	2.48×10^{-2}			12.7
	27	1.06×10^4	3.51×10^3		1.90E+00	3.48×10^{-2}			20.8
	31	1.25×10^4	3.27×10^3		2.69E+00	3.13×10^{-2}			14.7
	39	1.44×10^4	5.99×10^3		2.57E+00	5.70×10^{-2}			Survived
	21	1.32×10^4	4.97×10^3		1.43E+00	4.74×10^{-2}			Survived
	38	1.27×10^4	3.77×10^3		2.49E+00	3.60×10^{-2}			Survived

*$LD_{50} = 1.05 \times 10^5$ CFU (Source: Zaucha et al., 1998)
SD = standard deviation
GSD = geometric standard deviation

3.2 Clinical Observations, Body Weights, and Mortality

The majority of 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. One rabbit, Rabbit 33 (Group 4), was normal up to the time that it was found dead. Interestingly, Rabbit 38 (Group 4) showed clinical signs of disease including lethargy, anorexia, and respiratory abnormalities on Study Days 22–27 but returned to normal on Study Day 28 and survived to the end of the study. A complete list of individual clinical observations is presented in Appendix K.

Body weights were taken periodically over the course of the study as another indicator of disease. The body weights of the study rabbits remained consistent throughout the study. Individual body weights and statistical analysis are provided in Appendices L and H, respectively.

All of the rabbits in Groups 1 and 2 survived until the end of the study (Figure 2). One of the seven Group 3 animals (Rabbit 2) died 17.9 days after the first exposure. This animal received 14 of the 15 challenge doses and received an accumulated challenge dose of 1.86×10^4 CFUs over the course of the study. Four of the seven Group 4 rabbits succumbed to disease with a mean time to death of 14.80 ± 4.28 days. Table 7 shows the number of challenge doses and accumulated dose for each of the rabbits that succumbed to disease.

Table 7. Accumulated Challenge Dose Information for the Rabbits That Succumbed to Infection

1.1 Rabbit ID	Group	Number of Challenge Doses	Accumulated Dose	Time to Death (days)
2	3	14	1.86×10^4	17.9
6	4	9	5.77×10^4	10.9
33	4	10	9.75×10^4	12.7
27	4	15	1.51×10^5	20.8
31	4	11	1.37×10^5	14.7

The overall Fisher's exact test on the mortality was significant ($P = 0.0425$); however, there were no significant pairwise differences between the groups. An overall log-rank test was significant ($P = 0.0135$), indicating 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 comparisons, the time to death in Group 2 was significantly greater than that in Group 4. However, this relationship was no longer significant after adjusting for the multiple pairwise comparisons.

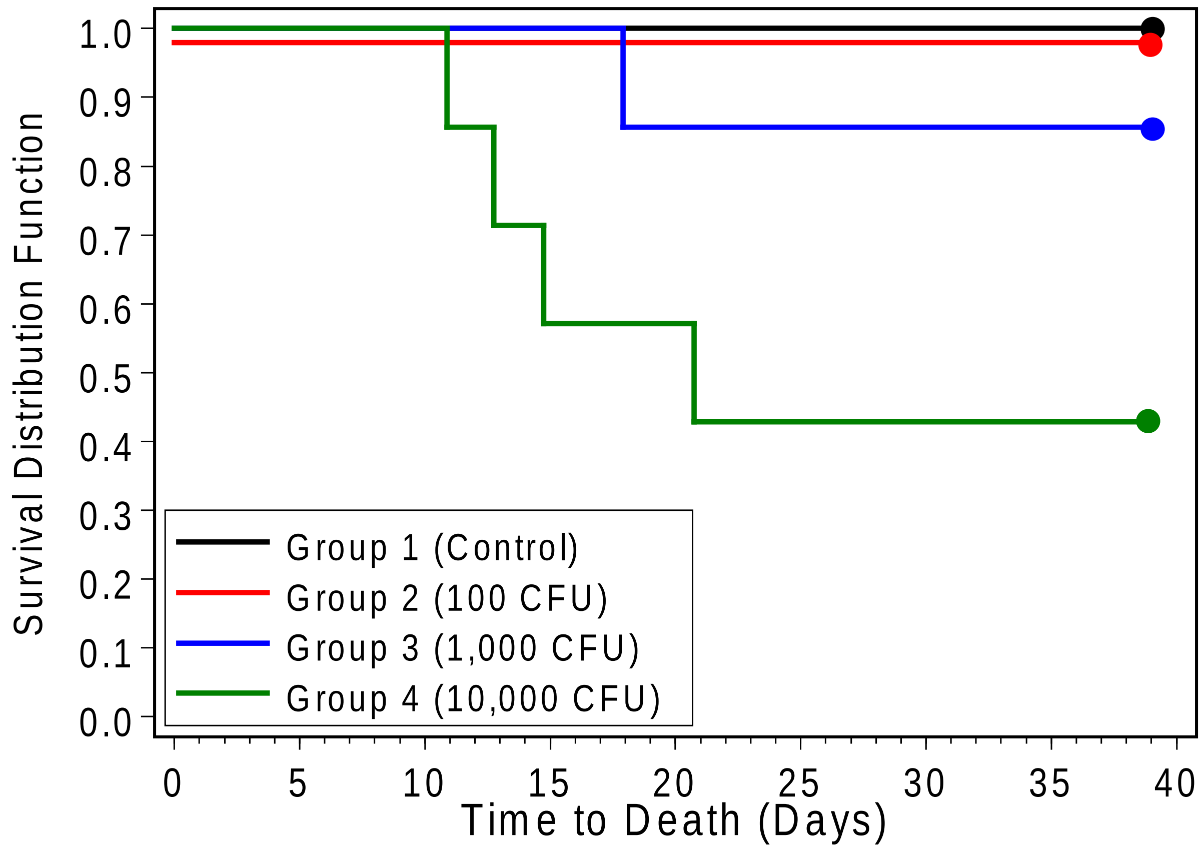

Figure 2. Kaplan-Meier curves representing time to death and survival data for each group.

A logistic regression model was fitted to the survival data and 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 -1.30 (P = 0.0288). The estimated accumulated inhaled dose LD_{50} was 8.1 x 10^3 CFU with a 95% Fieller confidence interval ranging from 2.3 x 10^3 CFU to 3.6 x 10^7 CFU. Individual mortality data are located in Appendix M, and complete statistical analysis can be found in Appendix G.

3.3 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 6-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). The standard deviation of each 6-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 6-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 last 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.

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 8 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. In addition, Table 8 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. The complete statistical analysis of the telemetry data is located in Appendix F.

Table 8. 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 Abnormality (Days)*	Fisher's Group Effect P-Value
Activity	1	2/5	0.40 (0.05, 0.85)	15.51	0.5161
	2	2/7	0.29 (0.04, 0.71)	7.38	
	3	3/7	0.43 (0.10, 0.82)	11.42	
	4	5/7	0.71 (0.29, 0.96)	6.00	
Heart Rate	1	5/5	1.00 (0.48, 1.00)	16.75	0.2855
	2	5/7	0.71 (0.29, 0.96)	19.10	
	3	7/7	1.00 (0.59, 1.00)	6.82	
	4	5/7	0.71 (0.29, 0.96)	7.10	
Respiratory Rate	1	3/5	0.60 (0.15, 0.95)	30.58	0.2096
	2	7/7	1.00 (0.59, 1.00)	22.11	
	3	4/7	0.57 (0.18, 0.90)	6.44	
	4	4/7	0.57 (0.18, 0.90)	7.44	
Temperature	1	3/5	0.6 (0.15, 0.95)	11.17	0.5542
	2	6/7	0.86 (0.42, 1.00)	23.42	
	3	4/7	0.57 (0.18, 0.90)	10.81	
	4	6/7	0.86 (0.42, 1.00)	12.08	

*Means exclude those animals that were never abnormal

Figure 3 illustrates the mean activity levels of the groups after challenge. Figure 4 shows the activity levels for each animal on study and highlights the variability within each group. By Study Day 8, all groups had experienced a significant decrease from baseline. This significant decrease from baseline activity continued intermittently in each group until Study Day 23, but was more prevalent in Group 4. On Study Day 17 at 6:00–12:00 and on Study Day 18 at 18:00–midnight, the mean decrease from baseline in Group 4 was significantly different from the mean change from baseline activity in Group 1. On Study Day 19 at 12:00–18:00, on Study Day 20 at midnight–6:00, 6:00–12:00, and 18:00–midnight, on Study Day 21 at midnight–6:00, on Study Day 24 at midnight–6:00, and on Study Day 37 midnight–6:00, the mean decrease from baseline activity in Group 4 was significantly different from the mean change from baseline in Group 2. On Study Day 37 at midnight–6:00, the mean decrease from baseline in Group 4 was significantly greater than that in Group 3.

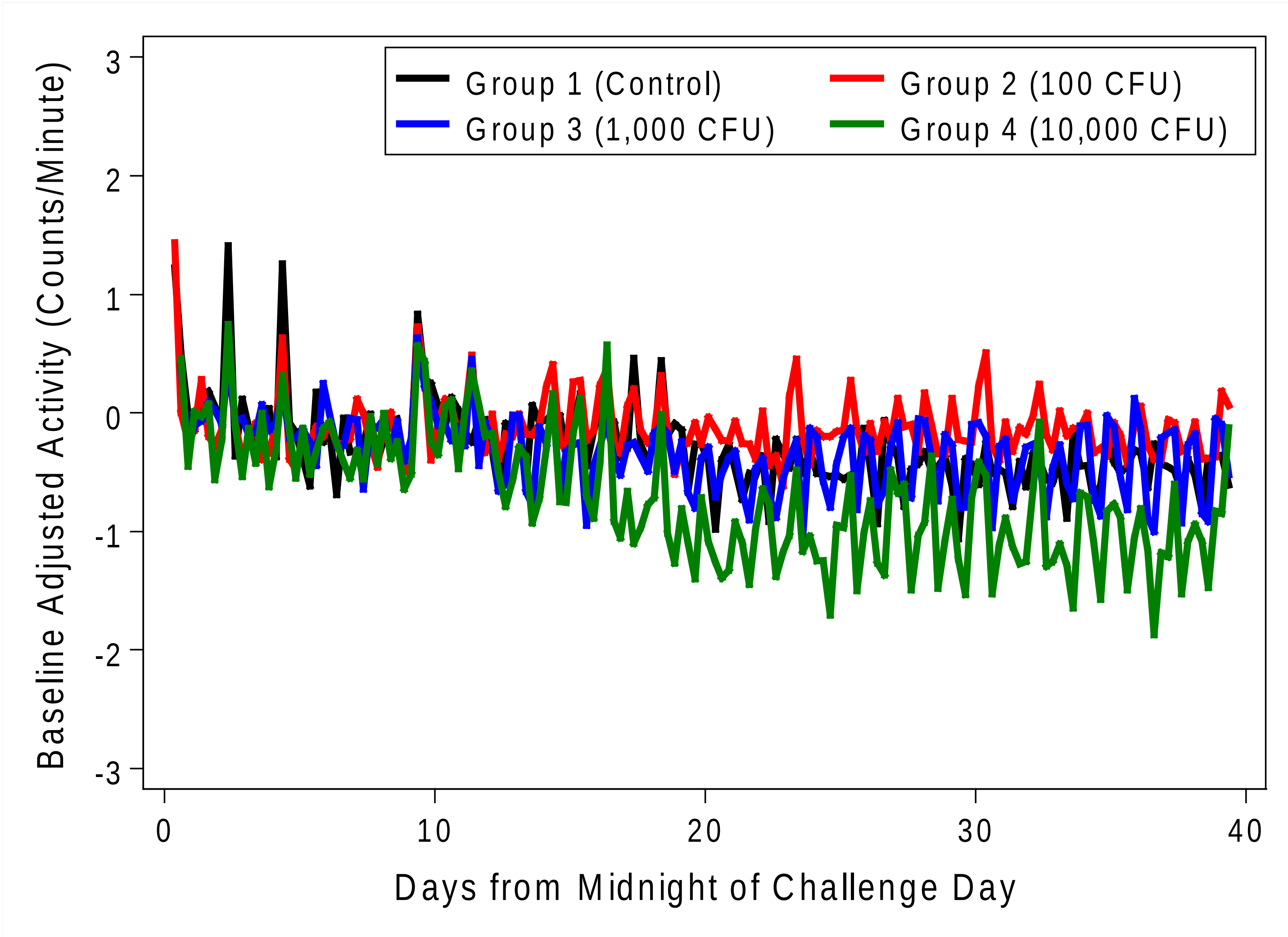
Group 1 (Control)
Group 2 (100 CFU)
Group 3 (1,000 CFU)
Group 4 (10,000 CFU)
Baseline Adjusted Activity (Counts/Minute)
3
2
1
0
-1
-2
-3
0
10
20
30
40
Days from Midnight of Challenge Day

Figure 3. Plot of mean baseline-adjusted activity (counts/min) for each group.

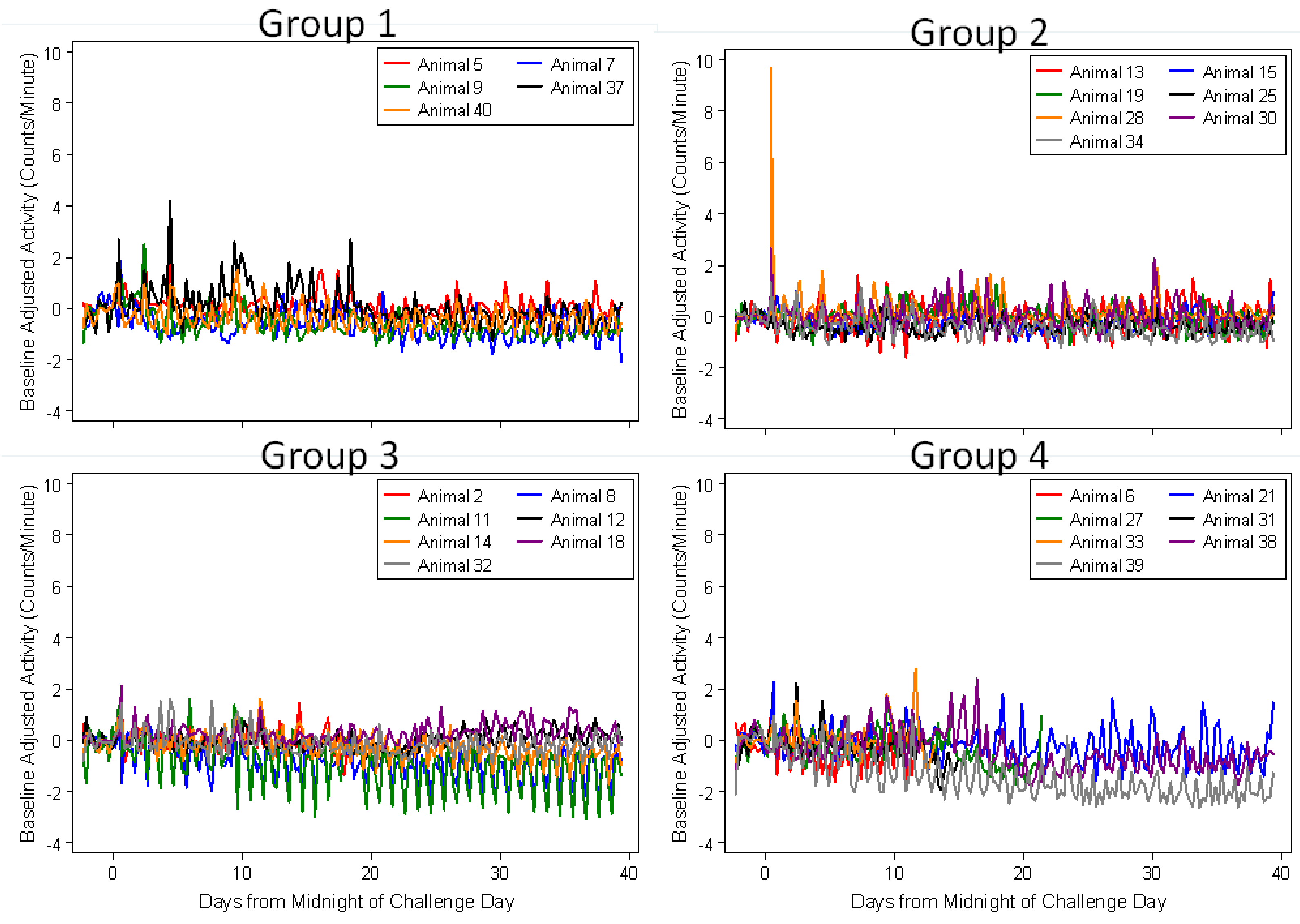

Figure 4. Plot of baseline-adjusted activity (counts/min) values for each rabbit.

Figure 5 illustrates the changes in heart rate in beats per minute (BPM) by group mean over the course of the study. Figure 6 shows the heart rate of each rabbit over the course of the study. By Study Day 1 at 12:00–18:00, all groups had experienced significant increases in heart rate from baseline. These significant increases continued intermittently for all groups until Study Day 5 at 6:00–12:00. By Study Day 6 at 12:00–18:00, all groups had experienced a significant decrease from baseline. The significant decreases in heart rate continued intermittently and with increasing frequency until the end of the study, with more prevalence in Groups 1 through 3 after Study Day 24. On Study Day 24 at 6:00–12:00 and 12:00–18:00, the decrease from baseline in Group 1 was significantly different from that in Groups 2, 3, and 4.

Figure 7 illustrates the mean respiratory rates of the groups after challenge in respiratory cycles per minute (RCPM). Figure 8 shows the respiratory rates for each rabbit over the course of the study. Each rabbit that succumbed to disease showed increased respiration rates. Animals that died are indicated by truncated data lines in Figure 8. Interestingly, Rabbit 38 showed an increase in respiration rates from approximately Day 21 to Day 26, which corresponds with the time frame in which the animal was bacteremic, neutrophilic, and toxemic.

By Study Day 1 at 12:00–18:00, all groups had experienced a significant increase in respiration rates from baseline. These significant increases from baseline continued intermittently throughout the study. In Groups 2 and 3, these significant increases were more prevalent especially after Study Day 15 through the end of the study. Group 4 was the only group that experienced significant decreases in respiration rates from baseline, which occurred on Study Day 5 at 6:00–12:00 and on Study Day 6 at 6:00–12:00. On Study Day 4 at midnight–6:00 the mean increase from baseline in Group 1 was significantly different from the change from baseline in Groups 2 and 3. In addition, on Study Day 4 at midnight–6:00, Study Day 5 at 6:00–12:00 and 12:00–18:00, Study Day 6 at 6:00–12:00, and Study Day 10 at midnight–6:00, the mean decrease from baseline respiration rate in Group 4 was significantly different from the change from baseline in Group 1. On Study Day 2 at 6:00–12:00, the mean increase from baseline in Group 2 was significantly different from the change from baseline in Group 3. On Study Day 13 at midnight–6:00, the mean increase from baseline in Group 4 was significantly different from the change from baseline in Group 2. On Study Day 1 at 18:00–midnight and on Study Day 5 at 6:00–12:00, the mean increase from baseline in Group 3 was significantly different from the change from baseline in Group 4.

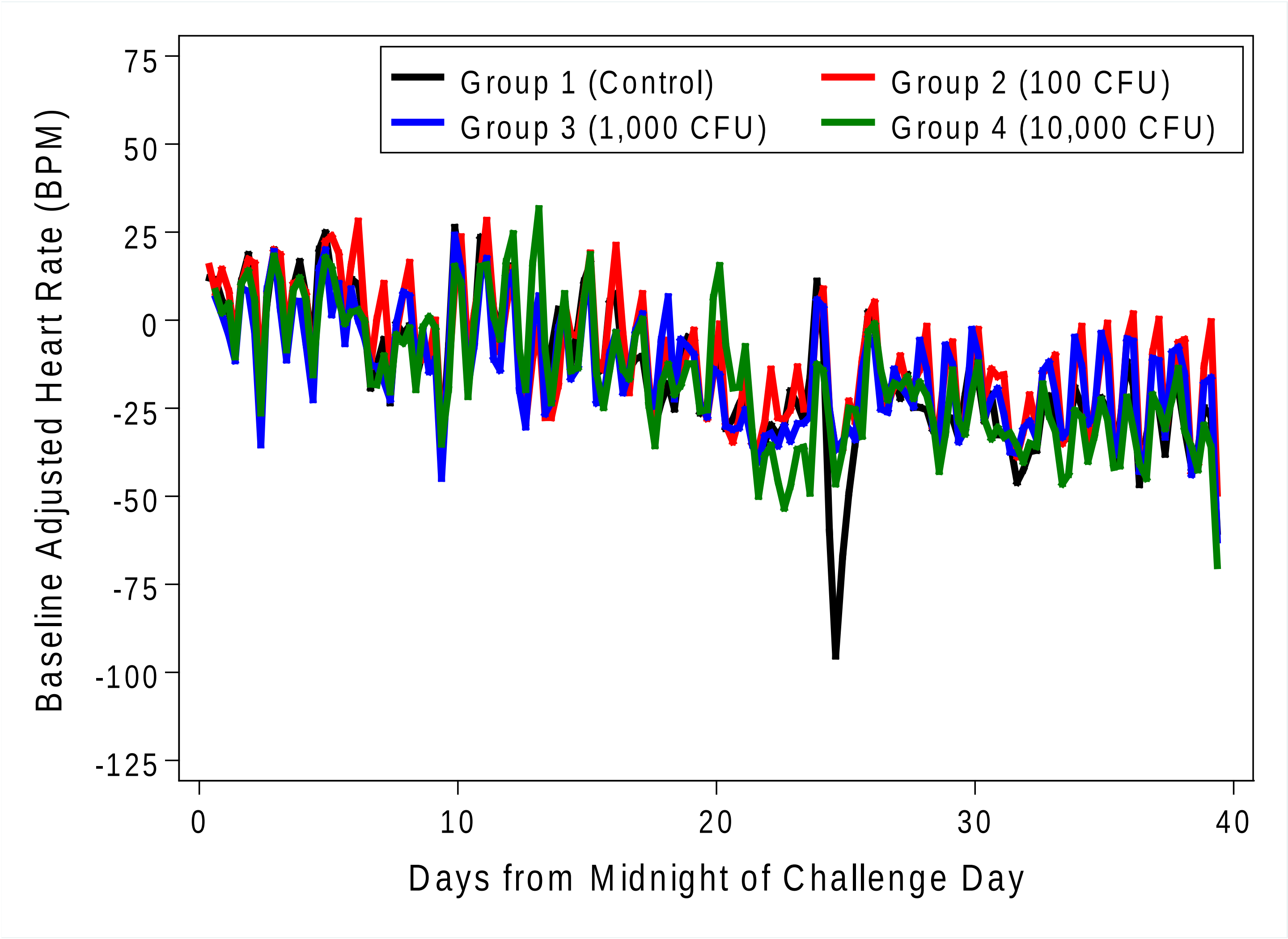

Figure 5. Plot of mean baseline-adjusted heart rate (BPM) for each group.

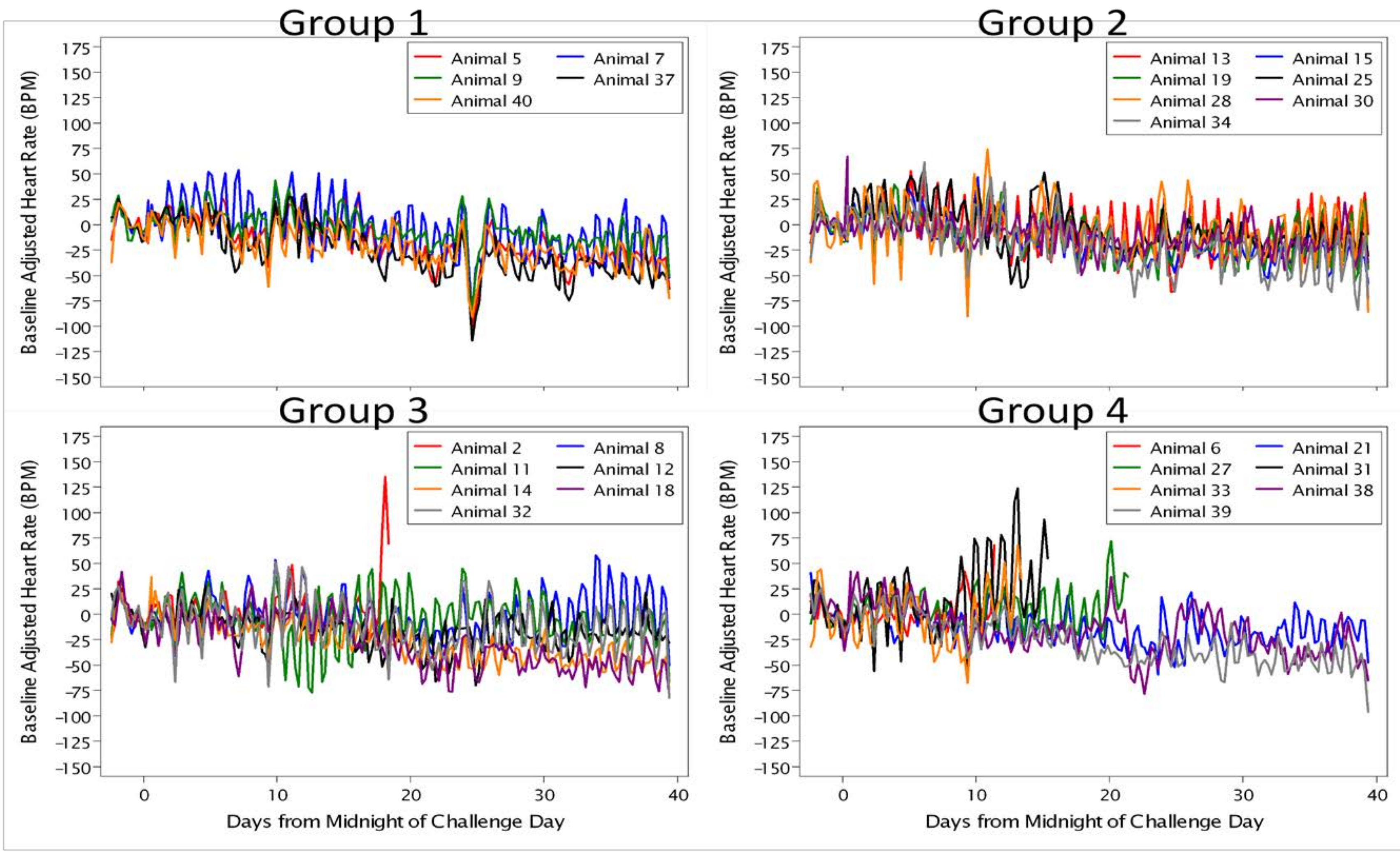

Figure 6. Plot of baseline-adjusted heart rate (BPM) for each rabbit.

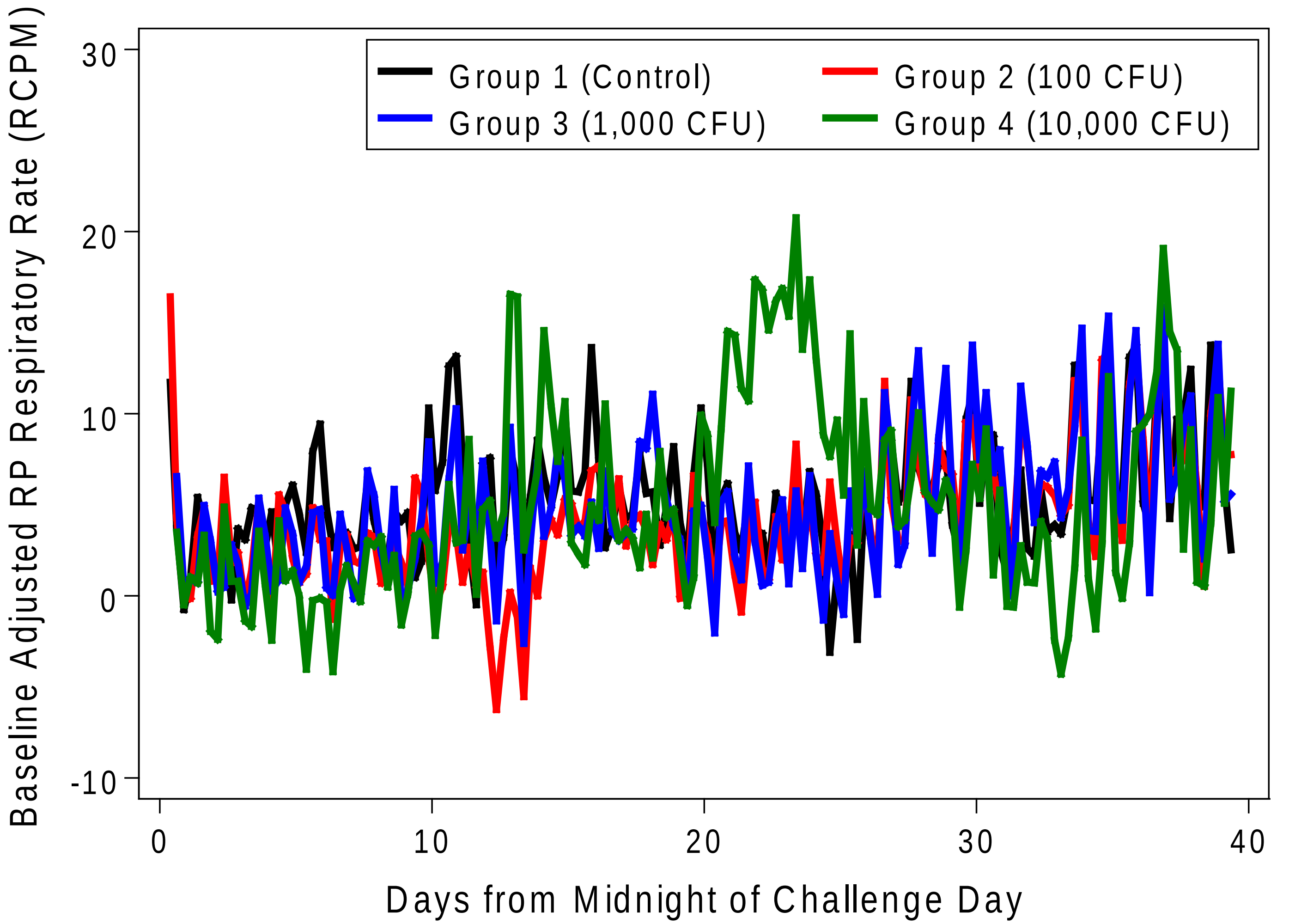

Figure 7. Plot of mean baseline-adjusted respiratory period (RP) respiratory rate (in RCPM) for each group.

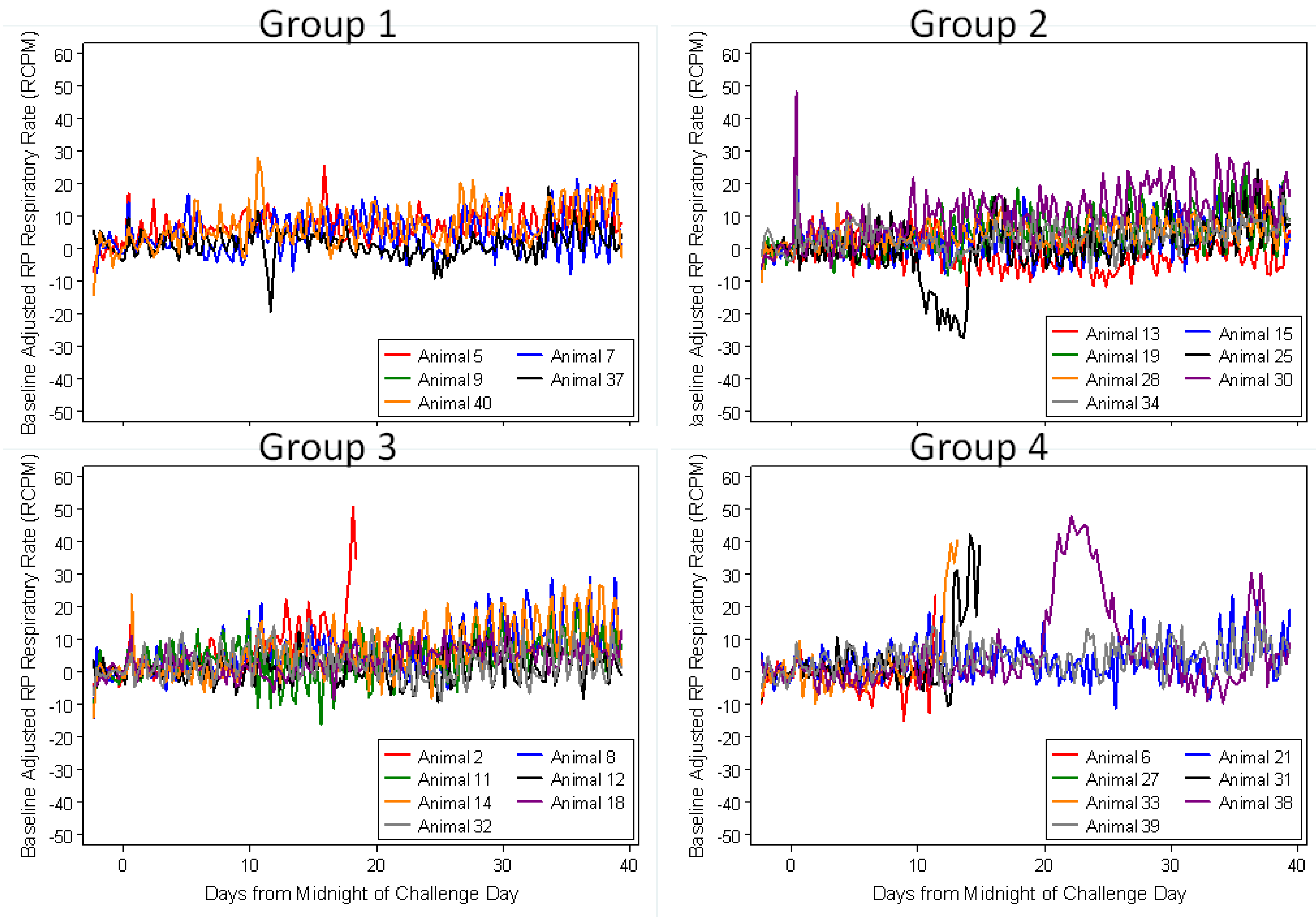

Figure 8. Plot of baseline-adjusted RP respiratory rate (in RCPM) for each animal.

Figure 9 shows the mean body temperatures of the groups after challenge. Figure 10 shows the body temperatures for each rabbit over the course of the study. All animals that succumbed to infection, except Rabbit 6 (Group 4), showed an increase in body temperature. Rabbit 38 also had a febrile response from Study Day 18 though 24, which corresponded with the time that the animal became bacteremic, toxemic, and neutrophilic, and had an increased respiration rate. Several animals showed sporadic decreases in body temperatures (Figure 10). These drops in temperature corresponded to blood draw days in which acepromazine was used as a sedative to facilitate blood draws from the ear. This sedative has been shown to decrease body temperature, and thus the decreases observed in the study are an artifact of sedation (Hobbs et al., 1991; Montané et al., 2003).

For Group 1, there were significant increases and decreases in body temperature from baseline beginning on Study Day 1 at 12:00–18:00 and continuing intermittently until Study Day 9 at 18:00–midnight. For Group 2, there were significant increases in body temperature from baseline starting on Study Day 0 at 18:00–midnight and continuing with decreasing frequency through Study Day 29 at midnight–6:00; relatively soon thereafter, significant decreases from baseline were observed beginning on Study Day 30 at 12:00–18:00 and continuing with increasing frequency through Study Day 38 at 12:00–18:00. For Group 3, there was a significant increase from baseline body temperature beginning on Study Day 1 at 12:00–18:00 and continuing intermittently through Study Day 38 at 18:00–midnight. For Group 4, there was a significant increase from baseline beginning on Study Day 1 at midnight–6:00 and continuing intermittently through Study Day 20 at 6:00–12:00. On Study Day 27 at 18:00–midnight, Study Day 33 at 18:00–midnight, and Study Day 34 at 18:00–midnight, the mean increase from baseline in Group 3 was significantly different from the change from baseline in Group 1. On Study Day 17 at midnight–6:00, Study Day 19 at 18:00–midnight, and Study Day 20 at 6:00–12:00, the mean increase from baseline in Group 4 was significantly different from the change from baseline in Group 1. On Study Day 36 at 12:00–18:00 and Study Day 37 at 12:00–18:00, the observed mean decrease from baseline in Group 4 was significantly different from the change from baseline body temperature in Group 1. On

Study Day 27 at 18:00–midnight, Study Day 29 at 18:00–midnight, and Study Day 32 at 12:00–18:00, the mean change from baseline in Group 2 was significantly different from the change from baseline in Group 3. On Study Day 3 at 6:00–12:00, Study Day 10 at midnight–6:00, Study Day 19 at 18:00–midnight, and Study Day 20 at 6:00–12:00, the mean change from baseline in Group 2 was significantly different from the change from baseline in Group 4. On Study Day 19 at 18:00–midnight, Study Day 27 at 18:00–midnight, and Study Day 36 at 12:00–18:00, the mean change from baseline in Group 3 was significantly different from the change from baseline in Group 4.

Measurements of inspiratory time, expiratory time, respiration integral, and peak amplitude were also conducted. See Appendix F for figures and complete statistical analysis.

3.4 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 Group 1 and 2 animals were below the LOD (4.9 nanogram/mL [ng/mL]) at all time points assayed. The Group 3 animal (Rabbit 2) that was found dead on Study Day 17 had 158.67 ng/mL of PA detected in the terminal blood sample. Only one of the four rabbits (Rabbit 27) in Group 4 that succumbed to disease had detectable levels of PA in the terminal sample (65330.90 ng/mL PA). Two of the Group 4 survivors had detectable levels of PA. Rabbit 38 had 7.70 and 6.28 ng/mL PA on Study Days 18 and 23, respectively. However, the toxemia resolved by Day 30. Rabbit 21 had PA levels of 4.97 ng/mL PA on Day 25; all other blood samples were below the LOD. Appendix N contains the PA ELISA results for each rabbit.

3.5 Bacteremia

All animals in Groups 1 and 2 were negative for *B. anthracis* bacteremia by culture on all study days. The terminal sample from the Group 3 animal (Rabbit 2) that succumbed to infection showed a bacterial load in the blood of 3.87×10^5 CFU/mL. The rest of the animals in this group never became bacteremic. The terminal samples of three out of the four rabbits that died in Group 4 were positive for bacteremia. Rabbits 33, 27, and 31 had terminal bacteremia levels of 4.13×10^5, 2.60×10^3, and 4.00×10^1, respectively. One of the nonsurvivors of this group (Rabbit 6) never showed a positive bacteremia culture and was found dead 10.9 days after the first challenge. Rabbit 38

(Group 4) became bacteremic on Study Day 18 (1.80×10^2), which resolved by the next blood collection time (Study Day 23). The other two rabbits that survived to the end of the study (Rabbits 39 and 21) never became bacteremic. Individual quantitative bacteremia culture results are located in Appendix O.

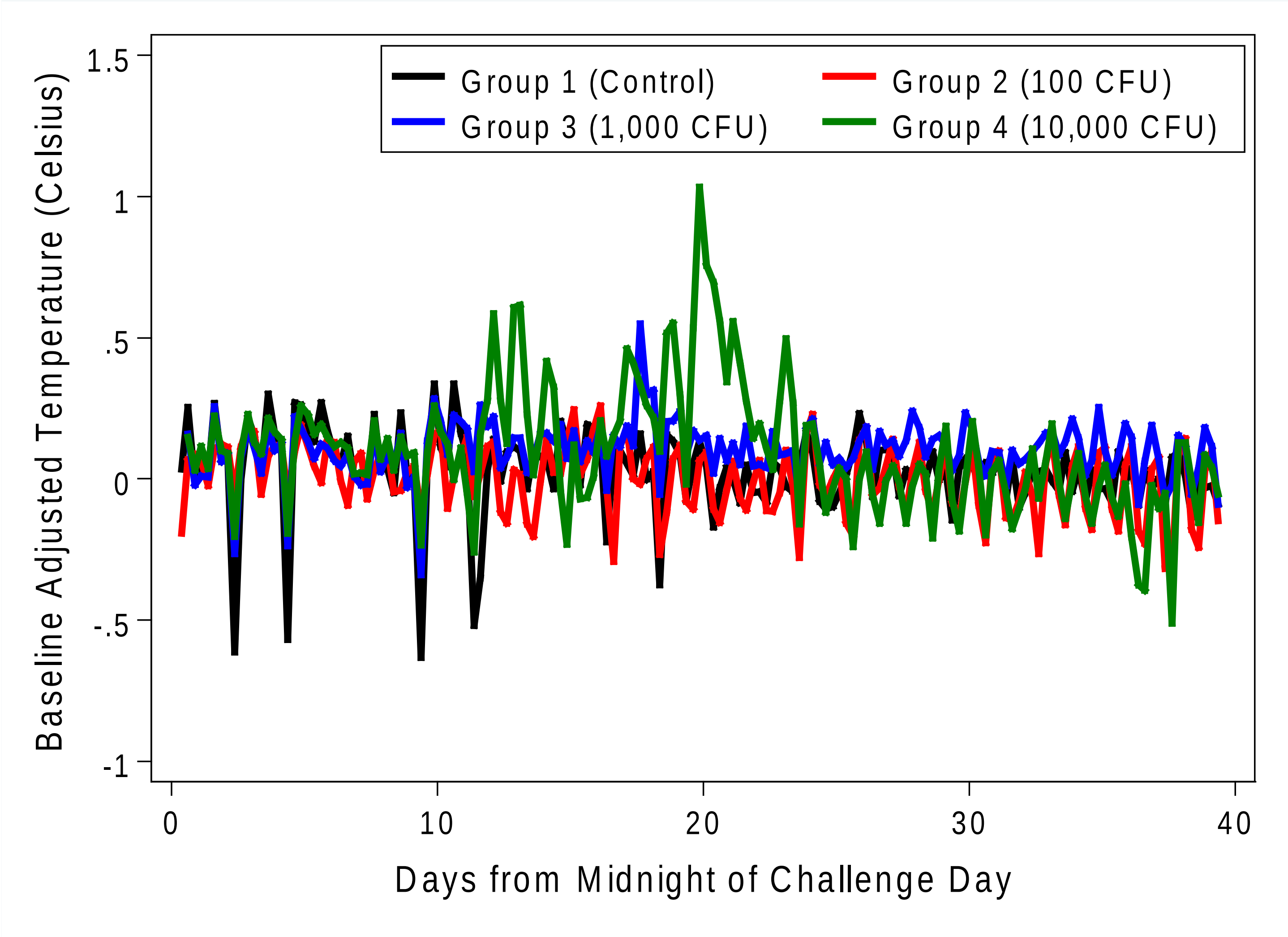

Figure 9. Plot of mean baseline-adjusted temperature values for each group.

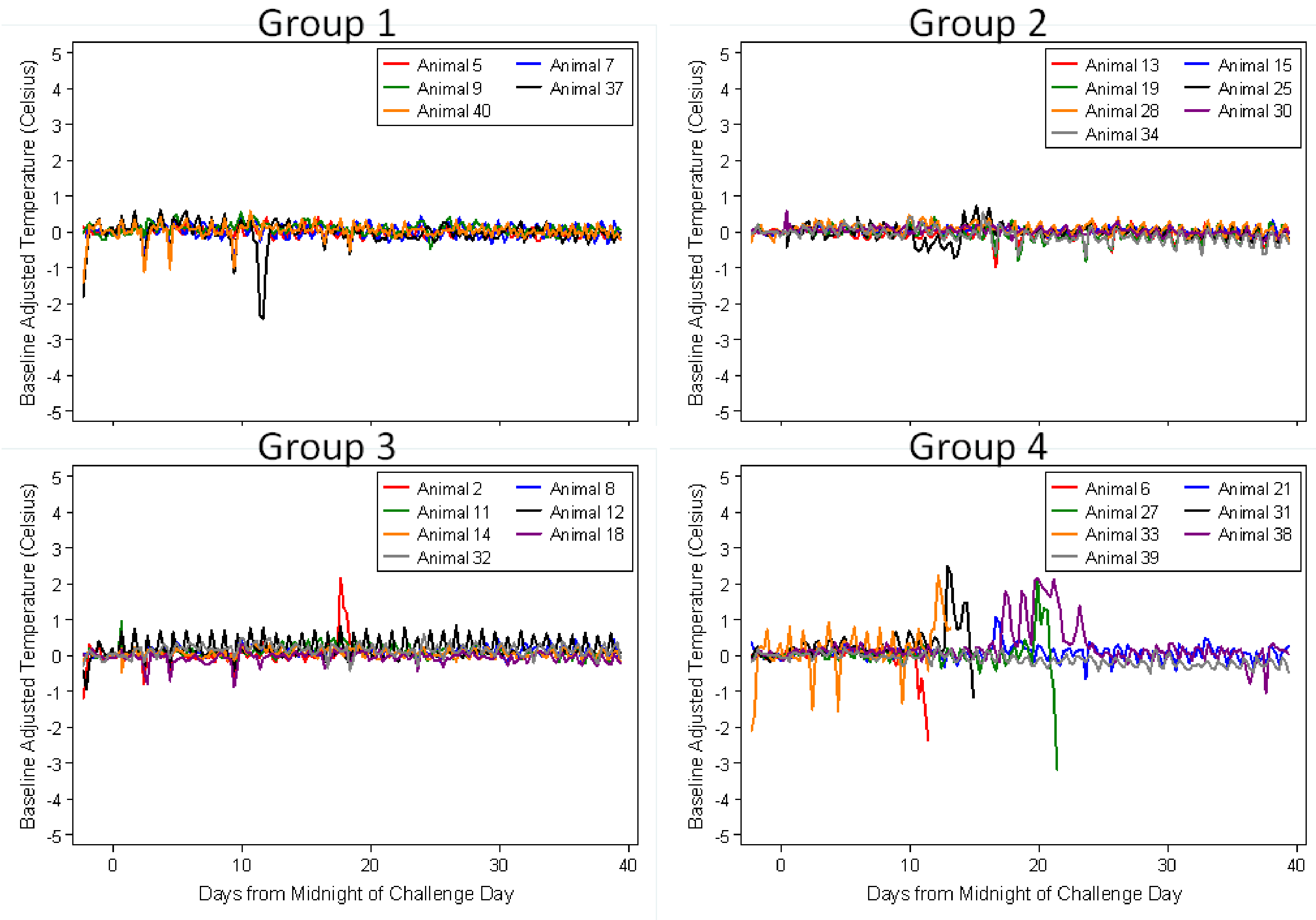

Figure 10. Baseline-adjusted temperature values for each rabbit.

Quantitative bacteremia was also assessed by qPCR targeting the *rpoB* gene. These results were consistent with the culture data for the terminal samples. Rabbits 2 (Group 3) , 27 (Group 4), 31 (Group 4), and 33 (Group 4) had 3.19 x 10^3, 2.52 x 10^3, 6.42 x 10^5, and 7.77 x 10^3 copies of *rpoB*/µL of blood, respectively. Rabbit 6 (Group 4) was negative for bacteremia by the qPCR method. Rabbit 38 (Group 4) was positive for bacteremia by the qPCR method on Study Days 23 (3.00 copies/µL) and 30 (5.00 copies/µL) despite only being positive by the culture method on Day 18. This is not surprising as the qPCR-based method does not distinguish between viable and killed bacterial cells. Individual qPCR results are contained in Appendix P.

3.6 TNA/IgG ELISA

Serum samples taken on Study Days -3, 4, 11, 18, 25, 32, and 39 were analyzed via TNA and anti-PA IgG ELISA to determine if the rabbits developed a humoral response to the repeated *B. anthracis* exposures. Only Rabbit 38 (Group 4) had detectable levels of antibodies by either method, no other animal seroconverted during the study. The TNA was used to determine the ED_{50} and NF_{50} of sera able to neutralize lethal toxin. The ED_{50} values for Rabbit 38 on Study Days 25, 32, and 39 were 5858, 12789, and 7250. The NF_{50} on these study days were 12.71, 26.44, and 14.82. The IgG ELISA results showed that Rabbit 38 had 1636.02, 2190.85, and 1728.47 µg/mL of circulating anti-PA IgG on Study Days 25, 32, and 39, respectively. Individual results for TNA and IgG ELISA are provided in Appendices Q and R, respectively.

3.7 Hematology and Clinical Chemistry

To further assess any physiological effects of low-dose exposure to *B. anthracis*, whole blood and plasma were assayed for a variety of hematological and CRP (refer to Section 2.11 for a complete list of parameters). Individual animal hematology and CRP results are presented in Appendices S and T, respectively, along with values for the normal ranges of hematology and CRP.

Analysis of variance (ANOVA) models were fitted separately to each hematology parameter and CRP to determine the effects of challenge dose and study day on group means. Appendix J contains the results of extensive statistical analyses of the hematological and CRP results.

3.7.1 Red Blood Cell Parameters

There were significant decreases in RBCs from baseline in Group 1 on Study Day 4, in

Group 2 on Study Day 16, and in Group 4 on Study Day 11 (Figure 11). There were significant group effects on Study Days 23 and 25. On Study Day 23, the mean decrease from baseline in Group 4 was significantly different than the mean increase from baseline in Group 3 ($P = 0.0489$, Tukey's test). On Study Day 25, the mean decrease from baseline in Group 4 was significantly different than the mean increases from baseline in Groups 1($P = 0.0023$, Tukey's test) and 2 ($P = 0.0229$, Tukey's test). While the changes were statistically significant, their biological relevance is limited as all rabbits remained in or very close to the normal range of 4.20–6.70 x 10^6 RBCs/µL (Figure 11).

There were also significant changes in the HGB concentrations in the blood (Figure 12). The decrease from baseline in Group 1 on Study Day 4 was significant ($P < 0.05$, ANOVA). There were also significant group effects on Study Days 23 and 25. On Study Day 23, the mean decrease from baseline in Group 4 was significantly different than the mean increases from baseline in Groups 1, 2, and 3 ($P < 0.05$, Tukey's test). On Study Day 25, the mean decrease from baseline in Group 4 was significantly different than the mean changes from baseline in Groups 1, 2, and 3 ($P < 0.05$, Tukey's test). Like the RBC counts, the HGB concentrations fell within or very near the normal range (9.5–14.5 grams per deciliter [g/dL]; Figure 12) and statistical differences were not likely to be clinically relevant.

3.7.2 Total and Differential White Blood Cell Parameters

The mammalian host responds to extracellular bacterial infection by increased hematopoiesis and neutrophilia. To determine if the rabbits responded to the multiple exposures of *B. anthracis*, complete WBC counts and differentials were performed. Interestingly, there were no significant shifts as a proportion of baseline and no significant differences between the groups on any postchallenge study day. While there was no significant mean increase in WBC counts in the groups, Rabbit 38 (Group 4) did show an increase in WBCs well above the normal range of 2.90 – 8.10 x 10^3 WBCs/µL (Figure 13). In fact, the WBC count reached 20.33 x 10^3 cells/µL on Study Day 23 but decreased back into the normal range by the end of the study.

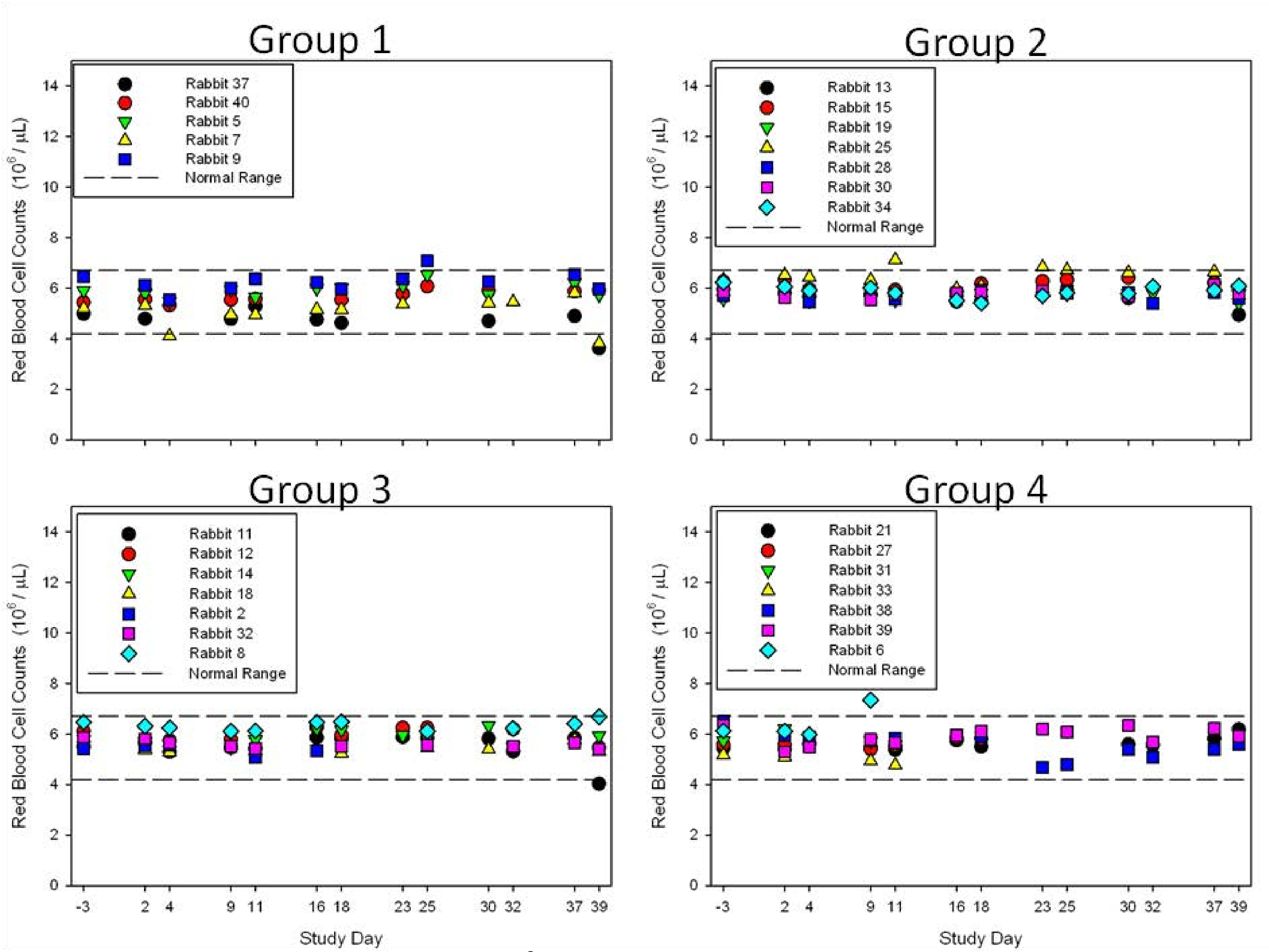

Figure 11. Plots of red blood cell counts (1 x 10^6 cells/µL).

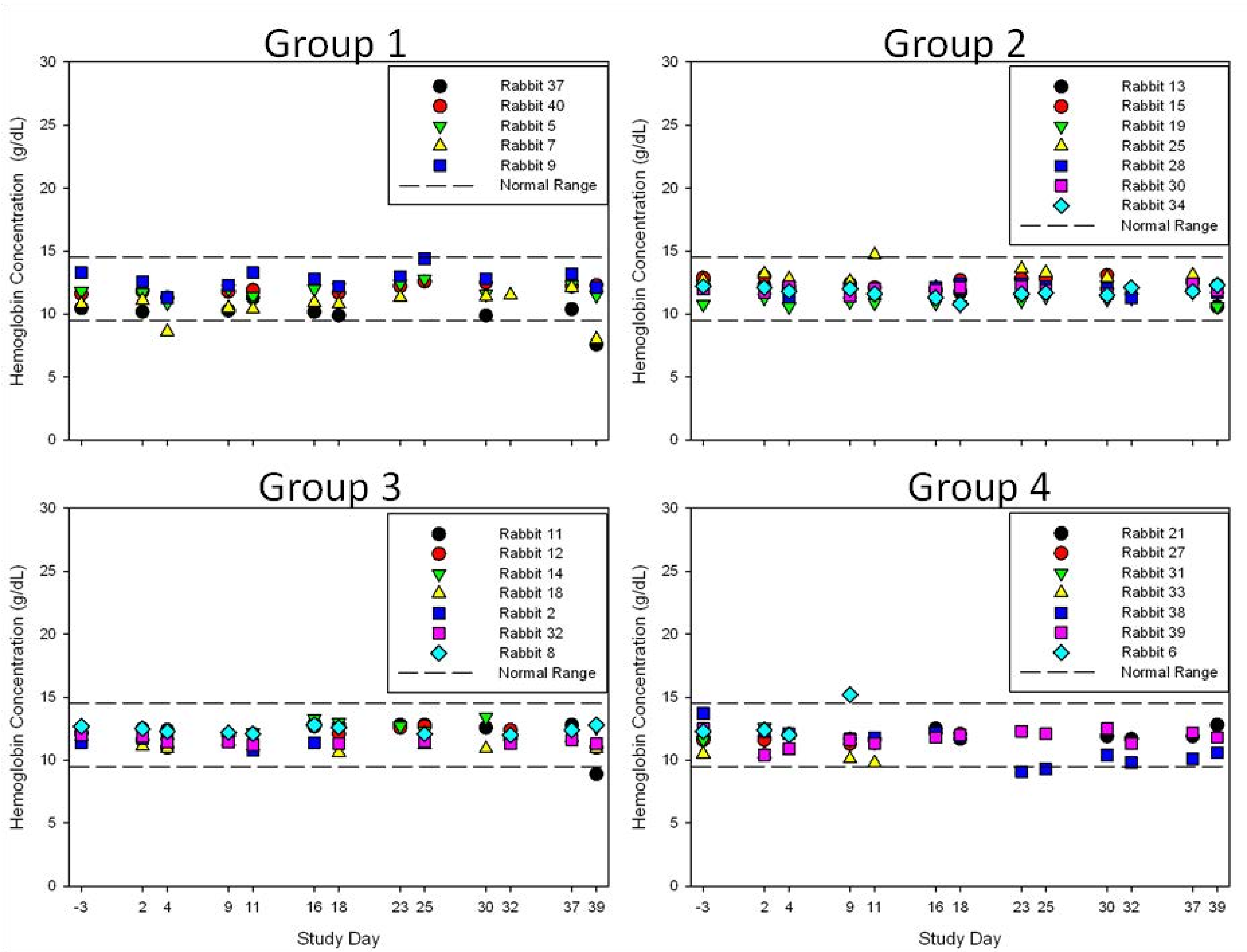

Figure 12. Plots of hemoglobin concentration (g/dL).

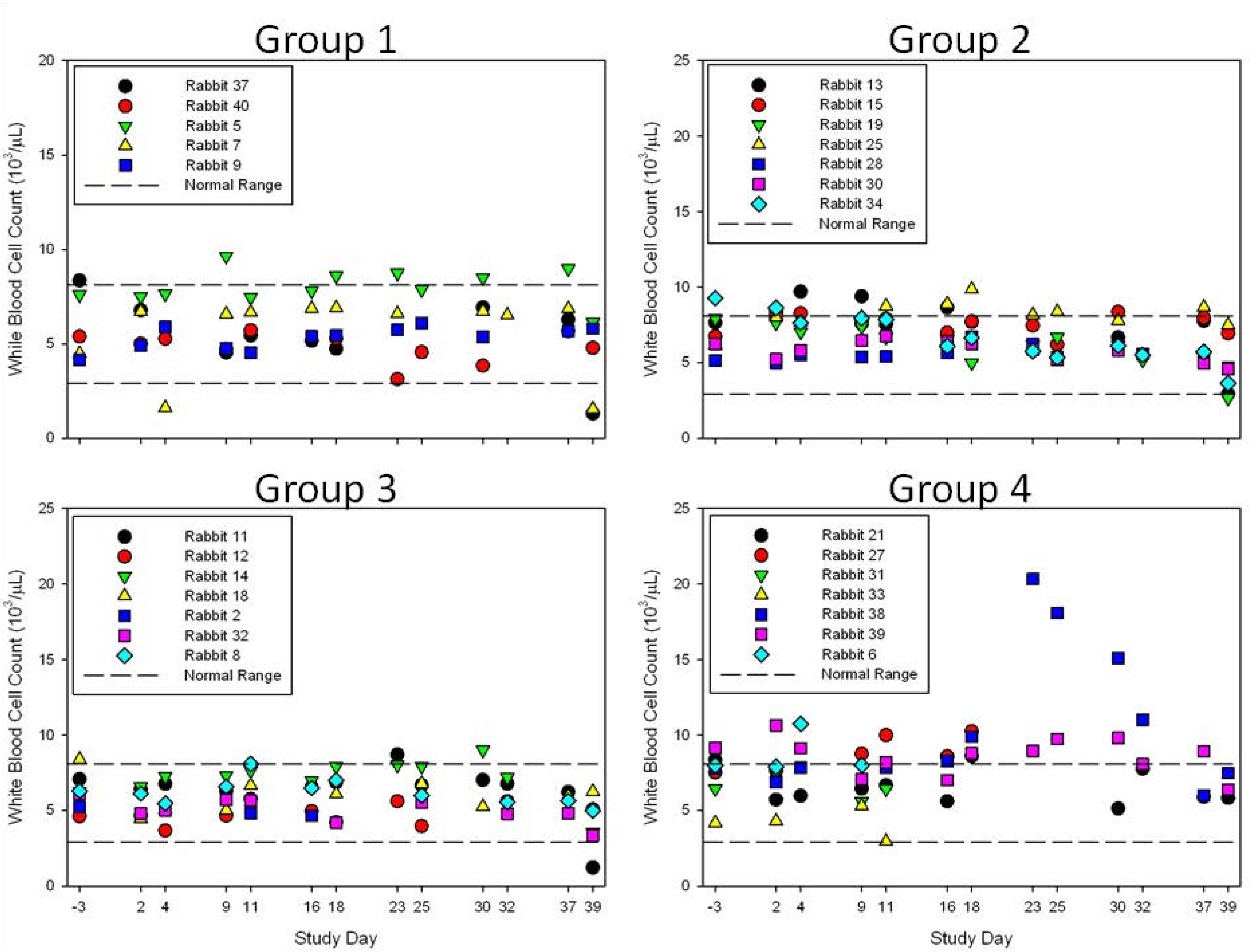

Figure 13. Plots of white blood cell counts (1 x 10^3 cells/µL).

There were no significant shifts in neutrophil counts as a proportion of baseline and no significant differences between the groups on any postchallenge study day (Figure 14). However, Rabbit 11 (Group 3, Day 23), Rabbit 6 (Group 4 , Day 4), Rabbit 27 (Group 4, Day 18), Rabbit 21 (Group 4, Day 32), and Rabbit 38 (Group 4, Days 23–32) showed levels of circulating neutrophils above the normal range of 0.8–2.9 x 10^3 cells/µL after the first challenge day (Figure 14). The neutrophilia was most pronounced in Rabbit 38, which was bacteremic on Study Day 18 and toxemic on Days 18 and 23. This animal was able to clear the infection and lived to the end of the study.

There were no significant differences in lymphocyte counts between the groups on any postchallenge study day (Figure 15). There was a significant decrease from baseline in Group 3 on Study Day 39. It is unlikely that this decrease was related to the multiple *B. anthracis* exposures as it did not occur until the last study day. Several rabbits experienced lymphophilia (levels above the normal range of 2.20–5.30 x 10^3 cells/µL) over the course of the study as illustrated in Figure 15. The effect of the exposures on the lymphocyte levels remains to be determined as there was no clear dose-response relationship in the measurements.

CRP is an indicator of stress and nonspecific inflammation. It can also be used as a marker for liver damage. There was a significant increase in CRP as a proportion of baseline in Group 4 on Study Day 2 ($P > 0.05$, ANOVA) (Figure 16). There were no significant differences between the groups on any postchallenge study day. The normal levels range from 0.25 to 0.29 milligram/deciliter (mg/dL) (Murty et al., 2010; Setorki et al., 2009). However, several animals had detectable levels of CRP (> 0.50 mg/dL) that were considered above the normal range (Figure 16). The increase in CRP levels did not correspond with morbidity or mortality and in most cases could have resulted from the stress of study activity. Rabbit 38 showed the highest levels of CRP between Study Days 18 and 25 topping out at 7.42 mg/dL on Study Day 23. The increase in CRP corresponded with bacteremia, toxemia, neutrophilia, and pyrexia indicating that the increase was in response to the *B. anthracis* infection.

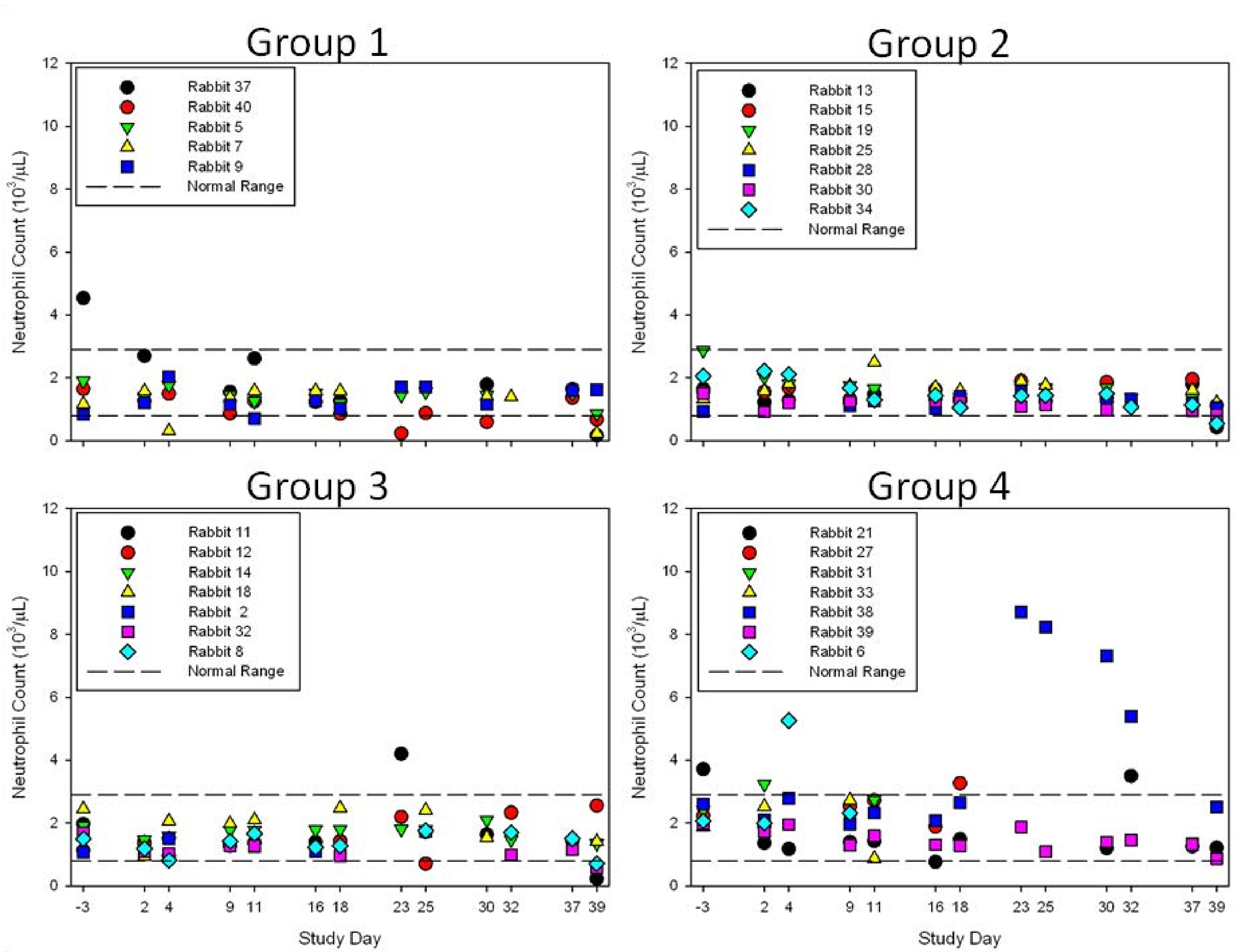

Figure 14. Plots of neutrophil counts (1 x 10^3 cells/μL).

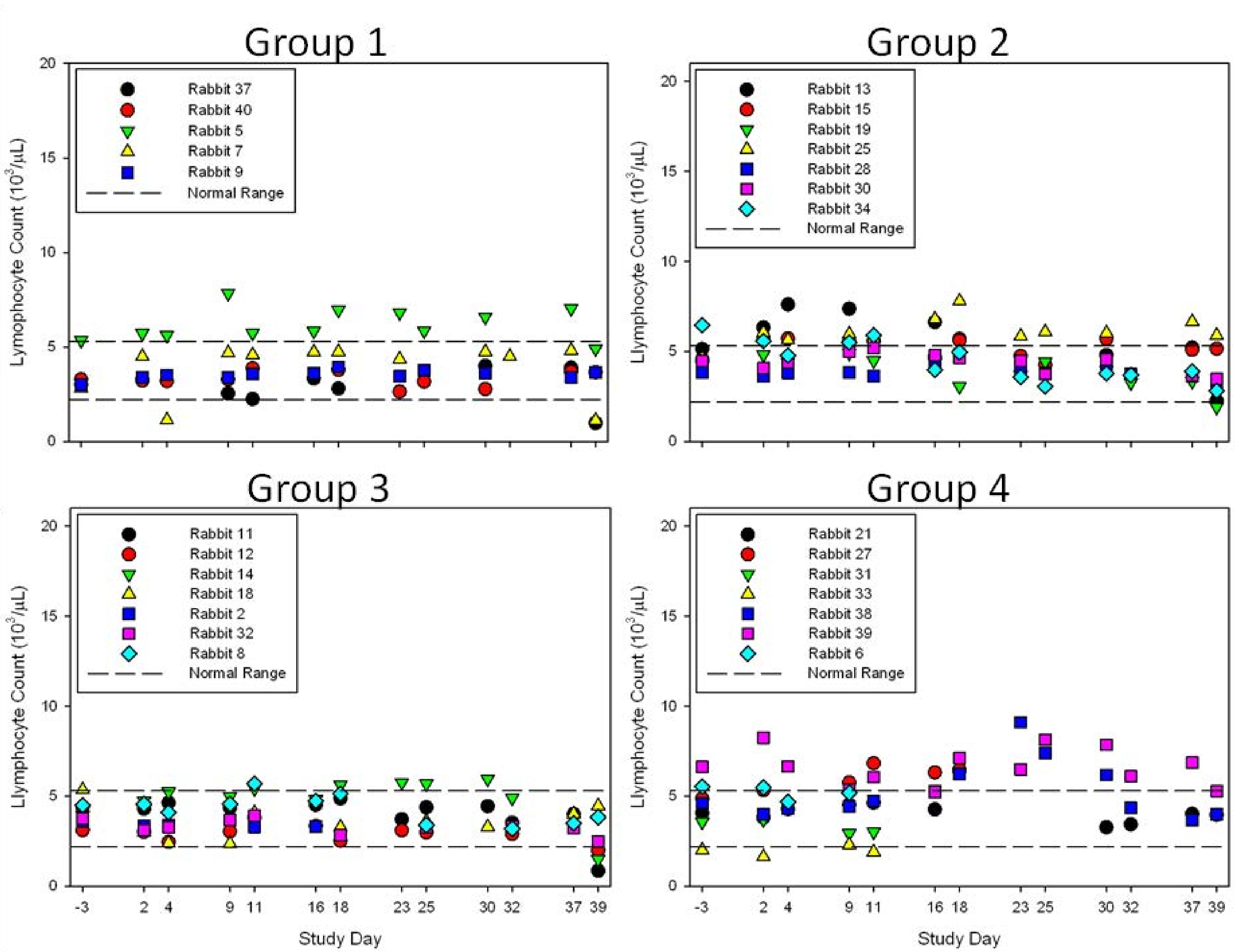

Figure 15. Plots of lymphocyte counts (1 x 10^3 cells/µL).

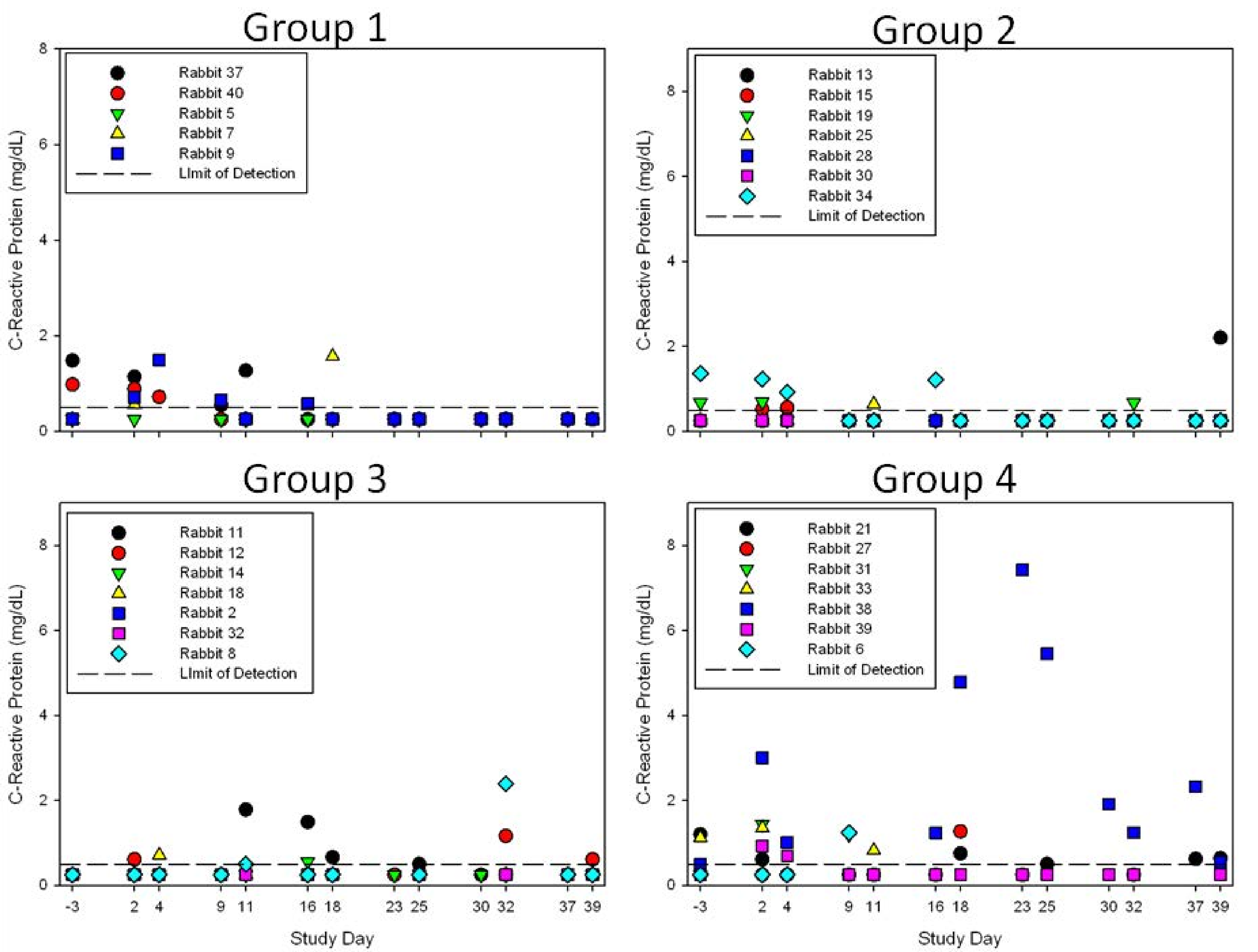

Figure 16. Plots of C-reactive protein levels (mg/dL).

4 Pathology

Complete necropsies were performed on all rabbits following spontaneous death or euthanasia, including rabbits surviving to study termination on Study Day 39. Protocol-specified tissues (lungs and gross lesions) were sampled and preserved in 10% neutral buffered formalin. Standard sections of these tissues from all rabbits were processed to slides, stained with hematoxylin and eosin, and interpreted by a board-certified veterinary pathologist.

Table 9 summarizes the pathological findings from each of the rabbits. The complete pathology report is located in Appendix U.

Gross lesions consistent with anthrax in rabbits (Zaucha, et al., 1998) included discoloration of the lungs, foci in the appendix, "accumulation" in the cecum, and/or enlargement of a mediastinal lymph node; these lesions were identified in Rabbits 12 (Group 3), 6 (Group 4), 33 (Group 4), and 27 (Group 4). These lesions correlated microscopically with hemorrhage, necrosis, edema/fibrin, and suppurative (largely heterophilic admixed with bacteria and/or necrotic debris) inflammation. Gross lesions in the lungs correlated with multiple foreign body granulomas/pyogranulomas (Rabbit 38) as shown microscopically in Figure 17. Multinucleated giant cells as well as foreign body granulomas/pyogranulomas were present in the lungs of challenged rabbits (survivors and non-survivors) but were not seen in control rabbits in this study. These multinucleated cells and granulomas/pyogranulomas surrounded foreign material (foreign bodies) consistent with organic debris [e.g. food particles or hair and debris from vascular access ports (Taketoh et al., 2009)].

Abdominal skin "lacerations" were diagnosed grossly in two rabbits (12, Group 3, and 38, Group 4). These lesions correlated microscopically with necrosuppurative inflammation but were not associated with bacteria. While anthrax may have been a contributing factor, these lesions were more likely due to trauma. Sections of left apical and right diaphragmatic lung lobes and gross lesions were examined microscopically for evidence of anthrax. Figure 18 shows normal lung tissue from Rabbit 37 (Group 1).

Microscopic findings consistent with anthrax (Zaucha et al., 1998) were present in tissues from all rabbits (survivors and non-survivors) in Groups 2, 3 and 4. Lesions typical of anthrax in this study included suppurative inflammation, necrosis, lymphocyte necrosis/depletion, hemorrhage, edema, and/or large rod-shaped bacteria (bacilli) in the lungs (Figure 19), cecum, appendix (Figure 20), and mediastinal lymph nodes (Figure 21). Lung lesions attributed to *B. anthracis* were primarily interstitial and consisted of minimal to mild suppurative interstitial inflammation and interstitial and/or intravascular bacteria.

Table 9. Summary of Individual Gross and Microscopic Observations

Target Dose	Animal Number/ Death Status*	Gross Findings	Microscopic Findings
Sham Challenge Control	1.2 40/FS		Lung: Unremarkable.
	7/FS		Lung: Unremarkable.
	5/FS		Lung: Perivascular eosinophils, minimal.
	9/FS		Lung: Perivascular eosinophils, minimal.
	37/FS		Lung: Unremarkable.
100 CFU	13/FS		Lung: Perivascular eosinophils, minimal.
	34/FS		Lung: Foreign body, mild. Lung: Multinucleated giant cells, mild.
	25/FS		Lung: Unremarkable.
	15/FS		Lung: Perivascular eosinophils, minimal.
	30/FS		Lung: Unremarkable.
	28/FS		Lung: Perivascular eosinophils, mild.
	19/FS		Lung: Unremarkable.
1000 CFU	14/FS		Lung: Perivascular eosinophils, minimal.
	11/FS		Lung: Perivascular eosinophils, minimal.
	2/FD		Lung: Hemorrhage, minimal. Lung: Inflammation, suppurative, minimal. Lung: Bacteria, minimal.
	8/FS		Unremarkable.
	12/FS	Skin: Laceration(s), hind limb, red, left hind limb, 40 x 20 mm	Lung: Foreign body, minimal. Lung: Multinucleated giant cells, mild. Skin: Inflammation, necrosuppurative, marked.
	306 (18)/FS		Lung: Unremarkable.
	307 (32)/FS		Lung: Perivascular eosinophils, minimal.

Table 9. Continued.

Target Dose	Animal Number/ Death Status[a]	Gross Findings	Microscopic Findings
10,000 CFU	6/FD	Cecum: Accumulation (gas). Samples of cecum, colon, jejunum, and appendix were collected to confirm lesion.	Cecum: Edema, mild. Cecum: Edema, hemorrhage and necrosis. Cecum: Hemorrhage, moderate. Cecum: Necrosis, moderate. Lung: Perivascular eosinophils, minimal.
	33/FD	Lymph node, Mediastinal: Enlarged, dark, 3x.	Lung: Bacteria, mild. Lung: Hemorrhage, minimal. Lung: Inflammation, suppurative, mild. Lung: Perivascular eosinophils, minimal. Lymph node, mediastinal: Bacteria, marked. Lymph node, mediastinal: Edema, fibrin, mild. Lymph node, mediastinal: Hemorrhage, minimal. Lymph node, mediastinal: Necrosis/depletion, lymphoid, marked.
	27FD	Appendix: Foci, multiple, red, up to 2 x 2 mm.	Appendix: Hemorrhage, mild. Appendix: Necrosis/depletion, lymphoid, moderate. Appendix: Infiltration cellular, macrophages, moderate. Appendix: Note: hemorrhage and necrosis. Lung: Bacteria, minimal. Lung: Inflammation, suppurative, minimal. Lung: Perivascular eosinophils, minimal.
	31/FD		Lung: Bacteria, mild. Lung: Inflammation, suppurative, minimal.
	39/FS		Lung: Foreign body, minimal. Lung: Multinucleated giant cells, minimal.
	21/FS		Lung: Unremarkable.
	38/FS	Lung: Discoloration(s), apical lobe, pale, firm. Skin: Laceration(s), abdominal, red, 20 x 15 mm.	Lung: Foreign body, moderate. Lung: Granuloma/pyogranuloma, moderate. Lung: Perivascular eosinophils, minimal. Skin: Inflammation, necrosuppurative, moderate. Skin: Thrombosis, artery, mild.

*FD = found dead, FS = final-phase sacrifice
mm = millimeters

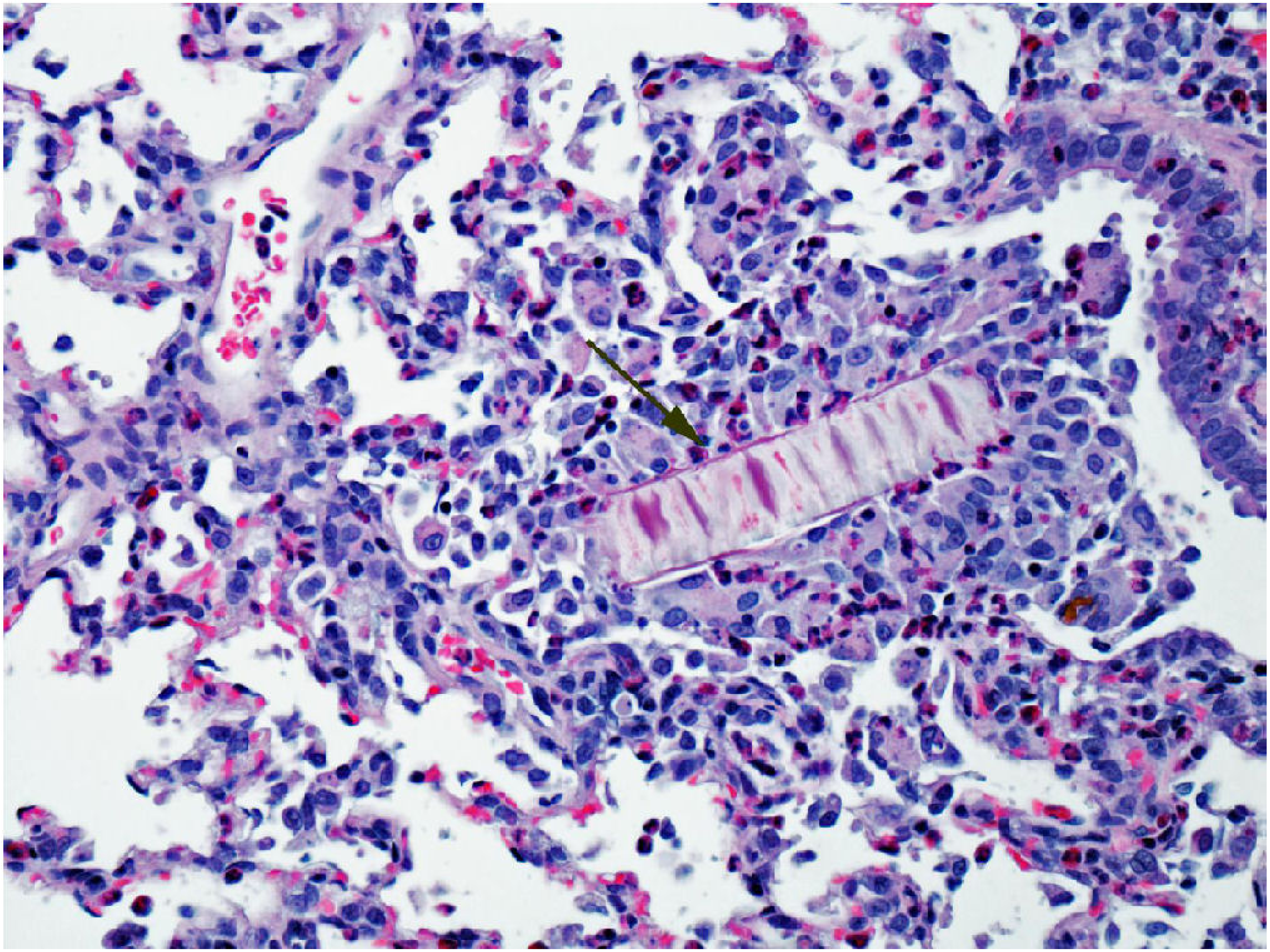

Figure 17. Animal 38 (Group 4): Lung, alveoli; pyogranulomatous (epithelioid macrophages, lymphocytes, and neutrophils) inflammatory reaction to a foreign body (arrow). Hematoxylin and eosin stain. 40X.

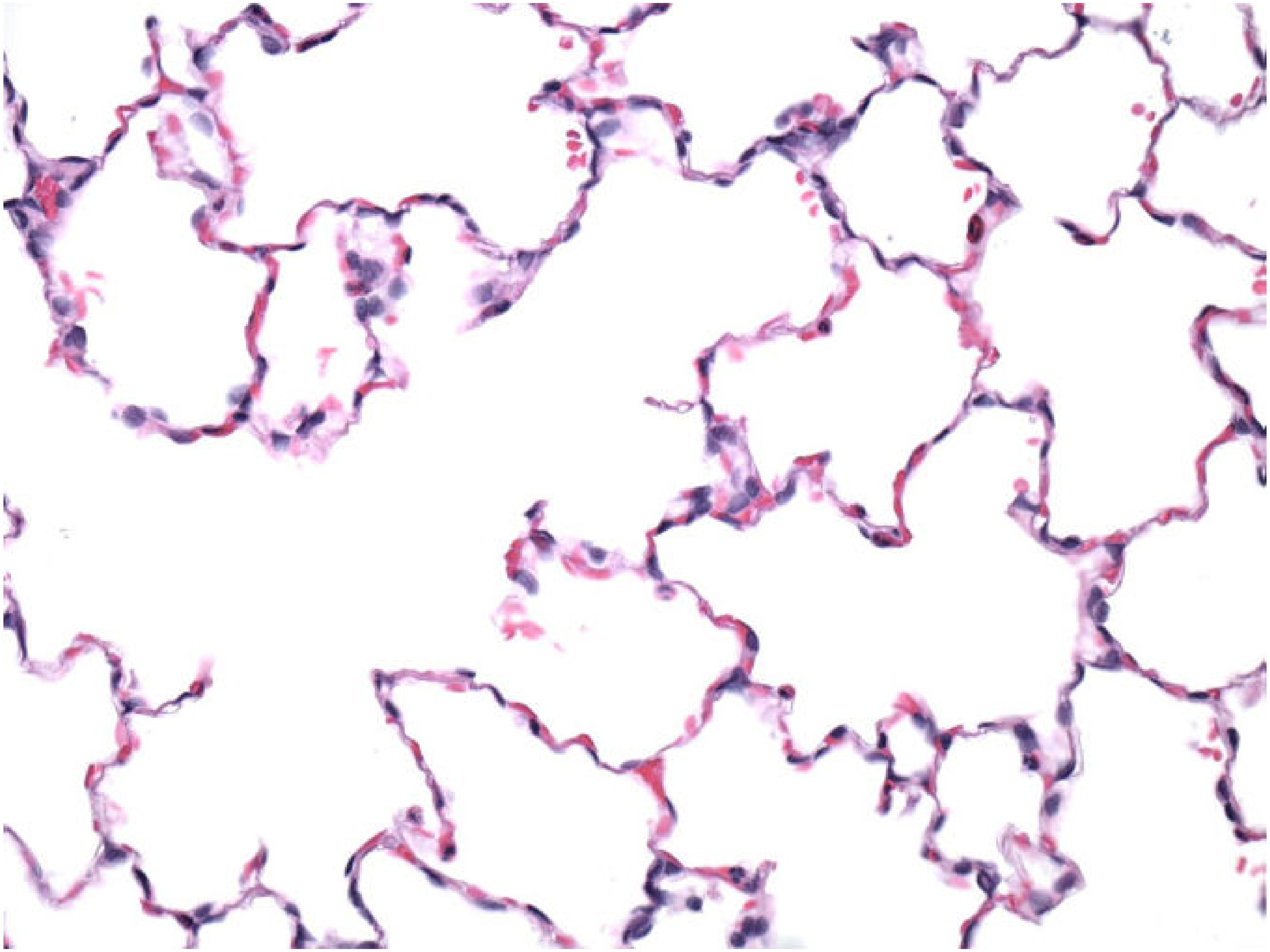

Figure 18. Animal 37 (Group 1): Lung; normal alveoli (control). Hematoxylin and eosin stain. 40X.

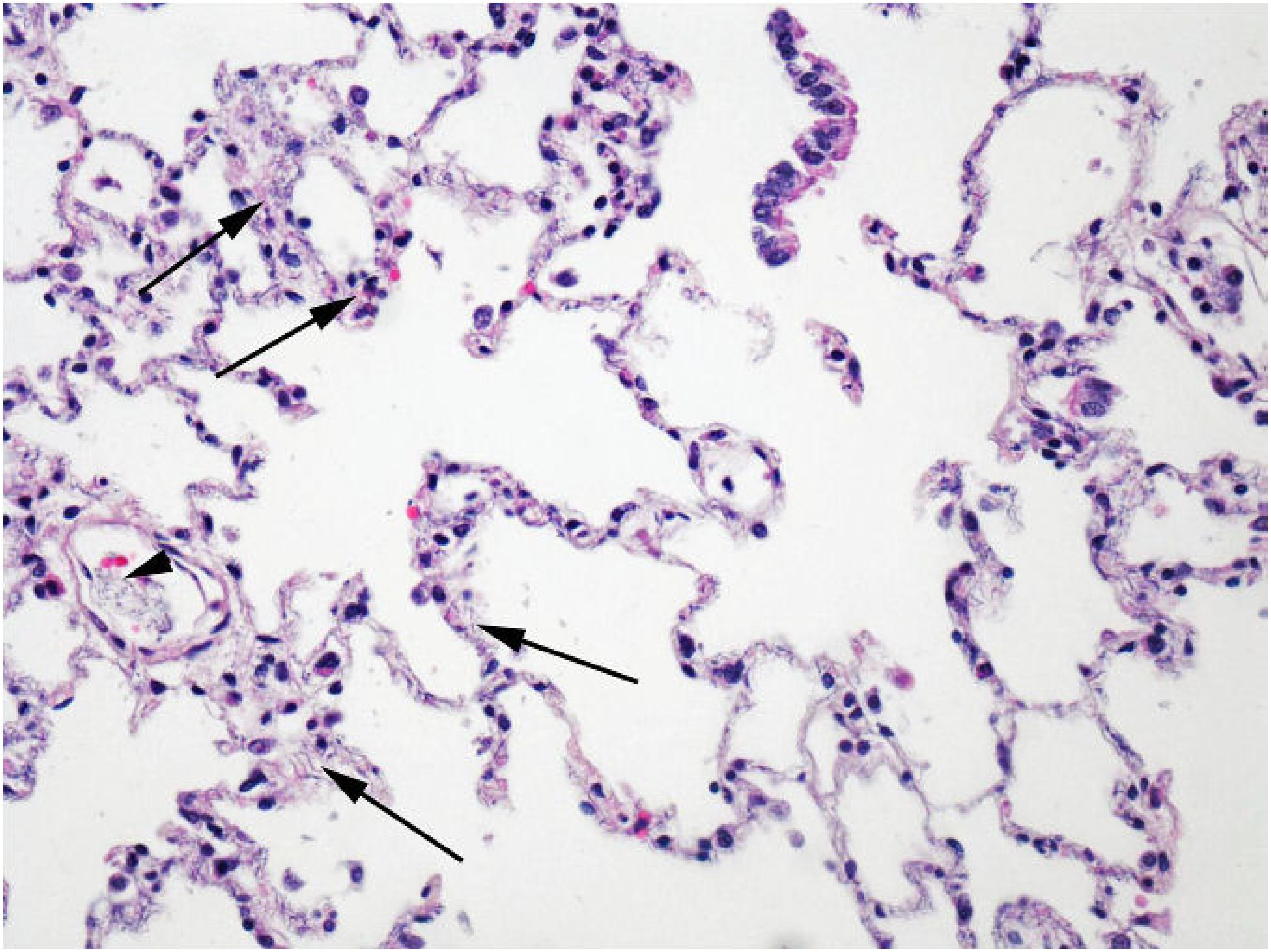

Figure 19. Animal 31 (Group 4): Lung; alveoli contain interstitial suppurative inflammation and anthrax bacilli (arrows). Alveolar vessels contain anthrax bacilli (arrowhead). Hematoxylin and eosin stain. 40X.

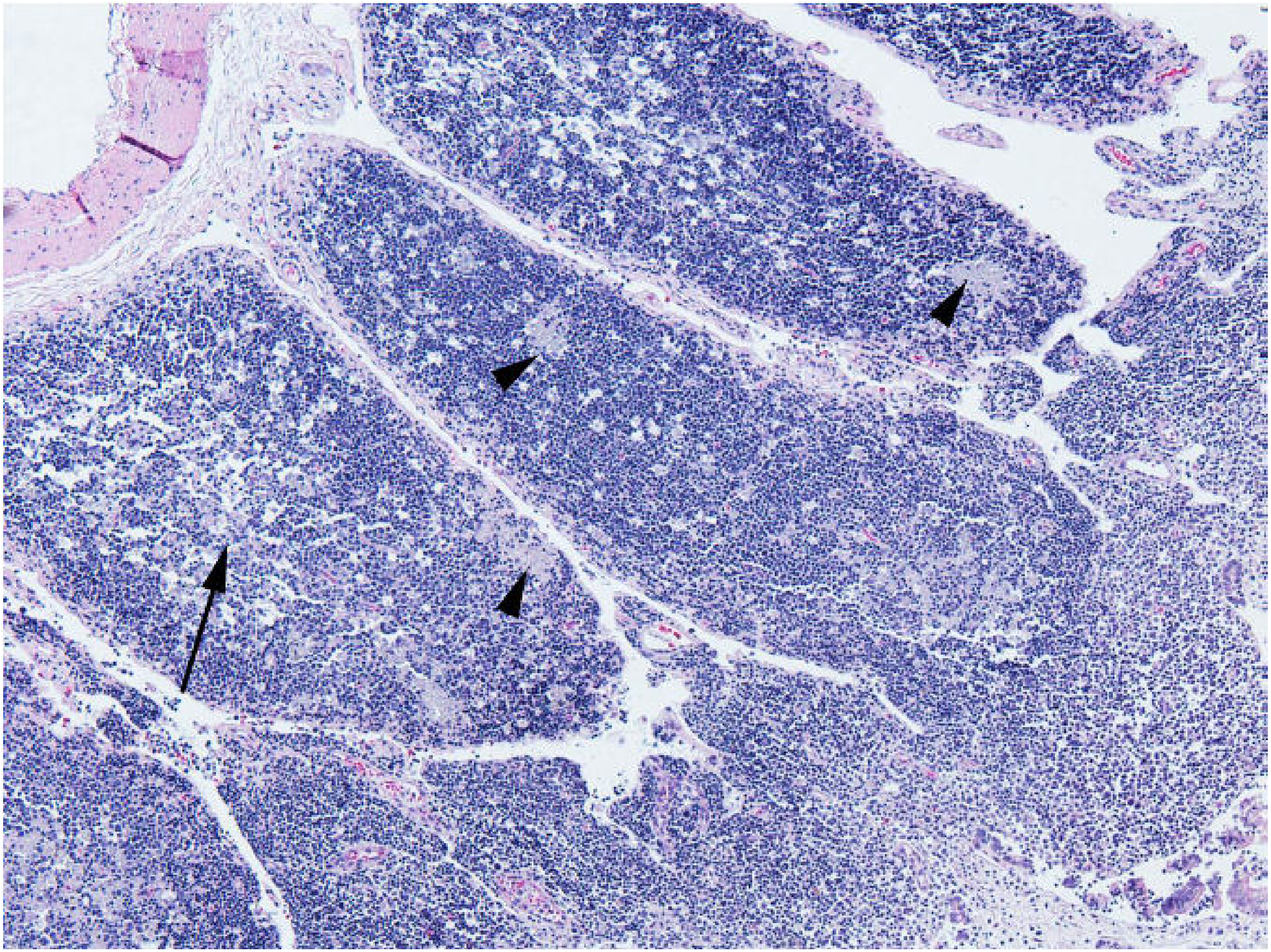

Figure 20. Animal 38 (Group 4): Appendix; lymphocytes undergoing excessive apoptosis (arrow) with macrophage infiltration (arrowheads). Hematoxylin and eosin stain. 10X.

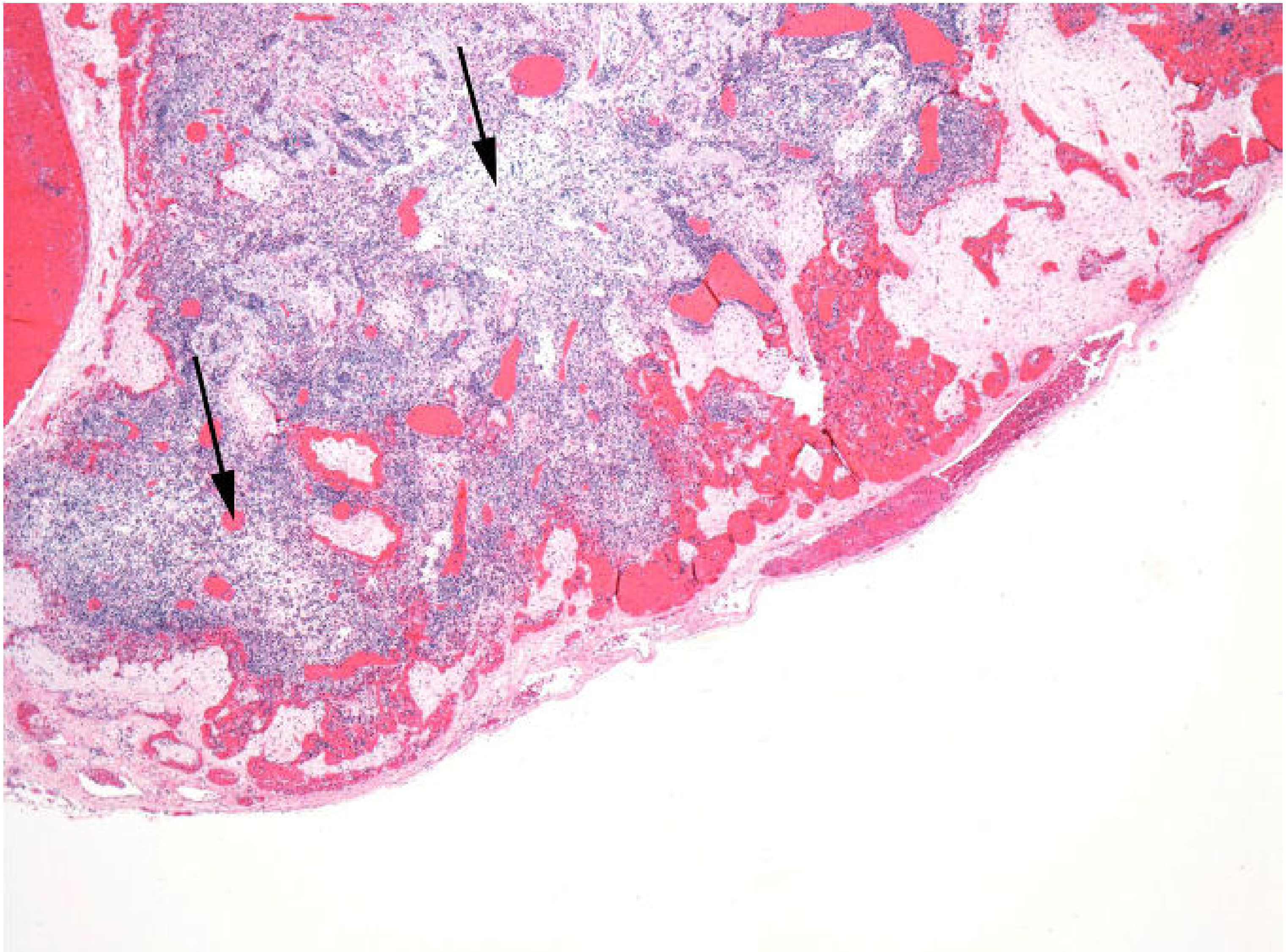

Figure 21. Animal 38 (Group 4): Lymph node, mediastinal; lymph node congestion and lymphoid follicles necrosis/depletion. Hematoxylin and **eosin stain. 4X.**

4.1 Benchmark Dose Analysis

The following models exhibited acceptable fits as measured by p-values and scaled residuals at BMDLs of interest for the average daily dose data: Dichotomous-Hill, Log_e Logistic, and Weibull (run as Exponential). Using existing EPA guidance (US EPA, 2008a), the log_e logistic model was identified as the best fitting model to the data. A $BMDL_{50}$ of 2.6×10^3 inhaled CFU and a $BMDL_{10}$ of 2.9×10^2 inhaled CFU were calculated using the model.

The following models exhibited acceptable fits as measured by p-values and scaled residuals at BMDLs of interest for the total aggregate dose data (i.e., cumulative dose over the course of the challenges): Dichotomous-Hill, Log_e Logistic, and Weibull (Run as Exponential). Using existing EPA guidance (US EPA, 2008a), the log_e logistic model was identified as the best fitting model to the data. A $BMDL_{50}$ of 4.4×10^4 total inhaled CFU and a $BMDL_{10}$ of 4.9×10^3 total inhaled CFU were calculated using the model. Using the average daily dose $BMDL_{10}$ value from the log_e logistic model, the calculated example values for the HED and HEC were 1,400 inhaled CFU and 87 CFU/cubic meter (m^3), respectively.

Given the relatively small animal numbers present in each dose group, the spacing of the doses, and modeling assumptions; the calculated BMDLs should be considered to be highly uncertain. The complete benchmark dose report, including the dosimetric adjustment, is provided in Appendix V.

4.2 Quality Assurance

The procedures set forth in the EPA-approved Quality Assurance Project Plan (QAPP); Comer (2010) prepared for this project were adhered to except in those instances that are documented as deviations (see Appendix B). In all, there were 15 study deviation reports (DR) and one investigation report (IR); no facility deviations occurred during the study. Each of these deviations is listed in Table 10 along with the impact on the quality of the data and results reported herein. Technical systems audits (TSATs) and data quality audits (DQAs) performed for this study along with dates performed, reported, and findings addressed by project management are listed in Table 11.

Table 10. Deviations and Impacts on Data Quality and Results

Deviation Number	Deviation Type	Impact on Data Quality and Results
DR-10019	Study	Positive. Decreasing the incubation time to a shorter period allows the plates to be counted at the same time as the non filtered samples.
DR-10063	Study	Minimal. It cannot be determined which incubator the plates were placed in, but the plates in both incubators were incubated for an appropriate amount of time and the plates for the animal in question returned satisfactory results.
DR-10263	Study	Minor. The small difference in the calibration values will not cause noticeable differences in the data. Additionally, postchallenge data are compared to the baseline data, which would offset any differences caused by an incorrect calibration value.
DR-10264	Study	Minor. Three days of baseline telemetry data will be used for comparison to postchallenge data during statistical analysis. 2–3 days of baseline data are commonly used for analysis and was the initial requirement of the draft protocol.
DR-10350	Study	None. A sterility check was performed on all samples prior to their removal from the Biosafety Level 3 laboratory (BL3); all serum aliquots were negative. This information was recorded on Microbio-463 and was confirmed by the Safety Officer before the samples were released from the BL3.
DR-10351	Study	Minimal. There is little possibility that the challenge organism could be present at the Day -3 time point. None of the other animals tested at this time had challenge organism colonies present. The quantitative bacteremia results for Day -3 will be reported as negative for Rabbits 33 and 31.
DR-10352	Study	Minimal. Residual samples will be pooled and sent to the client.
DR-10353	Study	None. All of the counts were zero.
DR-10444	Study	Minimal. The dilution of sham challenge control created was 1:50.2 instead of the SOP required 1:50. Since this resulted in a preparation that is more dilute, this may have had an effect on the performance of the sham challenge control within the assay.
DR-10496	Study	Minimal. The initial volume given was within the range specified in the protocol.
DR-10645	Study	Minimal. The permanent equipment used or the assay can be verified by other equipment use dates; however, the transportable equipment like pipettes cannot be verified.
DR-10646	Study	Minimal. a) The refrigerator information was recorded on the equipment form and can be verified that its use was within the calibration timeframe. b) Since the freeze thaw did not specify whether the sample was from the PA aliquot or retention, the exact freeze thaw for the sample could not be verified.
DR-10647	Study	These specifications improve the fidelity and consistency of the results reported by the assay.
DR-11043	Study	a. Minimal. The date of printing is captured on the records as 8/21/10, and Form No. ELISA-036 indicates which technician conducted the data analysis. b. Minimal. Based on the date of printing, data were analyzed on 8/27/10. Furthermore, all reportable values captured on Microbio-357 can be transcribed and verified; no reportable data are affected. c. Minimal. Although the date and operator cannot be confirmed, it has been verified that the data have been imported into the database. d. Minimal. Based on the information on the Sample Tracking System, it is most likely that the freeze/thaw (F/T) cycles should be: BMI032 = F/T 1, BMI012 = F/T 2, BMI009 = F/T 3, which is within the permissible number of F/T cycles form ELISA samples.

Table 10. Continued.

Deviation Number	Deviation Type	Impact on Data Quality and Results
DR-11044	Study	Minimal. There is less than a 0.2% difference between the actual dilution factor and that specified on ELISA-008. No data are significantly affected.
IR-419	NA	When using either new rPA lot, 17115A2A or 5051797, for coating there were inconsistencies with the binding of the currently qualified conjugate 05814. This was seen in preliminary analyses for study 1078 and it caused many failures with study plates. An investigation was undertaken to determine the most likely cause of the inconsistencies leading to failures and to determine a correction course of action to take. It was determined the conjugate 05814 had degraded and was, as a result, inconsistently binding. Proposed action was to use a different conjugate lot.

BL3 = Biosafety Level 3

Table 11. Technical System Audit (TSAT) and Data Quality Audit (DQA) Dates

Audit Name	Audit Type	Inspection Start Date	Date Reported	Response Date	Closure Date
Critical Phase: aerosol challenge, Day 2 blood collection, Day 2 weights, bacteremia, hematology, CRP, IgG ELISA, TNA, PCR, and PA ELISA	TSA	10/5/2010	11/8/2010	11/17/2010	2/24/2011
Telemetry Binder	DQA	10/7/2010	10/12/2010	10/20/2010	11/17/2010
Aerosol Binders 1–3, including the Aerosol Exposure Report and Final Data Tables. (Aerosol Exposure Events Tables audited 10%)	DQA	10/21/2010	10/21/2010	11/1/2010	12/17/2010
DNA Isolation and Real-Time PCR Binder	DQA	10/20/2010	10/26/2010	11/1/2010	12/17/2010
Hematology/CRP Binder, including summary sheets	DQA	10/29/2010	11/1/2010	11/2/2010	12/17/2010
α PA IgG ELISA Binders 1 and 2	DQA	11/17/2010	11/19/2010	11/23/2010	12/17/2010
Micro Binder	DQA	12/13/2010	12/14/2010	1/28/2011	2/24/2011
Pathology Binder	DQA	12/13/2010	12/14/2010	5/20/2011	6/8/2011
Vivo Binder	DQA	12/13/2010	12/14/2010	1/18/2011	2/24/2011
Inhalation Exposure Report	DQA	12/13/2010	12/14/2010	12/15/2010	12/17/2010
CircPA ELISA	DQA	12/14/2010	12/15/2010	1/17/2011	2/24/2011
TNA Binders 1–3, results table added 2/11/11	DQA	12/14/2010	12/15/2010	1/28/2011	2/24/2011

4.3 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.

5 Discussion and Conclusions

The previous study determined the physiological dose response to an acute inhalational exposure to various low-dose concentrations of *B. anthracis* spores (U.S. EPA, 2011). That study showed a dose effect on the physiological changes and that increases in respiration rate, heart rate, body temperature, and circulating neutrophils corresponded to decreased survival rates. Temperature was the most consistent indicator of disease outcome. All rabbits that had a febrile response died on study. The objective of this present study was to build on the data of the acute low challenge inhalational dose study and determine the physiological responses following multiple exposures to low-dose concentrations of *B. anthracis* spores. A summary of the findings is presented in Table 12.

Rabbits receiving multiple exposures to irradiated spores served as sham challenge controls and showed little physiological response. In fact, the only discernible reaction was a rise in CRP levels, which most probably was the result of stress due to study activity. This was similar to what was seen in the acute study when rabbits were exposed to 1.05×10^7 irradiated spores. This suggests that exposure to avirulent spore coat material either acutely or in multiple doses does not prompt a detectable physiological reaction.

All of the rabbits exposed to 15 doses of a mean of 2.91×10^2 CFU (Group 2) lived to the end of the study and showed minimal physiological changes due to the exposures. None of these rabbits was ever bacteremic or toxemic. The mean accumulated dose of inhaled CFU for the group after the 15 challenge days was 4.36×10^3 ($\pm 8.11 \times 10^2$) CFU. This average accumulated dose was higher than the previous study's acute inhaled dose of 2.06×10^3 CFU administered to 5 animals in which no measurable physiological effect was observed.

In the present study one rabbit (Rabbit 2) succumbed to disease in the 1.0×10^3 CFU targeted inhaled dose group. This animal's mean dose was 1.33×10^3 ($\pm 5.95 \times 10^2$) CFU and it was exposed to 1.86×10^4 total

CFU for 14 of the 15 days of challenge. This latter total exposure corresponded to a group of five animals that received a single dose of 2.54×10^4 ($\pm 5.21 \times 10^3$) CFU in the acute study in which two of the five rabbits died at 4 and 11 days post-challenge. Each animal presented with tachycardia, tachypnea, increased body temperature, neutrophilia, and bacteremia. Rabbit 2 in the present study succumbed to disease 17.9 days after the first challenge and also presented with tachycardia, tachypnea, increased body temperature, neutrophilia, bacteremia, and toxemia. These latter signs did not appear until just prior to death. Additionally, only the terminal blood draw was positive for bacteremia and toxemia. These results suggest that either an infection was not established until the majority of the challenges had taken place or the infection was localized and the rabbit did not respond in a systemic manner.

Four of the seven rabbits that received a mean inhaled dose of 1.0×10^4 ($\pm 4.64 \times 10^3$) CFU died during the study with a mean time to death of 14.80 ± 4.28 days. These rabbits received an accumulated challenge inhaled dose ranging from 5.77×10^4 to 2.16×10^5 CFU. This group of animals responded to the multiple challenges in the same manner as rabbits exposed to a single dose of 2.75×10^5 CFU in the previous acute study. This exposure resulted in four of the five challenged animals dying on study and presenting with tachycardia, tachypnea, increased body temperature, neutrophilia, bacteremia, and toxemia. However, only one of the four rabbits that died became toxemic in the current study. Nine of the eleven rabbits that died in the acute study (regardless of group) were toxemic. The reason for the lack of detection of circulating PA (the indicator of toxemia) is not known. This is the first study that the authors are aware of that used multiple low-dose exposures so there are no other comparable studies with which to relate this finding. It has been established that inhalational anthrax is a biphasic disease with a brief remission of bacteremia and toxemia (Boyer et al., 2009; Brachman, 1980). It is possible that these animals had circulating PA during the course of the infection, but the times of toxemia did not coincide with the blood collections. Most rabbits that succumb to anthrax have detectable PA levels in the late stage blood samples (U.S. EPA, 2011; Mabry et al., 2006; Kobiler et al., 2006). Therefore, more work is required to determine why the animals that succumbed to disease after multiple exposures were not toxemic.

The data generated in the acute study suggested an “all-or-none” outcome to disease. That is, once an infection was established (marked by bacteremia/toxemia and physiological changes) the disease progressed to a fulminant state and resulted in the death of the rabbit. In the current study, two rabbits showed signs of infection but lived to the end of the study. Rabbit 38 (Group 4) presented with all of the clinical signs mentioned above and was both bacteremic and toxemic during the study. However, the neutrophilic response was more robust than that previously seen and the animal seroconverted by Day 25. This suggests that the animal was able to mount a significant innate response to clear the infection and establish a humoral response that would counteract any residual circulating toxin. Another survivor in this group, Rabbit 21, presented with a fever, neutrophilia, and toxemia but never seroconverted. While the neutrophilia was substantially less than that of Rabbit 38, the animal survived to the end of the study. The infection may not have been as severe as that of Rabbit 38 as indicated by the lower neutrophilic response and the fact that Rabbit 21 never seroconverted.

All rabbits, including study survivors, underwent complete necropsies, and the lungs and any gross lesions were examined microscopically. All rabbits (survivors and non-survivors) had pathological findings consistent with inhalational anthrax in this model (Zaucha et al., 1998). Interestingly, multinucleated giant cells as well as foreign body granulomas/pyogranulomas were present in the lungs of challenged rabbits but were not seen in control rabbits in this study. In the acute study, multinucleated giant cells were noted in both exposed and control animals. However, the lesions were more severe in challenged rabbits. These multinucleated cells and granulomas/pyogranulomas surrounded foreign material (foreign bodies) consistent with organic debris (e.g., food particles or hair and debris from vascular access ports [Taketoh et al., 2009]). As with the acute study, these lesions could be the result of altered foreign particle clearance by alveolar macrophages (macrophage dysfunction). Macrophage dysfunction has been reported to occur in late sepsis (Pahuja et al., 2008). Prolonged bacteremia/sepsis attributed to anthrax could alter foreign particle clearance by alveolar macrophages, and lethal toxin has been shown to inhibit alveolar macrophage function (Ribot et al., 2006). However, foreign bodies were also found in animals that were not bacteremic and had no bacteria detected in the lungs during histopathological analysis. It is possible that a low-grade bacterial load was present in the lung and affected the function of alveolar macrophages in animals that had bacteria not detected by histopathology. While the foreign bodies were found only in challenged animals in the present study in contrast to the previous acute inhalation exposure study, the causal effect has not been established. Future studies may provide insight on the role of low *B. anthracis* challenge doses and failure to clear foreign bodies from the lungs.

Another pathology finding, perivascular eosinophils in the lungs, is likely attributable to vascular access port placement and has been observed in rodent studies (Taketoh et al., 2009). The observed necrosis in the skin is likely due to self-inflicted trauma.

The rabbit has been shown to be an acceptable model for human inhalational anthrax (Leffel and Pitt, 2006), and the findings from animals that died on this study were consistent with previous observations (Table 12). Dutch Belted rabbits exposed to 100 LD_{50} of *B. anthracis* spores also presented with tachycardia, tachypnea, pyrexia, leukocytosis, and neutrophilia (Lawrence et al., 2009). Yee et al. (2010) also showed that bacteremia and toxemia are associated with fever and hematological changes during inhalational anthrax disease progression in NZW rabbits after exposure to 150 times the LD_{50} of *B. anthracis* spores. Taken together with the data presented in this report, these findings suggest that disease progression and observed symptoms are independent of dose once a lethal infection has been established.

The challenge doses and mortality data presented here were used in benchmark dose analysis. While a dose-response relationship was derived through the successful fit of mathematical models to the study data, it should be noted the reported BMDLs and the measures derived from the BMDLs (i.e., HED and HEC) should be considered to be highly uncertain.

These dose-response data may also provide preliminary evidence that a threshold in the average daily dose may be present below which lethality is unlikely to occur in a healthy, adult male rabbit population. This is evidenced by the survival rates of the two lowest dose groups. Further testing of levels between these two doses may allow modeling of this potential threshold value.

When comparing the benchmark dose analyses of the data from this work and the previously-performed acute study (Hines et al., 2011), there are preliminary indications that a discernible relationship may exist between the measured endpoint of lethality and the administered dose, exposure duration, and number of doses. The basis for this hypothesis is that the total aggregate dose $BMDL_{10}$ of 4,900 CFU is approximately 3.5 times the $BMDL_{10}$ of 1,400 CFU from the acute study (i.e., single dose), and the total aggregate dose $BMDL_{50}$ of 44,000 CFU is approximately 3.4 times the $BMDL_{50}$ of 13,000 CFU from the same acute study. Given the limited availability of multiple low dose-response data sets for *B. anthracis* exposures, techniques to model the relationship between dose, concentration, and exposure duration may provide useful information to further characterize the hazard posed by acute and short-term exposure scenarios to low levels of *B. anthracis* contamination.

The data presented in this report suggest that rabbits exposed to multiple inhalation doses of a mean of 2.91×10^2 CFU of *B. anthracis* do not exhibit a measurable physiological response. Also, animals that exhibit responses and seroconvert may recover from infection.

While the work presented here starts to fill in the knowledge gaps in low dose *B. anthracis* exposures, there are some limitations that are difficult for overcome in a laboratory setting. For instance, an intentional release of spores will be more likely to use dried spores to increase dispersal and infectivity. This study used a wet preparation of spores in a very controlled environment. The challenge dose was determined mathematically and deposition of spores in the lungs was not determined. Evaluating deposition and accumulation of spores in the lungs will require serial euthanasia of animals and CFU counts of the lungs at different times post-exposure. Performing plate counts of the lungs was not in the scope of the presented work but may be included in future studies. Future studies with a serial pathology focus could also assist in confirming the sites involved in the initiation of infection.

The objective of this study was limited to identified physiological responses in the rabbit model of disease. This study assessed

PA in the blood, but did not look for other *B. anthracis*-specific biomarkers. Future studies may look for biomarkers, such as HtrA and NlpC/P60, as identified by Sela-Abramovich et al. (2009) or develop additional biomarkers for evaluation. In tandem with work to identify biomarkers suitable for modeling infection and disease, studies could also be conducted to inform the identify and measure critical biokinetic and biodynamic parameters to further inform development of physiologically based biokinetic models (PBBK) for anthrax pathogenesis.

Table 12. Summary of Study Findings

Group	ID	Mean Inhaled Dose (CFU/Animal)		# of Exposures	Sum of Doses (CFU/ Animal)	Heart Rate*	Respiratory Rate*	Body Temp*	White Blood Count†	Neutrophil †	CRP†	Bacteremia	Toxemia	Time to Death (day)
		Mean	SD											
	40	0	0	15	0	↔	↔	↔	↔	↓	↑	-	-	Survived
	7	0	0	15	0	↔	↔	↔	↓	↓	↑	-	-	Survived
1	5	0	0	15	0	↔	↔	↔	↑	↔	↔	-	-	Survived
	9	0	0	15	0	↔	↔	↔	↔	↔	↑	-	-	Survived
	37	0	0	15	0	↔	↔	↔	↓	↓	↑	-	-	Survived
	13	3.85 x 10^{2}	7.57 x 10^{2}	15	5.78 x 10^{3}	↔	↔	↔	↑	↔	↑	-	-	Survived
	34	3.17 x 10^{2}	4.48 x 10^{2}	15	4.76 x 10^{3}	↔	↔	↔	↑	↔	↑	-	-	Survived
	25	2.79 x 10^{2}	3.54 x 10^{2}	15	4.19 x 10^{3}	↔	↓	↔	↑	↔	↑	-	-	Survived
2	15	3.17 x 10^{2}	3.27 x 10^{2}	15	4.76 x 10^{3}	↔	↔	↔	↔	↔	↔	-	-	Survived
	30	2.72 x 10^{2}	2.33 x 10^{2}	15	4.07 x 10^{3}	↔	↑	↔	↔	↔	↔	-	-	Survived
	28	2.34 x 10^{2}	1.49 x 10^{2}	15	3.51 x 10^{3}	↔	↔	↔	↔	↔	↔	-	-	Survived
	19	2.32 x 10^{2}	1.28 x 10^{2}	15	3.48 x 10^{3}	↔	↔	↔	↓	↔	↑	-	-	Survived
	14	7.38 x 10^{2}	2.99 x 10^{2}	15	1.11 x 10^{4}	↔	↔	↔	↑	↔	↔	-	-	Survived
	11	1.12 x 10^{3}	5.01 x 10^{2}	15	1.68 x 10^{4}	↔	↔	↔	↑↓	↑	↑	-	-	Survived
	2	1.35 x 10^{3}	5.78 x 10^{2}	14	2.02 x 10^{4}	↑	↑	↑	↔	↔	↔	+	+	17.9
2	8	1.40 x 10^{3}	6.04 x 10^{2}	15	2.10 x 10^{4}	↔	↔	↔	↑	↔	↑	-	-	Survived
	12	1.30 x 10^{3}	4.90 x 10^{2}	15	1.95 x 10^{4}	↔	↔	↔	↔	↔	↑	-	+	Survived
	18	1.24 x 10^{3}	5.56 x 10^{2}	15	1.85 x 10^{4}	↔	↔	↔	↑	↔	↔	-	-	Survived
	32	1.89 x 10^{3}	1.87 x 10^{3}	15	2.83 x 10^{4}	↔	↔	↔	↔	↔	↔	-	-	Survived
	6	6.41 x 10^{3}	2.57 x 10^{3}	9	5.77 x 10^{4}	↑	↑	↑	↑	↑	↑	-	-	10.9
	33	9.75 x 10^{3}	2.58 x 10^{3}	10	9.75 x 10^{4}	↑	↑	↑	↔	↔	↑	+	-	12.7
	27	1.08 x 10^{4}	3.65 x 10^{3}	14	1.51 x 10^{5}	↑	↔	↑	↑	↔	↑	+	-	20.8
4	31	1.25 x 10^{4}	3.27 x 10^{3}	11	1.37 x 10^{5}	↑	↔	↑	↔	↑	↑	+	+	14.7
	39	1.44 x 10^{4}	5.99 x 10^{3}	15	2.16 x 10^{5}	↔	↔	↔	↑	↔	↑	-	-	Survived
	21	1.32 x 10^{4}	4.97 x 10^{3}	15	1.98 x 10^{5}	↔	↔	↑	↔	↑	↑	-	+	Survived
	38	1.27 x 10^{4}	3.77 x 10^{3}	15	1.91 x 10^{5}	↑	↑	↑	↑	↑	↑	+	+	Survived

↑= 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
*= Changes based on baseline
† = Changes based on normal ranges

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APPENDIX A
STUDY PROTOCOL

Rabbit Multiple Dose Anthrax Telemetry Study

Study No. 1078-CG920794

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For Gloria S. Sivko, Ph.D., D.V.M. Technical Reviewer	7-22-10 Date
Jason M. Mott, D.V.M., Ph.D. BBRC Associate Director	7-22-10 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 exposures 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 revealed 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 multiple exposures of varying doses of *Bacillus 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 and vascular access port (VAP) implantation surgery and histopathology 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 multiple exposures to *B. anthracis* Ames strain spores.

4.0 TEST SYSTEM

Animals: Thirty (30) male pathogen free New Zealand White (NZW) rabbits (*Oryctolagus cuniculus*) weighing at least 2.5 kg will be ordered from Covance (Denver, PA) for this study. Rabbit age will not be used as a criterion for placement on study. Twenty six (26) rabbits will be placed on study with the remaining four serving as replacements. A Battelle veterinarian will implant Data Sciences International model D70-PCT telemetric devices and femoral or jugular vascular access ports (VAPs) into the rabbits prior to 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. The four extra animals will also be implanted with telemetric devices and VAPs in case either apparatus fails in a study rabbit.

5.0 STUDY DESIGN

5.1 Randomization of Animals: Prior to challenge, rabbits will be randomized by body weight into three groups of seven and one control group of five. The rabbits within each group will also be randomized for challenge order (based on ear tag numbers) and 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 4 will be challenged last. Prior to challenge, any animal with a malfunctioning VAP or telemetric device will be replaced with one of the four extra animals.

5.2 Aerosol Challenge Generation and Monitoring: Each rabbit will be exposed to *B. anthracis* Ames Strain spores once a day for five straight working days each week for 3 straight weeks (up to 15 times each). On challenge days, rabbits will be placed into a plethysmography chamber, passed into a Class III cabinet system, and aerosol challenged with targeted doses of 100, 1,000, and 10,000 CFUs of *B. anthracis* (Ames strain) spores. The negative control group will be exposed to the equivalent of 10,000 CFUs of gamma-irradiated spores.

The first day of challenges will be designated as Study Day 0.

Aqueous suspensions 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. Negative control animals with be challenged in a separate hood line from the groups receiving viable spores to mitigate cross contamination. Impinger samples containing irradiated spores will be plated neat in triplicate to confirm that the animals were not exposed to viable spores.

Impinger samples from the groups receiving targeted doses of 100 or 1,000 CFUs will also be enumerated by a filter method according to SOP BBRC X-199. Briefly, 1 mL of the sample will be passed through a sterile 0.45 micron filter (Nalgene) and an Analytical Test Filter Funnel, (Fisher catalog number 145-0045). The filter will then be placed on top of a TSA plate and incubated for 24-72 hours at 37°C ± 2°C. The colonies that form on the filter will then be enumerated. Please note that colonies outside the range of 25-250 colonies/filter may be accepted at the discretion of the study director. 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.

Table 1. Study Design and Challenge Doses.

Group	Spore dose (CFU)	Number of Spore Challenges [1]	# of Rabbits
1 (neg) control*	10,000*	15	5
2	100	15	7
3	1,000	15	7
4	10,000	15	7

*Spores are inactivated/killed by irradiation. Challenges will occur in a separate aerosol system from the viable spore challenges.

[1] Will be challenged once a day for 5 straight working days (Monday thru Friday) each week for three straight weeks

5.3 Animal Weights: Animals will be weighed on Study Days 2, 9, 16, 23, 30, and 37 (Study Day 0 will be the first day of challenges). Animals will also be weighed prior to delivery to the BBRC and this weight will be used for randomization in to groups.

5.4 Blood Collection Schedule: On Study Days -3, 2, 4, 9, 11, 16, 18, 23, 25, 30, 32, and 37 blood will be collected into EDTA (~1.0 mL) and SST tubes (~2.0 – 2.5 mL) (Table 2). Collection time points for each rabbit will be relative to the very first challenge day (Challenge Day 0) on Week 1. If possible, a blood sample will be taken from animals found dead or prior to euthanasia and divided between the two types of tubes. On Study Day 39, all surviving rabbits will be terminally bled 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 collected in EDTA tubes will be stored at room temperature if utilized within 4 hours of collection. If not analyzed within 4 hours of collection, the blood will be stored at 2-8 °C. Sera will be stored at ≤ -70 °C until needed.

Blood samples will be collected from VAPs on Study Days for the entire study unless directed by the study director or the port malfunctions. If a port fails, the medial auricular artery, the marginal ear vein, or other appropriate vasculature may be utilized for blood collection if attainable. Rabbits will be sedated with Acepromazine prior to collecting blood from the ear. 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 2**	**Day 4**	**Day 9**	**Day 11**	**Day 16**	**Day 18**	**Day 23**	**Day 25**	**Day 30**	**Day 32**	**Day 37**	**Day 39**
EDTA (~ml)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	2.0
SST (~ml)	2.5	2.0	2.5	2.0	2.5	2.0	2.5	2.0	2.5	2.0	2.5	2.0	10.0
Total per day~(ml)	3.5	3.0	3.5	3.0	3.5	3.0	3.5	3.0	3.5	3.0	3.5	3.0	12.0*

*End of in-life blood sample, minimum draw volume.

5.5 VAP Maintenance: The VAPs will be maintained weekly until the end of the study or until the loss of patency. The skin over and ~3 inches around the VAP will be clipped weekly or as needed. Prior to flushing the port, clean the area three times with Nolvasan scrub and 70% alcohol. There will be 40 seconds between the last Nolvasan scrub and before final alcohol wash. Place the Huber needle into the VAP, then with a 3ml syringe pull the block. Pull blood samples, if required. Flush with 2mL of 0.9% saline and block with 1.0 mL of blocking solution (4.0 mL of 1000 IU of heparin in 16 mL of 50% dextrose.

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

Additionally, 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 based upon the schedule outlined in Table 3.

5.7 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).

5.8 Hematology and C-Reactive Protein: 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)

After hematological analysis is complete, plasma will be harvested from the residual sample according to SOP BBRC. V-033. The plasma sample will then be assayed for C-reactive protein levels (CRP) according to SOP BBRC. VI-077.

The assay priority list is below:
Whole Blood: Bacteremia via quantitative plating >>> Hematology >>> C-reactive protein >>> Bacteremia via Quantitative PCR .Although lower in priority, the PCR aliquot has to be removed before hematology to avoid cross contamination.
Sera: Circulating PA ELISA >>> Retention sample (maximum 750 uL if possible) >>> Anti-PA IgG ELISA and TNA.

All assays listed in Table 3 will be performed on terminal samples for rabbits that die on study with the exception of hematology and CRP. Hematology and CRP will not be performed on samples collected for rabbits that were found dead.

Table 3. Schedule of assays to be performed on each study day.

	Study Day													
Assay	**Day -3**	**Day 2**	**Day 4**	**Day 9**	**Day 11**	**Day 16**	**Day 18**	**Day 23**	**Day 25**	**Day 30**	**Day 32**	**Day 37**	**Day 39**	**Terminal**
Hematology	•	•	•	•	•	•	•	•	•	•	•	•	•	
C-reactive protein	•	•	•	•	•	•	•	•	•	•	•	•	•	
Quant. bact. culture	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Quant. bact. PCR	•	•	•	•	•	•	•	•	•	•	•	•	•	•
circ. PA ELISA	•	•	•	•	•	•	•	•	•	•	•	•	•	•
TNA, anti-PA IgG ELISA	•		•		•		•		•		•		•	•

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

5.10 Telemetric Monitoring: Rabbits will be surgically implanted with telemetry units (D70-PCT 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, Electrocardiogram (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 39 days after the first challenge day according to SOP BBRC.VI-087. ECG data will be collected but will not be analyzed, but will be available for future analysis if desired. 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.

5.11 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 39. The lungs and any gross lesions 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.* (1998) 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 trained life sciences technician, Battelle veterinarian, or by the Study Director will be euthanized.

Rabbits that are euthanized will be sedated with Acepromazine or other approved anesthetic and then administered a commercially prepared euthanasia solution at the prescribed dose.

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 SOP No. BBRC IV-008.

Humidity: The relative humidity of the animal rooms will be maintained according to Battelle SOP No. BBRC IV-008.

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 the rabbits, enrichment will be as described in Battelle SOP No. BBRC. VII-040.

7.0 ANALYTICAL AND STATISTICAL PLAN

7.1 Sample Size:

Groups of 7 animals are sufficient to detect group effects in a one-way analysis of variance (ANOVA) model with greater than 80% power, when a 2x 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 conducted or Cox proportional hazards model will be fitted.

For circulating PA levels, TNA/ELISA, hematology, CRP, and telemetric data, descriptive statistics will be produced for each animal at each sample collection time. As all negative control animals are expected to survive, baseline values for each animal 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 ANOVA model will be fitted to determine if parameters changed significantly from baseline and whether there were significant differences between groups.

To evaluate the change in health status due to challenge, the post-challenge telemetry endpoints may be adjusted to the 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 39.

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.

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 39 days post-last 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-029, "Standard Operating Procedure (SOP) for the Operation and Maintenance of the ABI Prism® 7900HT Fast Sequence Detection System."

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 Electrochemiluminescence (ECL) Screening Assay using Serum."

Battelle SOP Number MREF. VI-023, "Standard Operating Procedure (SOP) for the Use and Monitoring of Cold Storage Units (CSU)."

Battelle SOP Number MREF. VI-027, "Standard Operating Procedure (SOP) for the Operation, Calibration, and Maintenance of Electronic Balances."

Battelle SOP Number BBRC. VI-029, "Standard Operating Procedure (SOP) for the Use of the Mettler Toledo Balancelink."

Battelle SOP Number BBRC. VI-044, "Standard Operating Procedure (SOP) for the Operation and Maintenance of the Bio Medic Data System DAS-6007 Handheld Scanner."

Battelle SOP Number MREF. VI-054, "Standard Operating Procedure (SOP) for the Operation and Maintenance of the Dickson Model SP100 and SP150 Pro Temperature Data Loggers."

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 MREF. VI-084, "Standard Operating Procedure (SOP) for the Operation And Maintenance Of Primus General Purpose Steam Sterilizer Model: PSS5-A-MSSD."

Battelle SOP Number BBRC.VI-087, "Standard Operating Procedure (SOP) for Use and Maintenance of the Data Sciences International (DSI) Telemetry System."

Battelle SOP Number MREF. VI-101, "Standard Operating Procedure (SOP) for the General Use, Operation and Maintenance of Microscopes."

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

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 Psychological Well-Being of species other than Non-Human Primates."

Battelle SOP Number BBRC. VII-056, "Standard Operating Procedure (SOP) for the Slow Intravenous Administration by Infusion and Collection of Blood Samples From Venous Access Ports in Rabbits."

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-038, "Standard Operating Procedure (SOP) for the Operation and Maintenance of BBRC Infectious Waste Sterilizers."

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 MREF. X-074, "Standard Operating Procedure (SOP) for the Production of Bacillus Anthracis Spores."

Battelle SOP Number BBRC. X-075, "Standard Operating Procedure (SOP) for the Characterization and Qualification of Bacillus anthracis Spores."

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-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."

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

Battelle SOP Number MREF. X-160, "Standard Operating Procedure (SOP) for the Qualification of Negative Control Sera for Use in the Toxin Neutralization Assay (TNA) and Related Assays."

Battelle SOP Number BBRC. X-164, "Standard Operating Procedure (SOP) for the Qualification of Reference Serum for Use in the Toxin Neutralization Assay (TNA) and Related Assays."

Battelle SOP Number BBRC. X-166, "Standard Operating Procedure (SOP) for the Qualification of Reference Standard Plasmid for Use in Quantitative Real-Time Polymerase Chain Reaction (qPCR)."

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-186, "Standard Operating Procedure (SOP) for the Preparation and Analysis of Phenol Samples in Bacillus anthracis Spore Supernatant."

Battelle SOP Number BBRC. X-199, "Standard Operating Procedure (SOP for the Enumeration of Bacterial Colony Forming Units via the Membrane Filter Count Method."

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

Battelle SOP Number BBRC. XI-006, "Standard Operating Procedure (SOP) for the Calibration and Maintenance of Temperature/Humidity Measuring Instruments and Equipment."

Battelle SOP Number MREF. XI-007, "Standard Operating Procedure (SOP) for Labeling Reagents, Solutions, Test, Control and Reference Article/Substances, and Specimens."

Battelle SOP Number MREF. XI-025, "Standard Operating Procedure (SOP) for the General Preparation of Dilutions."

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. XIII-002, "Standard Operating Procedure (SOP) for the Maintenance and Decontamination of the Aerosol System."

Battelle SOP Number BBRC. XIII-005, "Standard Operating Procedure (SOP) for the Cleaning, Maintenance and Use of Collison Nebulizers and Impingers."

Battelle SOP Number MREF. XIII-006, "Standard Operating Procedure (SOP) for the Verification of Mass Flow Meters, Mass Flow Controllers, Magnehelics and Gauges."

Battelle SOP Number BBRC. XIII-008, "Standard Operating Procedure (SOP) for Programming the Buxco Biosystem XA Data Acquisition Software for Pulmonary Analysis during Animal Inhalation Studies."

Battelle SOP Number BBRC. XIII-009, "Standard Operating Procedure (SOP) for the Calibration and Operation of the Buxco Biosystem, Preamplifier System and Pressure Transducers."

Battelle SOP Number BBRC. XIII-010, "Standard Operating Procedure (SOP) for the Cleaning, Decontamination, and Maintenance of NHP and Rabbit Plethysmography Boxes."

Battelle SOP Number BBRC. XIII-011, "Standard Operating Procedure (SOP) for Using and Checking the Calibration of the Aerodynamic Particle Sizer 3321."

Battelle SOP Number BBRC. XIII-012, "Standard Operating Procedure (SOP) for Conducting Spray Factor Testing of Aerosolized Bacillus anthracis Spores Using the Battelle Aerosol Exposure System."

Battelle SOP Number BBRC. XIII-018, "Standard Operating Procedure (SOP) for the Calibration and Operation of the Buxco Biosystem XA (Windows Version 2.7.9)."

Battelle SOP Number BBRC. XIII-022, "Standard Operating Procedure (SOP) for the Operation of the Aerosol Challenge Database."

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.

APPENDIX B
STUDY DEVIATIONS AND INVESTIGATION REPORTS

BATTELLE BIOMEDICAL RESEARCH CENTER
DEVIATION FORM

Deviation No. (Assigned by QAU): DR-10019	CAQ No. (Assigned by QAU): NA

Standard or Procedure Deviated:

☒ **Protocol** (Number and Amendment No. if applicable): **1078**
☐ **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: 1078-CG920794**

Study Title: Rabbit Multiple Dose Anthrax Telemetry Study

Record Affected (describe Title, Binder name, location, Form no. etc.): Microbiology binder, form Microbio-455

Date of Deviation(s): July 26-~~July 30~~ and August ~~2~~-August 13 August 4 wd ~~RD~~ 8-9-10 5 wd RD 8-9-10

Description of Deviation:
Protocol states that filtered samples will be incubated for 24-72 hours before being read. For the time period of July 26-~~July 30~~ the protocol was deviated because samples were incubated for less than 24 hours, generally about 16-24 hours. Aug 4 wd ~~RD~~ 8/11/10

Root Cause of Deviation:
Filtered samples were incubated with non-filtered samples and were taken out and counted at the same time. Colonies were found to be of a size easy to count. Waiting beyond 24 hours would result in colonies too large to count.

Corrective Action: 5 wd ~~RD~~ 8/11/10
For the remainder of the aerosol challenge (August ~~2~~-13) the samples will continue to be counted following an incubation of 16-72 hours.

Impact of Deviation: Positive: Extending the incubation time to a shorter period allows the plates to be counted at the same time as the non filtered samples.

If deviation is planned, effective date: ~~8/2/10 to 8/13/10~~ 8-5-10 to 8-13-10 wd RD 8-9-10

Deviation Form Prepared by/Date: RD 8/2/10 [signature] 8-5-10

Deviation Reviewed and Corrective Action Accepted by/Date (Study Director or Responsible Individual): [signature] 8.5.10 [initials] 8.11.10

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
[signature] 8/5/10

Deviation Reviewed and Registered by QAU/Date: [signature] 8/11/10	☐ Category I ☒ Category II (See SOP XI-023 for details)

BATTELLE BIOMEDICAL RESEARCH CENTER
DEVIATION FORM

Deviation No. (Assigned by QAU): DR-10063	**CAQ No. (Assigned by QAU):** NA

Standard or Procedure Deviated:

☐ **Protocol (Number and Amendment No. if applicable):**
☑ **SOP (Number and Revision Number):** XI-009-03
☐ **Method (Number and Revision Number):**
☐ **GLP (Section):**
☐ **Other:**

Type of Deviation (check one):

☐ **Facility**

☑ **Study (fill out study info)** **Study Number:** 1078-CG920794

Study Title: Rabbit Multiple Dose Anthrax Telemetry Study

Record Affected (describe Title, Binder name, location, Form no. etc.): Microbiology binder, Form Microbio-380

Date of Deviation(s): 8/2/10

Description of Deviation: An animal ID was not listed as being included in either incubator.

Root Cause of Deviation: Animal ID 28 was inadvertently left off of the list of samples in both of the incubators listed on the paperwork.

Corrective Action: The technician reviewed SOP XI-009 and was reminded to verify that all animal IDs are accounted for on the incubation paperwork. AW 8.11.10

Impact of Deviation: Minimal. It cannot be determined which incubator the plates were placed in, but the plates in both incubators were incubated for an appropriate amount of time and the plates for the animal in question returned satisfactory results.

If deviation is planned, effective date: NA

Deviation Form Prepared by/Date: AW 8-11-10

Deviation Reviewed and Corrective Action Accepted by/Date (Study Director or Responsible Individual): [signature] 8-11-10

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
[signature] 8/13/10

Deviation Reviewed and Registered by QAU/Date: Zach Williams [signature]

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

BATTELLE BIOMEDICAL RESEARCH CENTER
DEVIATION FORM

Deviation No. (Assigned by QAU): DR-10263	**CAQ No. (Assigned by QAU):** NA

Standard or Procedure Deviated:

☐ **Protocol (Number and Amendment No. if applicable):**
☒ **SOP (Number and Revision Number):** BBRC VI-087
☐ **Method (Number and Revision Number):**
☐ **GLP (Section):**
☐ **Other:**

Type of Deviation (check one):

☐ **Facility**

☒ **Study (fill out study info)** **Study Number:** 1078-CG920794

Study Title: Rabbit Multiple Dose Anthrax Telemetry Study

Record Affected (describe Title, Binder name, location, Form no. etc.): Telemetry Data

Date of Deviation(s): 7/22/10

Description of Deviation: BBRC VI-087 states: Refer to Form No. Telemetry-006, or equivalent, for the transmitter serial number and calibration information.

On 7/22/10 the configuration was created with incorrect calibration values for IDs 30 and 38. The ECG calibration for ID 30 was entered as 22.6 mV when 22.3 mV should have been entered. Temperature calibration 3 of ID 38 was entered as 756.3, when 756.6 should have been entered.

Root Cause of Deviation: This was caused by operator error while entering the calibration values from the calibration cards into the telemetry computer. Data collection was required to start when the animals arrived to the facility, which did not allow sufficient time to enter and review all configuration values.

Corrective Action: When possible, transmitter calibration values will be entered prior to animal arrival to allow more time to enter and review calibration values.

Impact of Deviation: Minor. The small difference in the calibration values will not cause noticeable differences in the data. Additionally, post-challenge data is compared to the baseline data which would offset any differences caused by an incorrect calibration value.

If deviation is planned, effective date: NA

Deviation Form Prepared by/Date: [signature] 10/4/10

Deviation Reviewed and Corrective Action Accepted by/Date (Study Director or Responsible Individual): [signature] 10-6-10

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
[signature] 10/6/10

Deviation Reviewed and Registered by QAU/Date: [signature] 10/8/10

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

BATTELLE BIOMEDICAL RESEARCH CENTER
DEVIATION FORM

Deviation No. (Assigned by QAU): DR-10264	CAQ No. (Assigned by QAU): NA

Standard or Procedure Deviated:

☒ **Protocol** (**Number and Amendment No. if applicable**): BBRC Protocol 1078
☐ **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:** 1078-CG920794

Study Title: Rabbit Multiple Dose Anthrax Telemetry Study

Record Affected (describe Title, Binder name, location, Form no. etc.): 1078-CG920794 Telemetry Data

Date of Deviation(s):
7/23/10

Description of Deviation:
Protocol 1078 states "Body temperature, Electrocardiogram (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 39 days after the first challenge day according to SOP BBRC.VI-087."

Parameters were monitored for 3 days pre-challenge.

Root Cause of Deviation:
The draft protocol initially required 3 days of pre-challenge baseline data and was used when preparing the schedule for the start of data collection. Also, the study animals arrived to the BBRC 6 days pre-challenge, 1 day after baseline collection should have started.

Corrective Action:
Telemetry lead was reminded to verify start of data collection with study director when a final protocol is not available. NEG 9/14/10

Impact of Deviation:
Minor. Three days of baseline telemetry data will be used for comparison to post-challenge data during statistical analysis. 2-3 days of baseline data is commonly used for analysis and was the initial requirement of the draft protocol.

If deviation is planned, effective date: NA

Deviation Form Prepared by/Date: [signature]

Deviation Reviewed and Corrective Action Accepted by/Date (**Study Director or Responsible Individual**): [signature] 9.15.10

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
[signature] 9/15/10

BATTELLE BIOMEDICAL RESEARCH CENTER **DEVIATION FORM**		
Deviation No. (Assigned by QAU): DR-10264	**CAQ No. (Assigned by QAU):** NA	
Deviation Reviewed and Registered by QAU/Date: [signature] 10/8/10		☐ Category I ☒ Category II (See SOP XI-023 for details)

BATTELLE BIOMEDICAL RESEARCH CENTER
DEVIATION FORM

Deviation No. (Assigned by QAU): 10350	**CAQ No. (Assigned by QAU):** NA

Standard or Procedure Deviated:

☐ **Protocol (Number and Amendment No. if applicable):**
☒ **SOP (Number and Revision Number):** X-090-06
☐ **Method (Number and Revision Number):**
☐ **GLP (Section):**
☐ **Other:**

Type of Deviation (check one):

☐ **Facility**

☒ **Study (fill out study info)** **Study Number:** 1078-CG920794

Study Title: Rabbit Multiple Dose Anthrax Telemetry Study

Record Affected (describe Title, Binder name, location, Form no. etc.): Form No. BBRC BL3-026 and Microbio-435

Date of Deviation(s): 8/9/2010, 9/7/10, and 9/13/2010

Description of Deviation:
BBRC SOP X-090 Section V. B. states the following: "It is the responsibility of the Battelle Safety, Health and Emergency Response Representative, BSTI to provide approval for the release of tested sample(s) from the BSL-3 laboratory via form No. BBRC BL3-026, or equivalent." Thus, the deviation is as follows: On 8/9/2010, 9/7/10, and 9/13/2010 a technician passed serum out of the BL3 prior to receiving signature authority from the Safety Officer on Form No. BBRC BL3-026. The safety officer did, however, approve the sterility of the samples on Form No. Microbio-463 before release. On 8/10/10, 9/10/10, and 9/14/10, respectively, a signature was received on Form No. BBRC BL3-026 to authorize their release.

Root Cause of Deviation: Technician did not get a signature on BBRC-BL3-026 prior to removal of samples out of the BL3.

Corrective Action: Ensure that the safety officer signs Form No. BBRC-BL3-026 prior to pulling samples out of the BL3. Forms and/or SOP could be revised to eliminate the redundancy of the safety officer having to sign in two places.

Impact of Deviation: None. A sterility check was performed on all samples prior to their removal from the BL3; all serum aliquots were negative. This information was recorded on Microbio-463 and was confirmed by the Safety Officer before the samples were released from the BL3.

If deviation is planned, effective date: NA

Deviation Form Prepared by/Date: BB 10/27/10

Deviation Reviewed and Corrective Action Accepted by/Date (Study Director or Responsible Individual): [signature] 12-15-10

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
[signature] 12/15/10

Deviation Reviewed and Registered by QAU/Date: [signature] 2/1/11	☒ Category I ☐ Category II (See SOP XI-023 for details)

BATTELLE BIOMEDICAL RESEARCH CENTER
DEVIATION FORM

Deviation No. (Assigned by QAU): 10351	**CAQ No. (Assigned by QAU):** NA

Standard or Procedure Deviated:

☐ **Protocol (Number and Amendment No. if applicable):**
☒ **SOP (Number and Revision Number):** XI-009-03
☐ **Method (Number and Revision Number):**
☐ **GLP (Section):**
☐ **Other:**

Type of Deviation (check one):

☐ **Facility**

☒ **Study (fill out study info)** **Study Number:** 1078-CG920794

Study Title: Rabbit Multiple Dose Anthrax Telemetry Study

Record Affected (describe Title, Binder name, location, Form no. etc.): Study 1078-CG920794 Microbiology Binder. Enumerations for Quantitative Bacteremia; Form No. Microbio-472

Date of Deviation(s): 7/24/10

Description of Deviation: According to SOP XI-009 Section VA4: Study raw data and other data entries made in supporting records must be recorded clearly, accurately, legibly, completely and promptly in indelible black or blue ink. This includes, but is not limited to observations, calculations, measurements, materials used, test and control articles, critical reagents/solutions, equipment information (see Reference III.F. and Section V.A.10. of this SOP), and experimental and/or operational details to confirm the performance of the operation as specified. The technician did not accurately record the results of the challenge organism for animal IDs 33 and 31 Day -3 E1 quantitative bacteremia plates.

Root Cause of Deviation: The technician recorded a challenge organism colony as present on Day -3 E1 plates for animal IDs 33 and 31. As the results are written, it states there is one colony of the challenge organism and one contaminant present on one of the three plates for each animal ID. All three E1 plates for animal IDs 33 and 31 should read 0, with a comment on one plate each that contamination was present.

Corrective Action: Upon speaking with the Study Director, the technician has been asked to review SOP XI-009 for proper documentation and recording procedures. Proper documentation was also discussed between a study coordinator and the technician. The technician has been reminded that only challenge organism colonies present are recorded as the result for quantitative bacteremias. Contamination is documented with a footnote.

Impact of Deviation: Minimal. There is little possibility that the challenge organism could be present at the Day -3 time point. None of the other animals tested at this time had challenge organism colonies present. The quantitative bacteremia results for Day -3 will be reported as negative for animals 33 and 31.

If deviation is planned, effective date: NA

Deviation Form Prepared by/Date: KR 10/27/10 ①

Deviation Reviewed and Corrective Action Accepted by/Date (Study Director or Responsible Individual):
[signature] 12-15-10

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
[signature] 12/15/10

Deviation Reviewed and Registered by QAU/Date: [signature] 2/1/11	☒ Category I ☐ Category II (See SOP XI-023 for details)

① The responsible technician is no longer a Battelle employee and cannot sign this form. KR 10/27/10

BATTELLE BIOMEDICAL RESEARCH CENTER
DEVIATION FORM

Deviation No. (Assigned by QAU): 10352	CAQ No. (Assigned by QAU): NA

Standard or Procedure Deviated:

☒ **Protocol** (**Number and Amendment No. if applicable**): 1078-CG920794
☐ **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:** 1078-CG920794

Study Title: Rabbit Multiple Dose Anthrax Telemetry Study

Record Affected (describe Title, Binder name, location, Form no. etc.): Microbio-463

Date of Deviation(s): 8/13/2010

Description of Deviation:
On 8/13/2010 a technician did not make a retention aliquot for terminal animal 2, however, made an aliquot for Circulating PA, Anti-IgG ELISA and TNA.

Root Cause of Deviation:
The protocol does not specifically state a retention aliquot is required on terminal animals. The protocol states, "all assays listed in Table 3 will be performed on terminal samples for rabbits that die on study with the exception of hematology and CRP." Table 3 does not list retention as an aliquot/assay required on terminal samples. However, prior to Table 3 the protocol states a priority list for collected sera. This list states Circulating PA ELISA > Retention Sample > Anti-PA IgG ELISA and TNA. Because table 3 did not list retention as an aliquot/assay required, the technician only made aliquots for Circulating PA, Anti-IgG ELISA, and TNA.

Corrective Action:
Notify the study director of protocol ambiguities prior to the start of the study.

Impact of Deviation: Minimal. Residual samples will be pooled and sent to the client.

If deviation is planned, effective date: NA

Deviation Form Prepared by/Date: SB 11/17/10

Deviation Reviewed and Corrective Action Accepted by/Date (Study Director or Responsible Individual): [signature] 12-15-10

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
[signature] 12/15/10

Deviation Reviewed and Registered by QAU/Date: [signature] 2/1/11

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

BATTELLE BIOMEDICAL RESEARCH CENTER
DEVIATION FORM

Deviation No. (Assigned by QAU): 10353	CAQ No. (Assigned by QAU): NA

Standard or Procedure Deviated:

☐ **Protocol (Number and Amendment No. if applicable):**
☑ **SOP (Number and Revision Number):** BBRC XI-009-03
☐ **Method (Number and Revision Number):**
☐ **GLP (Section):**
☐ **Other:**

Type of Deviation (check one):

☐ **Facility**

☑ **Study (fill out study info)** **Study Number:** 1078-CG920794

Study Title: Rabbit Multiple Dose Anthrax Telemetry Study

Record Affected (describe Title, Binder name, location, Form no. etc.): Microbiology Binder, Form No. Microbio-472

Date of Deviation(s): 8/28/10

Description of Deviation: BBRC SOP XI-009-03 states that study raw data and other data entries made in supporting records must be recorded clearly, accurately, legibly, completely and promptly in indelible black or blue ink. This includes, but is not limited to observations, calculations, measurements, materials used, test and control articles, critical reagents/solutions, equipment information and experimental and/or operational details to confirm the performance of the operation as specified. On 8/28/10, all of the results were not documented correctly.

Root Cause of Deviation: The technician did not include footnote 2 in the appropriate results section on the form, meaning that all counts were zero.

Corrective Action: The technician was reminded to verify that all results are documented correctly before submitting paperwork. (The responsible technician is no longer a Battelle employee and cannot sign this form.)

Impact of Deviation: None. All of the counts were zero.

If deviation is planned, effective date: NA

Deviation Form Prepared by/Date: KR 10/27/10

Deviation Reviewed and Corrective Action Accepted by/Date (Study Director or Responsible Individual): [signature] 12-15-10

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
Marcus L. Cole 12/15/10

Deviation Reviewed and Registered by QAU/Date: [signature] 2/1/11

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

BATTELLE BIOMEDICAL RESEARCH CENTER
DEVIATION FORM

Deviation No. (Assigned by QAU): DR-10444 | **CAQ No. (Assigned by QAU):** NA

Standard or Procedure Deviated:

☐ **Protocol (Number and Amendment No. if applicable):**
☑ **SOP (Number and Revision Number): XI-009-03**
☐ **Method (Number and Revision Number):**
☐ **GLP (Section):**
☐ **Other:**

Type of Deviation (check one):

☐ **Facility**

☑ **Study (fill out study info)** **Study Number:** 1078-CG920794

Study Title: Rabbit Multiple Dose Anthrax Telemetry Study

Record Affected (describe Title, Binder name, location, Form no. etc.): S-MP, C-MP, and T-MP Preparation, form No. Microbio-447

Date of Deviation(s): 9/2/10, 9/3/10, 9/8/10

Description of Deviation: Section V.B.3.b of SOP XI-009 states that all quantitative data collected for a regulatory study must be recorded the same day the work was performed. Any omissions or errors discovered must be entered into the study record as a deviation. When preparing the negative control (Lot BMI012), a math error in the dilution calculation was noted. The negative control is to be prepared at a 1:50 dilution, with 1400uL total volume prepared. This would require 28uL of the neat negative control serum into 1372uL of diluent. 1378uL of diluent is recorded, yielding a 1:50.2 dilution.

Root Cause of Deviation: The technicians preparing controls did not notice the math error, and therefore did not correct it at the time of preparation. The correction was made several weeks after the work was performed. It is likely that the incorrect diluent volume recorded was actually the diluent volume used, but this cannot be verified with the information given.

Corrective Action: Technicians have been reminded to check all math, verify all dilutions, and back calculate all calculations prior to performing work on a study. It has been stressed that the accuracy of the dilutions created can make a difference between passing plates and unnecessary repeating of plates due to an incorrectly prepared critical reagent. KAS 11-21-10

Impact of Deviation: Minimal. The dilution of negative control created was 1:50.2 instead of the SOP required 1:50. Since this resulted in a preparation that is more dilute, this may have had an effect on the performance of the negative control within the assay.

If deviation is planned, effective date: NA

Deviation Form Prepared by/Date: [signature] 11/19/10

Deviation Reviewed and Corrective Action Accepted by/Date (~~Study Director or~~ Responsible Individual): [signature] 11-23-10

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
[signature] 11/23/10

Deviation Reviewed and Registered by QAU/Date: [signature] 11/23/10

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

BATTELLE BIOMEDICAL RESEARCH CENTER DEVIATION FORM	
Deviation No. (Assigned by QAU): DR-10496	**CAQ No. (Assigned by QAU):** NA

Standard or Procedure Deviated:

☐ **Protocol** (Number and Amendment No. if applicable):
☒ **SOP** (Number and Revision Number): **XI-009-03**
☐ **Method** (Number and Revision Number):
☐ **GLP (Section):**
☐ **Other:**

Type of Deviation (check one):

☐ **Facility**

☒ **Study (fill out study info)** **Study Number: 1078-C920794**

Study Title: Rabbit Multiple Dose Anthrax Telemetry Study

Record Affected (describe Title, Binder name, location, Form no. etc.): VIVO Binder, Form No. Animal Prep-038-00

Date of Deviation(s): 8/4/10

Description of Deviation:
Technician failed to sign that "Prior to Administration, the initial volume to be given was verified against the Acepromazine range specified in the protocol."

Root Cause of Deviation:
Technician failed to double check that all areas on the form were filled out at the end of study activities.

Corrective Action:
Technician will double check that all areas of forms are properly and completely filled out at the end of each study activity, technician was also asked to review SOP XI-009-03
DRN 9/28/10

Impact of Deviation: Minimal, due to the initial volume given was within the range specified in the protocol.

If deviation is planned, effective date:

Deviation Form Prepared by/Date: DRN 9/28/10

Deviation Reviewed and Corrective Action Accepted by/Date (Study Director or Responsible Individual): [signature] 11-15-10

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 11/15/10

Deviation Reviewed and Registered by QAU/Date: [signature] 12/14/10	☒ Category I ☐ Category II (See SOP XI-023 for details)

BATTELLE BIOMEDICAL RESEARCH CENTER
DEVIATION FORM

Deviation No. (Assigned by QAU): DR-10645	**CAQ No. (Assigned by QAU):** NA

Standard or Procedure Deviated:

- ☐ **Protocol (Number and Amendment No. if applicable):**
- ☒ **SOP (Number and Revision Number):** XI-009-03
- ☐ **Method (Number and Revision Number):**
- ☐ **GLP (Section):**
- ☐ **Other:**

Type of Deviation (check one):

☐ **Facility**

☒ **Study (fill out study info)** **Study Number:** 1078-CG920794

Study Title: Rabbit Multiple dose Anthrax Telemetry Study

Record Affected (describe Title, Binder name, location, Form no. etc.): 1078 PA ELISA binder; CircPA-007

Date of Deviation(s): 10/12/10

Description of Deviation:
According to SOP XI-009, all information and equipment must be recorded and verified at the time of use. The technician did not record the equipment used in 10/12/10.

Root Cause of Deviation:
The technician inadvertently missed printing the equipment form for recording equipment used.

Corrective Action:
The technician was reminded to confirm that all necessary paperwork is printed and to review paperwork thoroughly for accuracy and completion. ALS 11-11-10

Impact of Deviation: Minimal – The permanent equipment used for the assay can be verified per other equipment use dates; however, the transportable equipment like pipettes cannot be verified.

If deviation is planned, effective date: NA

Deviation Form Prepared by/Date: ALS 11-11-10

Deviation Reviewed and Corrective Action Accepted by/Date (Study Director or Responsible Individual): [signature] 11-30-10

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
[signature] 1/14/11

Deviation Reviewed and Registered by QAU/Date: [signature] 1/21/10 WD HM 1/21/11

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

BATTELLE BIOMEDICAL RESEARCH CENTER
DEVIATION FORM

Deviation No. (Assigned by QAU): DR-10646	CAQ No. (Assigned by QAU): NA

Standard or Procedure Deviated:

☐ **Protocol (Number and Amendment No. if applicable):**
☒ **SOP (Number and Revision Number):** XI-009-03
☐ **Method (Number and Revision Number):**
☐ **GLP (Section):**
☐ **Other:**

Type of Deviation (check one):

☐ **Facility**

☒ **Study (fill out study info)** **Study Number:** 1078-CG920794

Study Title: Rabbit Multiple dose Anthrax Telemetry Study

Record Affected (describe Title, Binder name, location, Form no. etc.): 1078 PA ELISA binder; Freeze thaw paperwork form MREF TNA-025

Date of Deviation(s): 10/4/10, 10/5/10, 10/7/10, 10/11/10

Description of Deviation:
According to SOP XI-009, all information and equipment, reagents, and sample IDs must be recorded and verified at the time of use.
a) The technician recorded the CSU that the samples were pulled from but did not record the refrigerator ~~were~~ where they were placed to thaw overnight. SE 1/21/11
b) Some retention samples were used to supplement the PA aliquot for re-analysis. The freeze thaw was recorded, but it wasn't specified if it was for the PA aliquot or the retention sample. Therefore, the retention sample freeze thaw could not be verified.

Root Cause of Deviation:
a) The technician inadvertently missed recording the refrigerator information on the freeze thaw forms.
b) The technician recorded on the process sheets that retention samples were also used but did not include the comment on the freeze thaw forms as well.

Corrective Action:
The technician was asked to review SOP-XI-009 and review all paperwork thoroughly for accuracy and completion. AIS 11-11-10

Impact of Deviation: Minimal – a) The refrigerator information was recorded on the equipment form and can be verified that its use was within the calibration timeframe. b) Since the freeze thaw did not specify whether the sample was from the PA aliquot or retention, the exact freeze thaw for the sample could not be verified.

If deviation is planned, effective date: NA

Deviation Form Prepared by/Date: AIS 11-11-10

Deviation Reviewed and Corrective Action Accepted by/Date (Study Director or Responsible Individual): [signature] 11.30.10

BATTELLE BIOMEDICAL RESEARCH CENTER DEVIATION FORM		
Deviation No. (Assigned by QAU): DR-10646	**CAQ No. (Assigned by QAU):** NA	
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 [signature] 1/14/11		
Deviation Reviewed and Registered by QAU/Date: [signature] 1/21/11		☒ Category I ☐ Category II (See SOP XI-023 for details)

BATTELLE BIOMEDICAL RESEARCH CENTER
DEVIATION FORM

Deviation No. (Assigned by QAU): DR-10047	**CAQ No. (Assigned by QAU):** NA

Standard or Procedure Deviated:

☐ **Protocol (Number and Amendment No. if applicable):**
☒ **SOP (Number and Revision Number):** X-180-03
☐ **Method (Number and Revision Number):**
☐ **GLP (Section):**
☐ **Other:**

Type of Deviation (check one):

☐ **Facility**

☒ **Study (fill out study info)** **Study Number:** 1078-CG920794

Study Title: Rabbit Multiple Dose Anthrax Telemetry Study

Record Affected (describe Title, Binder name, location, Form no. etc.): 1078 CircPA binder; All 1078 Softmax Pro Results packets

Date of Deviation(s): 5/4/2010

Description of Deviation: The current PA ELISA SOP X-180 is vague in its guidance for TS, QC, and NC censoring. The following provides clarification for censoring until the SOP may be appropriately updated:

Change No. #1 to Page 17, BBRC.X-180-03 SOP Acceptance Criteria, make the following change:

Section V.C.5.c.3.i of SOP X-180-03 states that for censoring the reference standard (RS) the following is to be done, "Up to two full dilution pairs (or four individual dilution points) between standard dilution 1 – 8 (or "S1" – "S8") of the RS may be censored or 'masked.' <u>Censor the RS as needed to ensure that the QCs meet the criteria described below.</u>"

<u>Instead (intended to replace the underlined portion above),</u> the RS will be censored only on plates in which two or more of the QC fail, and only if censoring improves the RS curve as judged by the overall percent recovery values moving closer to 100% rather than further away from 100% recovery (hence, worsening the RS curve). The RS should not be censored on plates in which the QCs fail but the RS is generally well behaved, and censoring only serves to worsen the percent recoveries. Also, the reference standard will not be manipulated on a plate in which 2 or 3 of the QCs pass the acceptance criteria.

++

Change No. #2 to Page 17, BBRC.X-180-03 SOP Acceptance Criteria, make the following changes:

Section V.C.5.c.3.ii of SOP X-180-03 currently states that for censoring the quality controls (QCs) and the test samples (TSs) the following is to be done (combining the guidance from both sections), "...the QCs/TSs may be censored as needed to obtain an intra-assay %CV of ≤ 30%. If the % CV is >30%, dilution points may be censored one at a time <u>(starting with the back calculated concentration furthest from the mean)</u> until the % CV is acceptable. It is appropriate to censor to only one dilution point if necessary."

<u>Instead (for the underlined portion above),</u> the QCs and TSs will be censored as follows (these guidelines are

Deviation No. (Assigned by QAU): DR-10647	CAQ No. (Assigned by QAU): NA

based on the premise that the asymptotes of the RS curve are less reliable for PA concentration interpolation compared to the central or "linear" portion of the RS curve, as defined by approximately S3 – S6):

1) If a sample demonstrates "high PA binding activity" as evident from high ODs tracking along the upper asymptote of the RS, then the most reliable data point(s) from which to interpolate the PA concentration would be starting at the bottom of the sample titration, since the bottom portion of the sample titration falls closest to the "linear" portion of the RS curve. Begin censoring using the "top-down" approach (starting with the first sample dilution at 1:1 and progress in order toward the 1:125 dilution). If during censoring, 2 points from the sample titration fall in the "linear" portion of the curve have >30% CV, then the sample is considered non-parallel and a concentration value should not be reported.

2) If a sample demonstrates "low PA binding activity" as evident from low ODs tracking along the lower asymptote of the RS, then the most reliable data point(s) from which to interpolate the PA concentration would be starting at the top of the sample titration, since the top portion of the sample titration falls closest to the "linear" portion of the RS curve. Begin censoring using the "bottom-up" approach (starting with the last sample dilution at 1:125 and progress in order toward the 1:1 dilution). If during censoring 2 points from the sample titration fall in the "linear" portion of the curve have >30% CV, then the sample is considered non-parallel and a concentration value should not be reported.

3) In the event that 2 – 4 points from the sample titration fall in the "linear" portion of the curve, utilize the following censoring logic:

    a. If 2 points from the sample titration fall in the "linear" portion of the curve have >30% CV, then the sample is considered non-parallel and a concentration value should not be reported.

    b. If the 3 - 4 points of the sample titration demonstrates a %CV of >30%, and the 3 - 4 sample titration points that fall into the linear range of the RS curve, then censor based on the titration point(s) that is furthest from the mean. In order to have a reportable value, at least two points must be used. If the CV of the 2 or 3 remaining values is ≤30%, then report the mean concentration of the 2 or 3 remaining values.

    c. If the ODs of the 2 remaining dilution points are within S3 – S6 and still have >30% CV, then the sample is considered non-parallel and a concentration value should not be reported.

4) Occasionally a sample titration demonstrates a "hook" either at the high or low end of the titration curve.

    a. "High-hooks" are found in PA samples of varying activity where the sample increases in OD signal between the 1:1 and 1:5 dilution or the 1:1 and 1:25, and then in turn decreases in OD signal between the 1:5 and 1:25 or between 1:25 and 1:125 signal. In general, the sample dilution ODs must decrease as diluted. Only dilutions showing this monotonic, downward trend in OD may be used to determine a reportable value. These types of high-hooks are caused by an un-explained matrix effect inherent to the individual sample (usually an individual animal) and it is appropriate to censor the "hook effect" by deleting the ODs from the non-monotonic dilution(s). Starting with the monotonic ODs, the TS censoring rules described above apply.

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DEVIATION FORM

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b. "Low-hooks" are found in low-activity PA samples where the sample signal is low (meaning, the lowest dilution of the sample begins in the linear range of the RS and higher dilutions reach the lower asymptote) in the 1:1 and usually the 1:5 dilution wells, but then aberrantly jumps in the 1:25 or the 1:125 dilution wells. The high dilution factors then inflate the erroneous signal to cause a large concentration to be reported. These types of low-hooks are caused by an un-explained noise effect likely due to a plate effect (improper washing by the automatic plate washer, cross contamination of wells, etc) and it is appropriate to censor the "hook effect" by deleting the ODs from the affected dilution (usually the 1:25 or 1:125, but occasionally it is the 1:5). As stated above, the sample dilution ODs must decrease as diluted. Only dilutions showing this monotonic, downward trend in OD may be used to determine a reportable value. After censoring the affected well, the TS censoring rules described above apply. In addition, low hooks may also affect the negative control sample. If a negative control sample displays OD values after the 1:1 dilution that are non-monotonic and result in a back-calculated concentration from the RS, they may be censored as per the rules described above.

++

Change No. #3 to Page 17, BBRC.X-180-03 SOP Acceptance Criteria, make the following addition:

Occasionally a precipitant is observed in the wells after the addition of ABTS. This cause of the precipitant (observed to also look like filaments) is not known and is currently under investigation. If the precipitant is observed in specific wells, it will be noted and the test operator may need to in turn censor the OD values from those wells.

Root Cause of Deviation: The censoring specifications for the RS described in SOP X-180-03 are somewhat vague and require clarification to ensure that censoring is performed properly, consistently, and in the spirit of good scientific judgment.

Corrective Action: SOP BBRC X-180 has been updated with the guidances outlined in this deviation.

Impact of Deviation: These specifications improve the fidelity and consistency of the results reported by the assay.

If deviation is planned, effective date: N/A

Deviation Form Prepared by/Date: Kristin Clement 02/02/11

Deviation Reviewed and Corrective Action Accepted by/Date (Study Director or Responsible Individual): [signature] 2/10/11

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
JAH 2/10/11

Deviation Reviewed and Registered by QAU/Date: [signature] 2/10/11

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

BATTELLE BIOMEDICAL RESEARCH CENTER
DEVIATION FORM

Deviation No. (Assigned by QAU): DR-11043	**CAQ No. (Assigned by QAU):** N/A

Standard or Procedure Deviated:

☐ **Protocol (Number and Amendment No. if applicable):**
☑ **SOP (Number and Revision Number):** XI-009-03
☐ **Method (Number and Revision Number):**
☐ **GLP (Section):**
☐ **Other:**

Type of Deviation (check one):

☐ **Facility**

☑ **Study (fill out study info)** **Study Number:** 1078-CG920794

Study Title: Rabbit Multiple Dose Anthrax Telemetry Study

Record Affected (describe Title, Binder name, location, Form no. etc.):

a. ELISA for Windows data packets for Plate IDs: 081910-654, 081910-655, 081910-656, and 081910-657.
b. Form No. Microbio-357 for plate IDs: 082510-668, 082510-669, 082610-672, 082610-673.
c. Form No. Microbio-357 for plate IDs: 082510-668, 082510-669, 082610-672, 082610-673.
d. Form No. Microbio-447 "ELISA Sample (S-MP, C-MP, and T-MP) Preparation Form

Date of Deviation(s):

a. 8/21/10
b. 8/27/10
c. Unknown
d. 8/25/10

Description of Deviation: BBRC XI-009 states that "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. The person entering or recording raw data must sign or initial each data entry and date it on the date of entry… Unless validated with the capability to archive electronic raw data, a hard copy (i.e., printout) of the electronic raw data is considered to be the raw data for archival. The operator must initial and date the hard copy…"

a. The operator did not initial and date the hard copy of data generated by ELISA for Windows at the time of analysis and printing.
b. The operator did not initial and date for the completion of the form at the time the work was performed.
c. The operator did not initial and date for the importing of data into the ELISA database.
d. The freeze/thaw cycle information was omitted for the following critical reagents (used as both controls and samples): BMI009, BMI012, and BMI032.

Root Cause of Deviation:

a. The operator inadvertently omitted her initials and date from the printouts.
b. The "Form Completed By/Date" field was erroneously populated with pre-typed information.
c. The staff member neglected to sign for importing results into the ELISA database.
d. The technician did not enter the information at the time the work was performed.

BATTELLE BIOMEDICAL RESEARCH CENTER
DEVIATION FORM

Deviation No. (Assigned by QAU): DR-11043	**CAQ No. (Assigned by QAU):** NA

Corrective Action:

a. The staff member is no longer employed at this facility – no corrective action can be taken.
b. The staff member has been reminded that the "Data Analyzed By/Date" section is transcribable and can therefore be pre-typed, while the signature for form completion is raw data that must be signed the date the work is performed.
c. The staff member was advised to review SOP XI-009 and sign for all work at the time of completion.
d. The technician was advised to record all information promptly at the time work is completed. New implementation of the sample tracking system is under development to assist in accurately tracking freeze thaw cycles of sample and critical reagents.

Impact of Deviation:

a. Minimal. The date of printing is captured on the records as 8/21/10, and Form No. ELISA-036 indicates which technician conducted the data analysis.
b. Minimal. Based on the date of printing, data were analyzed on 8/27/10. Furthermore, all reportable values captured on Microbio-357 can be transcribed and verified: no reportable data are affected.
c. Minimal. Although the date and operator cannot be confirmed, it has been verified that data have been imported into the database.
d. Minimal. Based on the information in the Sample Tracking System, it is most likely that the F/T cycles should be: BMI032 = F/T 1, BMI012 = F/T 2, BMI009 = F/T 3, which is within the permissible number of F/T cycles for ELISA samples.

If deviation is planned, effective date: n/a

Deviation Form Prepared by/Date: AMB 5-9-11

Deviation Reviewed and Corrective Action Accepted by/Date (Study Director or Responsible Individual): [signature] 5-25-11 TR 5/10/11

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 [signature] 5/25/11

Deviation Reviewed and Registered by QAU/Date: [signature] 5/25/11

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

BATTELLE BIOMEDICAL RESEARCH CENTER
DEVIATION FORM

Deviation No. (Assigned by QAU): DR-11044	**CAQ No. (Assigned by QAU):** NA

Standard or Procedure Deviated:

☐ **Protocol (Number and Amendment No. if applicable):**
☑ **SOP (Number and Revision Number):** X-101-08
☐ **Method (Number and Revision Number):**
☐ **GLP (Section):**
☐ **Other:**

Type of Deviation (check one):

☐ **Facility**

☑ **Study (fill out study info)** **Study Number:** 1078-CG920794

Study Title: Rabbit Multiple Dose Anthrax Telemetry Study

Record Affected (describe Title, Binder name, location, Form no. etc.): α-PA IgG ELISA IR-419 Data Binder

a. Form No. Microbio-447, "ELISA Sample (S-MP, C-MP, T-MP) Preparation form," For Lot No. 081310-JNG.
b. Form No. Microbio-447, "ELISA Sample (S-MP, C-MP, T-MP) Preparation form," For Lot No. 081310-GEM.

Date of Deviation(s): 8/13/2010

Description of Deviation: SOP X-101-08 section a. regarding Reference Standard Master Box (S-MP) preparation states: "Refer to Form No. ELISA-008 for directions on how to dilute a specific lot of species-specific reference sera. For each S-MP prepare an appropriate volume of 2X concentrated reference standard using MP diluent according to Form No. ELISA-008 for the specific lot being tested."

Form No. ELISA-008 specifies a starting plate dilution of 1:530 for BMI-009, the reference standard used for the assays in question. A 2X concentration would therefore require a dilution of 1:265. For the Batch Lot No. above, the primary dilution of the reference standard was conducted by transferring 4μL of neat sera into 1054μL of diluent – the total volume was listed as 1060μL even though the sum of the volumes is 1058μL. The actual primary dilution prepared was 1:264.5 for a final plate dilution of 1:529.

Root Cause of Deviation: A math error specifying 1054μL instead of 1056μL resulted in the slight variation in dilutional concentrations.

Corrective Action: Staff members were instructed to review all pre-typed information and calculations prior to performing study tasks. Because there is less than a 0.2% difference between dilution factors of 529 and 530, no additional action will be taken.

Impact of Deviation: Minimal, there is less than a 0.2% difference between the actual dilution factor and that specified in ELISA-008. No data are significantly affected.

If deviation is planned, effective date: n/a

Deviation Form Prepared by/Date: AMB 5·9·11

BATTELLE BIOMEDICAL RESEARCH CENTER DEVIATION FORM	
Deviation No. (Assigned by QAU): DR-11044	**CAQ No. (Assigned by QAU):** NA
Deviation Reviewed and Corrective Action Accepted by/Date (Study Director or Responsible Individual): [signature] 5-25-11 TR 5/11/11	
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 [signature] 5/25/11	
Deviation Reviewed and Registered by QAU/Date: [signature] 5/25/11	☒ Category I ☐ Category II (See SOP XI-023 for details)

BATTELLE BIOMEDICAL RESEARCH CENTER

INVESTIGATION REPORT FORM

STUDY # 1078-CG920794 IR# 419

Describe problem and reason for investigation: When using either new rPA lot, 17115A2A or 5051797, for coating there were inconsistencies with the binding of the currently qualified conjugate 05814. This was seen in preliminary analyses for study 1078 and it caused many failures with study plates. An investigation was undertaken to determine the most likely cause of the inconsistencies leading to failures and determine a correction course of action to take.

Check applicable boxes:

- ☑ Verified instrument/equipment setup and conditions.
- ☑ Performed instrument/equipment check.
- ☑ Verified sample/reagent/standard expiration date, storage and preparation.
- ☐ Notified Sponsor (if applicable)
- ☑ Verified instrument/equipment calibration.
- ☑ Verified analysis/operation procedures.
- ☑ Verified calculations.
- ☑ Verified all other operation specific requirements
- ☑ Verified operator training

Describe additional investigation:

First, we compared the old qualified conjugate 03068A and the new qualified conjugate 05814 on split plates coated with 5051797 on one half and 17115A2A on the other half. This was to test which rPA works best.

- This test plate layout was performed on 8/13/10 by GEM (plate 081210-617) & JNG (plate 081210-618). After looking at the results the study director determined that rPA 17115A2A should be used.

Second, we tested conjugate 03068A and 05814 at their approved dilutions and also tested 05814 at a higher dilution on plates coated with rPA 17115A2A. This was performed to determine the performance of the conjugates.

- This testing occurred on plates 081510-621 to -623 performed by KAS. All plates passed and conjugate seemed to bind well for each lot and both dilutions of 05814. The study director wanted to further test the effect of more dilutions.

Third, we tested 05814 at several dilutions using 03068A on the RS & QC's. This test was performed to get further data on the performance of the conjugates and determine if the conjugate 05814 needed to be re-qualified.

- These tests were performed on plates 081610-648 to -651 by KAS and plates 081910-654 to -657 by AH. The results showed no significant applicable improvement by increasing the dilution used. It was noted that ODs were closer to their expected values on plates that were more recently coated.

Fourth, we tested the possibility of plate age being a factor as we saw a better performance on plates with a shorter incubation period in the testing result from the third experiment.

- The shorter room temperature incubated plates, 082510-666 to -667, were used by GEM. Two other plates were coated overnight, 082510-668 to -669, and were run by KGM. Results did not show a significant difference due to plate age.

Lastly, we tested a direct comparison between a plate with 03068A and 05814. This was to decide which conjugate performs better and then to utilize that for 1078 study work.

- KGM compared conjugates on plates 082610-673 & -672 as did GZ on plates 082610-670 & -671. Study director decided that conjugate 03068A would be best for use on study 1078 and study work continued with that conjugate as a result.

Describe cause (if determined) and proposed action to be taken/recommendations:

BATTELLE BIOMEDICAL RESEARCH CENTER
INVESTIGATION REPORT FORM

STUDY # 1078-CG920794 **IR#** 419

It was determined the conjugate 05814 had degraded and was as a result, inconsistently binding. Proposed action was to use a different conjugate lot.

Completed by/date: KA 11-4-10 **Reviewed and accepted by/date:** TR 5/10/11

QAU Assigned IR # by/date JQ/8/16/10 [illegible] 5/25/11 **CAQ # Issued:** NA

Describe action taken:
Action taken was to switch to the older qualified conjugate 03068A and to begin qualifying a new conjugate.

Describe measures taken to prevent recurrence:
A suggestion was made that when it has been greater than 6 months since reagents have been used that we run a test plate of those reagents in order to determine that they are still functioning properly prior to starting study sample analyses.

Completed by/date: KA 11-4-10 **Reviewed and accepted by/date:** TR 5/10/11

QAU Registration by/date: [illegible] 5/25/11

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

Bacteriology Results Report

Sponsor: Battelle **Accession #: 2010-035209**

JM-8	**Received:**	28 Jul 2010
505 King Ave.	**Department:**	Approved by Richard D. Fister, 03 Aug 2010, 08:29*
Columbus, OH 43201		
USA		
Attn: Jason Comer	**Bill Method:**	Credit Card
Tel: 614-424-5825	**Test Specimen:**	Nasal Rabbit, Lot V100261001261

Bordetella Screen - Respiratory

Sample #: / Code :	1 / 40	2 / 7	3 / 5	4 / 9	5 / 37	6 / 13	7 / 34	8 / 25	9 / 15	10 / 30
B. bronchiseptica	-	-	-	-	-	-	-	-	-	-
Other	-	-	-	-	-	-	-	-	-	-

Sample #: / Code :	11 / 28	12 / 19	13 / 14	14 / 11	15 / 2	16 / 8	17 / 12	18 / 18	19 / 32	20 / 6
B. bronchiseptica	-	-	-	-	-	-	-	-	-	-
Other	-	-	-	-	-	-	-	-	-	-

Sample #: / Code :	21 / 33	22 / 27	23 / 31	24 / 39	25 / 21	26 / 38
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. 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.*

APPENDIX D
RANDOMIZATION REPORT

Battelle
The Business of Innovation

Project Number CG920794-1078STAT (3104)

Internal Distribution

RA Lordo/SIA Files (Judd)
NA Niemuth
GV Stark
HJ Mayfield
RMO

s:\BBRC\Anthrax\EPA\Study 1078-CG920794\
Study 1078 Animal Randomization & Sup Doc.doc

Date August 9, 2010

To **Jason Comer**

From Heather Mayfield

Subject **Study No. 1078-CG920794: Animal Randomization Report and Supporting Documentation**

Overview

This memorandum describes the animal randomization process and provides the supporting documentation for BBRC Study No. 1078-CG920794. The SAS® system (version 9.1.3) was used to transfer, process, and analyze the data.

Methods

Twenty-six (26) New Zealand White rabbits were available to be assigned to the study. Animals were randomly assigned to one of four groups (3 groups of 7 and 1 group of 5) by weight utilizing the SAS® PLAN procedure. Next, animals were assigned a random challenge order within each study group utilizing random numbers generated by the SAS® RANUNI function.

Data Inputs/Outputs

The Excel spreadsheet 1078 IDS AND WEIGHTS FOR RANDO.XLSX, containing the animal ID and weight for the animals available to be placed on study, was sent to SIA statisticians on July 19, 2010, by Jason Comer. The data were read in from the Excel spreadsheet with the SAS® program ANIMALRAND_1078_CG920794_071910.SAS used for the randomization. The allocation of animals to groups and challenge order was output by SAS® to create the Excel spreadsheet RANDOMIZATION_1078_071910.XLS.

Performance Test Methods/Results

A copy of the Excel spreadsheets 1078 IDS AND WEIGHTS FOR RANDO.XLSX and RANDOMIZATION_1078_071910.XLS, the SAS® program ANIMALRAND_1078_CG920794_071910.SAS, and the SAS® listing are attached. The correct transfer of data was verified by printing out the Excel spreadsheet 1078 IDS AND WEIGHTS FOR RANDO.XLSX and comparing it to a listing of the data in SAS®. The randomization was conducted with the SAS® PLAN procedure, which does not require performance testing.

[signatures on next page]

RESPONSIBLE INDIVIDUAL
Heather J Mayfield

RESPONSIBLE INDIVIDUAL

INDEPENDENT REVIEWER

HJM:bhf
Attachments

For Review and Approval

	Name	Initials	Date
Originator	Heather Mayfield	HJM	8/9/10
Concurrence	Greg Stark	GVS	8/10/10
Approved	Nancy Niemuth	N	8/9/10

Sent via: Interoffice mail

SUPPORTING DOCUMENTATION FOR

Study No. 1078-CG920794

ANIMAL RANDOMIZATION REPORT AND SUPPORTING DOCUMENTATION

Study No. 1078-CG920794

Rabbit ID	Body weight (kg)
2 ✓	2.514 ✓
5	2.619
6	2.91
7	2.757
8 ✓	2.891 ✓
9	2.88
11	2.575
12	2.819
13	2.656
14	2.718
15	2.873
18 ✓	2.707 ✓
19	2.554
21	2.842
25 ✓	2.917 ✓
27	2.595
28	2.806
30	2.757
31	2.667
32	3.025
33	2.532
34	2.707
37	2.411
38	2.859
39	2.774
40	3.013

Excel file 1078 IDS and weights for Rando.xlsx. Checkmarks denote the values matched with the data listing.
HTM 8/8/10

```
*******************************************************************************************
Program Name:   AnimalRand_1078_CG920794_071910.SAS
Purpose:        Randomize animals into treatment groups.
Project:        1078-CG920794
Charge Number(s):  CG920794-1078STAT
Date Created:     06/29/10
Input Data:
Output Data:      Listing of randomization scheme.
Other Files Used:
Comments:  26 rabbits will be randomized into three groups of seven and one control group of
five
              Jason Comer's 7/19/10 email ".  Please note that we lost several animals due to
                  complications with surgery and only have 26.  There will be no extras. "
        Rabbits in each groups will be assigned a random challenge order.
Author:         Heather Mayfield
*******************************************************************************************;
%let randloc=C:\Documents and Settings\mayfieldh\My Documents\BBRC\Proc Plan\Anthrax\EPA\1078-
CG920794;

libname randan "&randloc.";

title1 'Study Number 1078-CG920794';
%let stnum=1078;
%let date=071910;

options LS=120 PS=74 pageno=1;

proc printto print="&randloc.\AnimalRandomization_&stnum._Freq_&date..lst" new;
run;

*** read animal data ***;
libname ex1 odbc noprompt="DSN=Excel Files;
                 DBQ=&randloc.\1078 IDs and Weights for rando.xlsx";

Data animal&stnum._A;
   set EX1."Sheet1$A1:B27"N;
run;

data randan.animal_&stnum._&date.;
   set animal&stnum._A ;
run;

proc print data=randan.animal_&stnum._&date.;
title2'Animal Data';
run;
title2;

proc sort data=randan.animal_&stnum._&date. out=animals;
       by Body_weight__kg_;
run;

data middle other;
       set animals;
       if 10<_n_<17 then output middle;
              else output other;
run;
* Middle is middle weight block;
```

SAS program
HJM 8/8/10

```
*Other has light, medium-light, medium heavy, and heavy weight blocks. ;

data unrand;
       do block=1 to 4;
   do cell=1 to 5;
      if cell=1 then Group=1;
         else group=.;
      output;
   end;
   end;
run;

*********** Randomize to treatment groups*****************;
title2 'Animals Randomized to Groups';
proc plan ;*seed ;
       factors block=4 ordered cell=5;
       output data =unrand out = random;
run;
quit;
title2;

proc sort data=random;
       by block cell;
run;

data randomother(drop=block cell);
   merge other random;*merge w/o by correct here;
run;

data unrand2;
   do cell=1 to 6;
      if cell=1 then Group=1;
         else group=.;
      output;
   end;
run;

*********** Randomize to treatment groups*****************;
title2 'Animals Randomized to Groups';
proc plan ;*seed ;
       factors cell=6;
       output data =unrand2 out = random2;
run;
quit;
title2;

proc sort data=random2;
       by cell;
run;

data randommiddle(drop= cell);
   merge middle random2;*merge w/o by correct here;
run;

data group1 group234;
       set randommiddle randomother;
       if group=1 then output group1;
```

```
                else output group234;
run;

proc sort data=group234;
       by Body_weight__kg_;
run;

data unrand3;
       do block=1 to 7;
   do cell=1 to 3;
      group=cell+1;
      output;
   end;
   end;
run;

*********** Randomize to treatment groups******************;
title2 'Animals Randomized to Groups';
proc plan ;*seed ;
       factors block=7 ordered cell=3;
       output data =unrand3 out = random3;
run;
quit;
title2;

proc sort data=random3;
       by block cell;
run;

data randomgroup234(drop=block cell);
   merge group234 random3;*merge w/o by correct here;
run;

data animal_randomization&stnum._&date.;
       set group1 randomgroup234;
       chaldet=ranuni(-1);
run;

proc sort data=animal_randomization&stnum._&date.;
       by group chaldet;
run;

data randan.animal_randomization&stnum._&date.(drop=chaldet);
       set animal_randomization&stnum._&date.;
       retain Challenge_Order;
       by group chaldet;
       if first.group then Challenge_Order=1;
              else Challenge_Order=Challenge_Order+1;
run;

title2 'Frequency of Animals in Groups';
proc freq data=randan.animal_randomization&stnum._&date.;
   table group group*Challenge_Order;
run;
title2;

proc sort data=randan.animal_randomization&stnum._&date. out=randbygroup;
```

```
   by group Challenge_Order;
run;

libname libout odbc noprompt="DSN=Excel Files;
                      DBQ=&randloc.\Randomization_&stnum._&date..xls"
                      PRESERVE_COL_NAMES=yes;

data libout."By Group Order"n;
       set randbygroup;
       by group Challenge_Order;
run;

libname libout clear;

proc glm data=randbygroup;
       class group;
       model Body_weight__kg_=group;
run;

Proc printto;
run;
```

Animal Data

Obs	Rabbit_ ID	Body_ weight__ kg_
1	2 ✓	2.514 ✓
2	5	2.619
3	6	2.910
4	7	2.757
5	8 ✓	2.891 ✓
6	9	2.880
7	11	2.575
8	12	2.819
9	13	2.656
10	14	2.718
11	15	2.873
12	18 ✓	2.707 ✓
13	19	2.554
14	21	2.842
15	25 ✓	2.917 ✓
16	27	2.595
17	28	2.806
18	30	2.757
19	31	2.667
20	32	3.025
21	33	2.532
22	34	2.707
23	37	2.411
24	38	2.859
25	39	2.774
26	40	3.013

SAS output from Animalrand-1078-CG920794_071910.SAS
Checkmarks denote the values matched with the Excel file 1078 IDS and weights for Rando.xlsx.
HJM 8/8/10

Animals Randomized to Groups

The PLAN Procedure

Factor	Select	Levels	Order
block	4	4	Ordered
cell	5	5	Random

block	---cell--
1	1 5 2 4 3
2	2 3 4 5 1
3	5 3 1 2 4
4	4 5 3 2 1

Animals Randomized to Groups

The PLAN Procedure

Factor	Select	Levels	Order
cell	6	6	Random

----cell---

3 6 1 2 4 5

The PLAN Procedure

Factor	Select	Levels	Order
block	7	7	Ordered
cell	3	3	Random

block	--cell-		
1	3	1	2
2	3	1	2
3	2	3	1
4	2	1	3
5	1	2	3
6	2	3	1
7	2	3	1

Frequency of Animals in Groups

The FREQ Procedure

Group	Frequency	Percent	Cumulative Frequency	Cumulative Percent
1	5	19.23	5	19.23
2	7	26.92	12	46.15
3	7	26.92	19	73.08
4	7	26.92	26	100.00

Table of Group by Challenge_Order

Group / Challenge_Order (Frequency, Percent, Row Pct, Col Pct)	1	2	3	4	5	6	7	Total
1	1 3.85 20.00 25.00	1 3.85 20.00 25.00	1 3.85 20.00 25.00	1 3.85 20.00 25.00	1 3.85 20.00 25.00	0 0.00 0.00 0.00	0 0.00 0.00 0.00	5 19.23
2	1 3.85 14.29 25.00	1 3.85 14.29 25.00	1 3.85 14.29 25.00	1 3.85 14.29 25.00	1 3.85 14.29 25.00	1 3.85 14.29 33.33	1 3.85 14.29 33.33	7 26.92
3	1 3.85 14.29 25.00	1 3.85 14.29 25.00	1 3.85 14.29 25.00	1 3.85 14.29 25.00	1 3.85 14.29 25.00	1 3.85 14.29 33.33	1 3.85 14.29 33.33	7 26.92
4	1 3.85 14.29 25.00	1 3.85 14.29 25.00	1 3.85 14.29 25.00	1 3.85 14.29 25.00	1 3.85 14.29 25.00	1 3.85 14.29 33.33	1 3.85 14.29 33.33	7 26.92
Total	4 15.38	4 15.38	4 15.38	4 15.38	4 15.38	3 11.54	3 11.54	26 100.00

The GLM Procedure

Class Level Information

Class	Levels	Values
Group	4	1 2 3 4

Number of Observations Read	26
Number of Observations Used	26

The GLM Procedure

Dependent Variable: Body_weight__kg_ Body weight (kg)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.00118497	0.00039499	0.01	0.9977
Error	22	0.62616657	0.02846212		
Corrected Total	25	0.62735154			

R-Square	Coeff Var	Root MSE	Body_weight__kg_ Mean
0.001889	6.145293	0.168707	2.745308

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Group	3	0.00118497	0.00039499	0.01	0.9977

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Group	3	0.00118497	0.00039499	0.01	0.9977

There is no significant group effect on weight.
HJM 8/8/10

Study No. 1078-CG920794

Rabbit_ID	Body_weight__kg_	Group	Challenge_Order
40	3.013	1	1
7	2.757	1	2
5	2.619	1	3
9	2.88	1	4
37	2.411	1	5
13	2.656	2	1
34	2.707	2	2
25	2.917	2	3
15	2.873	2	4
30	2.757	2	5
28	2.806	2	6
19	2.554	2	7
14	2.718	3	1
11	2.575	3	2
2	2.514	3	3
8	2.891	3	4
12	2.819	3	5
18	2.707	3	6
32	3.025	3	7
6	2.91	4	1
33	2.532	4	2
27	2.595	4	3
31	2.667	4	4
39	2.774	4	5
21	2.842	4	6
38	2.859	4	7

Excel file HJM 8/8/10

By_Group_Order
Randomization_1078_071910.xls

APPENDIX E
AEROSOL REPORT

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List of Tables

List of Figures

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
SOP .... standard operating procedure
T .... exposure time
TATV .... total accumulated tidal volume
TSA .... tryptic soy agar
µm .... micrometer
V .... impinger sampler volume

1. Methods

Standard operating procedures (SOPs) were followed during animal aerosol exposure challenges. Aerosol procedures are described in SOP BBRC Number XIII-001. Procedures for using the Aerodynamic Particle Sizer® (APS) are described in SOP BBRC Number XIII-011. The procedures for operating the plethysmography system are described in SOP BBRC Numbers XIII-008 and XIII-009. A schematic of the exposure system is shown in Figure 1.

2. Experimental Setup and Test Conditions

The Battelle large animal exposure system can be divided into four subsystems plus an auxiliary plethysmography subsystem. A description of each subsystem is found below.

2.1 Aerosol Generation Subsystem

For this study both large animal systems were used, one for the non-viable spores and the other for the viable spores. For all intents and purposes these systems were operated identically with the same operation ranges for both. Air was supplied to the systems 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 a nebulizer bypass airflow of 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 53% to 83% for the non-viable spore system and 56 to 80% for the viable spore system. 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 non-viable *B. anthracis* spores, (spore lot Ames B36 irradiated prior to challenge days) or viable *B. anthracis* spores, (spore lot Ames B36). These nebulizers are designed to generate aerosols having an approximate mean diameter of 1-2 micrometer (µm). Each nebulizer was characterized for a pressure that results in an approximately 7.5 L/min flow, which normally is approximately 25 to 36 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 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 -0.2 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. Both systems used for this study were built to the same specifications.

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 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 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 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 mL. Concentrations are reported in terms of colony forming units per mL (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 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

Body plethysmography was performed real-time on each animal during agent challenge to measure important respiratory parameters. These parameters (tidal volume, total accumulated Tidal volume, 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 a sealed chamber called a plethysmograph. The data generated for each animal was used to determine the total accumulated tidal volume (TATV), which along with the aerosol concentration was used in calculating the inhaled dose. During the rabbit exposure, the anesthetized animal was placed in dorsal recumbence in a custom-made plexiglass plethysmograph with the head protruding out of a port that was sealed with rubber dental dam 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 plethysmography 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 for accurate TATV measurements.

3. Inhalation Results

The inhalation exposure system data for each exposure was 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. 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} for rabbits is 105,000 CFU, (Zaucha, et. al.1998). Tables 1 through 30 show the inhalation results for this study.

3.1 Impinger Sample Analysis

Impinger samples were enumerated by the spread plate method, SOP BBRC X-054 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 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 was determined using the equation below:

$$C = (A \cdot D) / 0.1 \text{ mL} \quad (1)$$

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

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

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

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 less than 1-5 mm 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 mm or lower, as opposed to larger aerosols is desired.

Figure 2 and Figure 3 show a log – probability plot representing the average of all APS particle size distributions obtained from exposure testing. The MMAD and geometric standard deviation (GSD) are also shown.

The MMAD for the log – probability plot (Figure 2 and Figure 3) was determined from averaging the cumulative median size (50% mass) from the aerosol size distributions obtained from the APS for all aerosol exposures. 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:

$$GSD = d84\%/d50\% \tag{3}$$

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).

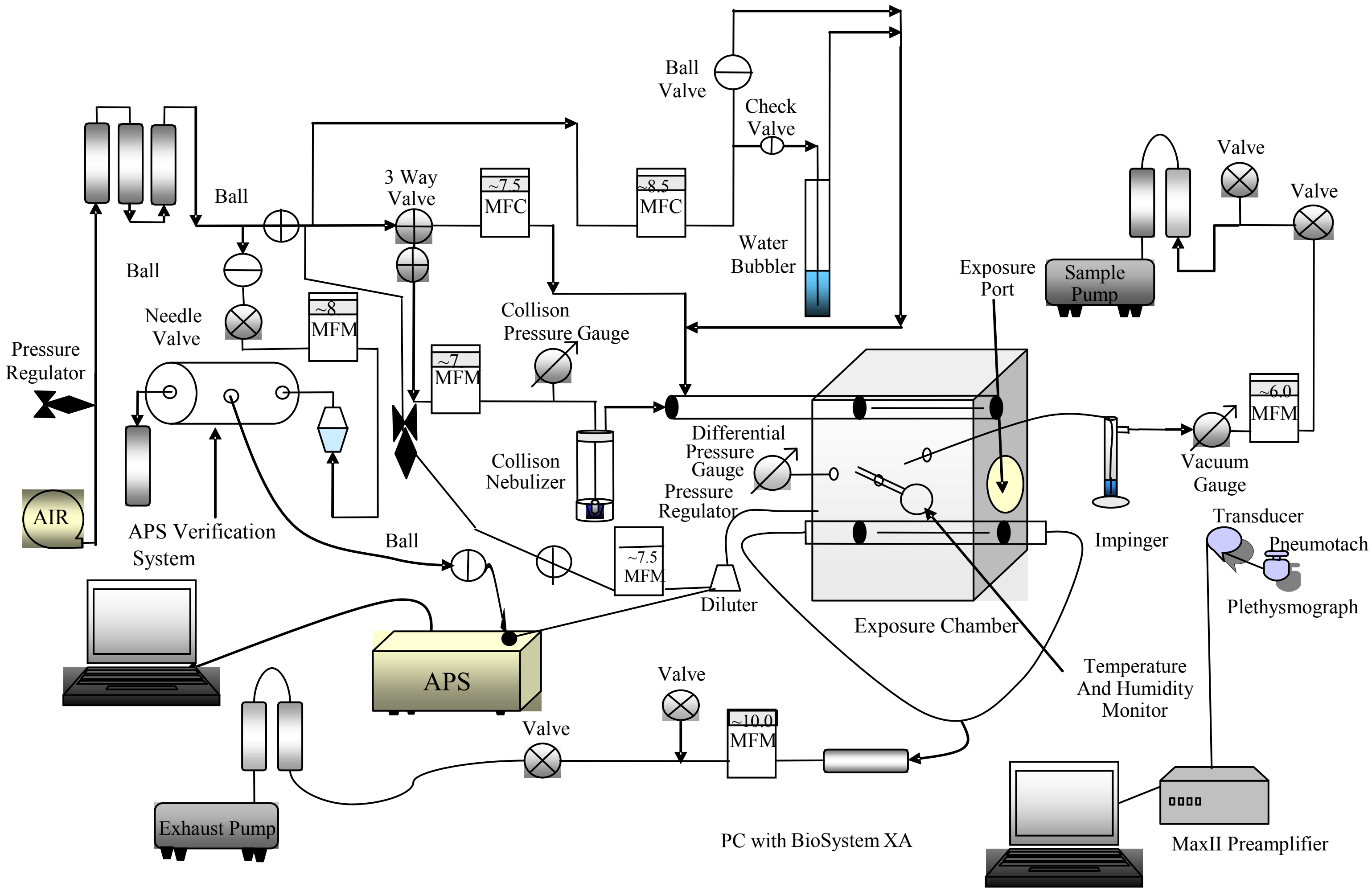

Figure 1. Exposure system diagram.

Non-Viable Spore Challenge Data

Table 1. Aerosol Data Summary Sheet (Day 1)

Study No. 1078-CG920794 Rabbits 07-26-10 Irradiated Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (ºF)	RH (%)
40	0.00E+00	0.00E+00	19.00	0.00E+00	6.3	7.00	0.00E+00	17.42	0	0.00E+00	NC	72.6	60.6
7	0.00E+00	0.00E+00	19.40	0.00E+00	6.2	8.67	0.00E+00	10.02	0	0.00E+00	NC	NA	NA
5	0.00E+00	0.00E+00	19.80	0.00E+00	6.3	7.17	0.00E+00	10.02	0	0.00E+00	NC	72.0	83.0
9	0.00E+00	0.00E+00	19.40	0.00E+00	6.3	7.00	0.00E+00	12.88	0	0.00E+00	NC	71.9	76.7
37	0.00E+00	0.00E+00	20.00	0.00E+00	6.3	7.00	0.00E+00	14.12	0	0.00E+00	NC	72.0	63.7

NA = Temp./RH probe malfunction. Readings not obtained.

NC = Not calculable due to zero impinger counts

Table 2. Aerosol Data Summary Sheet (Day 2)

Study No. 1078-CG920794 Rabbits 07-27-10 Irradiated Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (º F)	RH (%)
40	0.00E+00	0.00E+00	19.20	0.00E+00	6.3	7.00	0.00E+00	12.71	0	0.00E+00	NC	67.0	69.6
7	0.00E+00	0.00E+00	19.40	0.00E+00	6.3	7.00	0.00E+00	10.34	0	0.00E+00	NC	68.1	74.4
5	0.00E+00	0.00E+00	19.20	0.00E+00	6.3	7.00	0.00E+00	10.56	0	0.00E+00	NC	68.4	66.2
9	0.00E+00	0.00E+00	19.60	0.00E+00	6.3	7.00	0.00E+00	13.91	0	0.00E+00	NC	69.2	68.8
37	0.00E+00	0.00E+00	19.60	0.00E+00	6.3	7.00	0.00E+00	13.41	0	0.00E+00	NC	69.7	65.1

NC = Not calculable due to zero impinger counts

Table 3. Aerosol Data Summary Sheet (Day 3)

Study No. 1078-CG920794 Rabbits 07-28-10 Irradiated Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (^{0} F)	RH (%)
40	0.00E+00	0.00E+00	18.60	0.00E+00	6.3	12.12	0.00E+00	10.01	0	0.000	NC	68.1	67.9
7	0.00E+00	0.00E+00	18.40	0.00E+00	6.2	15.13	0.00E+00	10.02	0	0.000	NC	69.0	65.6
5	0.00E+00	0.00E+00	18.80	0.00E+00	6.3	7.95	0.00E+00	10.02	0	0.000	NC	69.8	65.9
9	0.00E+00	0.00E+00	19.00	0.00E+00	6.3	7.93	0.00E+00	10.01	0	0.000	NC	70.3	65.6
37	0.00E+00	0.00E+00	17.80	0.00E+00	6.3	15.75	0.00E+00	9.99	0	0.000	NC	70.6	61.9

NC = Not calculable due to zero impinger counts

Table 4. Aerosol Data Summary Sheet (Day 4)

Study No. 1078-CG920794 Rabbits 07-29-10 Irradiated Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (^{0} F)	RH (%)
40	0.00E+00	0.00E+00	19.20	0.00E+00	6.3	7.00	0.00E+00	17.00	0	0.00E+00	NC	67.9	76.9
7	0.00E+00	0.00E+00	19.40	0.00E+00	6.3	7.17	0.00E+00	10.02	0	0.00E+00	NC	68.7	74.4
5	0.00E+00	0.00E+00	19.20	0.00E+00	6.3	7.00	0.00E+00	12.77	0	0.00E+00	NC	69.2	70.2
9	0.00E+00	0.00E+00	19.20	0.00E+00	6.3	7.00	0.00E+00	12.83	0	0.00E+00	NC	69.7	70.5
37	0.00E+00	0.00E+00	19.40	0.00E+00	6.3	7.00	0.00E+00	11.19	0	0.00E+00	NC	70.2	73.2

NC = Not calculable due to zero impinger counts

Table 5. Aerosol Data Summary Sheet (Day 5)

Study No. 1078-CG920794 Rabbits 07-30-10 Irradiated Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (^{0} F)	RH (%)
40	0.00E+00	0.00E+00	18.00	0.00E+00	6.2	14.83	0.00E+00	10.02	0	0.00E+00	NC	67.4	71.9
7	0.00E+00	0.00E+00	18.40	0.00E+00	6.2	14.82	0.00E+00	10.01	0	0.00E+00	NC	68.3	70.4
5	0.00E+00	0.00E+00	19.40	0.00E+00	6.2	8.27	0.00E+00	10.03	0	0.00E+00	NC	69.1	72.7
9	0.00E+00	0.00E+00	19.20	0.00E+00	6.2	7.63	0.00E+00	10.03	0	0.00E+00	NC	69.6	71.9
37	0.00E+00	0.00E+00	18.40	0.00E+00	6.1	14.28	0.00E+00	10.04	0	0.00E+00	NC	69.9	67.5

NC = Not calculable due to zero impinger counts

Table 6. Aerosol Data Summary Sheet (Day 6)

Study No. 1078-CG920794 Rabbits 08-02-10 Irradiated Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (^{0} F)	RH (%)
40	0.00E+00	0.00E+00	18.50	0.00E+00	6.2	7.72	0.00E+00	10.02	0	0.00E+00	NC	69.7	52.9
7	0.00E+00	0.00E+00	19.00	0.00E+00	6.2	7.98	0.00E+00	10.01	0	0.00E+00	NC	70.0	61.0
5	0.00E+00	0.00E+00	19.50	0.00E+00	6.3	8.12	0.00E+00	10.01	0	0.00E+00	NC	70.2	60.3
9	0.00E+00	0.00E+00	19.25	0.00E+00	6.3	7.00	0.00E+00	13.09	0	0.00E+00	NC	70.7	63.6
37	0.00E+00	0.00E+00	19.25	0.00E+00	6.3	6.68	0.00E+00	17.15	0	0.00E+00	NC	70.9	61.1

NC = Not calculable due to zero impinger counts

Table 7. Aerosol Data Summary Sheet (Day 7)

Study No. 1078-CG920794 Rabbits 08-03-10 Irradiated Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (^{0} F)	RH (%)
40	0.00E+00	0.00E+00	18.50	0.00E+00	6.3	8.22	0.00E+00	10.00	0	0.00E+00	NC	66.7	70.6
7	0.00E+00	0.00E+00	19.25	0.00E+00	6.3	7.10	0.00E+00	10.02	0	0.00E+00	NC	66.7	74.4
5	0.00E+00	0.00E+00	19.25	0.00E+00	6.3	7.00	0.00E+00	11.41	0	0.00E+00	NC	68.4	73.8
9	0.00E+00	0.00E+00	19.25	0.00E+00	6.2	6.85	0.00E+00	17.01	0	0.00E+00	NC	69.1	78.4
37	0.00E+00	0.00E+00	19.00	0.00E+00	6.2	7.00	0.00E+00	14.08	0	0.00E+00	NC	69.3	72.8

NC = Not calculable due to zero impinger counts

Table 8. Aerosol Data Summary Sheet (Day 8)

Study No. 1078-CG920794 Rabbits 08-04-10 Irradiated Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (^{0} F)	RH (%)
40	0.00E+00	0.00E+00	18.50	0.00E+00	6.3	11.57	0.00E+00	10.00	0	0.00E+00	NC	68.5	64.1
7	0.00E+00	0.00E+00	18.50	0.00E+00	6.3	14.47	0.00E+00	10.00	0	0.00E+00	NC	69.1	62.5
5	0.00E+00	0.00E+00	19.00	0.00E+00	6.2	7.95	0.00E+00	10.00	0	0.00E+00	NC	69.7	63.9
9	0.00E+00	0.00E+00	19.25	0.00E+00	6.3	7.00	0.00E+00	11.24	0	0.00E+00	NC	70.2	64.7
37	0.00E+00	0.00E+00	18.50	0.00E+00	6.3	11.63	0.00E+00	10.00	0	0.00E+00	NC	70.4	60.3

NC = Not calculable due to zero impinger counts

Table 9. Aerosol Data Summary Sheet (Day 9)

Study No. 1078-CG920794 Rabbits 08-05-10 Irradiated Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (° F)	RH (%)
40	0.00E+00	0.00E+00	19.00	0.00E+00	6.2	7.00	0.00E+00	11.24	0	0.00E+00	NC	67.3	68.6
7	0.00E+00	0.00E+00	19.50	0.00E+00	6.2	7.00	0.00E+00	10.25	0	0.00E+00	NC	68.3	73.8
5	0.00E+00	0.00E+00	19.50	0.00E+00	6.2	7.00	0.00E+00	12.22	0	0.00E+00	NC	68.8	72.7
9	0.00E+00	0.00E+00	19.50	0.00E+00	6.2	7.00	0.00E+00	14.24	0	0.00E+00	NC	69.2	73.7
37	0.00E+00	0.00E+00	19.50	0.00E+00	6.2	7.15	0.00E+00	10.01	0	0.00E+00	NC	69.4	70.2

NC = Not calculable due to zero impinger counts

Table 10. Aerosol Data Summary Sheet (Day 10)

Study No. 1078-CG920794 Rabbits 08-06-10 Irradiated Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (° F)	RH (%)
40	0.00E+00	0.00E+00	18.60	0.00E+00	6.3	10.83	0.00E+00	10.01	0	0.00E+00	NC	67.8	68.2
7	0.00E+00	0.00E+00	19.00	0.00E+00	6.3	13.47	0.00E+00	10.00	0	0.00E+00	NC	68.5	66.0
5	0.00E+00	0.00E+00	19.60	0.00E+00	6.3	7.00	0.00E+00	10.57	0	0.00E+00	NC	69.1	69.3
9	0.00E+00	0.00E+00	19.60	0.00E+00	6.3	7.00	0.00E+00	14.88	0	0.00E+00	NC	69.7	73.7
37	0.00E+00	0.00E+00	19.00	0.00E+00	6.2	12.77	0.00E+00	10.01	0	0.00E+00	NC	69.7	62.0

NC = Not calculable due to zero impinger counts

Table 11. Aerosol Data Summary Sheet (Day 11)

Study No. 1078-CG920794 Rabbits 08-09-10 Irradiated Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (^{0} F)	RH (%)
40	0.00E+00	0.00E+00	18.80	0.00E+00	6.1	7.00	0.00E+00	11.65	0	0.00E+00	NC	69.5	67.9
7	0.00E+00	0.00E+00	19.60	0.00E+00	6.1	7.00	0.00E+00	10.87	0	0.00E+00	NC	69.9	69.1
5	0.00E+00	0.00E+00	19.40	0.00E+00	6.1	7.00	0.00E+00	10.99	0	0.00E+00	NC	70.1	69.9
9	0.00E+00	0.00E+00	19.40	0.00E+00	6.1	7.00	0.00E+00	10.42	0	0.00E+00	NC	70.5	74.3
37	0.00E+00	0.00E+00	19.40	0.00E+00	6.2	7.00	0.00E+00	11.64	0	0.00E+00	NC	70.8	70.0

NC = Not calculable due to zero impinger counts

Table 12. Aerosol Data Summary Sheet (Day 12)

Study No. 1078-CG920794 Rabbits 08-10-10 Irradiated Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (^{0} F)	RH (%)
40	0.00E+00	0.00E+00	19.20	0.00E+00	6.2	7.00	0.00E+00	16.43	0	0.00E+00	NC	67.5	76.2
7	0.00E+00	0.00E+00	19.60	0.00E+00	6.2	7.00	0.00E+00	10.51	0	0.00E+00	NC	67.9	73.8
5	0.00E+00	0.00E+00	19.60	0.00E+00	6.2	7.00	0.00E+00	10.83	0	0.00E+00	NC	68.4	71.0
9	0.00E+00	0.00E+00	19.60	0.00E+00	6.2	7.00	0.00E+00	14.41	0	0.00E+00	NC	68.9	72.7
37	0.00E+00	0.00E+00	19.60	0.00E+00	6.2	7.12	0.00E+00	10.00	0	0.00E+00	NC	69.3	72.3

NC = Not calculable due to zero impinger counts

Table 13. Aerosol Data Summary Sheet (Day 13)

Study No. 1078-CG920794 Rabbits 08-11-10 Irradiated Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (^{0} F)	RH (%)
40	0.00E+00	0.00E+00	18.60	0.00E+00	6.3	7.75	0.00E+00	10.01	0	0.00E+00	NC	67.1	74.5
7	0.00E+00	0.00E+00	19.20	0.00E+00	6.3	12.65	0.00E+00	10.03	0	0.00E+00	NC	67.8	70.0
5	0.00E+00	0.00E+00	19.40	0.00E+00	6.3	7.00	0.00E+00	10.45	0	0.00E+00	NC	68.4	71.2
9	0.00E+00	0.00E+00	19.40	0.00E+00	6.3	7.00	0.00E+00	13.63	0	0.00E+00	NC	69.0	73.7
37	0.00E+00	0.00E+00	18.00	0.00E+00	6.3	16.75	0.00E+00	10.01	0	0.00E+00	NC	69.4	67.1

NC = Not calculable due to zero impinger counts

Table 14. Aerosol Data Summary Sheet (Day 14)

Study No. 1078-CG920794 Rabbits 08-12-10 Irradiated Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (^{0} F)	RH (%)
40	0.00E+00	0.00E+00	18.80	0.00E+00	6.3	7.95	0.00E+00	10.09	0	0.00E+00	NC	68.8	76.6
7	0.00E+00	0.00E+00	19.40	0.00E+00	6.3	7.00	0.00E+00	10.16	0	0.00E+00	NC	69.0	74.7
5	0.00E+00	0.00E+00	19.20	0.00E+00	6.3	7.00	0.00E+00	11.15	0	0.00E+00	NC	69.2	71.2
9	0.00E+00	0.00E+00	19.40	0.00E+00	6.3	7.00	0.00E+00	14.35	0	0.00E+00	NC	69.8	76.8
37	0.00E+00	0.00E+00	19.60	0.00E+00	6.3	7.00	0.00E+00	11.34	0	0.00E+00	NC	69.9	70.9

NC = Not calculable due to zero impinger counts

Table 15. Aerosol Data Summary Sheet (Day 15)

Study No. 1078-CG920794 Rabbits 08-13-10 Irradiated Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (^{o} F)	RH (%)
40	0.00E+00	0.00E+00	18.40	0.00E+00	6.3	10.02	0.00E+00	10.02	0	0.00E+00	NC	67.0	68.6
7	0.00E+00	0.00E+00	19.20	0.00E+00	6.2	10.43	0.00E+00	10.02	0	0.00E+00	NC	67.8	68.6
5	0.00E+00	0.00E+00	19.80	0.00E+00	6.2	7.00	0.00E+00	10.30	0	0.00E+00	NC	68.7	70.1
9	0.00E+00	0.00E+00	19.40	0.00E+00	6.2	7.47	0.00E+00	14.34	0	0.00E+00	NC	68.9	70.5
37	0.00E+00	0.00E+00	19.00	0.00E+00	6.1	11.35	0.00E+00	10.01	0	0.00E+00	NC	69.5	67.3

NC = Not calculable due to zero impinger counts

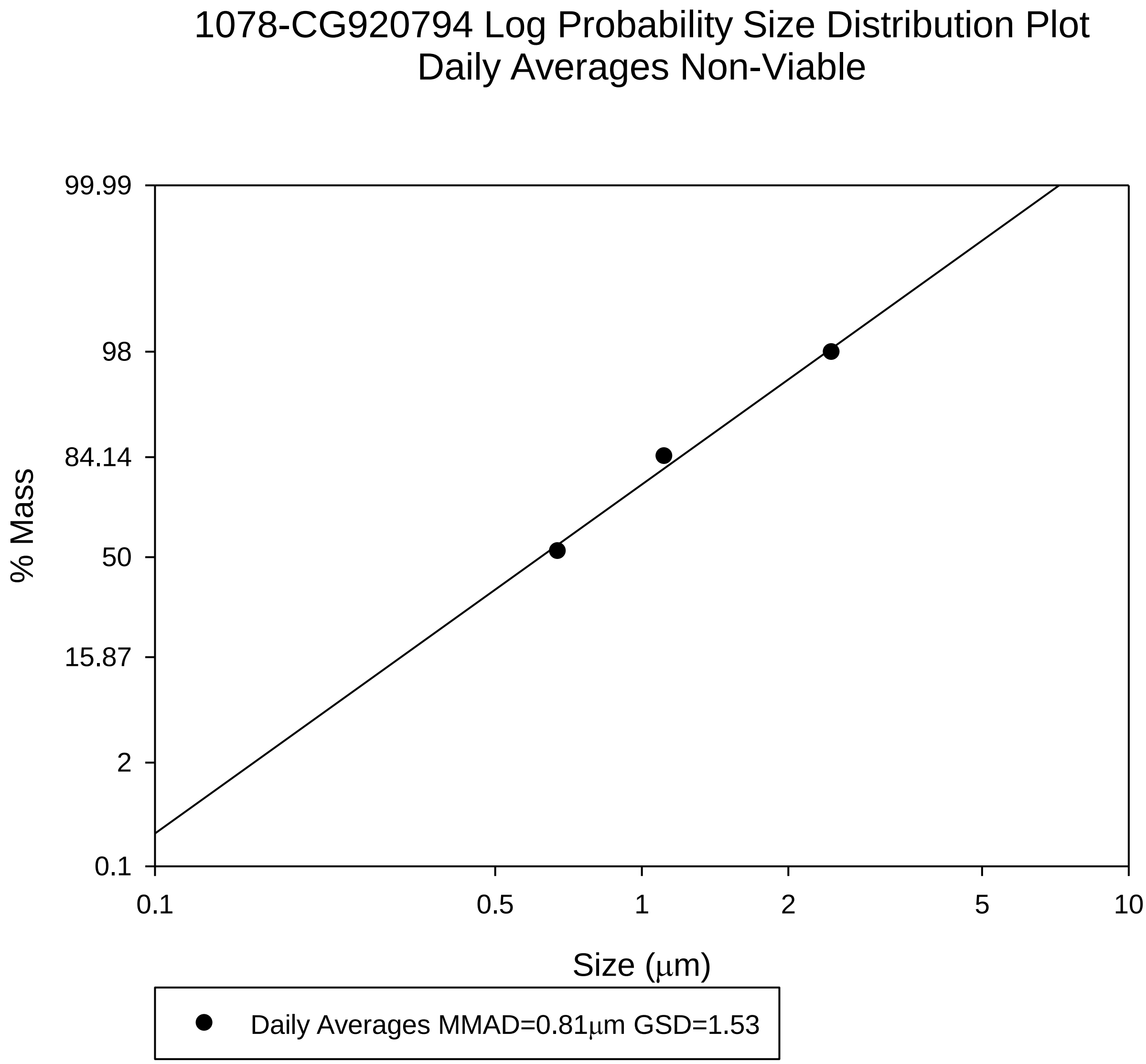

Figure 2. Log probability size distribution plot.

Viable Spore Challenge Data

Table 16. Aerosol Data Summary Sheet (Day 1)

Study No. 1078-CG920794 Rabbits 07-26-10 Viable Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (ºF)	RH (%)
13	1.32E+04	2.18E+01	19.80	4.32E+02	6.1	7.00	1.01E+01	12.45	1.26E+02	1.20E-03	7.66E-07	73.1	67.4
34	1.32E+04	1.98E+01	19.80	3.92E+02	6.1	5.78	1.11E+01	17.02	1.89E+02	1.80E-03	8.42E-07	73.5	65.6
25	1.32E+04	2.18E+01	19.60	4.27E+02	6.1	8.13	8.62E+00	10.01	8.62E+01	8.21E-04	6.53E-07	73.6	64.1
15	1.32E+04	2.46E+01	19.60	4.82E+02	6.1	7.87	1.00E+01	10.02	1.01E+02	9.58E-04	7.61E-07	73.8	61.8
30	1.32E+04	3.50E+01	19.20	6.72E+02	6.1	11.22	9.82E+00	10.01	9.83E+01	9.36E-04	7.44E-07	73.8	64.4
28	1.32E+04	1.38E+01	19.80	2.73E+02	6.0	7.00	6.51E+00	10.80	7.03E+01	6.69E-04	4.93E-07	74.5	68.6
19	1.32E+04	2.26E+01	19.60	4.43E+02	6.1	7.00	1.04E+01	11.54	1.20E+02	1.14E-03	7.86E-07	74.6	65.5
14	1.18E+05	1.86E+02	19.00	3.53E+03	6.1	9.10	6.37E+01	10.00	6.37E+02	6.06E-03	5.40E-07	74.7	62.7
11	1.18E+05	1.75E+02	19.40	3.40E+03	6.1	7.00	7.95E+01	10.25	8.15E+02	7.76E-03	6.74E-07	74.6	63.5
2	1.18E+05	3.64E+02	19.60	7.13E+03	6.1	8.75	1.34E+02	10.00	1.34E+03	1.27E-02	1.13E-06	74.6	63.8
8	1.18E+05	3.08E+02	19.60	6.04E+03	6.1	7.00	1.41E+02	13.61	1.92E+03	1.83E-02	1.20E-06	74.5	69.8
12	1.18E+05	1.83E+02	19.60	3.59E+03	6.1	7.00	8.40E+01	11.03	9.27E+02	8.82E-03	7.12E-07	74.5	66.5
18	1.18E+05	2.52E+02	19.60	4.94E+03	6.1	7.00	1.16E+02	12.23	1.41E+03	1.35E-02	9.80E-07	74.9	63.4
32	1.18E+05	2.82E+02	19.80	5.58E+03	6.1	7.00	1.31E+02	10.54	1.38E+03	1.31E-02	1.11E-06	74.8	61.8
6	1.15E+06	2.04E+03	19.20	3.92E+04	6.1	9.40	6.83E+02	10.01	6.84E+03	6.51E-02	5.94E-07	74.6	64.9
33	1.15E+06	3.02E+03	19.60	5.92E+04	6.1	7.25	1.34E+03	10.01	1.34E+04	1.28E-01	1.16E-06	74.6	65.9
27	1.15E+06	3.16E+03	19.40	6.13E+04	6.1	7.00	1.44E+03	12.07	1.73E+04	1.65E-01	1.25E-06	74.5	64.9
31	1.15E+06	2.96E+03	19.60	5.80E+04	6.1	7.00	1.36E+03	11.41	1.55E+04	1.48E-01	1.18E-06	74.6	64.3
39	1.15E+06	3.06E+03	19.40	5.94E+04	6.1	6.53	1.49E+03	17.02	2.54E+04	2.42E-01	1.30E-06	74.3	64.9
21	1.15E+06	3.06E+03	19.80	6.06E+04	6.1	7.00	1.42E+03	14.07	2.00E+04	1.90E-01	1.23E-06	75.3	74.8
38	1.15E+06	3.14E+03	19.40	6.09E+04	6.1	7.00	1.43E+03	10.42	1.49E+04	1.42E-01	1.24E-06	74.5	64.5

Table 17. Aerosol Data Summary Sheet (Day 2)

Study No. 1078-CG920794 Rabbits 07-27-10 Viable Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (ºF)	RH (%)
13	1.32E+04	8.84E+01	18.80	1.66E+03	5.9	10.00	2.82E+01	10.01	2.82E+02	2.69E-03	2.13E-06	71.2	65.5
34	1.32E+04	4.62E+01	19.20	8.87E+02	6.0	9.10	1.62E+01	10.01	1.63E+02	1.55E-03	1.23E-06	71.8	63.9
25	1.32E+04	4.28E+01	19.00	8.13E+02	6.0	8.88	1.53E+01	10.01	1.53E+02	1.46E-03	1.16E-06	71.9	70.4
15	1.32E+04	2.88E+01	19.60	5.64E+02	6.0	7.00	1.34E+01	12.55	1.69E+02	1.61E-03	1.02E-06	72.8	74.6
30	1.32E+04	3.68E+01	19.20	7.07E+02	6.0	7.98	1.48E+01	10.02	1.48E+02	1.41E-03	1.12E-06	72.8	67.9
28	1.32E+04	2.90E+01	19.60	5.68E+02	6.0	7.00	1.35E+01	10.65	1.44E+02	1.37E-03	1.03E-06	73.4	70.2
19	1.32E+04	4.26E+01	18.80	8.01E+02	6.0	7.00	1.91E+01	12.28	2.34E+02	2.23E-03	1.44E-06	73.8	74.4
14	1.18E+05	1.76E+02	18.80	3.31E+03	5.9	8.45	6.64E+01	10.03	6.66E+02	6.34E-03	5.62E-07	73.8	65.8
11	1.18E+05	3.38E+02	19.40	6.56E+03	6.0	8.55	1.28E+02	10.02	1.28E+03	1.22E-02	1.08E-06	73.8	66.2
2	1.18E+05	4.04E+02	19.40	7.84E+03	5.9	9.50	1.40E+02	10.02	1.40E+03	1.33E-02	1.19E-06	74.0	66.0
8	1.18E+05	3.42E+02	19.60	6.70E+03	5.9	7.05	1.61E+02	10.04	1.62E+03	1.54E-02	1.37E-06	74.1	67.2
12	1.18E+05	3.60E+02	19.40	6.98E+03	5.9	7.62	1.55E+02	10.14	1.58E+03	1.50E-02	1.32E-06	74.3	68.0
18	1.18E+05	1.81E+02	19.20	3.48E+03	6.0	7.00	8.27E+01	11.17	9.24E+02	8.80E-03	7.01E-07	74.5	69.3
32	1.18E+05	2.82E+02	19.60	5.53E+03	6.0	7.00	1.32E+02	10.34	1.36E+03	1.30E-02	1.12E-06	74.6	69.6
6	1.15E+06	2.80E+03	19.00	5.32E+04	6.0	8.37	1.06E+03	10.00	1.06E+04	1.01E-01	9.21E-07	74.8	63.9
33	1.15E+06	2.66E+03	19.40	5.16E+04	6.0	7.55	1.14E+03	10.02	1.14E+04	1.09E-01	9.91E-07	74.5	62.8
27	1.15E+06	2.96E+03	19.00	5.62E+04	5.9	7.70	1.24E+03	10.01	1.24E+04	1.18E-01	1.08E-06	74.7	68.2
31	1.15E+06	2.42E+03	19.60	4.74E+04	6.0	7.37	1.07E+03	10.01	1.07E+04	1.02E-01	9.33E-07	74.5	66.7
39	1.15E+06	1.73E+03	19.60	3.39E+04	5.9	6.12	9.39E+02	17.73	1.66E+04	1.59E-01	8.17E-07	74.6	69.4
21	1.15E+06	2.03E+03	19.80	4.02E+04	6.0	7.10	9.44E+02	10.01	9.44E+03	8.99E-02	8.20E-07	74.7	71.3
38	1.15E+06	3.32E+03	19.60	6.51E+04	6.0	7.72	1.40E+03	9.99	1.40E+04	1.34E-01	1.22E-06	74.6	64.5

Table 18. Aerosol Data Summary Sheet (Day 3)

Study No. 1078-CG920794 Rabbits 07-28-10 Viable Spores - Repeats

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (ºF)	RH (%)
13*	1.32E+04	6.08E+02	19.40	1.18E+04	6.0	7.00	2.81E+02	11.05	3.10E+03	2.96E-02	2.13E-05	73.8	60.9
34*	1.32E+04	4.38E+02	19.80	8.67E+03	6.0	7.53	1.92E+02	10.01	1.92E+03	1.83E-02	1.45E-05	74.0	60.8
25*	1.32E+04	3.86E+02	19.60	7.57E+03	6.0	8.18	1.54E+02	10.01	1.54E+03	1.47E-02	1.17E-05	74.1	66.2
15*	1.32E+04	5.10E+02	19.40	9.89E+03	6.0	11.33	1.46E+02	10.00	1.46E+03	1.39E-02	1.10E-05	74.7	69.8
30*	1.32E+04	3.20E+02	19.60	6.27E+03	6.0	9.88	1.06E+02	10.00	1.06E+03	1.01E-02	8.02E-06	74.6	64.1
28*	1.32E+04	1.17E+02	19.80	2.32E+03	6.0	7.00	5.52E+01	11.57	6.38E+02	6.08E-03	4.18E-06	75.2	68.8
19*	1.32E+04	9.18E+01	19.40	1.78E+03	6.0	7.00	4.24E+01	11.47	4.86E+02	4.63E-03	3.21E-06	75.1	64.8
14	1.18E+05	2.44E+02	19.60	4.78E+03	6.0	8.13	9.80E+01	10.01	9.81E+02	9.35E-03	8.31E-07	74.8	62.6
11	1.18E+05	3.64E+02	19.60	7.13E+03	6.0	7.00	1.70E+02	10.30	1.75E+03	1.67E-02	1.44E-06	74.9	68.3
2	1.18E+05	7.46E+02	19.40	1.45E+04	6.0	9.83	2.45E+02	10.01	2.46E+03	2.34E-02	2.08E-06	75.1	58.8
8	1.18E+05	3.42E+02	19.80	6.77E+03	5.9	7.00	1.64E+02	10.51	1.72E+03	1.64E-02	1.39E-06	75.2	69.7
12	1.18E+05	3.02E+02	19.60	5.92E+03	5.9	7.00	1.43E+02	11.28	1.62E+03	1.54E-02	1.21E-06	75.3	62.3
18	1.18E+05	4.70E+02	19.40	9.12E+03	6.0	9.05	1.68E+02	10.01	1.68E+03	1.60E-02	1.42E-06	75.2	56.4
32	1.18E+05	3.66E+02	19.80	7.25E+03	5.9	7.00	1.75E+02	13.22	2.32E+03	2.21E-02	1.49E-06	75.3	64.3
6	1.15E+06	1.47E+03	19.80	2.91E+04	5.9	7.37	6.69E+02	10.01	6.70E+03	6.38E-02	5.82E-07	75.4	63.7
33	1.15E+06	2.13E+03	19.40	4.13E+04	6.0	8.50	8.10E+02	10.01	8.11E+03	7.72E-02	7.05E-07	75.2	56.5
27	1.15E+06	2.50E+03	19.60	4.90E+04	5.9	7.27	1.14E+03	10.01	1.14E+04	1.09E-01	9.93E-07	75.3	66.8
31	1.15E+06	2.84E+03	19.60	5.57E+04	5.9	7.00	1.35E+03	11.98	1.61E+04	1.54E-01	1.17E-06	75.5	64.5
39	1.15E+06	3.48E+03	19.80	6.89E+04	6.0	7.00	1.64E+03	14.19	2.33E+04	2.22E-01	1.43E-06	75.7	66.0
21	1.15E+06	3.64E+03	19.60	7.13E+04	5.9	7.00	1.73E+03	11.71	2.02E+04	1.93E-01	1.50E-06	75.7	69.1
38	1.15E+06	3.50E+03	19.80	6.93E+04	6.0	7.40	1.56E+03	10.01	1.56E+04	1.49E-01	1.36E-06	75.5	64.1

* re-enumerations

Table 19. Aerosol Data Summary Sheet (Day 4)

Study No. 1078-CG920794 Rabbits 07-29-10 Viable Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (ºF)	RH (%)
13	1.32E+04	5.80E+01	19.60	1.14E+03	5.8	7.53	2.60E+01	10.00	2.60E+02	2.48E-03	1.97E-06	71.7	65.6
34	1.32E+04	5.14E+01	19.60	1.01E+03	5.8	7.00	2.48E+01	12.21	3.03E+02	2.89E-03	1.88E-06	72.3	67.0
25	1.32E+04	4.66E+01	19.80	9.23E+02	5.8	7.22	2.20E+01	10.01	2.21E+02	2.10E-03	1.67E-06	72.6	63.8
15	1.32E+04	4.54E+01	19.40	8.81E+02	5.8	7.00	2.17E+01	13.11	2.84E+02	2.71E-03	1.64E-06	73.3	71.0
30	1.32E+04	8.94E+01	19.40	1.73E+03	5.9	9.08	3.24E+01	10.01	3.24E+02	3.09E-03	2.45E-06	73.1	66.2
28	1.32E+04	7.32E+01	19.60	1.43E+03	5.8	7.00	3.53E+01	11.63	4.11E+02	3.91E-03	2.68E-06	73.9	70.2
19	1.32E+04	1.12E+02	19.40	2.17E+03	5.8	7.43	5.04E+01	10.01	5.05E+02	4.81E-03	3.82E-06	74.3	73.1
14	1.18E+05	3.10E+02	19.60	6.08E+03	5.8	7.85	1.33E+02	10.01	1.34E+03	1.27E-02	1.13E-06	74.0	65.5
11	1.18E+05	3.80E+02	19.60	7.45E+03	5.9	7.00	1.80E+02	11.08	2.00E+03	1.90E-02	1.53E-06	74.3	68.9
2	1.18E+05	5.92E+02	19.60	1.16E+04	5.9	7.58	2.59E+02	10.02	2.60E+03	2.48E-02	2.20E-06	74.2	70.3
8	1.18E+05	4.88E+02	19.80	9.66E+03	5.9	7.50	2.18E+02	10.01	2.19E+03	2.08E-02	1.85E-06	74.2	68.6
12	1.18E+05	4.60E+02	19.40	8.92E+03	5.9	7.28	2.08E+02	10.01	2.08E+03	1.98E-02	1.76E-06	74.3	66.1
18	1.18E+05	8.10E+02	19.40	1.57E+04	5.9	10.15	2.62E+02	10.04	2.63E+03	2.51E-02	2.22E-06	74.5	69.9
32	1.18E+05	4.36E+02	19.40	8.46E+03	5.9	7.00	2.05E+02	13.46	2.76E+03	2.63E-02	1.74E-06	74.7	68.1
6	1.15E+06	1.81E+03	19.60	3.55E+04	5.9	7.90	7.61E+02	10.01	7.62E+03	7.26E-02	6.62E-07	74.6	66.1
33	1.15E+06	1.61E+03	19.80	3.19E+04	5.9	7.00	7.72E+02	11.15	8.61E+03	8.20E-02	6.71E-07	74.6	69.1
27	1.15E+06	3.00E+03	19.40	5.82E+04	5.9	9.20	1.07E+03	10.01	1.07E+04	1.02E-01	9.32E-07	74.5	67.6
31	1.15E+06	2.74E+03	19.40	5.32E+04	5.9	7.00	1.29E+03	10.88	1.40E+04	1.33E-01	1.12E-06	74.5	65.6
39	1.15E+06	2.94E+03	19.80	5.82E+04	5.9	7.00	1.41E+03	13.22	1.86E+04	1.77E-01	1.23E-06	74.5	68.1
21	1.15E+06	2.05E+03	19.80	4.06E+04	5.9	7.00	9.83E+02	11.04	1.09E+04	1.03E-01	8.55E-07	74.7	69.1
38	1.15E+06	2.82E+03	19.60	5.53E+04	5.9	7.78	1.20E+03	10.01	1.21E+04	1.15E-01	1.05E-06	74.6	64.9

Table 20. Aerosol Data Summary Sheet (Day 5)

Study No. 1078-CG920794 Rabbits 07-30-10 Viable Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (ºF)	RH (%)
13	1.32E+04	8.38E+01	19.00	1.59E+03	6.0	7.00	3.79E+01	10.91	4.14E+02	3.94E-03	2.87E-06	72.3	67.3
34	1.32E+04	7.02E+01	19.40	1.36E+03	6.0	7.00	3.24E+01	10.65	3.45E+02	3.29E-03	2.46E-06	72.4	64.1
25	1.32E+04	4.82E+01	19.40	9.35E+02	6.0	7.17	2.17E+01	10.01	2.18E+02	2.07E-03	1.65E-06	72.6	65.9
15	1.32E+04	6.12E+01	19.60	1.20E+03	6.0	7.00	2.86E+01	13.62	3.89E+02	3.70E-03	2.16E-06	73.0	73.1
30	1.32E+04	9.84E+01	19.40	1.91E+03	6.0	8.60	3.70E+01	10.01	3.70E+02	3.53E-03	2.80E-06	73.0	68.0
28	1.32E+04	8.14E+01	19.40	1.58E+03	6.0	7.00	3.76E+01	11.01	4.14E+02	3.94E-03	2.85E-06	73.2	71.0
19	1.32E+04	8.08E+01	19.60	1.58E+03	6.0	7.00	3.77E+01	10.52	3.97E+02	3.78E-03	2.86E-06	73.9	76.0
14	1.18E+05	3.42E+02	19.40	6.63E+03	6.0	8.07	1.37E+02	10.01	1.37E+03	1.31E-02	1.16E-06	73.6	69.4
11	1.18E+05	2.76E+02	19.40	5.35E+03	6.0	7.00	1.27E+02	11.85	1.51E+03	1.44E-02	1.08E-06	73.6	67.3
2	1.18E+05	4.26E+02	19.20	8.18E+03	6.0	9.42	1.45E+02	10.01	1.45E+03	1.38E-02	1.23E-06	73.5	63.0
8	1.18E+05	3.70E+02	19.40	7.18E+03	6.0	7.00	1.71E+02	15.59	2.66E+03	2.54E-02	1.45E-06	73.8	69.2
12	1.18E+05	4.26E+02	19.60	8.35E+03	6.0	7.00	1.99E+02	10.88	2.16E+03	2.06E-02	1.68E-06	73.7	62.1
18	1.18E+05	3.08E+02	19.60	6.04E+03	6.0	7.27	1.38E+02	10.01	1.39E+03	1.32E-02	1.17E-06	75.0	64.5
32	1.18E+05	4.44E+02	19.40	8.61E+03	6.0	7.93	1.81E+02	10.01	1.81E+03	1.73E-02	1.53E-06	74.2	62.6
6	1.15E+06	1.42E+03	19.80	2.81E+04	6.0	8.12	5.77E+02	10.00	5.77E+03	5.50E-02	5.02E-07	74.3	67.2
33	1.15E+06	3.54E+03	19.20	6.80E+04	6.0	9.60	1.18E+03	10.00	1.18E+04	1.12E-01	1.03E-06	73.9	60.0
27	1.15E+06	2.84E+03	19.40	5.51E+04	6.0	8.32	1.10E+03	10.01	1.10E+04	1.05E-01	9.60E-07	74.4	72.3
31	1.15E+06	2.24E+03	19.80	4.44E+04	6.0	7.08	1.04E+03	12.31	1.29E+04	1.22E-01	9.08E-07	74.2	67.2
39	1.15E+06	1.93E+03	19.60	3.78E+04	6.0	7.00	9.01E+02	10.24	9.22E+03	8.78E-02	7.83E-07	74.2	67.1
21	1.15E+06	2.58E+03	19.80	5.11E+04	6.0	7.00	1.22E+03	12.78	1.55E+04	1.48E-01	1.06E-06	74.4	69.6
38	1.15E+06	2.78E+03	19.40	5.39E+04	6.0	7.00	1.28E+03	11.53	1.48E+04	1.41E-01	1.12E-06	74.4	63.0

Table 21. Aerosol Data Summary Sheet (Day 6)

Study No. 1078-CG920794 Rabbits 08-02-10 Viable Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (ºF)	RH (%)
13	1.32E+04	6.22E+01	18.75	1.17E+03	5.9	7.00	2.82E+01	10.07	2.84E+02	2.71E-03	2.14E-06	71.6	69.0
34	1.32E+04	5.88E+01	19.25	1.13E+03	5.9	8.12	2.36E+01	10.02	2.37E+02	2.25E-03	1.79E-06	71.9	60.7
25	1.32E+04	4.20E+01	19.25	8.09E+02	6.0	7.50	1.80E+01	10.02	1.80E+02	1.71E-03	1.36E-06	72.4	62.1
15	1.32E+04	4.16E+01	19.25	8.01E+02	6.0	7.00	1.91E+01	15.66	2.99E+02	2.84E-03	1.44E-06	73.5	78.6
30	1.32E+04	5.42E+01	19.40	1.05E+03	6.0	8.20	2.14E+01	10.01	2.14E+02	2.04E-03	1.62E-06	73.3	63.0
28	1.32E+04	3.86E+01	19.60	7.57E+02	6.0	7.45	1.69E+01	10.01	1.69E+02	1.61E-03	1.28E-06	74.0	69.0
19	1.32E+04	3.36E+01	19.60	6.59E+02	6.0	7.00	1.57E+01	11.48	1.80E+02	1.71E-03	1.19E-06	74.4	71.8
14	1.18E+05	1.16E+02	19.40	2.25E+03	6.0	7.70	4.87E+01	10.01	4.88E+02	4.64E-03	4.13E-07	74.2	64.0
11	1.18E+05	1.39E+02	19.40	2.70E+03	6.0	7.70	5.84E+01	10.00	5.84E+02	5.56E-03	4.95E-07	74.3	66.2
2	1.18E+05	2.28E+02	19.40	4.42E+03	6.0	7.53	9.79E+01	10.00	9.79E+02	9.32E-03	8.30E-07	74.6	68.3
8	1.18E+05	1.51E+02	19.60	2.96E+03	6.0	7.00	7.05E+01	11.79	8.31E+02	7.91E-03	5.97E-07	74.8	65.9
12	1.18E+05	2.76E+02	19.80	5.46E+03	6.0	7.50	1.21E+02	10.01	1.22E+03	1.16E-02	1.03E-06	74.8	65.5
18	1.18E+05	1.51E+02	19.40	2.93E+03	6.0	7.12	6.86E+01	11.25	7.71E+02	7.35E-03	5.81E-07	74.9	65.0
32	1.18E+05	3.16E+02	19.40	6.13E+03	6.0	8.12	1.26E+02	10.02	1.26E+03	1.20E-02	1.07E-06	75.0	67.7
6	1.15E+06	1.61E+03	19.00	3.06E+04	6.0	8.05	6.33E+02	10.01	6.34E+03	6.04E-02	5.51E-07	75.1	62.7
33	1.15E+06	1.90E+03	19.20	3.65E+04	6.0	8.07	7.53E+02	10.01	7.54E+03	7.18E-02	6.55E-07	74.3	70.3
27	1.15E+06	3.28E+03	19.00	6.23E+04	6.0	8.78	1.18E+03	10.03	1.19E+04	1.13E-01	1.03E-06	74.9	67.5
31	1.15E+06	1.92E+03	19.25	3.70E+04	6.0	7.00	8.80E+02	10.47	9.21E+03	8.77E-02	7.65E-07	75.1	64.0
39	1.15E+06	3.22E+03	19.25	6.20E+04	6.0	8.00	1.29E+03	10.02	1.29E+04	1.23E-01	1.12E-06	75.1	62.4
21	1.15E+06	1.97E+03	19.25	3.79E+04	5.9	7.53	8.54E+02	9.95	8.49E+03	8.09E-02	7.42E-07	75.5	67.3
38	1.15E+06	3.08E+03	19.25	5.93E+04	5.9	8.27	1.22E+03	10.03	1.22E+04	1.16E-01	1.06E-06	75.3	63.9

Table 22. Aerosol Data Summary Sheet (Day 7)

Study No. 1078-CG920794 Rabbits 08-03-10 Viable Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (ºF)	RH (%)
13	1.32E+04	5.66E+01	18.25	1.03E+03	6.0	8.03	2.14E+01	10.02	2.15E+02	2.05E-03	1.62E-06	71.2	66.7
34	1.32E+04	5.10E+01	19.25	9.82E+02	6.0	7.92	2.07E+01	10.02	2.07E+02	1.97E-03	1.57E-06	71.8	66.4
25	1.32E+04	4.92E+01	19.25	9.47E+02	6.0	7.97	1.98E+01	10.01	1.98E+02	1.89E-03	1.50E-06	72.2	64.6
15	1.32E+04	3.82E+01	19.25	7.35E+02	6.0	7.00	1.75E+01	12.99	2.27E+02	2.17E-03	1.33E-06	72.9	73.7
30	1.32E+04	4.38E+01	19.50	8.54E+02	6.0	9.22	1.54E+01	10.01	1.55E+02	1.47E-03	1.17E-06	72.8	65.4
28	1.32E+04	3.14E+01	19.50	6.12E+02	6.0	7.00	1.46E+01	10.60	1.55E+02	1.47E-03	1.10E-06	73.7	73.4
19	1.32E+04	3.28E+01	19.50	6.40E+02	6.0	7.00	1.52E+01	10.22	1.56E+02	1.48E-03	1.15E-06	73.9	70.2
14	1.18E+05	1.36E+02	19.50	2.65E+03	6.0	8.60	5.14E+01	10.01	5.14E+02	4.90E-03	4.36E-07	73.7	65.6
11	1.18E+05	1.36E+02	19.50	2.65E+03	6.0	8.12	5.44E+01	10.01	5.45E+02	5.19E-03	4.61E-07	73.7	64.7
2	1.18E+05	2.62E+02	19.75	5.17E+03	6.0	8.08	1.07E+02	10.00	1.07E+03	1.02E-02	9.05E-07	73.8	67.5
8	1.18E+05	1.55E+02	19.75	3.06E+03	6.1	7.00	7.17E+01	11.98	8.59E+02	8.18E-03	6.08E-07	74.2	69.4
12	1.18E+05	1.38E+02	19.75	2.73E+03	6.1	7.63	5.86E+01	10.01	5.86E+02	5.58E-03	4.96E-07	74.0	66.1
18	1.18E+05	1.62E+02	19.50	3.16E+03	6.1	7.93	6.53E+01	10.01	6.54E+02	6.23E-03	5.53E-07	74.1	66.8
32	1.18E+05	1.96E+02	19.25	3.77E+03	6.0	7.00	8.98E+01	11.81	1.06E+03	1.01E-02	7.61E-07	74.7	71.8
6	1.15E+06	1.85E+02	19.25	3.56E+03	6.0	8.65	6.86E+01	10.01	6.87E+02	6.54E-03	5.97E-08	74.5	64.4
33	1.15E+06	1.52E+03	19.50	2.96E+04	6.0	7.00	7.06E+02	10.15	7.16E+03	6.82E-02	6.14E-07	74.3	65.1
27	1.15E+06	2.13E+03	19.50	4.15E+04	6.0	7.80	8.88E+02	10.02	8.89E+03	8.47E-02	7.72E-07	74.5	65.8
31	1.15E+06	1.36E+03	19.50	2.65E+04	6.0	7.00	6.31E+02	11.11	7.02E+03	6.68E-02	5.49E-07	74.7	64.0
39	1.15E+06	1.67E+03	19.50	3.26E+04	6.0	7.00	7.75E+02	10.98	8.51E+03	8.11E-02	6.74E-07	74.8	64.9
21	1.15E+06	1.63E+03	19.50	3.18E+04	6.0	7.00	7.57E+02	11.13	8.42E+03	8.02E-02	6.58E-07	74.9	67.8
38	1.15E+06	2.11E+03	19.50	4.11E+04	6.0	7.00	9.80E+02	10.30	1.01E+04	9.61E-02	8.52E-07	74.7	65.0

Table 23. Aerosol Data Summary Sheet (Day 8)

Study No. 1078-CG920794 Rabbits 08-04-10 Viable Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (ºF)	RH (%)
13	1.32E+04	4.38E+01	18.75	8.21E+02	5.8	7.00	2.02E+01	10.04	2.03E+02	1.93E-03	1.53E-06	74.5	64.2
34	1.32E+04	3.92E+01	19.50	7.64E+02	5.8	7.00	1.88E+01	11.45	2.16E+02	2.05E-03	1.43E-06	74.4	58.0
25	1.32E+04	5.36E+01	19.25	1.03E+03	5.8	7.22	2.46E+01	10.01	2.47E+02	2.35E-03	1.87E-06	74.3	71.3
15	1.32E+04	5.62E+01	19.50	1.10E+03	5.8	7.00	2.70E+01	13.18	3.56E+02	3.39E-03	2.04E-06	74.7	75.6
30	1.32E+04	8.02E+01	19.00	1.52E+03	5.8	8.90	2.95E+01	10.00	2.95E+02	2.81E-03	2.24E-06	74.4	72.7
28	1.32E+04	4.02E+01	19.00	7.64E+02	5.8	7.00	1.88E+01	10.70	2.01E+02	1.92E-03	1.43E-06	74.6	73.4
19	1.32E+04	3.84E+01	19.00	7.30E+02	5.8	7.00	1.80E+01	12.66	2.28E+02	2.17E-03	1.36E-06	75.2	79.5
14	1.18E+05	1.22E+02	19.00	2.32E+03	5.8	7.13	5.61E+01	10.00	5.61E+02	5.34E-03	4.75E-07	74.8	67.7
11	1.18E+05	1.44E+02	19.00	2.74E+03	5.8	7.00	6.74E+01	10.20	6.87E+02	6.55E-03	5.71E-07	74.7	69.1
2	1.18E+05	1.47E+02	19.50	2.87E+03	5.8	7.00	7.06E+01	10.07	7.11E+02	6.77E-03	5.98E-07	74.6	65.0
8	1.18E+05	1.68E+02	19.25	3.23E+03	5.8	7.00	7.97E+01	11.46	9.13E+02	8.69E-03	6.75E-07	74.7	68.1
12	1.18E+05	1.53E+02	19.50	2.98E+03	5.8	7.00	7.35E+01	12.14	8.92E+02	8.50E-03	6.23E-07	74.7	67.8
18	1.18E+05	1.20E+02	19.25	2.31E+03	5.8	7.00	5.69E+01	11.35	6.46E+02	6.15E-03	4.82E-07	74.8	66.8
32	1.18E+05	1.51E+02	19.00	2.87E+03	5.8	7.00	7.07E+01	12.91	9.12E+02	8.69E-03	5.99E-07	74.9	65.5
6	1.15E+06	1.47E+03	19.50	2.87E+04	5.8	7.25	6.82E+02	10.01	6.82E+03	6.50E-02	5.93E-07	74.9	66.0
33	1.15E+06	1.49E+03	19.50	2.91E+04	5.8	7.00	7.16E+02	11.55	8.27E+03	7.87E-02	6.22E-07	74.8	64.3
27	1.15E+06	1.77E+03	19.00	3.36E+04	5.8	7.42	7.81E+02	10.01	7.82E+03	7.45E-02	6.80E-07	75.0	69.0
31	1.15E+06	1.34E+03	19.50	2.61E+04	5.8	7.00	6.44E+02	12.35	7.95E+03	7.57E-02	5.60E-07	75.0	64.1
39	1.15E+06	1.90E+03	19.50	3.71E+04	5.8	7.47	8.55E+02	10.01	8.56E+03	8.15E-02	7.44E-07	75.1	70.6
21	1.15E+06	1.80E+03	19.50	3.51E+04	5.8	7.00	8.65E+02	12.74	1.10E+04	1.05E-01	7.52E-07	75.3	72.4
38	1.15E+06	1.33E+03	19.50	2.59E+04	5.8	7.00	6.39E+02	10.38	6.63E+03	6.31E-02	5.55E-07	75.2	69.5

Table 24. Aerosol Data Summary Sheet (Day 9)

Study No. 1078-CG920794 Rabbits 08-05-10 Viable Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (ºF)	RH (%)
13	1.32E+04	3.98E+01	19.25	7.66E+02	6.0	7.60	1.68E+01	10.01	1.68E+02	1.60E-03	1.27E-06	71.9	73.7
34	1.32E+04	4.94E+01	19.00	9.39E+02	6.1	8.75	1.76E+01	10.01	1.76E+02	1.68E-03	1.33E-06	72.1	67.8
25	1.32E+04	5.88E+01	19.25	1.13E+03	6.0	8.33	2.26E+01	10.68	2.42E+02	2.30E-03	1.72E-06	72.5	66.5
15	1.32E+04	4.74E+01	19.25	9.12E+02	6.1	7.00	2.14E+01	10.54	2.25E+02	2.15E-03	1.62E-06	73.0	73.2
30	1.32E+04	6.04E+01	19.00	1.15E+03	6.0	9.43	2.03E+01	10.01	2.03E+02	1.93E-03	1.54E-06	73.1	70.8
28	1.32E+04	3.54E+01	19.50	6.90E+02	6.0	7.27	1.58E+01	10.00	1.58E+02	1.51E-03	1.20E-06	73.8	77.1
19	1.32E+04	3.62E+01	19.50	7.06E+02	6.0	7.77	1.51E+01	10.01	1.52E+02	1.44E-03	1.15E-06	73.8	76.1
14	1.18E+05	1.53E+02	19.00	2.91E+03	6.0	7.42	6.53E+01	10.01	6.54E+02	6.22E-03	5.53E-07	73.6	73.1
11	1.18E+05	2.50E+02	19.25	4.81E+03	6.0	7.00	1.15E+02	11.72	1.34E+03	1.28E-02	9.71E-07	73.9	74.9
2	1.18E+05	3.26E+02	19.00	6.19E+03	6.0	8.48	1.22E+02	10.02	1.22E+03	1.16E-02	1.03E-06	73.6	72.8
8	1.18E+05	3.06E+02	19.25	5.89E+03	6.0	7.92	1.24E+02	10.02	1.24E+03	1.18E-02	1.05E-06	73.8	73.7
12	1.18E+05	2.80E+02	19.25	5.39E+03	6.0	7.00	1.28E+02	10.54	1.35E+03	1.29E-02	1.09E-06	73.4	70.7
18	1.18E+05	3.18E+02	18.50	5.88E+03	6.0	7.08	1.38E+02	10.02	1.39E+03	1.32E-02	1.17E-06	73.7	72.6
32	1.18E+05	3.04E+02	19.25	5.85E+03	6.0	7.00	1.39E+02	11.06	1.54E+03	1.47E-02	1.18E-06	74.0	73.0
6	1.15E+06	1.56E+03	19.25	3.00E+04	6.0	7.98	6.27E+02	10.02	6.28E+03	5.99E-02	5.45E-07	73.9	68.9
33	1.15E+06	3.60E+03	19.50	7.02E+04	6.0	8.48	1.38E+03	10.01	1.38E+04	1.32E-01	1.20E-06	74.1	73.0
27	1.15E+06	3.80E+03	19.25	7.32E+04	6.0	8.22	1.48E+03	10.01	1.48E+04	1.41E-01	1.29E-06	74.2	73.0
31	1.15E+06	3.08E+03	19.50	6.01E+04	6.0	7.00	1.43E+03	10.79	1.54E+04	1.47E-01	1.24E-06	74.2	70.9
39	1.15E+06	3.00E+03	19.25	5.78E+04	6.0	7.00	1.38E+03	12.18	1.67E+04	1.60E-01	1.20E-06	74.5	73.0
21	1.15E+06	2.62E+03	19.50	5.11E+04	6.0	7.00	1.22E+03	12.99	1.58E+04	1.50E-01	1.06E-06	74.7	72.4
38	1.15E+06	2.98E+03	19.50	5.81E+04	6.0	7.25	1.34E+03	10.33	1.38E+04	1.31E-01	1.16E-06	74.6	68.5

Table 25. Aerosol Data Summary Sheet (Day 10)

Study No. 1078-CG920794 Rabbits 08-06-10 Viable Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (ºF)	RH (%)
13	1.32E+04	4.64E+01	19.00	8.82E+02	5.8	8.52	1.78E+01	10.52	1.88E+02	1.79E-03	1.35E-06	71.5	72.7
34	1.32E+04	5.24E+01	19.60	1.03E+03	5.9	7.35	2.37E+01	10.01	2.37E+02	2.26E-03	1.79E-06	71.7	68.7
25	1.32E+04	9.66E+01	18.40	1.78E+03	5.9	9.42	3.20E+01	10.01	3.20E+02	3.05E-03	2.42E-06	71.6	65.0
15	1.32E+04	6.90E+01	19.60	1.35E+03	5.9	7.97	2.88E+01	10.00	2.88E+02	2.74E-03	2.18E-06	72.3	76.4
30	1.32E+04	7.10E+01	19.25	1.37E+03	5.8	8.80	2.68E+01	10.02	2.68E+02	2.56E-03	2.03E-06	72.1	69.2
28	1.32E+04	5.88E+01	19.25	1.13E+03	5.8	8.47	2.30E+01	10.03	2.31E+02	2.20E-03	1.75E-06	72.6	74.7
19	1.32E+04	3.06E+01	19.50	5.97E+02	5.8	7.00	1.47E+01	12.81	1.88E+02	1.79E-03	1.11E-06	73.0	76.0
14	1.18E+05	1.73E+02	19.25	3.33E+03	5.8	8.23	6.98E+01	10.01	6.98E+02	6.65E-03	5.91E-07	72.9	72.6
11	1.18E+05	3.50E+02	19.40	6.79E+03	5.8	9.22	1.27E+02	10.01	1.27E+03	1.21E-02	1.08E-06	72.5	70.6
2	1.18E+05	4.84E+02	19.40	9.39E+03	5.8	10.00	1.62E+02	10.01	1.62E+03	1.54E-02	1.37E-06	72.8	69.2
8	1.18E+05	3.98E+02	19.60	7.80E+03	5.9	8.17	1.62E+02	10.01	1.62E+03	1.54E-02	1.37E-06	73.3	75.0
12	1.18E+05	3.04E+02	19.60	5.96E+03	5.9	7.60	1.33E+02	10.01	1.33E+03	1.27E-02	1.13E-06	73.6	71.1
18	1.18E+05	4.52E+02	19.20	8.68E+03	5.9	9.48	1.55E+02	10.01	1.55E+03	1.48E-02	1.31E-06	73.7	66.7
32	1.18E+05	3.30E+02	19.80	6.53E+03	5.9	7.00	1.58E+02	10.36	1.64E+03	1.56E-02	1.34E-06	73.8	69.6
33	1.15E+06	1.65E+03	19.60	3.23E+04	5.9	7.42	7.39E+02	10.01	7.39E+03	7.04E-02	6.42E-07	73.7	67.0
27	1.15E+06	4.34E+03	19.20	8.33E+04	5.9	9.27	1.52E+03	10.00	1.52E+04	1.45E-01	1.32E-06	73.8	72.7
31	1.15E+06	3.04E+03	19.60	5.96E+04	5.9	7.00	1.44E+03	10.84	1.56E+04	1.49E-01	1.25E-06	73.9	69.2
39	1.15E+06	5.08E+03	19.40	9.86E+04	5.9	9.15	1.83E+03	10.01	1.83E+04	1.74E-01	1.59E-06	73.8	67.2
21	1.15E+06	2.76E+03	19.40	5.35E+04	5.9	7.00	1.30E+03	11.14	1.44E+04	1.38E-01	1.13E-06	74.2	72.8
38	1.15E+06	5.24E+03	19.20	1.01E+05	5.9	9.15	1.86E+03	10.01	1.87E+04	1.78E-01	1.62E-06	73.9	67.8

Table 26. Aerosol Data Summary Sheet (Day 11)

Study No. 1078-CG920794 Rabbits 08-09-10 Viable Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (ºF)	RH (%)
13	1.32E+04	3.12E+01	19.00	5.93E+02	5.9	7.00	1.44E+01	10.40	1.49E+02	1.42E-03	1.09E-06	71.8	72.9
34	1.32E+04	3.36E+01	19.80	6.65E+02	6.0	7.00	1.58E+01	10.87	1.72E+02	1.64E-03	1.20E-06	72.0	70.9
25	1.32E+04	4.66E+01	19.60	9.13E+02	6.0	8.38	1.82E+01	10.01	1.82E+02	1.73E-03	1.38E-06	72.1	68.7
15	1.32E+04	4.30E+01	19.80	8.51E+02	5.9	8.52	1.69E+01	10.00	1.69E+02	1.61E-03	1.28E-06	73.0	80.0
30	1.32E+04	6.02E+01	19.40	1.17E+03	5.9	9.47	2.09E+01	10.01	2.09E+02	1.99E-03	1.58E-06	72.8	71.1
28	1.32E+04	3.36E+01	19.80	6.65E+02	5.9	7.00	1.61E+01	10.70	1.72E+02	1.64E-03	1.22E-06	73.5	74.5
19	1.32E+04	3.54E+01	19.60	6.94E+02	6.0	7.00	1.65E+01	10.91	1.80E+02	1.72E-03	1.25E-06	73.9	78.1
14	1.18E+05	1.30E+02	19.60	2.55E+03	6.0	7.00	6.07E+01	10.86	6.59E+02	6.27E-03	5.14E-07	73.6	68.7
11	1.18E+05	2.78E+02	19.80	5.50E+03	6.0	7.00	1.31E+02	13.61	1.78E+03	1.70E-02	1.11E-06	73.6	66.7
2	1.18E+05	1.56E+02	19.60	3.06E+03	5.9	7.00	7.40E+01	12.87	9.53E+02	9.07E-03	6.27E-07	73.7	73.2
8	1.18E+05	3.08E+02	19.60	6.04E+03	6.0	7.30	1.38E+02	10.01	1.38E+03	1.31E-02	1.17E-06	73.7	70.6
12	1.18E+05	2.92E+02	19.80	5.78E+03	5.9	7.00	1.40E+02	10.24	1.43E+03	1.37E-02	1.19E-06	73.6	68.1
18	1.18E+05	3.10E+02	19.80	6.14E+03	6.0	7.20	1.42E+02	10.00	1.42E+03	1.35E-02	1.20E-06	73.6	74.2
32	1.18E+05	2.68E+02	19.40	5.20E+03	6.0	7.00	1.24E+02	10.62	1.31E+03	1.25E-02	1.05E-06	73.8	67.6
27	1.15E+06	1.87E+03	19.80	3.70E+04	5.9	8.13	7.72E+02	10.18	7.86E+03	7.48E-02	6.71E-07	73.9	70.1
31	1.15E+06	2.19E+03	20.00	4.38E+04	6.0	7.00	1.04E+03	12.20	1.27E+04	1.21E-01	9.07E-07	74.0	64.2
39	1.15E+06	2.56E+03	19.80	5.07E+04	6.0	7.00	1.21E+03	16.12	1.95E+04	1.85E-01	1.05E-06	74.1	70.0
21	1.15E+06	3.96E+03	19.60	7.76E+04	5.9	7.00	1.88E+03	10.95	2.06E+04	1.96E-01	1.63E-06	74.2	73.3
38	1.15E+06	3.42E+03	19.40	6.63E+04	5.9	7.12	1.58E+03	10.01	1.58E+04	1.51E-01	1.37E-06	74.1	72.0

Table 27. Aerosol Data Summary Sheet (Day 12)

Study No. 1078-CG920794 Rabbits 08-10-10 Viable Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (ºF)	RH (%)
13	1.32E+04	1.92E+01	19.00	3.65E+02	5.9	7.00	8.83E+00	10.83	9.57E+01	9.11E-04	6.69E-07	70.6	72.0
34	1.32E+04	2.56E+01	19.60	5.02E+02	6.0	7.00	1.19E+01	11.29	1.35E+02	1.28E-03	9.05E-07	70.9	71.1
25	1.32E+04	3.92E+01	19.40	7.60E+02	5.9	7.87	1.64E+01	10.01	1.64E+02	1.56E-03	1.24E-06	71.6	71.3
15	1.32E+04	4.54E+01	19.60	8.90E+02	5.9	7.00	2.15E+01	10.23	2.20E+02	2.10E-03	1.63E-06	72.2	80.0
30	1.32E+04	6.26E+01	19.60	1.23E+03	5.9	8.22	2.53E+01	10.00	2.53E+02	2.41E-03	1.92E-06	71.2	68.2
28	1.32E+04	5.76E+01	19.60	1.13E+03	5.9	7.83	2.44E+01	10.01	2.45E+02	2.33E-03	1.85E-06	72.9	71.3
19	1.32E+04	3.92E+01	19.40	7.60E+02	5.9	7.13	1.81E+01	10.03	1.81E+02	1.73E-03	1.37E-06	73.2	73.0
14	1.18E+05	1.26E+02	19.60	2.47E+03	5.9	7.53	5.56E+01	10.00	5.56E+02	5.29E-03	4.71E-07	72.9	65.1
11	1.18E+05	1.42E+02	19.40	2.75E+03	5.9	7.73	6.04E+01	10.01	6.05E+02	5.76E-03	5.12E-07	73.0	66.1
2	1.18E+05	3.02E+02	19.40	5.86E+03	5.9	8.87	1.12E+02	10.01	1.12E+03	1.07E-02	9.49E-07	73.1	68.8
8	1.18E+05	1.55E+02	19.40	3.01E+03	5.9	7.00	7.28E+01	10.41	7.58E+02	7.22E-03	6.17E-07	73.2	69.1
12	1.18E+05	3.08E+02	19.60	6.04E+03	5.9	7.70	1.33E+02	10.02	1.33E+03	1.27E-02	1.13E-06	73.3	70.4
18	1.18E+05	1.31E+02	19.60	2.57E+03	5.9	7.00	6.22E+01	14.39	8.95E+02	8.52E-03	5.27E-07	73.6	74.2
32	1.18E+05	1.60E+02	19.40	3.10E+03	5.9	7.85	6.70E+01	10.01	6.71E+02	6.39E-03	5.68E-07	73.6	69.5
27	1.15E+06	1.53E+03	19.60	3.00E+04	5.9	8.32	6.11E+02	10.00	6.11E+03	5.82E-02	5.31E-07	73.4	67.7
39	1.15E+06	2.60E+03	19.60	5.10E+04	5.9	8.12	1.06E+03	10.01	1.06E+04	1.01E-01	9.25E-07	73.4	67.9
21	1.15E+06	1.70E+03	19.40	3.30E+04	5.9	7.28	7.68E+02	10.02	7.69E+03	7.33E-02	6.68E-07	73.8	74.4
38	1.15E+06	1.76E+03	19.20	3.38E+04	5.9	7.03	8.15E+02	10.02	8.16E+03	7.77E-02	7.08E-07	73.8	67.4

Table 28. Aerosol Data Summary Sheet (Day 13)

Study No. 1078-CG920794 Rabbits 08-11-10 Viable Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (ºF)	RH (%)
13	1.32E+04	4.32E+01	18.00	7.78E+02	5.8	14.02	9.56E+00	10.01	9.57E+01	9.12E-04	7.24E-07	71.7	64.8
34	1.32E+04	5.92E+01	18.60	1.10E+03	5.8	11.82	1.61E+01	10.02	1.61E+02	1.53E-03	1.22E-06	72.1	63.8
25	1.32E+04	3.04E+01	19.60	5.96E+02	5.8	7.47	1.38E+01	10.01	1.38E+02	1.31E-03	1.04E-06	72.6	67.8
15	1.32E+04	3.26E+01	19.60	6.39E+02	5.9	7.28	1.49E+01	10.01	1.49E+02	1.42E-03	1.13E-06	73.2	75.6
30	1.32E+04	7.18E+01	19.20	1.38E+03	5.9	9.78	2.39E+01	10.00	2.39E+02	2.28E-03	1.81E-06	72.8	66.0
28	1.32E+04	6.58E+01	19.40	1.28E+03	5.9	8.98	2.41E+01	10.00	2.41E+02	2.29E-03	1.83E-06	73.7	76.1
19	1.32E+04	7.18E+01	19.00	1.36E+03	5.9	10.22	2.26E+01	10.00	2.26E+02	2.15E-03	1.71E-06	73.6	65.3
14	1.18E+05	2.92E+02	19.20	5.61E+03	5.9	9.95	9.55E+01	10.00	9.55E+02	9.10E-03	8.09E-07	73.7	69.9
11	1.18E+05	2.70E+02	19.60	5.29E+03	5.9	8.72	1.03E+02	10.00	1.03E+03	9.80E-03	8.72E-07	73.4	66.7
2	1.18E+05	3.12E+02	19.60	6.12E+03	5.9	8.00	1.30E+02	10.01	1.30E+03	1.24E-02	1.10E-06	73.6	65.3
8	1.18E+05	3.30E+02	19.60	6.47E+03	5.9	7.17	1.53E+02	10.01	1.53E+03	1.46E-02	1.30E-06	73.6	62.7
12	1.18E+05	2.98E+02	19.40	5.78E+03	5.9	7.15	1.37E+02	10.01	1.37E+03	1.31E-02	1.16E-06	73.9	66.4
18	1.18E+05	3.02E+02	19.40	5.86E+03	5.9	8.38	1.18E+02	10.03	1.19E+03	1.13E-02	1.00E-06	73.9	61.1
32	1.18E+05	4.68E+02	19.20	8.99E+03	5.8	9.93	1.56E+02	10.01	1.56E+03	1.49E-02	1.32E-06	74.2	62.6
27	1.15E+06	2.52E+03	19.40	4.89E+04	5.8	7.48	1.13E+03	10.01	1.13E+04	1.07E-01	9.80E-07	74.3	68.6
39	1.15E+06	2.70E+03	19.60	5.29E+04	5.8	7.40	1.23E+03	10.02	1.24E+04	1.18E-01	1.07E-06	74.6	65.8
21	1.15E+06	2.80E+03	19.60	5.49E+04	5.8	7.00	1.35E+03	11.32	1.53E+04	1.46E-01	1.18E-06	74.9	70.8
38	1.15E+06	3.56E+03	19.60	6.98E+04	5.8	7.75	1.55E+03	10.00	1.55E+04	1.48E-01	1.35E-06	74.7	66.9

Table 29. Aerosol Data Summary Sheet (Day 14)

Study No. 1078-CG920794 Rabbits 08-12-10 Viable Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (ºF)	RH (%)
13	1.32E+04	1.18E+01	18.20	2.15E+02	5.9	7.00	5.20E+00	10.97	5.70E+01	5.43E-04	3.94E-07	71.0	73.1
34	1.32E+04	1.88E+01	19.20	3.61E+02	5.9	7.00	8.74E+00	10.31	9.01E+01	8.58E-04	6.62E-07	71.4	71.9
25	1.32E+04	2.48E+01	18.80	4.66E+02	6.0	7.95	9.77E+00	10.01	9.78E+01	9.32E-04	7.40E-07	71.4	71.5
15	1.32E+04	2.10E+01	19.00	3.99E+02	6.0	7.00	9.50E+00	14.10	1.34E+02	1.28E-03	7.20E-07	71.8	74.0
30	1.32E+04	1.76E+01	18.60	3.27E+02	6.0	9.47	5.76E+00	10.01	5.77E+01	5.49E-04	4.36E-07	71.7	67.5
28	1.32E+04	1.76E+01	19.20	3.38E+02	6.0	8.17	6.89E+00	10.01	6.90E+01	6.57E-04	5.22E-07	72.6	75.4
19	1.32E+04	1.78E+01	19.00	3.38E+02	6.1	8.10	6.84E+00	10.01	6.85E+01	6.53E-04	5.19E-07	72.8	75.0
14	1.18E+05	9.22E+01	18.80	1.73E+03	6.1	8.88	3.20E+01	10.00	3.20E+02	3.05E-03	2.71E-07	72.8	75.7
11	1.18E+05	1.06E+02	19.00	2.01E+03	6.1	7.72	4.28E+01	10.00	4.28E+02	4.07E-03	3.62E-07	72.5	67.3
2	1.18E+05	1.27E+02	18.80	2.39E+03	6.1	9.47	4.13E+01	10.00	4.13E+02	3.94E-03	3.50E-07	72.8	72.7
8	1.18E+05	8.62E+01	19.40	1.67E+03	6.2	7.87	3.43E+01	10.00	3.43E+02	3.26E-03	2.90E-07	72.8	73.0
12	1.18E+05	8.52E+01	18.80	1.60E+03	6.1	8.45	3.11E+01	10.00	3.11E+02	2.96E-03	2.63E-07	72.9	72.1
18	1.18E+05	1.17E+02	18.80	2.20E+03	6.1	9.32	3.87E+01	10.01	3.87E+02	3.69E-03	3.28E-07	73.0	74.7
32	1.18E+05	1.05E+02	18.60	1.95E+03	6.1	8.17	3.92E+01	10.04	3.93E+02	3.75E-03	3.32E-07	73.1	70.5
27	1.15E+06	8.96E+02	19.00	1.70E+04	6.0	7.30	3.89E+02	10.01	3.89E+03	3.71E-02	3.38E-07	73.2	70.2
39	1.15E+06	1.12E+03	18.80	2.11E+04	5.9	8.30	4.30E+02	10.02	4.31E+03	4.10E-02	3.74E-07	73.3	71.1
21	1.15E+06	9.80E+02	18.80	1.84E+04	6.0	7.00	4.39E+02	10.86	4.76E+03	4.54E-02	3.81E-07	73.7	73.7
38	1.15E+06	1.32E+03	18.80	2.48E+04	6.1	8.12	5.01E+02	10.01	5.02E+03	4.78E-02	4.36E-07	73.7	70.3

Table 30. Aerosol Data Summary Sheet (Day 15)

Study No. 1078-CG920794 Rabbits 08-13-10 Viable Spores

Rabbit ID	Neb (CFU/mL)	Impinger [ ] (CFU/mL)	Impinger Volume (mL)	Impinger (CFU)	Avg. Impinger Sample Rate (L/min)	Sample Time (min)	Aerosol [ ] (CFU/L)	Total Accum. Tidal Volume inhaled (L)	Inhaled Dose (CFU/animal)	Ames LD50 Equivalents	Spray Factor	Temp (ºF)	RH (%)
13	1.32E+04	7.48E+01	18.00	1.35E+03	6.0	15.92	1.41E+01	10.01	1.41E+02	1.34E-03	1.07E-06	71.0	65.1
34	1.32E+04	9.52E+01	18.80	1.79E+03	6.0	14.33	2.08E+01	10.00	2.08E+02	1.98E-03	1.58E-06	71.3	62.5
25	1.32E+04	5.34E+01	19.60	1.05E+03	6.0	8.60	2.03E+01	10.01	2.03E+02	1.93E-03	1.54E-06	71.8	71.7
15	1.32E+04	4.04E+01	19.40	7.84E+02	6.0	7.00	1.87E+01	15.65	2.92E+02	2.78E-03	1.41E-06	72.2	76.2
30	1.32E+04	4.78E+01	19.60	9.37E+02	6.0	8.72	1.79E+01	10.01	1.79E+02	1.71E-03	1.36E-06	72.1	70.9
28	1.32E+04	4.56E+01	19.60	8.94E+02	6.1	7.68	1.91E+01	10.01	1.91E+02	1.82E-03	1.45E-06	72.9	71.8
19	1.32E+04	7.38E+01	19.00	1.40E+03	6.1	13.13	1.75E+01	10.00	1.75E+02	1.67E-03	1.33E-06	72.6	60.6
14	1.18E+05	1.58E+02	19.60	3.10E+03	6.1	7.62	6.66E+01	10.01	6.67E+02	6.35E-03	5.65E-07	73.2	71.7
11	1.18E+05	3.02E+02	19.60	5.92E+03	6.1	8.27	1.17E+02	10.01	1.17E+03	1.12E-02	9.94E-07	72.9	67.3
8	1.18E+05	4.02E+02	19.80	7.96E+03	6.0	8.35	1.59E+02	10.00	1.59E+03	1.51E-02	1.35E-06	73.1	69.6
12	1.18E+05	3.60E+02	19.20	6.91E+03	6.0	8.40	1.37E+02	10.00	1.37E+03	1.31E-02	1.16E-06	73.1	63.4
18	1.18E+05	3.44E+02	19.40	6.67E+03	6.0	8.67	1.28E+02	10.01	1.28E+03	1.22E-02	1.09E-06	73.1	62.8
32	1.18E+05	3.36E+02	19.80	6.65E+03	6.0	7.00	1.58E+02	10.12	1.60E+03	1.53E-02	1.34E-06	73.4	68.2
27	1.15E+06	2.09E+03	19.40	4.05E+04	5.9	8.28	8.30E+02	10.01	8.31E+03	7.91E-02	7.22E-07	73.3	68.8
39	1.15E+06	2.28E+03	19.60	4.47E+04	6.0	7.25	1.03E+03	10.28	1.06E+04	1.01E-01	8.93E-07	73.4	69.5
21	1.15E+06	3.36E+03	19.80	6.65E+04	5.9	7.13	1.58E+03	10.01	1.58E+04	1.51E-01	1.38E-06	73.5	73.2
38	1.15E+06	2.82E+03	19.40	5.47E+04	5.9	7.00	1.32E+03	10.30	1.36E+04	1.30E-01	1.15E-06	73.5	68.1

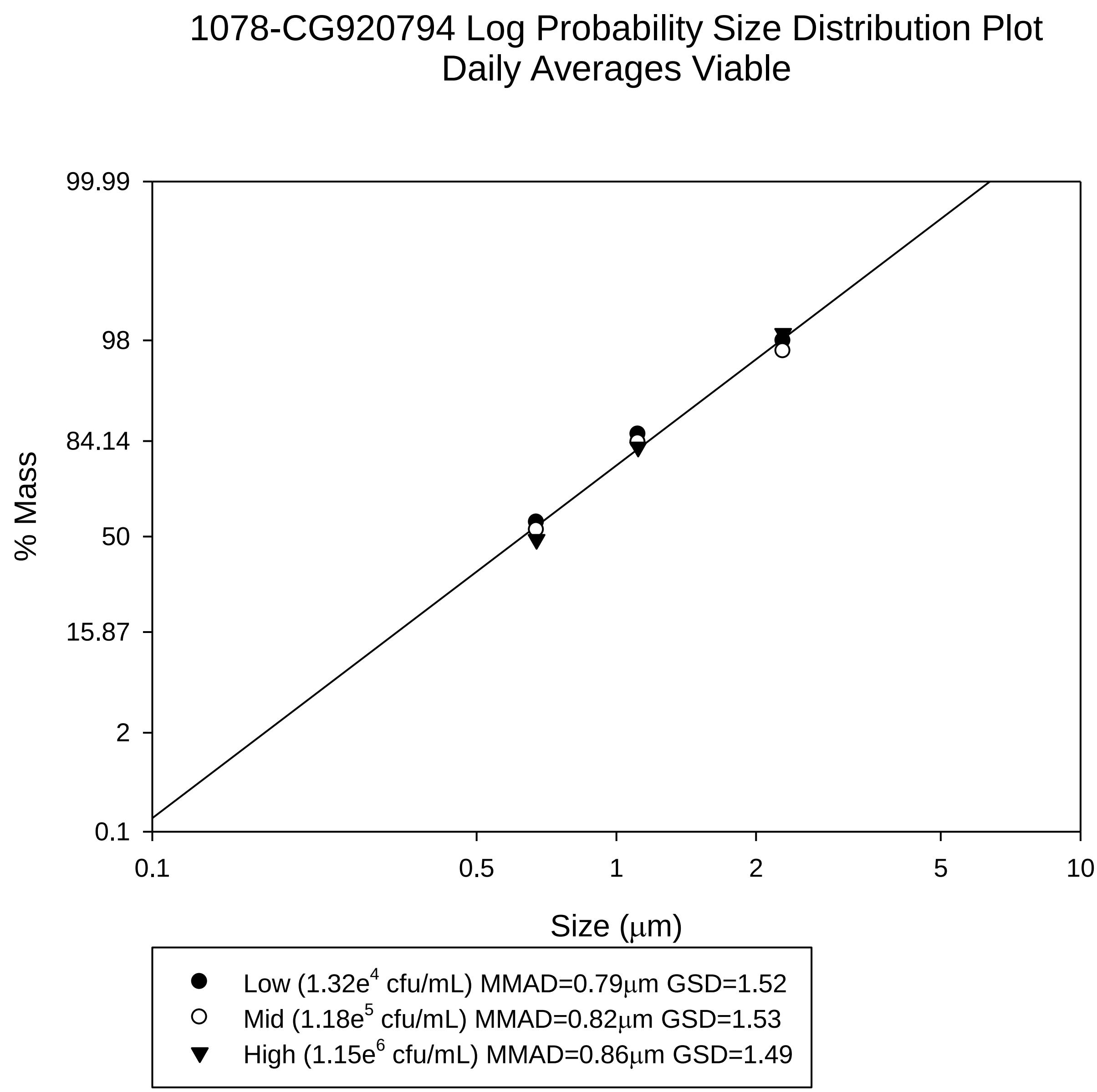

Figure 3. Log probability size distribution plot.

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APPENDIX F
STATISTICAL REPORT - TELEMETRY

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List of Tables

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List of Acronyms

ANOVA .... Analysis of variance
BBRC .... Battelle Biomedical Research Center
BPM .... Beats per minute
CFU .... Colony forming units
ECG... Electrocardiogram
N .... Number of animals
RCPM .... Respiratory cycles per minute
RP .... Respiratory period
NZW .... New Zealand White

1. Introduction

This report summarizes the statistical analysis of telemetry data collected under Battelle Biomedical Research Center (BBRC) Study No. 1078-CG920794. Twenty-six (26) pathogen-free New Zealand White (NZW) rabbits were randomly assigned to one of four groups of animals as shown in Table 1. Beginning on Study Day 0, animals were exposed to *Bacillus anthracis* (Ames strain) spores once a day for five straight working days each week for three straight weeks, at targeted doses shown in Table 1. The control group (Group 1) was exposed to gamma-irradiated spores.

Table 1. Study Design

Group	Number of Animals per Group	Target Spore Dose (CFU)	Number of Spore Challenges
1	5	10,000*	15
2	7	100	
3	7	1,000	
4	7	10,000	

CFU Colony forming units.
* These spores were inactivated by radiation.

Telemetry data were collected for activity, 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. Heart rate data was recreated from electrocardiogram (ECG) data; therefore, the collection times were rounded to the nearest 15-minute clock time. Approximately three days of baseline data were collected prior to the first challenge for each animal, while the post-challenge data were collected for surviving animals up to 39 days following the first 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.

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. The heart rate data was recreated from ECG data; therefore, the collection times were rounded to the nearest 15-minute clock time. 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-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 last 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.

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:

$$Y_{dij} = \mu + \text{Group}_i + \varepsilon_{ij}$$

where Y_{dij} is the baseline adjusted six-hour average telemetry value for the j*th* animal in Group i (i=1 to 4) at study time d, μ is an overall constant, and εij 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 tests 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 time combination are made at a joint 0.05 level of significance.

Estimates and exact binomial 95% confidence intervals for the proportion of animals that became abnormal were calculated within each group. 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.

All statistical analyses were conducted using Stata (StataCorp LP; College Station, TX; Version 11.1) and R (Version 2.9.2) software that has been performance tested by Battelle staff. All results were reported at the 0.05 level of significance.

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 8d 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 was 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 at some study times were based on smaller sample sizes due to missing data or due to animal deaths prior to the end of the study. A summary of the results from Tables 2 through 9 is discussed below for each parameter.

Activity (Table 2, Figures 1a-1d): By Study Day 8, all groups had experienced a significant decrease from baseline. This significant decrease from baseline continued intermittently in each group until Study Day 23, but was more prevalent in Group 4. All significant pairwise group comparisons involved Group 4. On Study Day 17 at 6AM-Noon and Study Day 18 at 6PM-Midnight, the mean decrease from baseline in Group 4 was significantly different from the mean change from baseline in Group 1. On Study Day 19 at Noon-6PM, on Study Day 20 at Midnight-6AM, 6AM-Noon, and 6PM-Midnight, on Study Day 21 at Midnight-6AM, on Study Day 24 at Midnight-6AM, and on Study Day Midnight-6AM, the mean decrease from baseline in Group 4 was significantly different from the mean change from baseline in Group 2. On Study Day 37 at Midnight-6AM, the mean decrease from baseline in Group 4 was significantly greater than that in Group 3.

Heart Rate (Table 3, Figures 2a-2d): By Study Day 1 at Noon-6PM, all groups had experienced significant increases from baseline. These significant increases continued intermittently for all groups until Study Day 5 at 6AM-Noon. By Study Day 6 at Noon-6PM, all groups had experienced a significant decrease from baseline. The significant decreases continued intermittently and with increasing frequency until the end of the study, with more prevalence in Groups 1 through 3 after Study Day 24. On Study Day 24 at 6AM-Noon and Noon-6PM, the mean decrease from baseline in Group 1 was significantly greater than those in Groups 2 through 4.

RP Expiratory Time (Table 4, Figures 3a-3d): Significant decreases from baseline only occurred in Groups 2 and 3 beginning on Study Day 0 at 6AM-Noon and continuing intermittently until Study Day 39 at Midnight-6AM, but with increasing frequency after Study Day 25. On Study Day 16 at Midnight-6AM, the mean decrease from baseline in Group 2 was significantly greater than that in Group 3.

RP Inspiratory Time (Table 5, Figures 4a-4d): Significant decreases from baseline occurred in Groups 1, 2, and 3 without any detectable pattern. In addition, there were significant increases from baseline at three study times for either Group 2 or 3. On Study Day 1 at Midnight-6AM, Study Day 2 at Midnight-6AM, and Study Day 5 at Midnight-6AM, the mean decrease from baseline in Group 1 was significantly different from the mean increase from baseline in Group 2. On Study Day 19 at 6AM-Noon, the mean decrease from baseline in Group 1 was significantly different from the increase from baseline in Group 3. On Study Day 1 at Midnight-6AM, Study Day 14 at Midnight-6AM, and Study Day 25 at 6AM-Noon and 6PM-Midnight, the mean decrease from baseline in Group 4 was significantly different from the mean change from baseline in Group 2. On Study Day 25 at 6AM-Noon and 6PM-Midnight, the mean increase from baseline in Group 3 was significantly different from the mean decrease from baseline in Group 4.

RP Integral (Table 6, Figures 5a-5d): In Group 3, there were significant increases from baseline starting on Study Day 5 at 6PM-Midnight and continuing intermittently through Study Day 17 at 6PM-Midnight and less frequently from Study Day 30 at Midnight-6AM through Study Day 38 at Midnight-6AM. In Group 2, there was a significant increase from baseline starting on Study Day 32 at 6AM-Noon and continuing intermittently through Study Day 39 at 6AM-Noon. In Group 4, there were significant increases from baseline on Study Day 9 at 6PM-Midnight, Study Day 10 at Midnight-6AM, and Study Day 13 at Midnight-6AM. On Study Day 5 at 6PM-Midnight and Study Day 6 at Noon-6PM and 6PM-Midnight, the mean increase from baseline in Group 3 was significantly different from the mean decrease from baseline in Group 2. On Study Day 14 at Midnight-6AM and 6PM-Midnight and Study Day 15 at Midnight-6AM, the mean increase from baseline in Group 3 was significantly different from the mean decrease from baseline in Group 1.

RP Peak Amplitude (Table 7, Figures 6a-6d): In Group 2, there was typically a significant increase from baseline beginning on Study Day 36 at 6AM-Noon and ending at Study Day 39 at 6AM-Noon. In Group 3, there was a significant increase from baseline starting on Study Day 5 at Midnight-6AM and continuing consistently through Study Day 17 at Midnight-6AM. On Study Day 5 at 6PM-Midnight, Study Day 6 at Midnight-6AM, Study Day 11 at Midnight-6AM, Study Day 11 at 6AM-Noon, Study Day 12 at 6AM-Noon, and Study Day 14 Midnight-6AM through Study Day 15 6AM-Noon, the mean increase from baseline in Group 3 was significantly different from the mean decrease from baseline in Group 1. On Study Day 5 at 6PM-Midnight, Study Day 6 at Midnight-6AM and 6AM-Noon, Study Day 14 at Midnight-6AM and Noon-6PM, and Study Day 15 at 6AM-Noon the mean increase from baseline in Group 3 was significantly different from the mean change from baseline in Group 2. On Study Day 5 at 6PM-Midnight, the mean decrease from baseline in Group 2 was significantly different from the mean increase from baseline in Group 4. On Study Day 14 at Noon-6PM, the mean increase from baseline in Group 3 was significantly greater than that in Group 4.

RP Respiratory Rate (Table 8, Figures 7a-7d): By Study Day 1 at Noon-6PM, all groups had experienced a significant increase from baseline. These significant increases from baseline continued intermittently throughout the study. In Groups 2 and 3, these significant increases were more prevalent especially after Study Day 15 through the end of the study. Group 4 was the only group that experienced significant decreases from baseline which occurred on Study Day 5 at 6AM-Noon and on Study Day 6 at 6AM-Noon. On Study Day 4 at Midnight-6AM, the mean increase from baseline in Group 1 was significantly different from the mean changes from baseline in Groups 2 and 3. Also, on Study Day 4 at Midnight-6AM, Study Day 5 at 6AM-Noon and Noon-6PM, Study Day 6 at 6AM-Noon, and Study Day 10 at Midnight-6AM, the mean decrease from baseline in Group 4 was significantly different from the mean increase from baseline in Group 1. On Study Day 2 at 6AM-Noon, the mean increase from baseline in Group 2 was significantly greater than that in Group 3. On Study Day 13 at Midnight-6AM, the mean increase from baseline in Group 4 was significantly different from the mean decrease from baseline in Group 2. On Study Day 1 at 6PM-Midnight and on Study Day 5 at 6AM-Noon, the mean increase from baseline in Group 3 was significantly different from the mean decrease from baseline in Group 4.

Temperature (Table 9, Figures 8a-8d): In Group 1, there were significant increases and decreases from baseline beginning on Study Day 1 at Noon-6PM and continuing intermittently until Study Day 9 at 6PM-Midnight. In Group 2, there were significant increases from baseline starting on Study Day 0 at 6PM-Midnight and continuing with decreasing frequency through Study Day 29 at Midnight-6AM. Also in Group 2, significant decreases from baseline were observed beginning on Study Day 30 at Noon-6PM and continuing with increasing frequency through Study Day 38 at Noon-6PM. In Group 3, there was a significant increase from baseline beginning on Study Day 1 at Noon-6PM and continuing intermittently through Study Day 38 at 6PM-Midnight. In Group 4, there was a significant increase from baseline beginning on Study Day 1 at Midnight-6AM and continuing intermittently through Study Day 20 at 6AM-Noon. On Study Day 27 at 6PM-Midnight, Study Day 33 at 6PM-Midnight, and Study Day 34 at 6PM-Midnight, the mean increase from baseline in Group 3 was significantly different from the mean decrease from baseline in Group 1. On Study Day 17 at Midnight-6AM, Study Day 19 at 6PM-Midnight, and Study Day 20 at 6AM-Noon, the mean increase from baseline in Group 4 was significantly different from the mean change from baseline in Group 1. On Study Day 36 at Noon-6PM and Study Day 37 at Noon-6PM, the mean decrease from baseline in Group 4 was significantly different from the mean increase from baseline in Group 1. On Study Day 27 at 6PM-Midnight, Study Day 29 at 6PM-Midnight, and Study Day 32 at Noon-6PM, the mean increase from baseline in Group 3 was significantly different from the mean change from baseline in Group 2. On Study Day 3 at 6AM-Noon, Study Day 10 at Midnight-6AM, Study Day 19 at 6PM-Midnight, and Study Day 20 at 6AM-Noon, the mean increase from baseline in Group 4 was significantly different from the mean change from baseline in Group 2. On Study Day 19 at 6PM-Midnight, Study Day 27 at 6PM-Midnight, and Study Day 36 at Noon-6PM, the mean change from baseline in Group 3 was significantly different from the mean change from baseline in Group 4.

Table 10 contains the proportion of animals that were abnormal at any time during the study by group for each parameter, as well as the mean duration of abnormality for the groups that had 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. The proportions of animals that became abnormal were not significantly different between the groups for any of the telemetry parameters.

Table 11 contains the results of the overall log-rank tests for each parameter comparing the times to abnormality between the groups. The times to abnormality were not significantly different between the groups for any of the telemetry parameters. Figures 17 through 24 display the Kaplan-Meier curves associated with time to abnormality for activity, heart rate, 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 or if an animal survived the length of the study then the animal's time to death would be censored at the end of study.

Table 12 contains the results of the overall log-rank tests for each parameter comparing the duration of abnormality between the groups. The durations of abnormality were not significantly different between the groups for any of the telemetry parameters. Figures 25 through 32 display the Kaplan-Meier curves associated with duration of abnormality for activity, heart rate, RP expiratory time, RP inspiratory time, RP integral, RP peak amplitude, and RP respiratory rate, and temperature, 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 the time of death or at the end of the study, then the duration of abnormality for that animal would be unobserved.

4. Conclusions

For activity, most significant shifts from baseline were decreases and all significant pairwise group comparisons involved the targeted 10,000 CFU dose group (Group 4). On Study Day 17 at 6AM-Noon, on Study Day 19 at Noon-6PM, on Study Day 20 at Midnight-6AM, 6AM-Noon, and 6PM-Midnight, on Study Day 21 at Midnight-6AM, on Study Day 24 at Midnight-6AM, and on Study Day 37 at Midnight-6AM, the mean decrease from baseline activity in the targeted 10,000 CFU dose group (Group 4) was significantly different from the mean change from baseline activity in the targeted 100 CFU dose group (Group 2). Prior to Study Day 6, most significant shifts from baseline for heart rate were increases, while most after Study Day 6 were decreases. On Study Day 24 at 6AM-Noon and Noon-6PM, the mean decrease from baseline heart rate in the control group (Group 1) was significantly greater than those in the challenged groups (Groups 2 through 4). There were no significant shifts from baseline RP expiratory time in the control group (Group 1) or the targeted 10,000 CFU dose group (Group 4). There were no significant shifts from baseline RP inspiratory time in the targeted 10,000 CFU dose group (Group 4). There were no significant shifts from baseline RP integral in the control group (Group 1) and all significant pairwise group comparisons involved the targeted 1,000 CFU dose group (Group 3). There were no significant shifts from baseline RP peak amplitude in the control group (Group 1) or the targeted 10,000 CFU dose group (Group 4) and all but one significant pairwise group comparisons involved the targeted 1,000 CFU dose group (Group 3). There were significant increases from baseline RP respiratory rate in all groups at some time during the study; however, they were more prevalent in the targeted 100 CFU and 1,000 CFU dose groups (Groups 2 and 3, repectively). There were significant increases from baseline temperature in all groups at some time during the study and all significant pairwise group comparisons involved either the targeted 1,000 CFU or 10,000 CFU dose groups (Groups 3 or 4, respectively). In terms of the proportion of animals that became abnormal, time to abnormality, and duration of abnormality, there were no significant differences between the groups for any of the telemetry parameters.

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

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value[#]
		1	2	3	4		
0	6AM - Noon	1.26	2.59	NA	NA	0.4460	
	Noon - 6PM	0.51	0.00	0.45	0.43	0.4940	
	6PM - Midnight	-0.02	-0.24	-0.31	-0.43	0.5805	
1	Midnight - 6AM	-0.13	-0.14	-0.11	0.00	0.7176	
	6AM - Noon	0.12	0.28	-0.06	-0.01	0.5958	
	Noon - 6PM	0.18	-0.20	0.09	0.08	0.6716	
	6PM - Midnight	0.06	-0.29	0.02	-0.56↓	0.0685	
2	Midnight - 6AM	-0.09	-0.14	-0.09	-0.22	0.9197	
	6AM - Noon	1.38↑	0.45	0.42	0.75↑	0.1959	
	Noon - 6PM	-0.36	-0.08	-0.08	-0.11	0.9103	
	6PM - Midnight	0.12	-0.39	-0.05	-0.54↓	0.0622	
3	Midnight - 6AM	-0.11	-0.24	-0.19	-0.18	0.9560	
	6AM - Noon	-0.24	-0.09	-0.17	-0.42↓	0.5066	
	Noon - 6PM	-0.34	-0.40	0.07	0.00	0.5777	
	6PM - Midnight	0.03	-0.34	-0.14	-0.62↓	0.2685	
4	Midnight - 6AM	-0.37	-0.11	-0.08	-0.33	0.4573	
	6AM - Noon	1.20	0.62	0.20	0.30	0.4668	
	Noon - 6PM	-0.09	-0.39	-0.22	-0.16	0.9126	
	6PM - Midnight	-0.20	-0.48	-0.20	-0.55↓	0.5694	
5	Midnight - 6AM	-0.40	-0.24	-0.14	-0.15	0.8237	
	6AM - Noon	-0.62	-0.25	-0.25	-0.51↓	0.5463	
	Noon - 6PM	0.17	-0.12	-0.44	-0.32	0.3937	
	6PM - Midnight	-0.27	-0.20	0.25	-0.13	0.4168	
6	Midnight - 6AM	-0.16	-0.08	-0.01	-0.10	0.9590	
	6AM - Noon	-0.69↓	-0.27	-0.28	-0.27	0.3443	
	Noon - 6PM	-0.06	-0.25	-0.27	-0.42	0.7470	
	6PM - Midnight	-0.35	-0.22	-0.04	-0.55↓	0.1947	
7	Midnight - 6AM	-0.33	0.11	-0.06	-0.36	0.4427	
	6AM - Noon	-0.25	0.00	-0.64↓	-0.56↓	0.1750	
	Noon - 6PM	-0.02	-0.26	-0.11	-0.03	0.9354	
	6PM - Midnight	-0.38	-0.46↓	-0.12	-0.42	0.5552	
8	Midnight - 6AM	-0.23	-0.07	-0.05	-0.04	0.9380	
	6AM - Noon	-0.09	0.00	-0.33	-0.38	0.7363	
	Noon - 6PM	-0.05	-0.29	-0.06	-0.24	0.8428	
	6PM - Midnight	-0.43	-0.50	-0.41	-0.59↓	0.9500	
9	Midnight - 6AM	-0.28	-0.21	-0.22	-0.52↓	0.6484	
	6AM - Noon	0.81	0.71	0.63	0.57	0.9675	
	Noon - 6PM	0.26	0.29	0.05	0.44	0.9087	
	6PM - Midnight	0.24	-0.40	0.10	-0.24	0.4261	
10	Midnight - 6AM	0.07	-0.09	-0.11	-0.36	0.6555	
	6AM - Noon	-0.12	0.11	-0.10	0.05	0.8385	
	Noon - 6PM	0.12	0.06	-0.22	0.11	0.8751	
	6PM - Midnight	0.00	-0.19	-0.12	-0.47	0.7284	

Table 2. (Continued)

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
11	Midnight - 6AM	-0.16	-0.04	-0.28	-0.01	0.7960	
	6AM - Noon	-0.25	0.49	0.46	0.31	0.3689	
	Noon - 6PM	-0.06	-0.26	-0.45	0.07	0.8382	
	6PM - Midnight	-0.07	-0.33	-0.08	-0.19	0.8054	
12	Midnight - 6AM	-0.32	-0.01	-0.23	-0.17	0.7057	
	6AM - Noon	-0.66↓	-0.39	-0.66↓	-0.50↓	0.6539	
	Noon - 6PM	-0.09	-0.18	-0.60	-0.78↓	0.2988	
	6PM - Midnight	-0.16	-0.20	-0.02	-0.56	0.3682	
13	Midnight - 6AM	-0.07	-0.01	-0.02	-0.30	0.7930	
	6AM - Noon	-0.60	-0.18	-0.67↓	-0.37	0.4480	
	Noon - 6PM	0.06	-0.19	-0.78	-0.92	0.2120	
	6PM - Midnight	-0.10	-0.10	-0.12	-0.71	0.3396	
14	Midnight - 6AM	-0.07	0.23	-0.26	-0.29	0.4274	
	6AM - Noon	-0.05	0.40	0.11	0.18	0.8571	
	Noon - 6PM	-0.03	-0.25	-0.69	-0.75	0.4608	
	6PM - Midnight	-0.35	-0.32	-0.32	-0.76↓	0.5255	
15	Midnight - 6AM	-0.12	0.26	-0.27	-0.19	0.5335	
	6AM - Noon	0.14	0.27	-0.26	0.08	0.5665	
	Noon - 6PM	-0.44	-0.23	-0.94↓	-0.69	0.4288	
	6PM - Midnight	-0.18	-0.15	-0.45	-0.89	0.2617	
16	Midnight - 6AM	-0.03	0.23	-0.28	-0.43	0.3379	
	6AM - Noon	-0.12	0.38	-0.08	0.57	0.3295	
	Noon - 6PM	-0.08	-0.25	-0.38	-0.91	0.4652	
	6PM - Midnight	-0.38	-0.34	-0.52↓	-1.05↓	0.1082	
17	Midnight - 6AM	-0.29	0.06	-0.27	-0.71	0.3538	
	6AM - Noon	0.43	0.20	-0.26	-1.11↓	0.0162 *	1.54 (4<1) 0.0166 1.31 (4<2) 0.0321
	Noon - 6PM	-0.28	-0.14	-0.37	-0.96↓	0.1565	
	6PM - Midnight	-0.47	-0.25	-0.49	-0.80	0.5931	
18	Midnight - 6AM	-0.32	-0.17	-0.18	-0.73	0.3686	
	6AM - Noon	0.44	0.31	-0.14	-0.03	0.7492	
	Noon - 6PM	-0.19	-0.14	-0.20	-1.01↓	0.0606	
	6PM - Midnight	-0.10	-0.51↓	-0.49	-1.26↓	0.0205 *	1.16 (4<1) 0.0123
19	Midnight - 6AM	-0.16	-0.29	-0.24	-0.82↓	0.2426	
	6AM - Noon	-0.67↓	-0.25	-0.67↓	-1.10↓	0.0784	
	Noon - 6PM	-0.28	-0.08	-0.80↓	-1.40↓	0.0396 *	1.31 (4<2) 0.0381
	6PM - Midnight	-0.28	-0.27	-0.38	-0.73	0.7050	
20	Midnight - 6AM	-0.43	-0.04	-0.29	-1.14↓	0.0201 *	1.10 (4<2) 0.0124
	6AM - Noon	-0.98↓	-0.14	-0.71↓	-1.30↓	0.0171 *	1.16 (4<2) 0.0172
	Noon - 6PM	-0.41	-0.23	-0.52	-1.36↓	0.1634	
	6PM - Midnight	-0.35	-0.24	-0.38	-1.30↓	0.0196 *	1.06 (4<2) 0.0167

Table 2. (Continued)

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value[#]
		1	2	3	4		
21	Midnight - 6AM	-0.48	-0.07	-0.33	-0.95↓	0.0483 *	0.88 (4<2) 0.0318
	6AM - Noon	-0.75↓	-0.26	-0.66↓	-0.61	0.4936	
	Noon - 6PM	-0.51	-0.26	-0.90↓	-1.45	0.0734	
	6PM - Midnight	-0.53	-0.39	-0.47	-0.98	0.3777	
22	Midnight - 6AM	-0.33	0.01	-0.38	-0.64	0.4265	
	6AM - Noon	-0.92↓	-0.44	-0.69↓	-0.77	0.5307	
	Noon - 6PM	-0.22	-0.36	-0.88↓	-1.38	0.1501	
	6PM - Midnight	-0.35	-0.62↓	-0.56↓	-1.18↓	0.1421	
23	Midnight - 6AM	-0.44	0.14	-0.39	-1.03	0.1066	
	6AM - Noon	-0.24	0.45	-0.25	-0.50	0.0764	
	Noon - 6PM	-0.52	-0.22	-0.99↓	-1.17	0.2569	
	6PM - Midnight	-0.35	-0.44	-0.13	-1.04	0.2137	
24	Midnight - 6AM	-0.51	-0.15	-0.20	-1.24	0.0389 *	1.09 (4<2) 0.0348
	6AM - Noon	-0.54	-0.20	-0.57	-1.25	0.1635	
	Noon - 6PM	-0.60	-0.20	-0.79	-1.70	0.1377	
	6PM - Midnight	-0.55	-0.16	-0.43	-0.95	0.2810	
25	Midnight - 6AM	-0.56	-0.14	-0.21	-0.97	0.2423	
	6AM - Noon	-0.52	0.27	-0.13	-0.53	0.1632	
	Noon - 6PM	-0.66	-0.15	-0.81	-1.50	0.1756	
	6PM - Midnight	-0.14	-0.33	-0.19	-1.01	0.1083	
26	Midnight - 6AM	-0.46	-0.09	-0.23	-0.74	0.5828	
	6AM - Noon	-0.94↓	-0.33	-0.78↓	-1.27	0.1259	
	Noon - 6PM	-0.07	-0.08	-0.65	-1.37	0.1449	
	6PM - Midnight	-0.26	-0.22	-0.28	-0.49	0.9716	
27	Midnight - 6AM	-0.42	0.12	-0.09	-0.67	0.3134	
	6AM - Noon	-0.79↓	-0.12	-0.69↓	-0.61	0.1906	
	Noon - 6PM	-0.48	-0.10	-0.72	-1.49	0.1929	
	6PM - Midnight	-0.44	-0.33	-0.05	-1.04	0.1025	
28	Midnight - 6AM	-0.36	0.16	-0.07	-0.92	0.1339	
	6AM - Noon	-0.49	-0.10	-0.38	-0.36	0.7820	
	Noon - 6PM	-0.46	-0.34	-0.74	-1.48	0.3427	
	6PM - Midnight	-0.38	-0.35	-0.19	-1.07	0.1999	
29	Midnight - 6AM	-0.61	0.12	-0.27	-0.73	0.0677	
	6AM - Noon	-1.07↓	-0.22	-0.80↓	-1.24	0.0681	
	Noon - 6PM	-0.39	-0.24	-0.79	-1.53	0.2153	
	6PM - Midnight	-0.44	-0.25	-0.10	-0.72	0.7038	
30	Midnight - 6AM	-0.61	0.23	-0.09	-0.42	0.3361	
	6AM - Noon	-0.28	0.51	-0.19	-0.53	0.2561	
	Noon - 6PM	-0.51	-0.28	-0.97↓	-1.52	0.1909	
	6PM - Midnight	-0.48	-0.40	-0.28	-1.11	0.2642	
31	Midnight - 6AM	-0.51	-0.08	-0.23	-0.89	0.3883	
	6AM - Noon	-0.80	-0.33	-0.74↓	-1.13	0.3839	
	Noon - 6PM	-0.44	-0.13	-0.52	-1.28	0.4368	
	6PM - Midnight	-0.63	-0.18	-0.29	-1.26	0.1548	

Table 2. (Continued)

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
32	Midnight - 6AM	-0.36	-0.02	-0.26	-0.65	0.4727	
	6AM - Noon	-0.42	0.25	-0.40	-0.08	0.4141	
	Noon - 6PM	-0.57	-0.18	-0.88	-1.29	0.3110	
	6PM - Midnight	-0.62	-0.31	-0.46	-1.26	0.0918	
33	Midnight - 6AM	-0.41	0.01	-0.27	-1.11	0.1943	
	6AM - Noon	-0.89↓	-0.19	-0.60	-1.29	0.1484	
	Noon - 6PM	-0.20	-0.13	-0.72	-1.65	0.1651	
	6PM - Midnight	-0.47	-0.13	-0.11	-0.68	0.7059	
34	Midnight - 6AM	-0.45	-0.01	-0.11	-0.71	0.4405	
	6AM - Noon	-0.74	-0.34	-0.70↓	-1.08	0.3990	
	Noon - 6PM	-0.63	-0.30	-0.87	-1.57	0.2993	
	6PM - Midnight	-0.25	-0.37	-0.03	-0.83	0.3355	
35	Midnight - 6AM	-0.43	-0.09	-0.12	-0.77	0.4592	
	6AM - Noon	-0.51	-0.19	-0.54	-0.89	0.5453	
	Noon - 6PM	-0.45	-0.43	-0.81	-1.49	0.4024	
	6PM - Midnight	-0.33	-0.10	0.12	-1.05	0.0954	
36	Midnight - 6AM	-0.35	0.05	-0.14	-0.81	0.2472	
	6AM - Noon	-0.63	-0.28	-0.89↓	-1.16	0.0963	
	Noon - 6PM	-0.27	-0.39	-1.00↓	-1.88	0.0758	
	6PM - Midnight	-0.44	-0.40	-0.21	-1.18	0.1611	
37	Midnight - 6AM	-0.47	-0.06	-0.17	-1.21	0.0197 *	1.16 (4<2) 0.0155 1.04 (4<3) 0.0358
	6AM - Noon	-0.49	-0.08	-0.15	-0.60	0.6323	
	Noon - 6PM	-0.67	-0.33	-0.93↓	-1.53	0.2759	
	6PM - Midnight	-0.40	-0.29	-0.27	-1.09	0.0855	
38	Midnight - 6AM	-0.54	-0.08	-0.18	-0.94	0.0941	
	6AM - Noon	-0.82↓	-0.39	-0.85↓	-1.09	0.2692	
	Noon - 6PM	-0.39	-0.38	-0.91↓	-1.47	0.2685	
	6PM - Midnight	-0.39	-0.37	-0.05	-0.83	0.4049	
39	Midnight - 6AM	-0.36	0.18	-0.10	-0.85	0.1937	
	6AM - Noon	-0.65	0.06	-0.53	-0.13	0.4250	

\# Cells contain all pairwise comparisons that were 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<2)" indicates that the mean baseline adjusted value in Group 2 was significantly greater than that in Group 1], and (3) the Tukey-adjusted p-value.

NA Data was not available for this group at this study time.

↑, ↓ "↑" indicates that the mean at the study time was significantly greater than that at baseline, while "↓" indicates that the mean at the study time was significantly less than that at baseline (at the 0.05 level).

* 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)

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value[#]
		1	2	3	4		
0	6AM - Noon	11.69	22.39	NA	NA	0.4463	
	Noon - 6PM	11.51	7.89	6.14	8.86	0.8343	
	6PM - Midnight	5.85	14.33↑	1.48	2.32	0.3218	
1	Midnight - 6AM	2.39	8.29	-4.38	4.58	0.4670	
	6AM - Noon	-6.54	-6.58	-11.49↓	-10.33↓	0.7360	
	Noon - 6PM	11.16↑	10.22↑	9.10↑	10.42↑	0.9822	
	6PM - Midnight	18.63↑	17.21↑	8.28	14.03↑	0.5074	
2	Midnight - 6AM	9.58	16.10↑	-3.38	5.81	0.0960	
	6AM - Noon	-13.61	-18.28↓	-35.25↓	-26.39↓	0.1988	
	Noon - 6PM	4.90	8.49	8.99	7.11	0.9534	
	6PM - Midnight	19.96↑	20.02↑	19.48↑	18.15↑	0.9939	
3	Midnight - 6AM	9.99	18.66↑	3.23	11.26	0.1912	
	6AM - Noon	-5.54	-4.42	-11.21	-8.30	0.7519	
	Noon - 6PM	9.99	10.30	5.58	8.69	0.8699	
	6PM - Midnight	16.66	11.44	5.24	11.90	0.7603	
4	Midnight - 6AM	6.91	7.84	-6.91	5.77	0.3249	
	6AM - Noon	-8.06	-12.93	-22.34↓	-15.53	0.5722	
	Noon - 6PM	20.22↑	8.97	14.76↑	6.01	0.3102	
	6PM - Midnight	24.82↑	22.48↑	19.97↑	17.64↑	0.9076	
5	Midnight - 6AM	12.07	23.90↑	1.63	15.15	0.1792	
	6AM - Noon	11.40	19.05↑	10.43↑	5.68	0.0919	
	Noon - 6PM	5.22	2.66	-6.71	-0.79	0.2043	
	6PM - Midnight	11.68	15.69	8.92	2.26	0.5647	
6	Midnight - 6AM	10.31	28.23↑	0.06	2.81	0.1022	
	6AM - Noon	-0.76	1.99	-5.15	-0.27	0.4592	
	Noon - 6PM	-19.64↓	-12.96↓	-12.58↓	-16.74↓	0.3634	
	6PM - Midnight	-10.48	0.55	-13.16	-16.03	0.4951	
7	Midnight - 6AM	-3.12	10.48	-17.33	-8.24	0.2276	
	6AM - Noon	-22.29↓	-12.04↓	-22.48↓	-20.13↓	0.3760	
	Noon - 6PM	-0.77	-3.97	-0.80	-4.02	0.9057	
	6PM - Midnight	-4.01	7.71	8.03	-6.29	0.3325	
8	Midnight - 6AM	-1.48	16.33	6.99	-2.08	0.2658	
	6AM - Noon	-16.89↓	-8.72	-12.40↓	-19.68↓	0.3688	
	Noon - 6PM	-9.11	-11.06↓	-2.91	-2.58	0.4336	
	6PM - Midnight	-10.12	-10.99	-14.53	0.98	0.5906	
9	Midnight - 6AM	-10.54	0.14	-10.96	-2.26	0.7381	
	6AM - Noon	-38.06↓	-29.37↓	-44.99↓	-35.18↓	0.7464	
	Noon - 6PM	-7.31	-15.15↓	-8.96	-19.65↓	0.3869	
	6PM - Midnight	26.41	9.36	24.15↑	15.29	0.5795	

Table 3. (Continued)

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
10	Midnight - 6AM	14.27	23.74↑	14.54	10.74	0.7014	
	6AM - Noon	-12.56	-10.18	-18.90↓	-21.64↓	0.4178	
	Noon - 6PM	-3.13	1.93	-7.00	-0.39	0.8770	
	6PM - Midnight	23.47	13.04	11.69	15.22	0.9107	
11	Midnight - 6AM	22.05	28.32↑	17.55	15.84	0.7850	
	6AM - Noon	3.17	2.79	-10.94	3.40	0.5551	
	Noon - 6PM	-2.90	-8.92	-13.67	-5.48	0.8380	
	6PM - Midnight	12.83	3.50	9.34	16.63	0.8104	
12	Midnight - 6AM	15.45	14.79	13.56	24.68↑	0.8218	
	6AM - Noon	-11.43	-16.97↓	-19.97↓	-8.41	0.5901	
	Noon - 6PM	-18.63	-29.10↓	-30.29↓	-19.58↓	0.5794	
	6PM - Midnight	-0.61	-11.00	-1.92	16.22	0.4173	
13	Midnight - 6AM	4.34	-2.44	6.82	31.68	0.2790	
	6AM - Noon	-17.47	-27.59↓	-26.62↓	-3.23	0.1084	
	Noon - 6PM	-8.14	-27.55↓	-23.13↓	-23.38↓	0.2634	
	6PM - Midnight	3.06	-18.64↓	-2.22	-6.87	0.1888	
14	Midnight - 6AM	2.73	5.16	4.72	7.59	0.9861	
	6AM - Noon	-12.14	-4.06	-16.92↓	-14.38	0.4534	
	Noon - 6PM	-2.16	-4.44	-13.41	-13.45	0.6713	
	6PM - Midnight	11.14	6.67	4.62	6.29	0.9758	
15	Midnight - 6AM	16.24	19.06	8.96	18.58	0.8736	
	6AM - Noon	-15.18	-11.12	-23.69↓	-5.71	0.5048	
	Noon - 6PM	-13.53	-13.80	-22.04↓	-24.72	0.6048	
	6PM - Midnight	5.40	4.33	-9.22	-13.94	0.3558	
16	Midnight - 6AM	7.30	21.21↑	-3.44	-3.48	0.1851	
	6AM - Noon	-12.04	-4.08	-20.57↓	-13.98	0.1472	
	Noon - 6PM	-13.85↓	-20.52↓	-14.97↓	-16.48	0.7082	
	6PM - Midnight	-11.06	-2.91	-3.06	-4.09	0.9136	
17	Midnight - 6AM	-10.22	7.62	1.90	0.35	0.5236	
	6AM - Noon	-22.09↓	-16.66↓	-19.59↓	-23.99↓	0.6790	
	Noon - 6PM	-30.09↓	-31.34↓	-24.28↓	-35.45↓	0.5061	
	6PM - Midnight	-23.35	-18.35	-5.42	-18.19	0.7521	
18	Midnight - 6AM	-18.06	-5.78	6.68	-12.64	0.7285	
	6AM - Noon	-25.10	-18.09	-18.77	-20.99	0.9660	
	Noon - 6PM	-10.54	-15.67↓	-5.44	-18.51	0.3791	
	6PM - Midnight	-4.90	-8.88	-7.48	-12.32	0.9525	
19	Midnight - 6AM	-5.85	-2.82	-9.81	-12.31	0.9081	
	6AM - Noon	-26.22↓	-23.43↓	-23.79↓	-25.80↓	0.9423	
	Noon - 6PM	-23.69↓	-27.95↓	-27.46↓	-25.47↓	0.9359	
	6PM - Midnight	-10.83	-15.52	-13.90	6.43	0.5033	

Table 3. (Continued)

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
20	Midnight - 6AM	-10.21	-1.05	-15.40	15.45	0.3356	
	6AM - Noon	-30.69↓	-29.95↓	-30.01↓	-7.15	0.1087	
	Noon - 6PM	-28.12↓	-34.41↓	-30.97↓	-19.12	0.4607	
	6PM - Midnight	-23.87	-28.06↓	-30.60↓	-18.99	0.8502	
21	Midnight - 6AM	-20.05	-13.95	-25.32	-7.55	0.7164	
	6AM - Noon	-35.13↓	-35.04↓	-35.45↓	-21.19	0.5978	
	Noon - 6PM	-37.29↓	-36.16↓	-40.18↓	-49.97↓	0.4116	
	6PM - Midnight	-34.68↓	-28.78↓	-32.85↓	-38.45	0.9456	
22	Midnight - 6AM	-29.88↓	-14.00	-32.12↓	-35.69	0.4073	
	6AM - Noon	-32.78↓	-27.74↓	-35.61↓	-45.81↓	0.2593	
	Noon - 6PM	-29.94↓	-28.29↓	-30.06↓	-53.35↓	0.2238	
	6PM - Midnight	-20.06	-25.42↓	-34.20↓	-47.06	0.4128	
23	Midnight - 6AM	-23.92	-13.34	-29.46↓	-36.81	0.4478	
	6AM - Noon	-28.66↓	-25.47↓	-30.01↓	-36.12↓	0.5818	
	Noon - 6PM	-16.24	-25.15↓	-28.91↓	-49.35	0.2923	
	6PM - Midnight	11.09	2.39	5.80	-12.61	0.6263	
24	Midnight - 6AM	-5.25	8.92	3.86	-14.31	0.3754	
	6AM - Noon	-60.19↓	-25.11↓	-25.30↓	-28.92	0.0020 *	-35.07 (1<2) 0.0027 -34.89 (1<3) 0.0039 -31.26 (1<4) 0.0340
	Noon - 6PM	-95.46↓	-36.71↓	-36.64↓	-46.36↓	<0.0001 *	-58.75 (1<2) <0.0001 -58.82 (1<3) <0.0001 -49.11 (1<4) 0.0024
	6PM - Midnight	-67.06↓	-36.25↓	-34.23↓	-36.98	0.1475	
25	Midnight - 6AM	-49.08↓	-23.05↓	-30.97↓	-25.07	0.2511	
	6AM - Noon	-35.25↓	-28.85↓	-33.63↓	-25.81	0.8643	
	Noon - 6PM	-12.51	-11.23	-13.85	-32.99	0.4163	
	6PM - Midnight	2.28	1.27	-4.76	-3.22	0.9571	
26	Midnight - 6AM	-1.50	5.16	-7.54	-1.14	0.7488	
	6AM - Noon	-16.97	-20.97↓	-25.11↓	-14.07	0.7008	
	Noon - 6PM	-21.42↓	-24.05↓	-26.15↓	-22.50	0.9710	
	6PM - Midnight	-16.05	-18.74↓	-14.01	-17.86	0.9752	
27	Midnight - 6AM	-21.99	-10.16	-18.59	-19.11	0.8226	
	6AM - Noon	-15.41	-19.22↓	-21.44↓	-16.22	0.8905	
	Noon - 6PM	-24.75↓	-18.21↓	-24.45↓	-22.12	0.8785	
	6PM - Midnight	-24.76	-13.92	-5.82	-17.74	0.4884	
28	Midnight - 6AM	-25.36	-2.10	-14.17	-21.49	0.3753	
	6AM - Noon	-31.10↓	-29.53↓	-30.48↓	-28.58	0.9980	
	Noon - 6PM	-29.05↓	-28.00↓	-35.70↓	-42.92↓	0.5428	
	6PM - Midnight	-27.08↓	-13.46	-7.12	-32.26	0.2266	

Table 3. (Continued)

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
29	Midnight - 6AM	-27.86↓	-6.16	-12.62	-14.22	0.3165	
	6AM - Noon	-33.71↓	-30.78↓	-34.46↓	-28.61	0.9067	
	Noon - 6PM	-21.32↓	-27.57↓	-31.48↓	-32.22	0.7266	
	6PM - Midnight	-8.87	-8.97	-2.56	-20.62	0.7451	
30	Midnight - 6AM	-16.80	-2.67	-9.60	-12.07	0.6872	
	6AM - Noon	-28.57↓	-23.03↓	-27.85↓	-27.89	0.9268	
	Noon - 6PM	-20.98	-13.88	-22.08↓	-33.57	0.4893	
	6PM - Midnight	-32.50↓	-15.81	-19.51↓	-30.42	0.3678	
31	Midnight - 6AM	-32.82↓	-15.48	-26.81↓	-33.09	0.4163	
	6AM - Noon	-34.99↓	-35.60↓	-37.38↓	-32.09↓	0.9453	
	Noon - 6PM	-45.94↓	-38.63↓	-37.87↓	-35.75↓	0.6937	
	6PM - Midnight	-42.50↓	-31.72↓	-30.75↓	-40.17	0.7865	
32	Midnight - 6AM	-37.28↓	-21.19↓	-28.70↓	-34.94	0.6436	
	6AM - Noon	-37.11↓	-30.78↓	-33.94↓	-35.72↓	0.8689	
	Noon - 6PM	-23.35↓	-14.92	-14.41	-18.08	0.7999	
	6PM - Midnight	-21.63	-13.19	-11.94	-27.16	0.6045	
33	Midnight - 6AM	-27.40	-9.99	-20.74	-31.57	0.5081	
	6AM - Noon	-33.45↓	-34.86↓	-33.23↓	-46.36↓	0.4498	
	Noon - 6PM	-33.71↓	-32.50↓	-31.08↓	-43.76↓	0.6880	
	6PM - Midnight	-19.31	-12.72	-4.86	-25.63	0.6669	
34	Midnight - 6AM	-24.45	-1.72	-12.97	-27.69	0.4180	
	6AM - Noon	-34.98↓	-35.40↓	-29.51↓	-40.05↓	0.7996	
	Noon - 6PM	-28.52↓	-25.43↓	-27.43↓	-33.16	0.9470	
	6PM - Midnight	-22.11	-10.03	-3.68	-22.43	0.5495	
35	Midnight - 6AM	-25.75	-0.88	-10.41	-28.31	0.2195	
	6AM - Noon	-39.97↓	-34.58↓	-38.16↓	-41.70↓	0.8987	
	Noon - 6PM	-28.99↓	-30.81↓	-31.94↓	-41.38	0.8217	
	6PM - Midnight	-11.88	-5.26	-5.39	-21.84	0.7304	
36	Midnight - 6AM	-18.54	1.72	-5.84	-30.78	0.2296	
	6AM - Noon	-46.67↓	-37.43↓	-42.91↓	-40.77↓	0.7754	
	Noon - 6PM	-33.01↓	-35.97↓	-36.76↓	-44.83	0.8611	
	6PM - Midnight	-22.73	-9.05	-10.71	-21.21	0.7220	
37	Midnight - 6AM	-25.12	0.23	-11.43	-25.18	0.2560	
	6AM - Noon	-38.12↓	-32.48↓	-33.24↓	-29.82	0.9354	
	Noon - 6PM	-19.01	-19.64↓	-9.13	-22.23	0.7427	
	6PM - Midnight	-19.70	-6.47	-7.78	-13.76	0.7258	

Table 3. (Continued)

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
38	Midnight - 6AM	-31.01↓	-5.40	-15.20	-31.23	0.1913	
	6AM - Noon	-41.96↓	-43.37↓	-43.74↓	-36.13	0.9056	
	Noon - 6PM	-36.56↓	-41.05↓	-39.42↓	-42.42	0.9782	
	6PM - Midnight	-24.70	-12.93	-17.92	-29.89	0.7239	
39	Midnight - 6AM	-27.32	-0.45	-16.32	-36.07	0.1444	
	6AM - Noon	-60.17↓	-48.13↓	-62.30↓	-69.60↓	0.4570	

Cells contain all pairwise comparisons that were 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<2)" indicates that the mean baseline adjusted value in Group 2 was significantly greater than that in Group 1], and (3) the Tukey-adjusted p-value.

NA Data was not available for this group at this study time.

↑, ↓ "↑" indicates that the mean at the study time was significantly greater than that at baseline, while "↓" indicates that the mean at the study time was significantly less than that at baseline (at the 0.05 level).

* Group effect was significant at the 0.05 level.

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

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
0	6AM - Noon	-0.07	-0.23↓	NA	NA	0.2303	
	Noon - 6PM	-0.05	-0.06↓	-0.09↓	-0.03	0.4301	
	6PM - Midnight	0.04	-0.02	-0.02	0.02	0.1971	
1	Midnight - 6AM	0.00	-0.01	-0.01	0.01	0.8226	
	6AM - Noon	0.00	-0.03	0.00	0.02	0.5398	
	Noon - 6PM	-0.04	-0.05↓	-0.03	-0.03	0.8870	
	6PM - Midnight	-0.02	-0.01	-0.05	0.05	0.1079	
2	Midnight - 6AM	0.02	0.00	0.02	0.04	0.7157	
	6AM - Noon	0.06	-0.05	0.06	-0.01	0.0367 *	
	Noon - 6PM	0.04	-0.03	0.00	-0.01	0.5082	
	6PM - Midnight	-0.04	-0.03	-0.04	0.00	0.7861	
3	Midnight - 6AM	-0.04	0.00	0.00	0.04	0.5450	
	6AM - Noon	0.00	0.00	0.02	0.04	0.8434	
	Noon - 6PM	-0.01	-0.07	-0.04	-0.02	0.6614	
	6PM - Midnight	-0.06	0.00	-0.02	-0.01	0.5643	
4	Midnight - 6AM	-0.04	0.02	0.03	0.06	0.1275	
	6AM - Noon	0.04	-0.03	0.05	-0.02	0.2461	
	Noon - 6PM	-0.02	-0.04	-0.01	0.01	0.8102	
	6PM - Midnight	-0.05	-0.01	-0.04	-0.03	0.9354	
5	Midnight - 6AM	-0.02	-0.01	0.00	-0.01	0.9831	
	6AM - Noon	0.00	-0.02	-0.01	0.03	0.7613	
	Noon - 6PM	-0.04	-0.06	-0.03	0.01	0.4432	
	6PM - Midnight	-0.09	-0.02	-0.07	0.00	0.3260	
6	Midnight - 6AM	-0.06	-0.03	0.01	0.02	0.5559	
	6AM - Noon	0.02	0.03	0.03	0.05	0.9243	
	Noon - 6PM	0.02	-0.02	-0.04	0.01	0.4250	
	6PM - Midnight	0.00	-0.03	-0.02	-0.01	0.9640	
7	Midnight - 6AM	-0.02	-0.02	0.04	-0.01	0.5966	
	6AM - Noon	0.04	0.00	0.03	0.01	0.8399	
	Noon - 6PM	-0.02	-0.03	-0.07	-0.03	0.6721	
	6PM - Midnight	-0.02	-0.03	-0.07	-0.02	0.6873	
8	Midnight - 6AM	-0.01	-0.02	-0.03	-0.05	0.7643	
	6AM - Noon	0.02	0.00	0.01	0.00	0.9881	
	Noon - 6PM	-0.03	-0.02	-0.05	-0.01	0.8660	
	6PM - Midnight	-0.02	0.01	0.00	0.01	0.9823	
9	Midnight - 6AM	-0.02	0.01	-0.01	-0.04	0.6680	
	6AM - Noon	0.09	-0.05	0.03	-0.02	0.0953	
	Noon - 6PM	0.01	-0.07	-0.04	-0.04	0.6906	
	6PM - Midnight	-0.10	-0.03	-0.12↓	-0.07	0.3885	
10	Midnight - 6AM	-0.09	-0.01	-0.04	-0.01	0.4767	
	6AM - Noon	-0.04	0.03	-0.02	-0.02	0.5682	
	Noon - 6PM	-0.11	-0.02	-0.06	-0.09	0.6847	
	6PM - Midnight	-0.10	0.01	-0.11	-0.08	0.5270	

Table 4. (Continued)

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
11	Midnight - 6AM	-0.04	0.00	-0.04	-0.08	0.7490	
	6AM - Noon	0.06	0.00	0.02	-0.06	0.4971	
	Noon - 6PM	0.03	0.02	-0.01	-0.01	0.9792	
	6PM - Midnight	-0.07	0.07	-0.09	-0.09	0.2976	
12	Midnight - 6AM	-0.07	0.08	-0.05	-0.11	0.3435	
	6AM - Noon	0.01	0.12	0.02	0.00	0.4570	
	Noon - 6PM	-0.04	0.08	-0.03	-0.04	0.6093	
	6PM - Midnight	-0.11	0.04	-0.09	-0.16	0.2805	
13	Midnight - 6AM	-0.11	0.07	-0.02	-0.21	0.2110	
	6AM - Noon	-0.01	0.14	0.06	-0.02	0.6623	
	Noon - 6PM	-0.06	0.03	-0.02	-0.07	0.8478	
	6PM - Midnight	-0.11	0.06	-0.07	-0.12	0.4752	
14	Midnight - 6AM	-0.12	-0.06	-0.04	-0.14	0.3849	
	6AM - Noon	0.00	-0.06	-0.06	-0.03	0.5038	
	Noon - 6PM	-0.04	-0.06	-0.06	-0.03	0.9432	
	6PM - Midnight	-0.10	-0.08	-0.06	-0.08	0.9283	
15	Midnight - 6AM	-0.08	-0.11↓	-0.05	-0.03	0.5403	
	6AM - Noon	-0.03	-0.06↓	-0.04	-0.01	0.5476	
	Noon - 6PM	-0.04	-0.06	-0.03	0.02	0.5431	
	6PM - Midnight	-0.12	-0.08	-0.04	-0.04	0.5476	
16	Midnight - 6AM	-0.09	-0.13↓	0.00	-0.03	0.0366 *	-0.13 (2<3) 0.0313
	6AM - Noon	0.05	-0.05	-0.04	-0.10	0.2376	
	Noon - 6PM	-0.01	-0.01	0.03	-0.04	0.8787	
	6PM - Midnight	-0.03	-0.07	-0.02	-0.01	0.6204	
17	Midnight - 6AM	-0.03	-0.03	-0.06	-0.01	0.9223	
	6AM - Noon	0.00	-0.03	-0.04	-0.01	0.8942	
	Noon - 6PM	-0.05	-0.06	-0.06	0.02	0.6032	
	6PM - Midnight	-0.09	-0.05	-0.07	-0.03	0.8045	
18	Midnight - 6AM	-0.12	-0.03	-0.11↓	0.00	0.2417	
	6AM - Noon	0.03	-0.01	-0.05	-0.06	0.6218	
	Noon - 6PM	-0.03	-0.03	-0.05	-0.02	0.9732	
	6PM - Midnight	-0.06	-0.05	-0.04	-0.01	0.9266	
19	Midnight - 6AM	-0.05	-0.02	-0.07	0.00	0.6676	
	6AM - Noon	0.02	0.00	-0.01	0.02	0.9212	
	Noon - 6PM	-0.03	-0.08	-0.09	0.03	0.3969	
	6PM - Midnight	-0.08	-0.05	-0.07	-0.07	0.9724	
20	Midnight - 6AM	-0.06	-0.08	-0.03	-0.07	0.9248	
	6AM - Noon	0.05	-0.02	0.01	0.02	0.7217	
	Noon - 6PM	0.01	-0.06	-0.06	-0.02	0.6195	
	6PM - Midnight	-0.05	-0.05	-0.07	-0.07	0.9788	
21	Midnight - 6AM	-0.03	-0.03	-0.05	-0.08	0.8881	
	6AM - Noon	0.01	0.01	-0.04	-0.05	0.7225	
	Noon - 6PM	-0.05	-0.05	-0.08	-0.04	0.9254	
	6PM - Midnight	-0.06	-0.05	-0.03	-0.09	0.8662	

Table 4. (Continued)

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
22	Midnight - 6AM	-0.07	-0.05	-0.01	-0.09	0.7908	
	6AM - Noon	-0.04	-0.02	-0.02	-0.08	0.6902	
	Noon - 6PM	-0.03	-0.06	-0.06	-0.06	0.9532	
	6PM - Midnight	-0.06	-0.03	-0.09	-0.06	0.8090	
23	Midnight - 6AM	-0.05	-0.05	-0.01	-0.06	0.9382	
	6AM - Noon	-0.02	-0.08↓	-0.06	-0.14	0.3185	
	Noon - 6PM	0.02	0.00	-0.02	-0.02	0.9679	
	6PM - Midnight	-0.10	-0.05	-0.11	-0.09	0.8372	
24	Midnight - 6AM	-0.11	-0.05	-0.05	-0.04	0.7805	
	6AM - Noon	-0.02	-0.02	-0.02	-0.02	0.9994	
	Noon - 6PM	0.08	-0.07	-0.05	-0.01	0.1963	
	6PM - Midnight	-0.03	-0.05	-0.01	-0.03	0.9221	
25	Midnight - 6AM	0.02	-0.07	0.00	-0.01	0.7197	
	6AM - Noon	-0.01	-0.04	-0.06	-0.08	0.7461	
	Noon - 6PM	0.00	-0.04	-0.06	0.07	0.4628	
	6PM - Midnight	-0.09	-0.09	-0.09	-0.07	0.9931	
26	Midnight - 6AM	-0.08	-0.07	-0.05	0.00	0.7799	
	6AM - Noon	-0.01	-0.04	-0.01	0.01	0.8455	
	Noon - 6PM	-0.04	-0.13↓	-0.12↓	-0.02	0.2646	
	6PM - Midnight	-0.07	-0.06	-0.11	-0.03	0.7308	
27	Midnight - 6AM	-0.07	-0.05	-0.03	-0.01	0.8745	
	6AM - Noon	-0.03	-0.03	-0.07↓	-0.01	0.5612	
	Noon - 6PM	-0.06	-0.12↓	-0.09	-0.03	0.6285	
	6PM - Midnight	-0.07	-0.07	-0.15↓	-0.06	0.5994	
28	Midnight - 6AM	-0.06	-0.07	-0.11	-0.03	0.7659	
	6AM - Noon	-0.02	-0.03	-0.03	0.02	0.8981	
	Noon - 6PM	-0.01	-0.08	-0.08	-0.01	0.6086	
	6PM - Midnight	-0.06	-0.08	-0.15↓	-0.03	0.4187	
29	Midnight - 6AM	0.00	-0.08	-0.06	-0.05	0.8161	
	6AM - Noon	0.05	-0.03	-0.02	0.05	0.3640	
	Noon - 6PM	-0.05	-0.08↓	-0.07	0.02	0.3569	
	6PM - Midnight	-0.08	-0.09	-0.16↓	-0.03	0.5360	
30	Midnight - 6AM	-0.02	-0.11	-0.11	-0.04	0.6069	
	6AM - Noon	0.03	-0.07	-0.10↓	-0.06	0.2239	
	Noon - 6PM	-0.03	-0.06	-0.06	0.05	0.2726	
	6PM - Midnight	0.01	-0.06	-0.10	-0.02	0.5025	
31	Midnight - 6AM	0.03	-0.05	-0.03	0.03	0.4989	
	6AM - Noon	0.02	-0.02	-0.01	0.04	0.7265	
	Noon - 6PM	-0.03	-0.11↓	-0.11↓	-0.01	0.2634	
	6PM - Midnight	0.00	-0.12↓	-0.10	0.00	0.3400	

Table 4. (Continued)

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
32	Midnight - 6AM	0.00	-0.07	-0.05	0.02	0.6238	
	6AM - Noon	0.04	-0.05	-0.05	-0.02	0.3253	
	Noon - 6PM	0.03	-0.07	-0.05	0.01	0.4048	
	6PM - Midnight	-0.03	-0.08	-0.11	0.08	0.1941	
33	Midnight - 6AM	-0.03	-0.09	-0.07	0.07	0.2705	
	6AM - Noon	0.00	-0.05	-0.06	0.08	0.1092	
	Noon - 6PM	-0.09	-0.12↓	-0.12↓	-0.01	0.5301	
	6PM - Midnight	-0.12	-0.11	-0.17↓	-0.06	0.6770	
34	Midnight - 6AM	-0.02	-0.08	-0.09	-0.04	0.8377	
	6AM - Noon	-0.02	-0.02	-0.04	-0.01	0.9443	
	Noon - 6PM	-0.07	-0.13↓	-0.14↓	0.01	0.1304	
	6PM - Midnight	-0.11	-0.13↓	-0.18↓	-0.10	0.8093	
35	Midnight - 6AM	-0.05	-0.08	-0.10	-0.04	0.8315	
	6AM - Noon	-0.02	-0.03	-0.07	0.02	0.3695	
	Noon - 6PM	-0.09	-0.10↓	-0.12↓	-0.02	0.5036	
	6PM - Midnight	-0.14	-0.13↓	-0.18↓	-0.07	0.7201	
36	Midnight - 6AM	-0.05	-0.12↓	-0.09	-0.09	0.8136	
	6AM - Noon	0.03	-0.04	-0.02	-0.07	0.6481	
	Noon - 6PM	-0.08	-0.08	-0.10	-0.10	0.9879	
	6PM - Midnight	-0.10	-0.13↓	-0.18↓	-0.19	0.6769	
37	Midnight - 6AM	-0.08	-0.09↓	-0.07	-0.08	0.9764	
	6AM - Noon	0.00	-0.05	-0.04	-0.09	0.6534	
	Noon - 6PM	-0.04	-0.06	-0.07	0.02	0.7602	
	6PM - Midnight	-0.09	-0.10	-0.12	-0.10	0.9773	
38	Midnight - 6AM	-0.06	-0.09↓	-0.05	-0.04	0.8254	
	6AM - Noon	0.01	0.00	-0.02	-0.01	0.9400	
	Noon - 6PM	-0.08	-0.08↓	-0.12↓	-0.02	0.4239	
	6PM - Midnight	-0.10	-0.14↓	-0.16↓	-0.14	0.8677	
39	Midnight - 6AM	-0.05	-0.11↓	-0.09	-0.11	0.7425	
	6AM - Noon	0.06	-0.07	-0.06	-0.12	0.0754	

\# Cells contain all pairwise comparisons that were 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<2)" indicates that the mean baseline adjusted value in Group 2 was significantly greater than that in Group 1], and (3) the Tukey-adjusted p-value.

NA Data was not available for this group at this study time.

↑, ↓ "↑" indicates that the mean at the study time was significantly greater than that at baseline, while "↓" indicates that the mean at the study time was significantly less than that at baseline (at the 0.05 level).

* Group effect was significant at the 0.05 level.

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

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
0	6AM - Noon	-0.14	-0.09	NA	NA	0.4084	
	Noon - 6PM	-0.03	-0.02	-0.02	-0.01	0.9169	
	6PM - Midnight	-0.04	0.01	0.02	-0.02	0.1500	
1	Midnight - 6AM	-0.04	0.03↑	0.00	-0.03	0.0031 *	-0.07 (1<2) 0.0068 0.06 (4<2) 0.0085
	6AM - Noon	-0.09↓	-0.02	-0.01	-0.02	0.0630	
	Noon - 6PM	-0.03	-0.04↓	-0.06↓	-0.03	0.2686	
	6PM - Midnight	-0.07	-0.01	-0.01	-0.02	0.4263	
2	Midnight - 6AM	-0.08	0.02	-0.02	0.00	0.0312 *	-0.10 (1<2) 0.0224
	6AM - Noon	-0.13↓	-0.04	-0.04	-0.05	0.1400	
	Noon - 6PM	-0.04	-0.01	-0.04	-0.01	0.5472	
	6PM - Midnight	-0.08	-0.02	0.01	-0.03	0.1229	
3	Midnight - 6AM	-0.04	0.02	0.02	-0.01	0.2823	
	6AM - Noon	-0.09↓	-0.02	-0.01	-0.01	0.0786	
	Noon - 6PM	-0.07	-0.02	-0.04	-0.04	0.6780	
	6PM - Midnight	-0.02	-0.03	-0.03	-0.03	0.9902	
4	Midnight - 6AM	-0.07	0.01	-0.02	-0.02	0.2826	
	6AM - Noon	-0.09	-0.04	-0.05	-0.04	0.6357	
	Noon - 6PM	-0.07	-0.04	-0.06↓	-0.03	0.7331	
	6PM - Midnight	-0.08	-0.02	-0.02	-0.02	0.3752	
5	Midnight - 6AM	-0.08	0.02	-0.02	0.01	0.0341 *	-0.10 (1<2) 0.0310
	6AM - Noon	-0.07	0.01	-0.01	0.05	0.1248	
	Noon - 6PM	-0.09	-0.03	-0.04	-0.01	0.3963	
	6PM - Midnight	-0.09	-0.04	-0.01	-0.01	0.3215	
6	Midnight - 6AM	-0.06	0.00	-0.02	-0.02	0.5143	
	6AM - Noon	-0.06	0.00	0.00	0.04	0.3257	
	Noon - 6PM	-0.06	-0.02	-0.03	0.01	0.4677	
	6PM - Midnight	-0.08	-0.03	0.00	-0.04	0.4628	
7	Midnight - 6AM	-0.05	0.01	-0.01	0.00	0.7323	
	6AM - Noon	-0.08	-0.01	0.00	0.00	0.3132	
	Noon - 6PM	-0.08	-0.03	-0.01	-0.03	0.5928	
	6PM - Midnight	-0.09	-0.03	-0.02	-0.04	0.5639	
8	Midnight - 6AM	-0.07	0.02	-0.01	-0.02	0.3222	
	6AM - Noon	-0.06	-0.02	0.00	0.01	0.5108	
	Noon - 6PM	-0.04	-0.03	-0.03	-0.01	0.8671	
	6PM - Midnight	-0.09	-0.03	0.03	0.02	0.1288	
9	Midnight - 6AM	-0.10	0.00	0.04	0.05	0.1181	
	6AM - Noon	-0.08	-0.04	-0.02	0.01	0.1682	
	Noon - 6PM	-0.03	-0.01	-0.01	0.00	0.9030	
	6PM - Midnight	-0.11	-0.01	-0.01	-0.02	0.2840	
10	Midnight - 6AM	-0.05	0.05	0.03	0.07	0.2746	
	6AM - Noon	-0.09	0.00	0.01	0.02	0.1313	
	Noon - 6PM	-0.05	-0.01	-0.02	0.01	0.8389	
	6PM - Midnight	-0.14	-0.04	-0.05	0.01	0.2063	

Table 5. (Continued)

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
11	Midnight - 6AM	-0.12	0.05	-0.02	0.03	0.0737	
	6AM - Noon	-0.10	0.00	-0.07	-0.07	0.3943	
	Noon - 6PM	0.03	0.02	-0.03	0.03	0.8508	
	6PM - Midnight	-0.09	-0.01	-0.01	-0.03	0.7892	
12	Midnight - 6AM	-0.10	0.08	-0.01	0.03	0.0882	
	6AM - Noon	-0.05	0.08	0.02	0.01	0.2401	
	Noon - 6PM	-0.02	0.05	-0.03	0.04	0.5779	
	6PM - Midnight	-0.06	0.05	-0.04	-0.11	0.2586	
13	Midnight - 6AM	-0.03	0.10	-0.03	-0.07	0.0679	
	6AM - Noon	0.00	0.10↑	0.00	0.02	0.2204	
	Noon - 6PM	-0.03	0.03	-0.04	0.01	0.6897	
	6PM - Midnight	-0.08	0.05	-0.03	-0.03	0.3016	
14	Midnight - 6AM	-0.03	0.03	-0.03	-0.09	0.0385 *	0.12 (4<2) 0.0260
	6AM - Noon	-0.09	0.00	0.00	-0.07	0.2292	
	Noon - 6PM	-0.06	0.00	-0.04	-0.08	0.4134	
	6PM - Midnight	-0.10	-0.02	-0.02	-0.13	0.1307	
15	Midnight - 6AM	-0.07	0.02	-0.02	-0.07	0.1584	
	6AM - Noon	-0.07	0.01	0.00	-0.02	0.0727	
	Noon - 6PM	-0.07	-0.01	-0.01	-0.04	0.7181	
	6PM - Midnight	-0.13	-0.05	-0.03	-0.09	0.3214	
16	Midnight - 6AM	-0.06	0.01	-0.05	-0.05	0.3952	
	6AM - Noon	-0.10	-0.01	-0.03	-0.08	0.2175	
	Noon - 6PM	-0.05	-0.03	-0.06↓	-0.04	0.8022	
	6PM - Midnight	-0.11↓	-0.04	-0.02	-0.07	0.2491	
17	Midnight - 6AM	-0.08	0.02	-0.01	-0.08	0.2390	
	6AM - Noon	-0.08	-0.01	0.00	-0.03	0.1904	
	Noon - 6PM	-0.06	-0.02	-0.07	-0.05	0.7530	
	6PM - Midnight	-0.03	-0.02	-0.03	-0.07	0.8462	
18	Midnight - 6AM	-0.01	0.01	-0.05	-0.03	0.7084	
	6AM - Noon	-0.08	-0.02	-0.01	-0.06	0.3543	
	Noon - 6PM	-0.05	-0.01	-0.03	-0.06	0.5249	
	6PM - Midnight	-0.10	-0.03	-0.02	-0.10	0.1938	
19	Midnight - 6AM	-0.04	0.05	-0.01	-0.06	0.0964	
	6AM - Noon	-0.06	0.03	0.04	-0.02	0.0249 *	-0.10 (1<3) 0.0299
	Noon - 6PM	-0.08	-0.03	0.02	-0.05	0.2050	
	6PM - Midnight	-0.11	-0.03	0.00	-0.12	0.0932	
20	Midnight - 6AM	-0.10	0.04	-0.02	-0.07	0.0535	
	6AM - Noon	-0.07	0.02	0.07	-0.06	0.0378 *	
	Noon - 6PM	-0.09	-0.01	-0.01	-0.10	0.1857	
	6PM - Midnight	-0.07	0.00	-0.01	-0.11	0.3121	
21	Midnight - 6AM	-0.05	0.03	0.01	-0.08	0.3505	
	6AM - Noon	-0.06	0.03	0.04	-0.05	0.2119	
	Noon - 6PM	-0.03	-0.01	-0.03	-0.05	0.9162	
	6PM - Midnight	-0.01	-0.02	0.01	-0.10	0.4589	

Table 5. (Continued)

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
22	Midnight - 6AM	-0.01	0.04	0.01	-0.07	0.4444	
	6AM - Noon	-0.01	0.01	0.04	-0.05	0.5177	
	Noon - 6PM	-0.04	-0.01	0.02	-0.09	0.4248	
	6PM - Midnight	-0.04	0.01	0.02	-0.12	0.2119	
23	Midnight - 6AM	-0.04	0.03	0.02	-0.10	0.1516	
	6AM - Noon	-0.04	-0.03	0.03	-0.11	0.1277	
	Noon - 6PM	-0.06	-0.04	0.01	-0.07	0.4634	
	6PM - Midnight	-0.06	-0.04	0.01	-0.17	0.0985	
24	Midnight - 6AM	-0.02	0.05	0.00	-0.10	0.1742	
	6AM - Noon	-0.02	0.00	0.08↑	-0.06	0.0515	
	Noon - 6PM	0.00	-0.02	0.00	-0.05	0.8675	
	6PM - Midnight	-0.01	0.01	0.02	-0.11	0.2122	
25	Midnight - 6AM	-0.01	0.09↑	0.04	-0.06	0.1041	
	6AM - Noon	0.00	0.02	0.03	-0.11	0.0095 *	0.14 (4<3) 0.0085 0.13 (4<2) 0.0117
	Noon - 6PM	0.04	-0.01	0.00	-0.05	0.5853	
	6PM - Midnight	-0.04	-0.02	0.01	-0.14	0.0237 *	0.15 (4<3) 0.0158 0.12 (4<2) 0.0471
26	Midnight - 6AM	0.02	0.03	-0.01	-0.05	0.2159	
	6AM - Noon	-0.02	0.03	0.04	-0.05	0.2756	
	Noon - 6PM	-0.09	-0.06	-0.04	-0.10	0.6564	
	6PM - Midnight	-0.08	-0.02	0.00	-0.13	0.1726	
27	Midnight - 6AM	-0.03	0.02	0.00	-0.07	0.2388	
	6AM - Noon	-0.06	-0.01	0.03	-0.05	0.1525	
	Noon - 6PM	-0.10	-0.07↓	-0.04	-0.06	0.6111	
	6PM - Midnight	-0.06	-0.04	-0.05	-0.14	0.4807	
28	Midnight - 6AM	-0.05	0.01	-0.02	-0.06	0.5183	
	6AM - Noon	-0.06	-0.02	0.02	-0.07	0.3274	
	Noon - 6PM	-0.08	-0.05	-0.04	-0.04	0.8391	
	6PM - Midnight	-0.08	-0.03	-0.05	-0.09	0.8045	
29	Midnight - 6AM	-0.09	0.00	-0.03	-0.06	0.3043	
	6AM - Noon	-0.08	-0.01	0.03	-0.02	0.2763	
	Noon - 6PM	-0.09	-0.06	-0.02	-0.02	0.3490	
	6PM - Midnight	-0.12	-0.07	-0.05	-0.12	0.4837	
30	Midnight - 6AM	-0.07	0.02	-0.02	-0.05	0.5654	
	6AM - Noon	-0.12	-0.02	-0.04	-0.06	0.2767	
	Noon - 6PM	-0.09	-0.05	-0.03	-0.03	0.5762	
	6PM - Midnight	-0.06	-0.03	-0.02	-0.08	0.5923	
31	Midnight - 6AM	-0.07	0.03	-0.01	0.02	0.4929	
	6AM - Noon	-0.07	-0.02	0.04	-0.01	0.3242	
	Noon - 6PM	-0.06	-0.05	-0.04	-0.03	0.9264	
	6PM - Midnight	-0.06	-0.01	-0.01	0.01	0.7792	

Table 5. (Continued)

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
32	Midnight - 6AM	-0.04	0.03	-0.01	-0.04	0.5303	
	6AM - Noon	-0.09	-0.02	-0.01	-0.01	0.5198	
	Noon - 6PM	-0.05	-0.02	-0.03	-0.03	0.7734	
	6PM - Midnight	-0.06	-0.01	0.00	0.01	0.4454	
33	Midnight - 6AM	-0.04	0.04	0.00	0.06	0.1151	
	6AM - Noon	-0.07	-0.01	-0.01	0.01	0.4687	
	Noon - 6PM	-0.07	-0.06↓	0.01	0.00	0.0434 *	
	6PM - Midnight	-0.09	-0.06	-0.04	-0.11	0.5531	
34	Midnight - 6AM	-0.08	0.03	0.02	0.03	0.0511	
	6AM - Noon	-0.05	-0.01	0.02	0.08	0.2730	
	Noon - 6PM	-0.07	-0.07↓	-0.01	-0.04	0.2723	
	6PM - Midnight	-0.08	-0.08↓	-0.06	-0.13	0.6033	
35	Midnight - 6AM	-0.03	0.01	0.00	0.03	0.7210	
	6AM - Noon	-0.05	0.00	0.04	0.02	0.6614	
	Noon - 6PM	-0.07	-0.06	-0.03	0.01	0.4681	
	6PM - Midnight	-0.11	-0.07	-0.04	-0.10	0.6187	
36	Midnight - 6AM	-0.05	0.01	-0.03	-0.05	0.4939	
	6AM - Noon	-0.05	0.01	0.06	-0.01	0.2266	
	Noon - 6PM	-0.07	-0.06	-0.04	-0.05	0.9188	
	6PM - Midnight	-0.11	-0.09↓	-0.05	-0.14	0.3941	
37	Midnight - 6AM	-0.01	-0.01	-0.01	-0.11	0.3860	
	6AM - Noon	-0.08	-0.03	0.00	-0.08	0.3076	
	Noon - 6PM	-0.06	-0.04	-0.05	-0.03	0.9634	
	6PM - Midnight	-0.12	-0.05	-0.03	-0.06	0.4805	
38	Midnight - 6AM	-0.02	0.01	0.00	0.07	0.4444	
	6AM - Noon	-0.07	0.01	0.03	0.05	0.2755	
	Noon - 6PM	-0.10	-0.06	0.00	-0.02	0.2160	
	6PM - Midnight	-0.08	-0.05	-0.03	-0.07	0.8748	
39	Midnight - 6AM	-0.07	0.01	-0.01	0.01	0.4562	
	6AM - Noon	-0.06	-0.02	0.01	-0.01	0.4787	

\# Cells contain all pairwise comparisons that were 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<2)" indicates that the mean baseline adjusted value in Group 2 was significantly greater than that in Group 1], and (3) the Tukey-adjusted p-value.

NA Data was not available for this group at this study time.

↑, ↓ "↑" indicates that the mean at the study time was significantly greater than that at baseline, while "↓" indicates that the mean at the study time was significantly less than that at baseline (at the 0.05 level).

* Group effect was significant at the 0.05 level.

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

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
0	6AM - Noon	0.35	4.76	NA	NA	0.1812	
	Noon - 6PM	-0.66	1.71	1.90	0.52	0.4913	
	6PM - Midnight	-1.40	0.65	1.03	-0.63	0.0747	
1	Midnight - 6AM	-0.81	1.17	0.38	-0.45	0.6685	
	6AM - Noon	-0.14	1.30	0.93	0.17	0.8957	
	Noon - 6PM	0.62	1.78	1.21	0.42	0.9487	
	6PM - Midnight	-0.30	1.06	0.30	-0.10	0.9363	
2	Midnight - 6AM	-1.13	-0.07	-0.92	-0.28	0.9481	
	6AM - Noon	-1.05	1.09	-1.12	0.17	0.7318	
	Noon - 6PM	0.03	0.57	0.78	0.79	0.9789	
	6PM - Midnight	-0.47	-0.14	1.19	0.94	0.6368	
3	Midnight - 6AM	-0.72	-1.81	0.37	0.60	0.3831	
	6AM - Noon	-0.56	-1.30	0.31	-0.27	0.5939	
	Noon - 6PM	0.32	-0.78	1.27	0.21	0.4438	
	6PM - Midnight	0.06	-2.86	1.15	0.86	0.0900	
4	Midnight - 6AM	-0.52	-8.14	0.93	0.27	0.1326	
	6AM - Noon	-1.10	0.21	0.00	-0.09	0.7677	
	Noon - 6PM	-0.73	-0.76	1.44	-0.19	0.1732	
	6PM - Midnight	-0.62	-1.50	1.55	0.74	0.0650	
5	Midnight - 6AM	-0.41	-0.65	1.28	1.47	0.2615	
	6AM - Noon	-1.02	-0.84	0.88	0.13	0.3145	
	Noon - 6PM	-0.25	0.92	1.79	0.61	0.4989	
	6PM - Midnight	-0.18	-1.12	2.29↑	1.09	0.0269 *	-3.41 (2<3) 0.0207
6	Midnight - 6AM	0.06	-0.89	2.03	1.38	0.2048	
	6AM - Noon	-0.50	-1.50	1.31	0.25	0.1087	
	Noon - 6PM	0.13	-0.35	2.14↑	0.36	0.0197 *	-2.49 (2<3) 0.0160
	6PM - Midnight	-0.12	-0.29	2.10↑	0.73	0.0222 *	-2.39 (2<3) 0.0210
7	Midnight - 6AM	0.00	0.95	1.19	1.83	0.7453	
	6AM - Noon	0.25	1.59	1.27	1.22	0.7779	
	Noon - 6PM	0.05	2.16↑	2.44↑	1.25	0.3632	
	6PM - Midnight	0.03	1.45	2.13↑	1.24	0.5186	
8	Midnight - 6AM	-0.09	1.22	2.73↑	1.95	0.2415	
	6AM - Noon	0.13	1.63	1.65	0.97	0.6511	
	Noon - 6PM	0.28	1.87	2.41	1.31	0.6472	
	6PM - Midnight	-0.39	1.46	1.93	0.66	0.4848	
9	Midnight - 6AM	-0.73	0.59	2.93↑	1.28	0.0804	
	6AM - Noon	-1.33	1.65	1.34	0.77	0.2486	
	Noon - 6PM	-0.11	2.70	2.97↑	2.07	0.4536	
	6PM - Midnight	0.34	1.09	3.44↑	2.51↑	0.1248	
10	Midnight - 6AM	-0.31	0.18	3.33↑	2.84↑	0.0621	
	6AM - Noon	0.42	1.14	2.16↑	1.82	0.4953	
	Noon - 6PM	0.01	0.90	2.61	1.89	0.5419	
	6PM - Midnight	-0.83	0.50	3.56↑	1.96	0.1735	

Table 6. (Continued)

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
11	Midnight - 6AM	-1.55	0.32	2.95↑	3.06	0.0790	
	6AM - Noon	-1.58	1.00	2.59↑	2.35	0.0860	
	Noon - 6PM	-0.42	2.15	2.40	1.43	0.6323	
	6PM - Midnight	-1.20	1.37	3.33↑	2.93	0.1722	
12	Midnight - 6AM	-1.58	0.22	2.97	4.30	0.0886	
	6AM - Noon	-1.91	0.18	1.81	1.41	0.0643	
	Noon - 6PM	-0.57	1.84	2.52	1.98	0.6224	
	6PM - Midnight	-1.19	1.36	3.34↑	3.43	0.1156	
13	Midnight - 6AM	-0.78	1.06	2.86	4.58↑	0.0830	
	6AM - Noon	-0.76	1.06	2.15↑	0.74	0.1208	
	Noon - 6PM	-0.53	2.18	2.12	0.54	0.6394	
	6PM - Midnight	-0.72	1.34	2.96↑	0.35	0.1296	
14	Midnight - 6AM	-1.26	0.73	3.15↑	2.28	0.0216 *	-4.41 (1<3) 0.0186
	6AM - Noon	-0.93	1.43	2.72↑	1.32	0.1013	
	Noon - 6PM	-0.49	0.42	3.09↑	0.48	0.0690	
	6PM - Midnight	-1.17	0.51	3.03↑	1.00	0.0491 *	-4.21 (1<3) 0.0389
15	Midnight - 6AM	-1.52	0.89	3.62↑	1.23	0.0568	-5.14 (1<3) 0.0399
	6AM - Noon	-0.72	1.20	2.40↑	0.93	0.1357	
	Noon - 6PM	-0.75	0.65	2.15↑	-0.18	0.1888	
	6PM - Midnight	-0.31	0.88	2.44	0.36	0.4784	
16	Midnight - 6AM	-1.12	1.36	2.50↑	0.99	0.2143	
	6AM - Noon	-1.28	0.87	1.63	1.39	0.3791	
	Noon - 6PM	0.01	-0.03	2.25	0.33	0.6026	
	6PM - Midnight	-0.90	1.02	2.90↑	-0.28	0.0540	
17	Midnight - 6AM	-0.97	-0.41	3.22↑	0.73	0.0634	
	6AM - Noon	-1.16	-0.02	1.85	0.60	0.3028	
	Noon - 6PM	0.00	1.56	2.29	0.05	0.6138	
	6PM - Midnight	0.29	1.84	2.91↑	1.10	0.5666	
18	Midnight - 6AM	0.70	1.48	2.89	2.15	0.7281	
	6AM - Noon	-0.92	1.39	1.88	2.26	0.7004	
	Noon - 6PM	-0.85	1.96	3.46	0.50	0.6296	
	6PM - Midnight	-1.04	2.18	3.19	0.52	0.5116	
19	Midnight - 6AM	-1.67	2.26	3.68	0.92	0.1942	
	6AM - Noon	-2.16	2.51	2.47	0.54	0.3178	
	Noon - 6PM	-1.58	3.98	3.65	0.96	0.3605	
	6PM - Midnight	-0.98	3.31	3.94	1.62	0.3295	
20	Midnight - 6AM	-1.27	3.31	3.22	2.73	0.3502	
	6AM - Noon	-2.19	2.97	2.56	1.85	0.2120	
	Noon - 6PM	-0.60	4.10	3.10	2.08	0.5093	
	6PM - Midnight	-0.66	3.56	3.69	2.50	0.4056	

Table 6. (Continued)

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
21	Midnight - 6AM	-0.69	2.12	3.82↑	3.55	0.2721	
	6AM - Noon	-0.84	3.09	2.59	2.83	0.4047	
	Noon - 6PM	-0.30	4.17	3.97	2.15	0.4475	
	6PM - Midnight	0.18	3.25	3.73	2.36	0.5588	
22	Midnight - 6AM	0.25	2.68	3.37	3.07	0.5870	
	6AM - Noon	-0.51	3.00	2.99	2.73	0.4826	
	Noon - 6PM	0.18	3.66	3.48	2.25	0.6880	
	6PM - Midnight	-0.01	3.25	4.13	2.59	0.5423	
23	Midnight - 6AM	-0.20	2.26	3.51	3.00	0.5444	
	6AM - Noon	-0.97	3.21	3.29	3.59	0.2969	
	Noon - 6PM	-0.77	2.67	2.56	2.31	0.6400	
	6PM - Midnight	-0.39	2.87	4.10	2.61	0.4464	
24	Midnight - 6AM	-0.10	2.39	3.63	3.22	0.5721	
	6AM - Noon	0.10	2.60	2.47	2.45	0.7901	
	Noon - 6PM	0.15	3.76	2.77	2.32	0.7271	
	6PM - Midnight	0.60	3.07	3.22	2.56	0.7392	
25	Midnight - 6AM	0.58	2.78	3.59	2.97	0.6950	
	6AM - Noon	0.25	2.65	3.21	3.44	0.5233	
	Noon - 6PM	0.19	3.82	3.48	2.39	0.6770	
	6PM - Midnight	0.28	3.07	4.20	2.21	0.6360	
26	Midnight - 6AM	0.38	2.85	3.47	2.39	0.7022	
	6AM - Noon	-0.26	2.82	3.03	2.04	0.6514	
	Noon - 6PM	-0.33	4.27	3.99	1.96	0.4875	
	6PM - Midnight	0.22	3.14	3.77	1.73	0.6464	
27	Midnight - 6AM	0.60	2.85	2.83	1.75	0.8006	
	6AM - Noon	-0.61	2.62	3.04	1.52	0.5241	
	Noon - 6PM	0.16	3.85	3.50	1.41	0.6109	
	6PM - Midnight	0.07	2.89	4.11	0.87	0.5225	
28	Midnight - 6AM	0.40	2.82	4.29	1.19	0.4981	
	6AM - Noon	0.11	3.38	2.51	0.69	0.6248	
	Noon - 6PM	0.26	3.52	3.77	0.55	0.6190	
	6PM - Midnight	0.03	2.81	4.20	0.97	0.4428	
29	Midnight - 6AM	-0.45	2.87	3.96	1.35	0.4377	
	6AM - Noon	-0.23	2.94	2.87	0.36	0.5066	
	Noon - 6PM	-0.02	3.56	3.69	-0.45	0.3226	
	6PM - Midnight	-0.48	2.60	4.48	0.12	0.3728	
30	Midnight - 6AM	-0.01	3.29	4.55↑	1.19	0.3082	
	6AM - Noon	-0.25	3.20	3.50	0.43	0.2926	
	Noon - 6PM	0.48	3.00	3.46	-0.29	0.4901	
	6PM - Midnight	0.01	3.36	3.76	-0.35	0.4258	
31	Midnight - 6AM	0.10	3.27	3.97	0.12	0.3607	
	6AM - Noon	0.01	3.21	2.69	-0.66	0.3882	
	Noon - 6PM	0.76	4.25	3.84	-0.64	0.4341	
	6PM - Midnight	0.20	4.07	4.07	-0.59	0.3092	

Table 6. (Continued)

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
32	Midnight - 6AM	0.41	3.40	4.34↑	0.02	0.3170	
	6AM - Noon	-0.17	3.58↑	2.80	0.69	0.4108	
	Noon - 6PM	0.24	3.83	3.80	-0.06	0.4437	
	6PM - Midnight	0.16	3.41	4.38	-0.33	0.2929	
33	Midnight - 6AM	0.33	3.55	4.45↑	0.32	0.3524	
	6AM - Noon	0.10	3.58↑	3.38	0.26	0.3506	
	Noon - 6PM	0.78	4.72	3.93	-0.10	0.4430	
	6PM - Midnight	0.58	3.59	4.96↑	0.96	0.4372	
34	Midnight - 6AM	0.17	3.26	4.00	1.19	0.4613	
	6AM - Noon	0.38	2.96	3.39	0.65	0.5666	
	Noon - 6PM	0.99	4.60↑	4.02	0.76	0.5153	
	6PM - Midnight	1.03	3.74	4.85	1.58	0.5757	
35	Midnight - 6AM	0.57	2.63	3.99	1.94	0.6672	
	6AM - Noon	0.46	3.03	2.73	0.95	0.6838	
	Noon - 6PM	1.11	4.21	4.09	1.12	0.6057	
	6PM - Midnight	1.11	3.99	4.73	1.47	0.5579	
36	Midnight - 6AM	0.52	3.95	4.16	2.20	0.5583	
	6AM - Noon	0.02	3.45↑	2.93	1.49	0.4121	
	Noon - 6PM	1.14	4.51↑	3.83	1.08	0.4843	
	6PM - Midnight	0.95	4.02↑	4.74↑	1.62	0.4391	
37	Midnight - 6AM	0.60	3.02	3.99	1.70	0.5780	
	6AM - Noon	0.73	3.44↑	3.03	1.23	0.5348	
	Noon - 6PM	0.69	4.24	3.81	0.51	0.5250	
	6PM - Midnight	0.89	3.47	4.44↑	2.23	0.5592	
38	Midnight - 6AM	0.62	3.16	4.21↑	2.14	0.5283	
	6AM - Noon	-0.15	2.65	3.21	1.49	0.4359	
	Noon - 6PM	1.38	4.81↑	4.26	2.00	0.6799	
	6PM - Midnight	0.79	4.01	4.96	2.48	0.5698	
39	Midnight - 6AM	0.98	3.44	4.35	3.16	0.6710	
	6AM - Noon	0.83	3.65↑	3.21	2.76	0.6287	

Cells contain all pairwise comparisons that were 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<2)" indicates that the mean baseline adjusted value in Group 2 was significantly greater than that in Group 1], and (3) the Tukey-adjusted p-value.

NA Data was not available for this group at this study time.

↑, ↓ "↑" indicates that the mean at the study time was significantly greater than that at baseline, while "↓" indicates that the mean at the study time was significantly less than that at baseline (at the 0.05 level).

* Group effect was significant at the 0.05 level.

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

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
0	6AM - Noon	-0.51	4.98	NA	NA	0.2621	
	Noon - 6PM	-2.14	1.60	2.15	0.36	0.5696	
	6PM - Midnight	-1.98	1.29	1.47	-0.14	0.2381	
1	Midnight - 6AM	-1.06	2.67	0.42	-0.35	0.5938	
	6AM - Noon	-0.07	1.20	1.37	0.82	0.9818	
	Noon - 6PM	0.91	2.00	1.36	0.20	0.9733	
	6PM - Midnight	-0.64	1.64	0.37	-0.35	0.9519	
2	Midnight - 6AM	-1.56	-0.51	-0.93	-0.34	0.9927	
	6AM - Noon	-1.11	0.21	-1.19	0.26	0.9673	
	Noon - 6PM	0.28	0.71	0.76	1.23	0.9843	
	6PM - Midnight	-1.35	-1.43	1.43	0.78	0.5443	
3	Midnight - 6AM	-1.23	-3.38	1.11	0.62	0.3362	
	6AM - Noon	-0.73	-3.48	0.78	-0.13	0.2575	
	Noon - 6PM	0.24	-2.80	0.89	-0.32	0.3686	
	6PM - Midnight	-0.26	-5.70	1.61	0.86	0.0922	
4	Midnight - 6AM	-0.97	-11.00	1.81	0.60	0.1225	
	6AM - Noon	-1.24	0.89	1.27	0.32	0.8297	
	Noon - 6PM	-1.23	-0.63	1.67	-0.06	0.6034	
	6PM - Midnight	-1.50	-1.95	1.87	0.66	0.1090	
5	Midnight - 6AM	-1.02	-0.25	2.59↑	1.50	0.1242	
	6AM - Noon	-1.92	-0.48	1.26	0.60	0.3344	
	Noon - 6PM	-0.99	1.55	2.62↑	1.08	0.1093	
	6PM - Midnight	-1.27	-2.07	3.55↑	1.72	0.0012 *	-4.82 (1<3) 0.0193 -3.79 (2<4) 0.0424 -5.62 (2<3) 0.0013
6	Midnight - 6AM	-0.46	-1.88	3.51↑	1.70	0.0499 *	-5.39 (2<3) 0.0410
	6AM - Noon	-0.72	-1.40	2.36↑	0.83	0.0469 *	-3.76 (2<3) 0.0402
	Noon - 6PM	0.11	0.22	3.13↑	0.48	0.1278	
	6PM - Midnight	-0.45	0.18	3.46↑	1.16	0.1136	
7	Midnight - 6AM	-0.31	1.33	2.57	2.08	0.8405	
	6AM - Noon	0.70	2.88	2.36	1.83	0.9072	
	Noon - 6PM	-0.30	4.79	3.46	1.75	0.5706	
	6PM - Midnight	-0.38	3.22	3.09	1.55	0.7223	
8	Midnight - 6AM	-0.17	2.71	3.97↑	1.73	0.4692	
	6AM - Noon	0.42	3.26	3.17	1.50	0.6988	
	Noon - 6PM	0.39	3.68	3.23	1.60	0.7245	
	6PM - Midnight	-0.88	2.74	3.44	1.01	0.4224	
9	Midnight - 6AM	-1.61	0.85	4.09↑	1.06	0.0467 *	-5.70 (1<3) 0.0357
	6AM - Noon	-1.34	2.06	3.09	1.65	0.2485	
	Noon - 6PM	0.36	4.44	4.91↑	3.07	0.4949	
	6PM - Midnight	-0.43	1.54	4.41↑	2.93	0.3063	

Table 7. (Continued)

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
10	Midnight - 6AM	-0.93	0.36	5.18↑	3.69	0.0450 *	
	6AM - Noon	0.62	2.05	3.43↑	2.86	0.5045	
	Noon - 6PM	-0.50	0.90	4.00↑	2.24	0.3129	
	6PM - Midnight	-2.64	-0.08	4.49	1.96	0.2095	
11	Midnight - 6AM	-2.94	0.03	4.21	3.22	0.0920	
	6AM - Noon	-2.43	0.84	4.23↑	2.32	0.0264 *	-6.65 (1<3) 0.0186
	Noon - 6PM	-0.68	3.78	3.96	1.58	0.4788	
	6PM - Midnight	-2.94	1.93	4.73	2.38	0.1839	
12	Midnight - 6AM	-3.71	0.73	4.49	4.35	0.0642	
	6AM - Noon	-3.00	1.54	3.47↑	1.00	0.0177 *	-6.48 (1<3) 0.0099
	Noon - 6PM	-1.27	4.16	3.66	2.73	0.4265	
	6PM - Midnight	-2.82	2.75	4.35	2.94	0.1779	
13	Midnight - 6AM	-2.17	1.90	3.77↑	3.38	0.1282	
	6AM - Noon	-1.38	2.85	3.61↑	0.82	0.1834	
	Noon - 6PM	-1.60	4.73	2.94	-0.06	0.2648	
	6PM - Midnight	-2.04	2.72	4.08↑	-0.66	0.0745	
14	Midnight - 6AM	-3.09	0.17	4.51↑	1.41	0.0023 *	-7.59 (1<3) 0.0015 -4.34 (2<3) 0.0352
	6AM - Noon	-1.83	1.35	4.37↑	1.06	0.0087 *	-6.19 (1<3) 0.0054
	Noon - 6PM	-1.34	0.38	5.07↑	0.29	0.0034 *	4.79 (4<3) 0.0423 -6.42 (1<3) 0.0053 -4.70 (2<3) 0.0171
	6PM - Midnight	-3.08	0.42	4.58↑	0.61	0.0233 *	-7.65 (1<3) 0.0162
15	Midnight - 6AM	-3.09	-0.23	5.42↑	0.39	0.0123 *	-8.50 (1<3) 0.0118
	6AM - Noon	-1.50	0.90	4.28↑	0.52	0.0019 *	-5.78 (1<3) 0.0016 -3.38 (2<3) 0.0307
	Noon - 6PM	-2.09	1.11	3.50↑	-0.37	0.1365	
	6PM - Midnight	-1.41	0.37	3.59	-0.68	0.2837	
16	Midnight - 6AM	-2.66	0.66	3.77	-0.07	0.1690	
	6AM - Noon	-2.03	0.80	2.96	0.38	0.1583	
	Noon - 6PM	0.04	0.36	4.20↑	-0.59	0.2158	
	6PM - Midnight	-1.52	1.08	4.57↑	-1.47	0.1299	
17	Midnight - 6AM	-1.92	-0.56	4.85↑	-0.65	0.0422 *	
	6AM - Noon	-1.96	0.25	3.28	0.19	0.2195	
	Noon - 6PM	-0.47	3.28	3.10	-0.72	0.5976	
	6PM - Midnight	0.23	3.86	3.71	0.99	0.7957	
18	Midnight - 6AM	0.42	2.77	3.49	2.64	0.8998	
	6AM - Noon	-0.80	3.82	2.52	2.55	0.7815	
	Noon - 6PM	-1.66	4.47	5.73	-0.10	0.4846	
	6PM - Midnight	-2.62	4.09	5.03	-0.43	0.5108	
19	Midnight - 6AM	-2.99	4.66	5.81	0.32	0.2881	
	6AM - Noon	-3.57	5.11	4.69	0.36	0.2830	
	Noon - 6PM	-3.08	7.23	6.06	0.87	0.3233	
	6PM - Midnight	-2.69	6.37	5.96	1.08	0.3767	

Table 7. (Continued)

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
20	Midnight - 6AM	-2.70	6.26	5.20	2.52	0.3105	
	6AM - Noon	-3.20	5.93	4.95	2.23	0.2650	
	Noon - 6PM	-1.05	7.39	5.81	2.50	0.4183	
	6PM - Midnight	-1.53	6.84	5.81	2.61	0.4278	
21	Midnight - 6AM	-1.71	4.37	6.04	3.92	0.3665	
	6AM - Noon	-1.32	6.01	5.00	3.59	0.4171	
	Noon - 6PM	-0.73	7.60↑	6.46	2.57	0.3499	
	6PM - Midnight	-0.14	6.50	5.90	1.61	0.5626	
22	Midnight - 6AM	-0.02	4.88	5.68	2.26	0.5481	
	6AM - Noon	-0.71	5.58	5.59	2.36	0.4734	
	Noon - 6PM	0.06	7.16	6.05	2.00	0.5134	
	6PM - Midnight	-0.40	6.58	6.53	2.66	0.5755	
23	Midnight - 6AM	-0.37	4.22	5.81	2.60	0.5670	
	6AM - Noon	-1.54	5.73	5.63	3.55	0.3438	
	Noon - 6PM	-0.71	6.45	4.77	2.62	0.5544	
	6PM - Midnight	-1.39	4.97	5.89	2.12	0.5606	
24	Midnight - 6AM	-1.03	4.29	5.83	3.12	0.5806	
	6AM - Noon	0.34	4.61	4.71	3.00	0.7817	
	Noon - 6PM	1.23	6.72	4.65	2.77	0.7494	
	6PM - Midnight	1.25	6.36	5.69	2.74	0.7542	
25	Midnight - 6AM	0.97	6.05	6.11	3.33	0.6979	
	6AM - Noon	0.62	5.44	5.68	3.84	0.5919	
	Noon - 6PM	0.82	7.80	5.83	3.33	0.6025	
	6PM - Midnight	-0.08	5.46	6.47	1.67	0.6359	
26	Midnight - 6AM	0.35	5.11	5.59	2.59	0.7108	
	6AM - Noon	-0.23	5.17	5.33	2.39	0.6204	
	Noon - 6PM	-0.83	7.17	5.96	1.87	0.4023	
	6PM - Midnight	-0.40	6.26	5.92	0.94	0.5520	
27	Midnight - 6AM	0.39	5.25	4.74	0.76	0.6288	
	6AM - Noon	-1.16	4.64	4.94	0.76	0.4606	
	Noon - 6PM	-0.45	6.82	5.26	0.30	0.4342	
	6PM - Midnight	-0.43	5.50	5.82	-1.30	0.5028	
28	Midnight - 6AM	0.06	4.93	6.23	-1.23	0.4191	
	6AM - Noon	0.28	5.80	4.27	-1.47	0.4524	
	Noon - 6PM	0.23	5.84	6.13	-1.93	0.3922	
	6PM - Midnight	-0.45	4.52	6.21	-1.11	0.4424	
29	Midnight - 6AM	-0.71	4.80	6.06	-1.54	0.3910	
	6AM - Noon	0.02	5.23	5.13	-2.17	0.3411	
	Noon - 6PM	-0.48	6.16	6.12	-3.53	0.1946	
	6PM - Midnight	-1.49	4.48	6.19	-3.21	0.3645	
30	Midnight - 6AM	-0.40	5.41	6.60	-2.02	0.2406	
	6AM - Noon	-0.34	5.53	5.42	-3.26	0.1864	
	Noon - 6PM	0.23	6.14	5.67	-3.65	0.2548	
	6PM - Midnight	0.00	6.18	5.44	-4.02	0.3635	

Table 7. (Continued)

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
31	Midnight - 6AM	0.10	5.71	6.02	-3.86	0.2616	
	6AM - Noon	-0.05	5.67	5.09	-3.97	0.2369	
	Noon - 6PM	1.25	7.27	5.86	-4.29	0.2295	
	6PM - Midnight	0.24	7.54	5.87	-3.47	0.2868	
32	Midnight - 6AM	0.41	6.01	6.66	-3.37	0.2368	
	6AM - Noon	-0.10	6.39	4.84	-1.90	0.2761	
	Noon - 6PM	0.43	7.35	6.30	-3.12	0.2593	
	6PM - Midnight	-0.11	6.67	6.78	-2.69	0.2939	
33	Midnight - 6AM	0.10	6.25	6.91	-1.81	0.3354	
	6AM - Noon	0.17	6.50	5.94	-1.32	0.2905	
	Noon - 6PM	0.39	7.99	6.59	-2.46	0.2967	
	6PM - Midnight	-0.11	6.23	7.35	-1.20	0.4290	
34	Midnight - 6AM	-0.48	5.56	6.23	-0.41	0.3808	
	6AM - Noon	0.57	5.16	5.88	-0.77	0.5022	
	Noon - 6PM	1.23	7.72	6.35	-0.67	0.4276	
	6PM - Midnight	0.91	6.19	6.91	-0.29	0.5764	
35	Midnight - 6AM	0.36	4.51	6.30	0.69	0.6187	
	6AM - Noon	0.75	5.19	5.00	0.30	0.6329	
	Noon - 6PM	1.38	6.78	6.55	0.08	0.5370	
	6PM - Midnight	0.96	6.92	6.75	-0.23	0.5306	
36	Midnight - 6AM	0.31	6.45	6.47	0.77	0.4756	
	6AM - Noon	0.47	6.21↑	5.64	-0.10	0.3422	
	Noon - 6PM	1.37	7.72↑	6.22	-0.57	0.3398	
	6PM - Midnight	0.78	6.65	6.78	-0.23	0.4209	
37	Midnight - 6AM	0.40	5.03	6.29	-0.06	0.4579	
	6AM - Noon	0.90	6.41↑	5.28	-0.77	0.3745	
	Noon - 6PM	0.77	8.03	6.23	-0.01	0.4492	
	6PM - Midnight	0.57	6.34	6.50	2.08	0.6136	
38	Midnight - 6AM	0.28	5.56	6.26	1.83	0.5756	
	6AM - Noon	-0.57	5.55	5.67	1.42	0.3914	
	Noon - 6PM	1.54	8.48↑	6.91	2.15	0.5782	
	6PM - Midnight	0.47	6.93	7.50	2.26	0.5844	
39	Midnight - 6AM	0.97	5.70	6.75	3.25	0.6659	
	6AM - Noon	1.42	6.34↑	5.83	3.58	0.6493	

Cells contain all pairwise comparisons that were 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<2)" indicates that the mean baseline adjusted value in Group 2 was significantly greater than that in Group 1], and (3) the Tukey-adjusted p-value.

NA Data was not available for this group at this study time.

↑, ↓ "↑" indicates that the mean at the study time was significantly greater than that at baseline, while "↓" indicates that the mean at the study time was significantly less than that at baseline (at the 0.05 level).

* Group effect was significant at the 0.05 level.

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

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
0	6AM - Noon	11.90	21.83↑	NA	NA	0.3139	
	Noon - 6PM	3.71	4.30↑	6.52↑	3.36	0.3481	
	6PM - Midnight	-0.76	0.37	0.32	-0.49	0.8891	
1	Midnight - 6AM	0.87	-0.14	0.94	1.00	0.6979	
	6AM - Noon	5.33↑	3.31↑	0.73	0.71	0.0538	
	Noon - 6PM	4.01	4.84↑	4.94↑	3.30↑	0.7367	
	6PM - Midnight	2.63	0.81	3.07↑	-1.93	0.0486 *	5.00 (4<3) 0.0435
2	Midnight - 6AM	1.43	0.84	0.22	-2.41	0.1226	
	6AM - Noon	3.41	6.57↑	0.47	4.84↑	0.0678	6.10 (3<2) 0.0499
	Noon - 6PM	-0.25	1.88	2.83	0.84	0.5052	
	6PM - Midnight	3.65	2.33	1.76	0.79	0.6709	
3	Midnight - 6AM	3.09	-0.42	-0.58	-1.39	0.2065	
	6AM - Noon	4.74	1.34	0.10	-1.70	0.1233	
	Noon - 6PM	4.57	5.08↑	5.32↑	3.53	0.8615	
	6PM - Midnight	2.72	0.99	3.28↑	0.53	0.4479	
4	Midnight - 6AM	4.61↑	-1.57	0.26	-2.42	0.0016 *	7.03 (4<1) 0.0013 4.35 (3<1) 0.0431 6.18 (2<1) 0.0059
	6AM - Noon	1.35	5.79↑	0.85	4.12	0.2209	
	Noon - 6PM	5.02	4.65↑	4.79↑	0.78	0.4114	
	6PM - Midnight	6.06	2.09	3.42	1.38	0.4909	
5	Midnight - 6AM	4.54	0.73	0.76	0.02	0.4900	
	6AM - Noon	2.38	1.18	1.64	-4.05↓	0.0221 *	5.69 (4<3) 0.0402 6.43 (4<1) 0.0470
	Noon - 6PM	7.92↑	4.83↑	4.55↑	-0.25	0.0352 *	8.17 (4<1) 0.0281
	6PM - Midnight	9.43↑	3.12	4.75	-0.15	0.0869	
6	Midnight - 6AM	4.78	3.02	0.43	-0.34	0.3217	
	6AM - Noon	2.64	-1.30	0.04	-4.18↓	0.0396 *	6.82 (4<1) 0.0307
	Noon - 6PM	2.82	2.55	4.49↑	0.33	0.1803	
	6PM - Midnight	3.36	3.33	2.66	1.65	0.9083	
7	Midnight - 6AM	2.42	1.90	-0.18	0.65	0.7577	
	6AM - Noon	2.28	1.66	0.20	-0.31	0.6334	
	Noon - 6PM	6.36	3.45	6.82↑	3.05	0.4097	
	6PM - Midnight	4.09	3.23	5.51↑	2.73	0.7524	
8	Midnight - 6AM	2.43	0.70	2.38	3.23	0.6438	
	6AM - Noon	1.99	1.40	0.72	0.50	0.7934	
	Noon - 6PM	4.64	2.87	5.76↑	2.11	0.4707	
	6PM - Midnight	4.14	1.80	-0.43	-1.59	0.3683	
9	Midnight - 6AM	4.54	0.55	0.34	0.09	0.3597	
	6AM - Noon	0.73	6.49↑	2.01	3.25	0.2301	
	Noon - 6PM	1.90	4.98	4.32	3.48	0.8739	
	6PM - Midnight	10.32↑	2.03	8.48↑	2.81	0.0592	

Table 8. (Continued)

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
10	Midnight - 6AM	5.78	-0.55	0.72	-2.19	0.0255*	7.97 (4<1) 0.0180
	6AM - Noon	7.34	0.66	1.94	1.57	0.1826	
	Noon - 6PM	12.62	3.91	6.42	6.12	0.5096	
	6PM - Midnight	13.15	3.43	10.25↑	2.93	0.1656	
11	Midnight - 6AM	7.39	0.75	2.53	3.04	0.4343	
	6AM - Noon	2.66	2.78	4.89	9.22↑	0.4464	
	Noon - 6PM	-0.50	0.54	2.75	0.11	0.9468	
	6PM - Midnight	7.25	1.27	7.41	4.74	0.5703	
12	Midnight - 6AM	7.57	-2.80	3.35	5.21	0.2382	
	6AM - Noon	1.30	-6.24	-1.35	3.20	0.3467	
	Noon - 6PM	3.23	-2.30	4.38	4.56	0.6896	
	6PM - Midnight	8.04	0.17	9.25	16.54↑	0.0812	
13	Midnight - 6AM	6.23	-1.19	3.29	18.43↑	0.0343 *	-19.62 (2<4) 0.0236
	6AM - Noon	0.46	-5.56	-2.62	2.52	0.3283	
	Noon - 6PM	5.39	1.50	2.95	4.64	0.9176	
	6PM - Midnight	8.56	-0.01	7.29	6.91	0.3389	
14	Midnight - 6AM	6.48	3.10	3.27	14.55	0.2354	
	6AM - Noon	4.86	4.21	4.78	10.39	0.7155	
	Noon - 6PM	6.53	3.34	8.03↑	7.45	0.7287	
	6PM - Midnight	10.03	5.32	6.39	12.15	0.6306	
15	Midnight - 6AM	5.77	5.05	3.32	2.92	0.8512	
	6AM - Noon	5.68	3.71↑	3.78↑	2.25	0.7041	
	Noon - 6PM	6.75	4.10	3.34	1.68	0.8449	
	6PM - Midnight	13.62↑	6.86	5.12	4.98	0.3649	
16	Midnight - 6AM	7.45	7.12↑	2.61	4.14	0.4385	
	6AM - Noon	2.54	6.03↑	6.77↑	10.55	0.2870	
	Noon - 6PM	3.80	4.15	3.59	4.73	0.9974	
	6PM - Midnight	6.02	6.40↑	3.03	3.04	0.6346	
17	Midnight - 6AM	4.15	2.72	3.42	3.61	0.9811	
	6AM - Noon	4.51	4.14↑	3.67	3.17	0.9752	
	Noon - 6PM	7.80	4.44	8.45↑	1.54	0.2862	
	6PM - Midnight	5.64	3.65	8.10↑	4.47	0.7956	
18	Midnight - 6AM	5.70	1.70	11.06↑	2.08	0.4634	
	6AM - Noon	2.76	3.79	8.04	7.94	0.7404	
	Noon - 6PM	4.82	3.11	5.30	4.29	0.8893	
	6PM - Midnight	8.18	4.50	3.82	4.73	0.7075	
19	Midnight - 6AM	4.01	-0.15	3.09	2.32	0.5708	
	6AM - Noon	2.01	0.00	-0.44	-0.52	0.8192	
	Noon - 6PM	6.68	6.59↑	4.65	1.02	0.5732	
	6PM - Midnight	10.32↑	4.56	4.96	9.90	0.3029	

Table 8. (Continued)

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
20	Midnight - 6AM	7.15	3.17	2.12	8.87	0.4799	
	6AM - Noon	0.65	0.42	-2.05	4.05	0.4420	
	Noon - 6PM	5.35	3.96	5.12	9.17	0.7190	
	6PM - Midnight	6.15	4.13	5.71	14.50	0.3985	
21	Midnight - 6AM	3.43	1.15	2.16	14.31	0.3165	
	6AM - Noon	2.50	-0.90	0.90	11.44	0.3152	
	Noon - 6PM	5.25	3.65	7.14	10.70	0.6919	
	6PM - Midnight	3.68	5.13	2.95	17.35	0.2429	
22	Midnight - 6AM	3.41	1.49	0.58	16.81	0.2131	
	6AM - Noon	1.47	0.94	0.76	14.59	0.2522	
	Noon - 6PM	5.60	4.32	3.80	16.19	0.3129	
	6PM - Midnight	4.62	2.01	5.25	16.86	0.2978	
23	Midnight - 6AM	3.65	2.82	0.66	15.36	0.3130	
	6AM - Noon	6.93	8.27↑	5.53	21.11↑	0.1001	
	Noon - 6PM	2.82	2.53	2.01	13.61	0.4231	
	6PM - Midnight	6.81	4.96	6.60	17.35	0.2741	
24	Midnight - 6AM	5.59	2.27	2.15	12.96	0.3718	
	6AM - Noon	2.71	1.31	-1.33	8.84	0.4409	
	Noon - 6PM	-3.11	6.24	3.40	7.66	0.3641	
	6PM - Midnight	1.20	2.84	0.74	9.64	0.4388	
25	Midnight - 6AM	-0.13	0.45	-1.02	5.53	0.5580	
	6AM - Noon	3.35	3.18	5.79	14.37	0.0837	
	Noon - 6PM	-2.40	2.89	3.04	2.79	0.7673	
	6PM - Midnight	4.85	6.43	6.86	10.66	0.7778	
26	Midnight - 6AM	3.07	3.36	2.91	4.77	0.9730	
	6AM - Noon	1.91	0.95	0.11	4.45	0.5126	
	Noon - 6PM	9.45	11.75↑	11.15↑	8.57	0.9090	
	6PM - Midnight	8.61	5.25	7.69	9.05	0.8984	
27	Midnight - 6AM	5.14	2.99	1.73	3.80	0.8649	
	6AM - Noon	5.81	2.97	2.77	4.14	0.7125	
	Noon - 6PM	11.80↑	10.77↑	8.96↑	6.50	0.7072	
	6PM - Midnight	7.06	7.29↑	13.44↑	10.05	0.4228	
28	Midnight - 6AM	5.90	5.31	7.53↑	5.75	0.9336	
	6AM - Noon	5.95	4.72	2.33	5.15	0.7503	
	Noon - 6PM	7.11	8.26↑	8.68↑	4.73	0.8558	
	6PM - Midnight	7.73	7.04	12.49↑	6.33	0.5815	
29	Midnight - 6AM	3.89	6.67	5.31	5.41	0.9629	
	6AM - Noon	2.22	2.87	1.32	-0.61	0.7498	
	Noon - 6PM	9.69↑	9.53↑	6.67↑	2.49	0.1905	
	6PM - Midnight	11.13	9.74↑	13.77↑	7.21	0.7287	
30	Midnight - 6AM	5.10	6.54	7.49	5.35	0.9652	
	6AM - Noon	8.62	7.50↑	11.19↑	9.17	0.6959	
	Noon - 6PM	8.79	6.80↑	6.78↑	1.14	0.3584	
	6PM - Midnight	2.65	4.76	8.00↑	5.81	0.6558	

Table 8. (Continued)

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
31	Midnight - 6AM	1.23	2.85	3.28	-0.57	0.7923	
	6AM - Noon	3.63	3.08	-0.02	-0.64	0.3504	
	Noon - 6PM	6.89	9.29↑	11.50↑	2.74	0.3735	
	6PM - Midnight	2.61	8.06↑	8.29	0.73	0.4830	
32	Midnight - 6AM	2.15	4.32	4.04	0.70	0.8419	
	6AM - Noon	5.80↑	6.14↑	6.87↑	4.05	0.7111	
	Noon - 6PM	3.48	5.98	6.52	3.08	0.8271	
	6PM - Midnight	3.88	5.49	7.34↑	-2.45	0.1783	
33	Midnight - 6AM	3.40	4.42	4.31	-4.28	0.1249	
	6AM - Noon	5.54	4.96↑	5.86↑	-2.32	0.1122	
	Noon - 6PM	12.65↑	11.82↑	9.21↑	1.57	0.1805	
	6PM - Midnight	11.61	10.45↑	14.67↑	8.54	0.7835	
34	Midnight - 6AM	5.25	4.07	3.57	0.99	0.8494	
	6AM - Noon	5.20	2.17	3.55	-1.82	0.2567	
	Noon - 6PM	10.00	12.95↑	11.36↑	2.90	0.2697	
	6PM - Midnight	11.16	13.43↑	15.38↑	12.02	0.9096	
35	Midnight - 6AM	4.08	5.49	5.86	1.30	0.7199	
	6AM - Noon	5.40	3.07	4.18	-0.15	0.5491	
	Noon - 6PM	13.12↑	11.60↑	11.08↑	2.89	0.2178	
	6PM - Midnight	13.72	13.77↑	14.58↑	9.02	0.8610	
36	Midnight - 6AM	5.11	8.53↑	7.43	9.42	0.8989	
	6AM - Noon	3.79	3.57	0.16	9.99	0.4376	
	Noon - 6PM	11.51	10.38↑	9.10↑	12.36	0.9279	
	6PM - Midnight	12.24	14.18↑	15.85↑	19.07	0.7787	
37	Midnight - 6AM	4.27	6.79↑	5.31	14.50	0.2801	
	6AM - Noon	9.67	6.99↑	6.54↑	13.68	0.3791	
	Noon - 6PM	9.80	7.49	9.22↑	2.58	0.6999	
	6PM - Midnight	12.45	9.42↑	10.99↑	9.13	0.9306	
38	Midnight - 6AM	4.58	5.88↑	4.45	0.76	0.4507	
	6AM - Noon	5.12	0.53	2.02	0.55	0.5528	
	Noon - 6PM	13.75↑	10.02↑	9.32↑	3.97	0.3516	
	6PM - Midnight	11.54	12.86↑	13.80↑	10.91	0.9682	
39	Midnight - 6AM	6.11	7.71↑	5.19	5.11	0.8653	
	6AM - Noon	2.17	7.71↑	5.58↑	11.26	0.1662	

\# Cells contain all pairwise comparisons that were 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<2)" indicates that the mean baseline adjusted value in Group 2 was significantly greater than that in Group 1], and (3) the Tukey-adjusted p-value.

NA Data was not available for this group at this study time.

↑, ↓ "↑" indicates that the mean at the study time was significantly greater than that at baseline, while "↓" indicates that the mean at the study time was significantly less than that at baseline (at the 0.05 level).

* Group effect was significant at the 0.05 level.

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

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
0	6AM - Noon	0.04	-0.07	NA	NA	0.5990	
	Noon - 6PM	0.25	0.07	0.16	0.13	0.5434	
	6PM - Midnight	-0.02	0.08↑	-0.02	0.03	0.0786	
1	Midnight - 6AM	0.03	0.06	0.01	0.11↑	0.0908	
	6AM - Noon	0.01	-0.02	0.01	0.04	0.6618	
	Noon - 6PM	0.27↑	0.11	0.26↑	0.22↑	0.4428	
	6PM - Midnight	0.06	0.12↑	0.06	0.10↑	0.6318	
2	Midnight - 6AM	0.09	0.11↑	0.09↑	0.09↑	0.9495	
	6AM - Noon	-0.61↓	-0.06	-0.27	-0.21	0.1610	
	Noon - 6PM	0.00	0.11	0.08	0.09	0.8955	
	6PM - Midnight	0.22↑	0.19↑	0.18↑	0.23↑	0.8553	
3	Midnight - 6AM	0.16↑	0.17↑	0.11↑	0.13↑	0.6936	
	6AM - Noon	0.03	-0.06	0.02	0.09↑	0.0574	-0.14 (2<4) 0.0351
	Noon - 6PM	0.30	0.07	0.18	0.22	0.5019	
	6PM - Midnight	0.16	0.12	0.10	0.17↑	0.7641	
4	Midnight - 6AM	0.10	0.12↑	0.13↑	0.14↑	0.9155	
	6AM - Noon	-0.53↓	-0.06	-0.24	-0.19	0.3005	
	Noon - 6PM	0.27↑	0.10	0.22↑	0.14	0.3763	
	6PM - Midnight	0.28↑	0.19↑	0.18↑	0.26↑	0.6149	
5	Midnight - 6AM	0.17↑	0.11↑	0.13↑	0.22↑	0.1659	
	6AM - Noon	0.13	0.05	0.07	0.16↑	0.3521	
	Noon - 6PM	0.27	-0.01	0.12	0.19	0.2582	
	6PM - Midnight	0.17	0.14↑	0.11	0.15↑	0.9172	
6	Midnight - 6AM	0.08	0.13↑	0.07	0.12↑	0.7150	
	6AM - Noon	0.05	-0.01	0.05	0.12↑	0.3470	
	Noon - 6PM	0.12	-0.09	0.09	0.09	0.2664	
	6PM - Midnight	0.01	0.06	0.01	0.00	0.8981	
7	Midnight - 6AM	0.03	0.09	-0.02	0.03	0.4432	
	6AM - Noon	-0.05	-0.07	-0.02	0.00	0.6543	
	Noon - 6PM	0.23	0.03	0.17	0.21	0.4452	
	6PM - Midnight	0.04	0.09	0.03	0.07	0.7558	
8	Midnight - 6AM	0.05	0.11↑	0.08	0.14↑	0.4638	
	6AM - Noon	-0.05	-0.05	0.04	0.03	0.4791	
	Noon - 6PM	0.23	-0.04	0.16	0.16	0.2057	
	6PM - Midnight	0.02	0.02	-0.03	0.08	0.7395	
9	Midnight - 6AM	0.03	0.05	0.01	0.09	0.6412	
	6AM - Noon	-0.61↓	-0.12	-0.34	-0.24	0.1969	
	Noon - 6PM	0.06	0.01	0.11	0.09	0.8065	
	6PM - Midnight	0.34↑	0.16	0.28↑	0.26↑	0.4239	

Table 9. (Continued)

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
10	Midnight - 6AM	0.12	0.17↑	0.21↑	0.17↑	0.8211	
	6AM - Noon	0.04	-0.10	0.08	0.12	0.0321 *	-0.22 (2<4) 0.0297
	Noon - 6PM	0.34	0.01	0.23	0.00	0.3599	
	6PM - Midnight	0.18	0.08	0.20	0.11	0.8763	
11	Midnight - 6AM	0.09	0.15	0.18	-0.05	0.6568	
	6AM - Noon	-0.53	-0.06	0.03	-0.34	0.4677	
	Noon - 6PM	-0.35	0.02	0.26	0.16	0.3183	
	6PM - Midnight	0.04	0.11	0.19	0.27	0.5869	
12	Midnight - 6AM	0.14	0.13	0.22	0.58↑	0.2359	
	6AM - Noon	-0.01	-0.12	0.04	0.27	0.3476	
	Noon - 6PM	0.10	-0.16	0.08	0.12	0.3749	
	6PM - Midnight	0.11	0.03	0.15	0.60↑	0.1901	
13	Midnight - 6AM	0.10	0.01	0.14	0.61↑	0.1208	
	6AM - Noon	-0.03	-0.16	0.02	0.23	0.2299	
	Noon - 6PM	0.10	-0.20	0.07	0.01	0.2459	
	6PM - Midnight	0.09	-0.01	0.16	0.17	0.3584	
14	Midnight - 6AM	0.07	0.14	0.16	0.39	0.4600	
	6AM - Noon	-0.03	0.03	0.13	0.30	0.3682	
	Noon - 6PM	0.20	0.02	0.19↑	-0.05	0.0627	
	6PM - Midnight	0.15	0.14	0.07	-0.25	0.0892	
15	Midnight - 6AM	0.15	0.24↑	0.17↑	0.12	0.6932	
	6AM - Noon	-0.02	0.01	0.06	-0.09	0.5297	
	Noon - 6PM	0.19	0.07	0.13	-0.07	0.3906	
	6PM - Midnight	0.18	0.17↑	0.10	0.01	0.2725	
16	Midnight - 6AM	0.15	0.26↑	0.19↑	0.21	0.6898	
	6AM - Noon	-0.23	-0.04	-0.04	0.08	0.2623	
	Noon - 6PM	0.09	-0.29	0.15	0.13	0.1899	
	6PM - Midnight	0.10	0.11	0.12	0.20	0.9313	
17	Midnight - 6AM	0.06	0.18↑	0.19↑	0.44↑	0.0563	-0.38 (1<4) 0.0378
	6AM - Noon	0.01	0.00	0.12	0.39	0.4248	
	Noon - 6PM	0.16	-0.02	0.55↑	0.33	0.3072	
	6PM - Midnight	0.01	0.07	0.30↑	0.25	0.3649	
18	Midnight - 6AM	0.03	0.11	0.31↑	0.22	0.2852	
	6AM - Noon	-0.37	-0.26	-0.04	0.10	0.1052	
	Noon - 6PM	0.15	-0.12	0.20	0.52	0.1263	
	6PM - Midnight	0.13	0.07	0.20	0.55↑	0.1615	
19	Midnight - 6AM	0.10	0.12	0.24↑	0.29	0.3111	
	6AM - Noon	-0.08	-0.08	0.08	-0.02	0.2477	
	Noon - 6PM	0.05	-0.11	0.17	0.53	0.2366	
	6PM - Midnight	0.13	0.07	0.14	1.11↑	0.0181 *	-0.97 (3<4) 0.0360 -1.04 (2<4) 0.0187 -0.98 (1<4) 0.0437

Table 9. (Continued)

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
20	Midnight - 6AM	0.05	0.10	0.15	0.77↑	0.0458 *	
	6AM - Noon	-0.17	-0.11	0.02	0.76↑	0.0148 *	-0.87 (2<4) 0.0192 -0.93 (1<4) 0.0189
	Noon - 6PM	-0.03	-0.15	0.14	0.63	0.0722	
	6PM - Midnight	0.05	-0.04	0.06	0.30	0.5870	
21	Midnight - 6AM	0.00	0.06	0.12	0.32	0.8311	
	6AM - Noon	-0.08	-0.07	0.05	-0.45	0.8189	
	Noon - 6PM	0.05	-0.11	0.19	0.28	0.3525	
	6PM - Midnight	-0.05	-0.01	0.05	0.15	0.3563	
22	Midnight - 6AM	-0.04	0.06	0.05	0.20	0.1439	
	6AM - Noon	-0.08	-0.11	0.04	0.11	0.0483 *	
	Noon - 6PM	0.06	-0.12	0.17	0.03	0.1957	
	6PM - Midnight	0.04	-0.05	0.08	0.28	0.1446	
23	Midnight - 6AM	-0.01	0.10	0.09	0.50	0.1358	
	6AM - Noon	-0.04	-0.03	0.10	0.28	0.1213	
	Noon - 6PM	0.05	-0.28	-0.06	-0.16	0.5372	
	6PM - Midnight	0.18	0.16↑	0.18↑	0.19	0.9913	
24	Midnight - 6AM	0.10	0.23↑	0.21↑	0.20	0.6619	
	6AM - Noon	-0.08	-0.02	0.05	0.05	0.5484	
	Noon - 6PM	-0.14	-0.06	0.13	-0.12	0.2227	
	6PM - Midnight	-0.10	0.00	0.05	-0.04	0.1913	
25	Midnight - 6AM	-0.05	0.04	0.07	0.04	0.5211	
	6AM - Noon	0.01	-0.16	0.04	0.00	0.3194	
	Noon - 6PM	0.10	-0.20	0.07	-0.24	0.1261	
	6PM - Midnight	0.23↑	0.14↑	0.13	0.00	0.1445	
26	Midnight - 6AM	0.14	0.18↑	0.18↑	0.10	0.8542	
	6AM - Noon	-0.06	-0.06	0.04	-0.06	0.4563	
	Noon - 6PM	0.10	-0.03	0.17	-0.16	0.2066	
	6PM - Midnight	0.10	0.04	0.12	-0.01	0.6616	
27	Midnight - 6AM	0.11	0.14↑	0.14	0.05	0.7726	
	6AM - Noon	-0.06	-0.03	0.08	-0.01	0.3985	
	Noon - 6PM	0.03	-0.12	0.14	-0.16	0.1189	
	6PM - Midnight	-0.01	0.03	0.24↑	-0.03	0.0133 *	-0.25 (1<3) 0.0275 -0.21 (2<3) 0.0403 0.27 (4<3) 0.0456
28	Midnight - 6AM	0.09	0.13	0.18↑	0.05	0.5951	
	6AM - Noon	0.00	-0.04	0.08	0.02	0.6234	
	Noon - 6PM	0.10	-0.12	0.14	-0.21	0.0624	
	6PM - Midnight	0.00	-0.01	0.15↑	0.03	0.1878	
29	Midnight - 6AM	0.03	0.12↑	0.12↑	0.19	0.2550	
	6AM - Noon	-0.14	-0.09	0.03	-0.07	0.1856	
	Noon - 6PM	0.04	-0.13	0.09	-0.18	0.1871	
	6PM - Midnight	0.07	-0.01	0.23↑	0.00	0.0396 *	-0.24 (2<3) 0.0357

Table 9. (Continued)

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
30	Midnight - 6AM	0.06	0.11	0.18↑	0.20	0.4022	
	6AM - Noon	-0.01	-0.09	0.06	0.04	0.5075	
	Noon - 6PM	0.06	-0.23↓	0.01	-0.20	0.1738	
	6PM - Midnight	0.02	0.05	0.10	0.02	0.7775	
31	Midnight - 6AM	0.06	0.10	0.09	0.06	0.9760	
	6AM - Noon	-0.07	-0.14	-0.03	-0.05	0.6086	
	Noon - 6PM	0.06	-0.15	0.10	-0.17	0.1040	
	6PM - Midnight	-0.10	-0.08	0.05	-0.10	0.3340	
32	Midnight - 6AM	-0.03	0.02	0.07	-0.03	0.6402	
	6AM - Noon	0.03	-0.06	0.10	0.11	0.2000	
	Noon - 6PM	0.03	-0.26↓	0.13	-0.07	0.0356 *	-0.39 (2<3) 0.0275
	6PM - Midnight	0.03	0.06	0.16	0.06	0.6590	
33	Midnight - 6AM	-0.01	0.15↑	0.17↑	0.20	0.2074	
	6AM - Noon	-0.04	-0.06	0.09	-0.01	0.2961	
	Noon - 6PM	0.09	-0.16	0.13	-0.14	0.0490 *	
	6PM - Midnight	-0.05	0.04	0.21↑	0.00	0.0469 *	-0.26 (1<3) 0.0429
34	Midnight - 6AM	0.04	0.12	0.14	0.09	0.6841	
	6AM - Noon	-0.08	-0.10	0.01	-0.06	0.4703	
	Noon - 6PM	0.03	-0.18	0.06	-0.16	0.1777	
	6PM - Midnight	-0.03	0.09	0.25↑	-0.03	0.0182 *	-0.28 (1<3) 0.0222
35	Midnight - 6AM	-0.03	0.11	0.10	0.05	0.4614	
	6AM - Noon	-0.08	-0.11	0.01	-0.07	0.4668	
	Noon - 6PM	0.09	-0.18	0.08	-0.13	0.0661	
	6PM - Midnight	-0.01	0.05	0.20↑	-0.02	0.1109	
36	Midnight - 6AM	0.04	0.12	0.15	-0.21	0.0577	
	6AM - Noon	-0.09	-0.18↓	-0.09	-0.37	0.1312	
	Noon - 6PM	0.03	-0.23↓	0.06	-0.40	0.0086 *	0.43 (4<1) 0.0378 0.46 (4<3) 0.0201
	6PM - Midnight	-0.03	0.04	0.19↑	-0.02	0.1282	
37	Midnight - 6AM	0.01	0.08	0.07	-0.10	0.1417	
	6AM - Noon	-0.09	-0.32↓	-0.07	-0.04	0.0904	
	Noon - 6PM	0.08	-0.31↓	-0.02	-0.51	0.0252 *	0.59 (4<1) 0.0454
	6PM - Midnight	0.10	0.08	0.15↑	0.13	0.7430	
38	Midnight - 6AM	0.01	0.14	0.12	0.13	0.5262	
	6AM - Noon	-0.13	-0.18↓	-0.05	-0.01	0.3728	
	Noon - 6PM	0.01	-0.24↓	0.04	-0.15	0.1405	
	6PM - Midnight	-0.03	0.03	0.18↑	0.08	0.1363	

Table 9. (Continued)

Study Day	Time	Mean Baseline Adjusted Value, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value#
		1	2	3	4		
39	Midnight - 6AM	-0.02	0.10	0.12	0.04	0.4091	
	6AM - Noon	-0.10	-0.14	-0.09	-0.05	0.8824	

Cells contain all pairwise comparisons that were 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<2)" indicates that the mean baseline adjusted value in Group 2 was significantly greater than that in Group 1], and (3) the Tukey-adjusted p-value.

NA Data was not available for this group at this study time.

↑, ↓ "↑" indicates that the mean at the study time was significantly greater than that at baseline, while "↓" indicates that the mean at the study time was significantly less than that at baseline (at the 0.05 level).

* Group effect was significant at the 0.05 level.

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	2/5	0.40 (0.05, 0.85)	15.51	0.5161
	2	2/7	0.29 (0.04, 0.71)	7.38	
	3	3/7	0.43 (0.10, 0.82)	11.42	
	4	5/7	0.71 (0.29, 0.96)	6.00	
Heart Rate	1	5/5	1.00 (0.48, 1.00)	16.75	0.2855
	2	5/7	0.71 (0.29, 0.96)	19.10	
	3	7/7	1.00 (0.59, 1.00)	6.82	
	4	5/7	0.71 (0.29, 0.96)	7.10	
RP Expiratory Time	1	3/5	0.60 (0.15, 0.95)	25.08	0.7925
	2	6/7	0.86 (0.42, 1.00)	21.63	
	3	5/7	0.71 (0.29, 0.96)	15.60	
	4	4/7	0.57 (0.18, 0.90)	9.94	
RP Inspiratory Time	1	2/5	0.40 (0.05, 0.85)	11.00	0.8224
	2	4/7	0.57 (0.18, 0.90)	22.19	
	3	5/7	0.71 (0.29, 0.96)	12.90	
	4	3/7	0.43 (0.10, 0.82)	6.58	
RP Integral	1	3/5	0.60 (0.15, 0.95)	27.17	0.7925
	2	6/7	0.86 (0.42, 1.00)	26.96	
	3	5/7	0.71 (0.29, 0.96)	12.35	
	4	4/7	0.57 (0.18, 0.90)	11.38	
RP Peak Amplitude	1	4/5	0.80 (0.28, 0.99)	21.50	0.68788
	2	6/7	0.86 (0.42, 1.00)	28.54	
	3	7/7	1.00 (0.59, 1.00)	22.46	
	4	5/7	0.71 (0.29, 0.96)	8.00	
RP Respiratory Rate	1	3/5	0.60 (0.15, 0.95)	30.58	0.2096
	2	7/7	1.00 (0.59, 1.00)	22.11	
	3	4/7	0.57 (0.18, 0.90)	6.44	
	4	4/7	0.57 (0.18, 0.90)	7.44	
Temperature	1	3/5	0.6 (0.15, 0.95)	11.17	0.5542
	2	6/7	0.86 (0.42, 1.00)	23.42	
	3	4/7	0.57 (0.18, 0.90)	10.81	
	4	6/7	0.86 (0.42, 1.00)	12.08	

N Number of animals.
Means exclude those animals that were never abnormal.

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

Parameter	Group Effect P-Value
Activity	0.2687
Heart Rate	0.5098
RP Expiratory Time	0.9260
RP Inspiratory Time	0.8017
RP Integral	0.7576
RP Peak Amplitude	0.5836
RP Respiratory Rate	0.1549
Temperature	0.6605

* Group effect was significant at the 0.05 level.

Table 12. Results of Overall Log-Rank Tests Comparing the Duration of Abnormality between Groups by Parameter

Parameter	Group Effect P-Value
Activity	0.1384
Heart Rate	0.4335
RP Expiratory Time	0.9781
RP Inspiratory Time	0.5030
RP Integral	0.7944
RP Peak Amplitude	0.2260
RP Respiratory Rate	0.0710
Temperature	0.3558

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

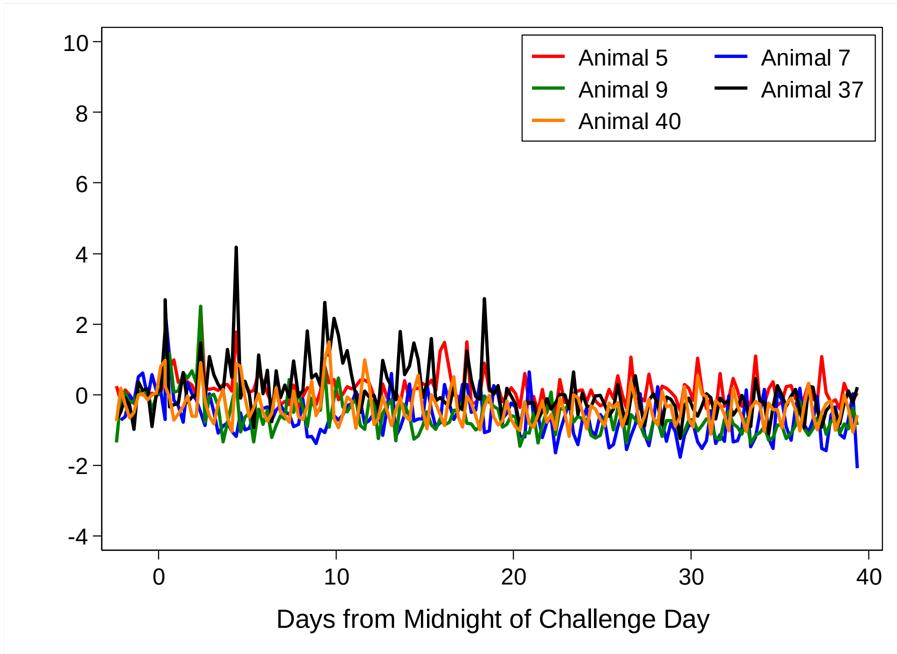


Figure 1a. **Plot of baseline adjusted Activity (counts/minute) for each animal in Group 1.**

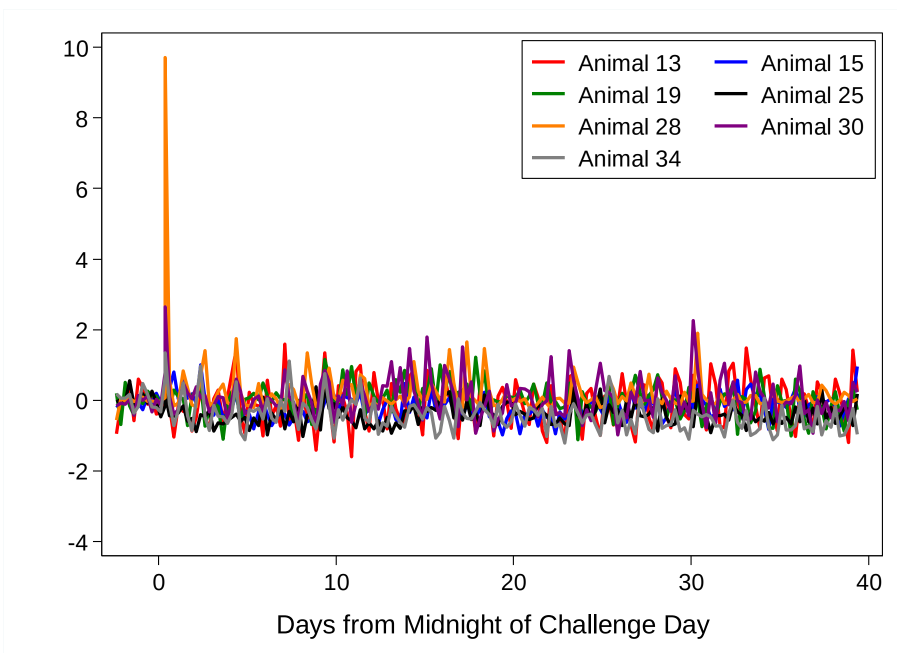


Figure 1b. **Plot of baseline adjusted Activity (counts/minute) for each animal in Group 2.**

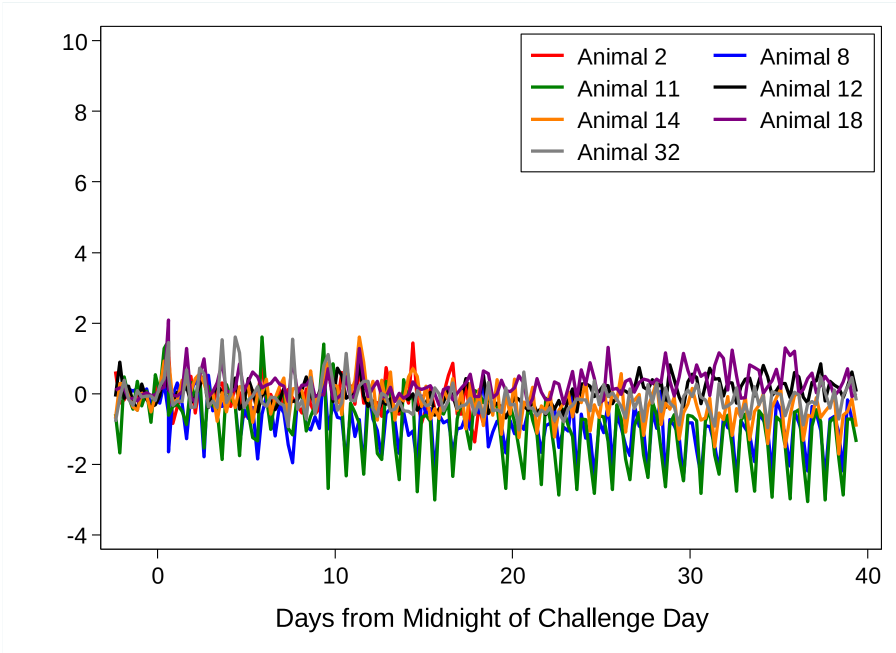


Figure 1c. **Plot of baseline adjusted Activity (counts/minute) for each animal in Group 3.**

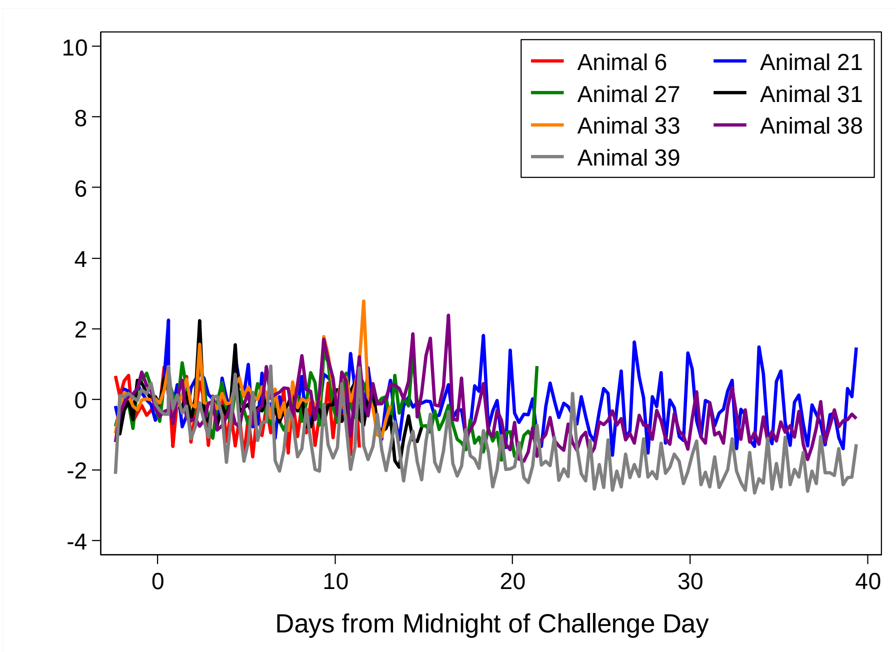


Figure 1d. **Plot of baseline adjusted Activity (counts/minute) for each animal in Group 4.**

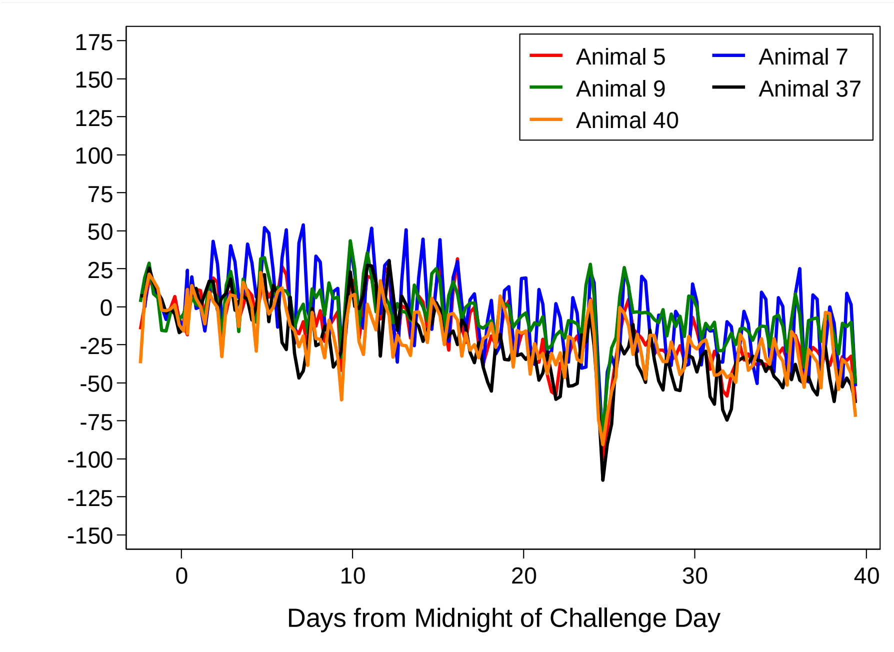


Figure 2a. **Plot of baseline adjusted Heart Rate (BPM) for each animal in Group 1.**

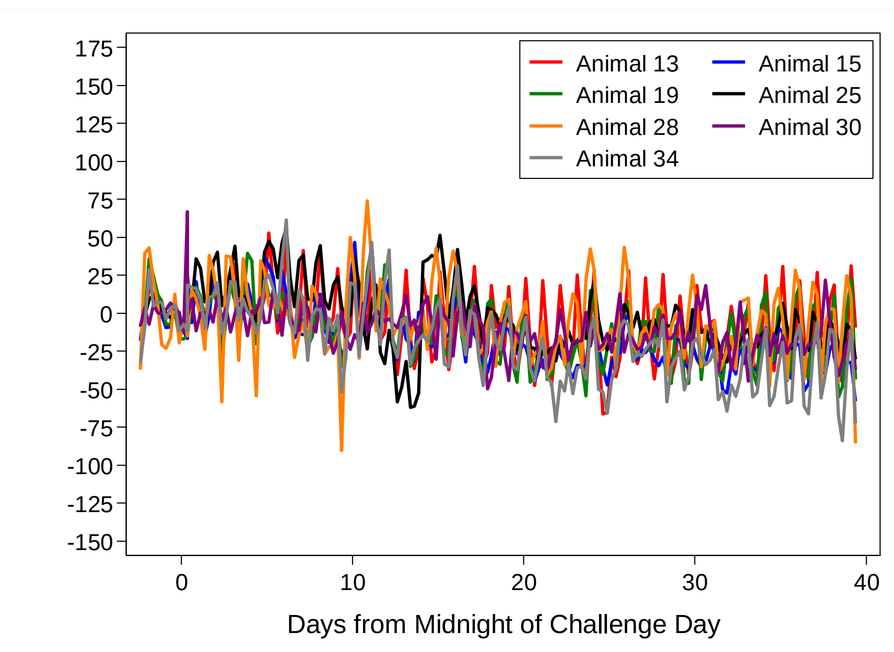


Figure 2b. **Plot of baseline adjusted Heart Rate (BPM) for each animal in Group 2.**

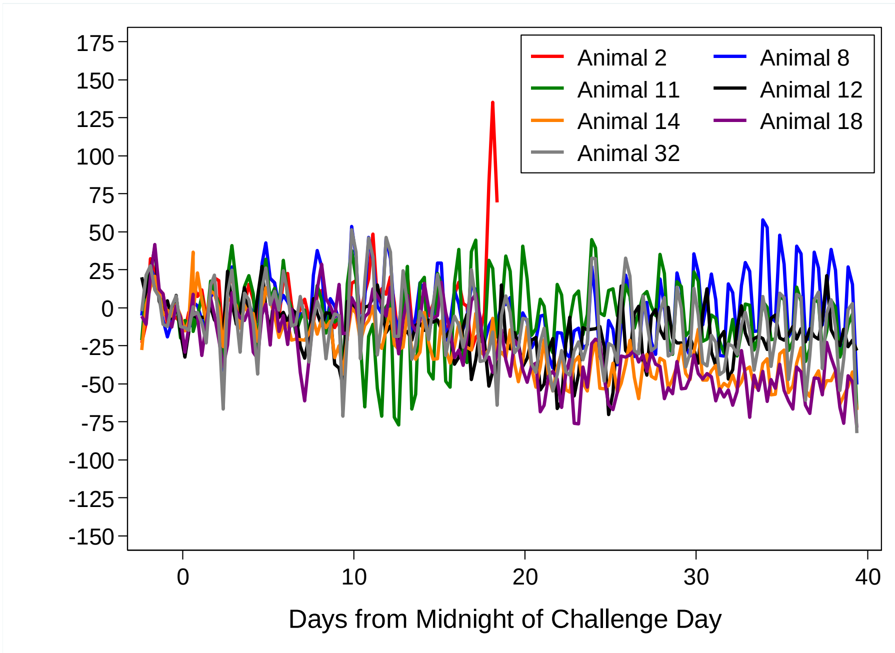


Figure 2c. Plot of baseline adjusted Heart Rate (BPM) for each animal in Group 3.

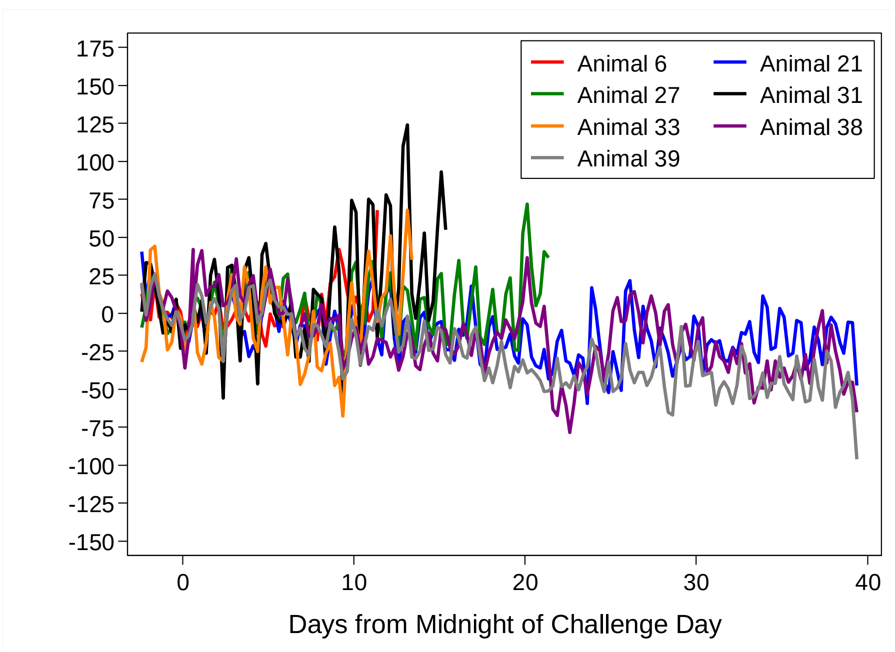


Figure 2d. Plot of baseline adjusted Heart Rate (BPM) for each animal in Group 4.

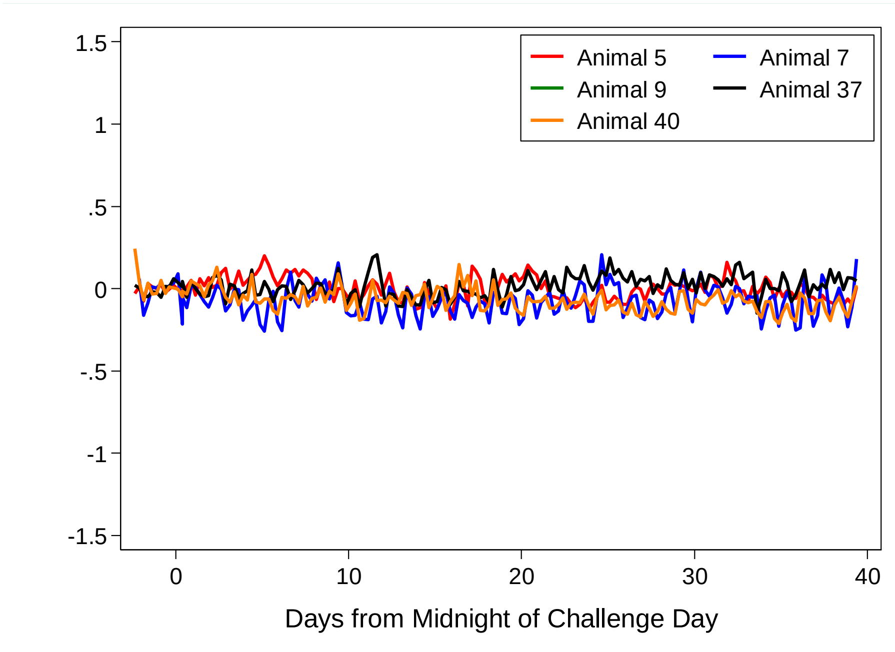


Figure 3a. **Plot of baseline adjusted RP Expiratory Time (seconds) for each animal in Group 1.**

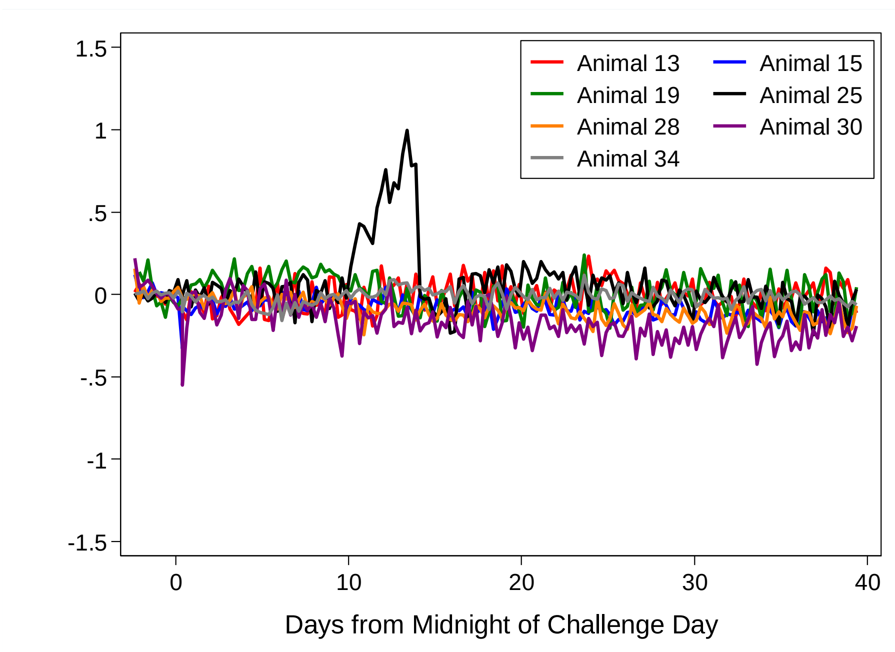


Figure 3b. **Plot of baseline adjusted RP Expiratory Time (seconds) for each animal in Group 2.**

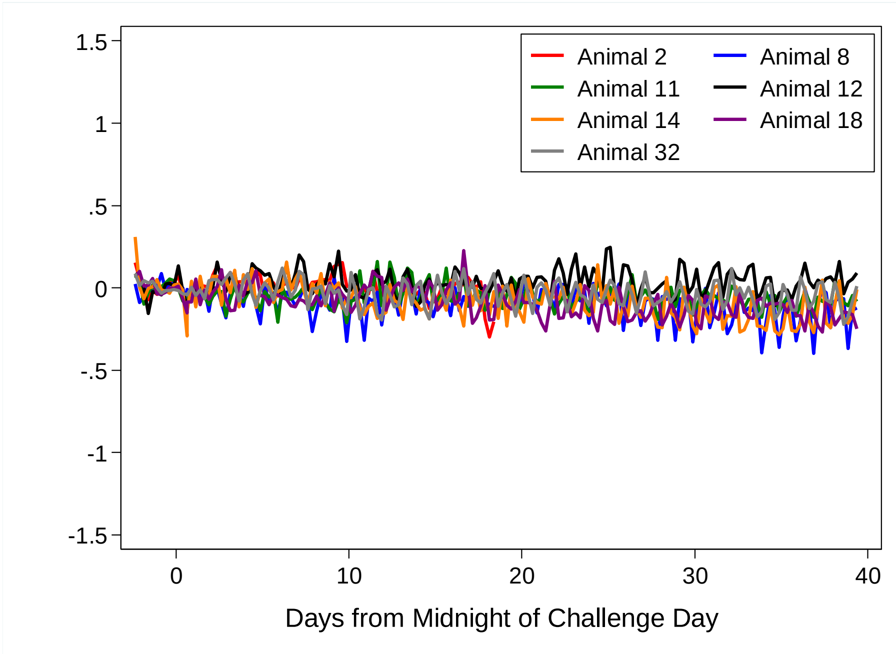


Figure 3c. **Plot of baseline adjusted RP Expiratory Time (seconds) for each animal in Group 3.**

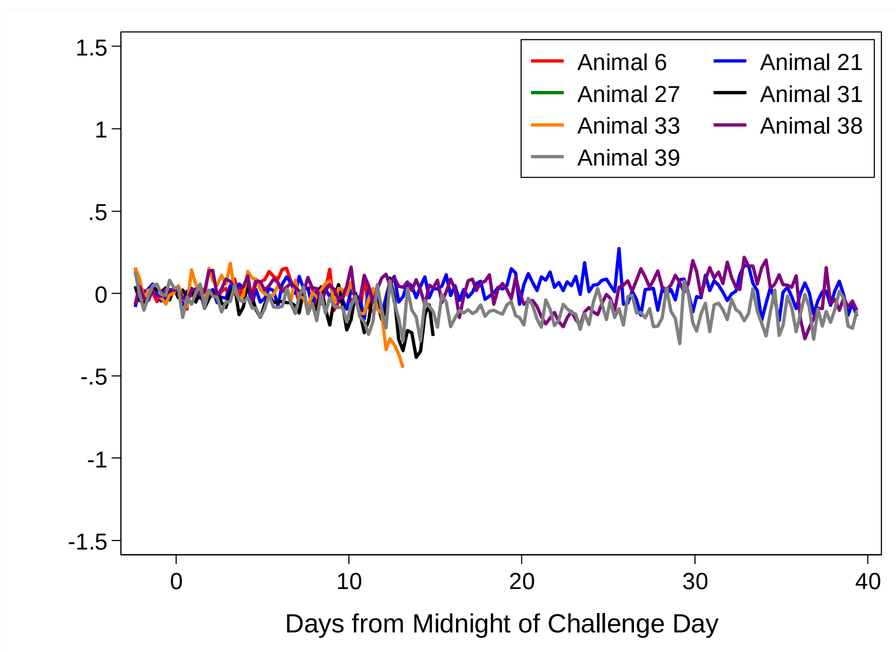


Figure 3d. **Plot of baseline adjusted RP Expiratory Time (seconds) for each animal in Group 4.**

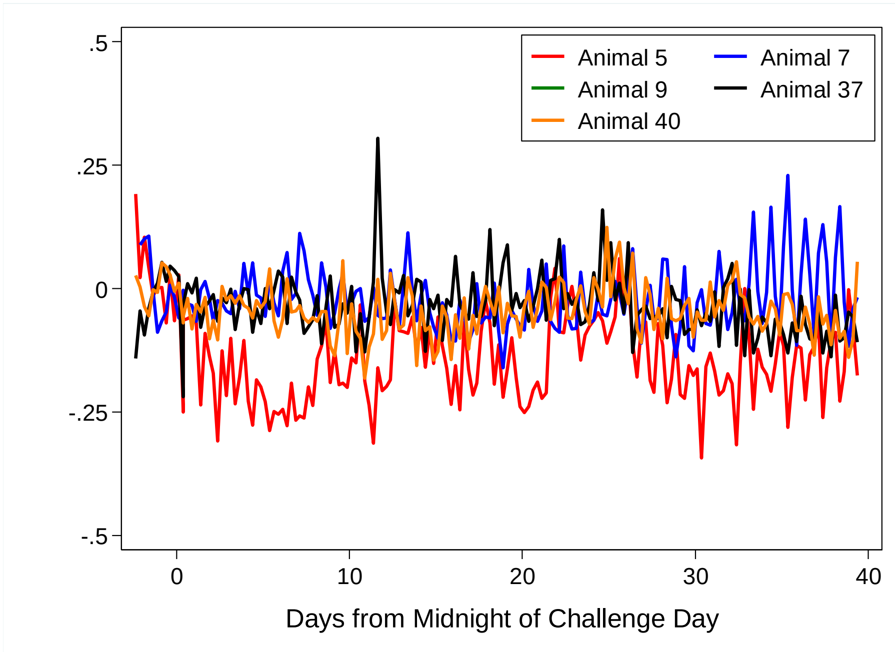


Figure 4a. **Plot of baseline adjusted RP Inspiratory Time (seconds) for each animal in Group 1.**

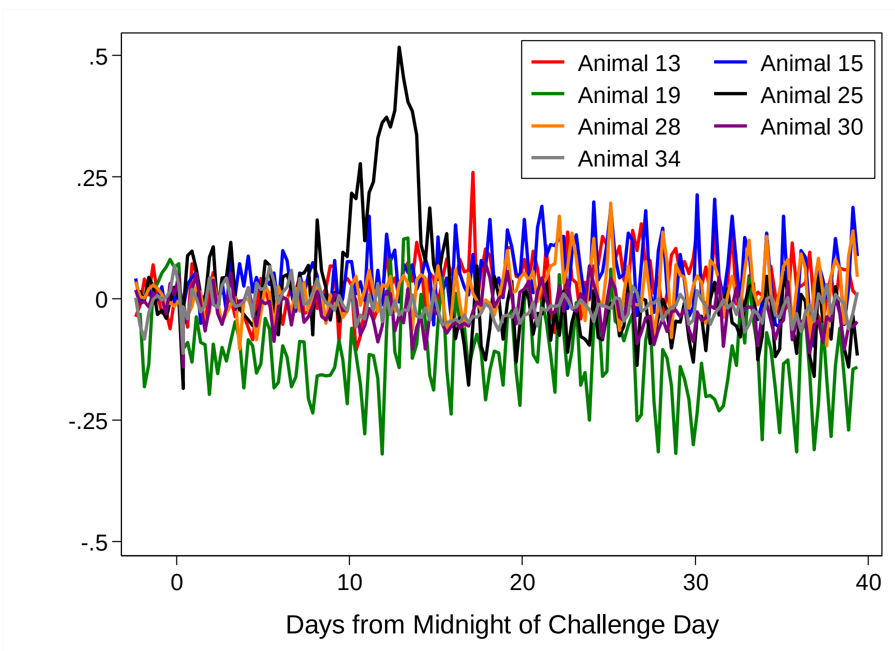


Figure 4b. **Plot of baseline adjusted RP Inspiratory Time (seconds) for each animal in Group 2.**

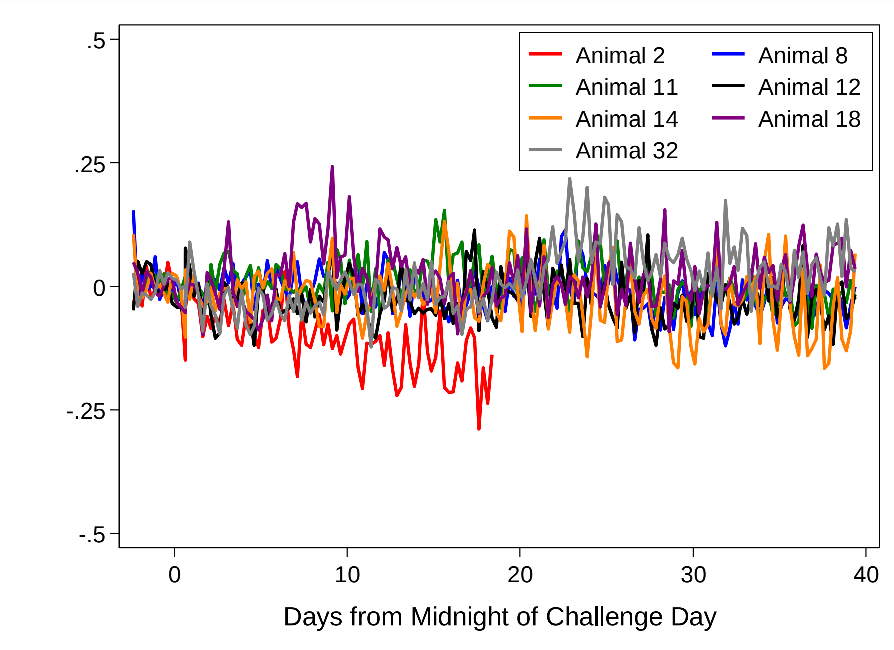


Figure 4c. Plot of baseline adjusted RP Inspiratory Time (seconds) for each animal in Group 3.

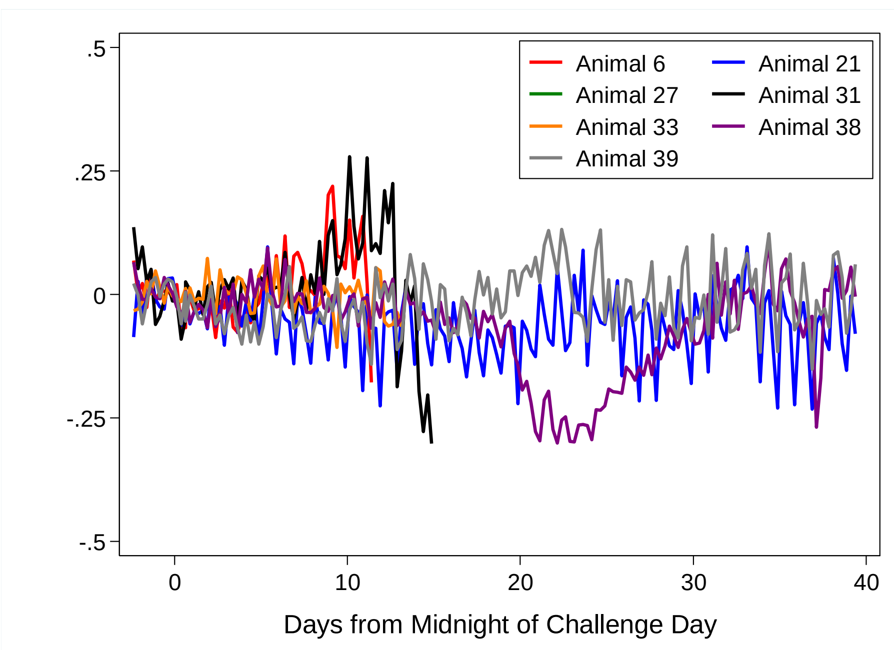


Figure 4d. Plot of baseline adjusted RP Inspiratory Time (seconds) for each animal in Group 4.

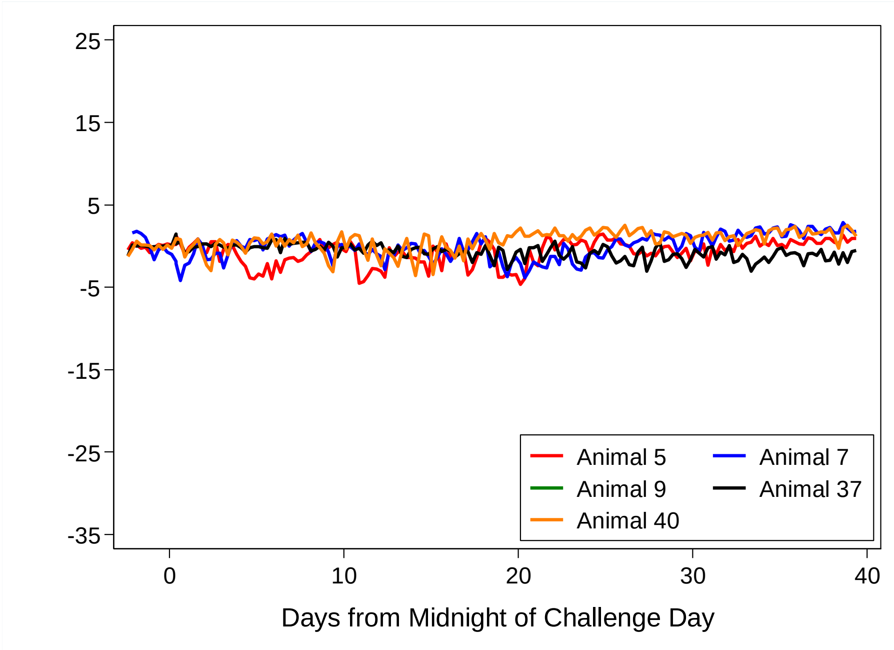


Figure 5a. **Plot of baseline adjusted RP Integral (mmHg-seconds) for each animal in Group 1.**

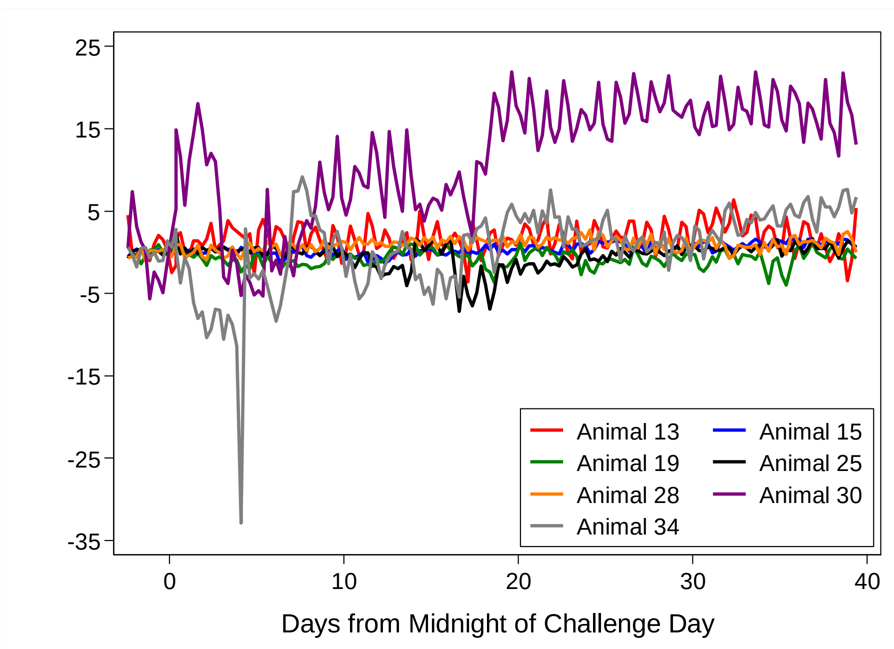


Figure 5b. **Plot of baseline adjusted RP Integral (mmHg-seconds) for each animal in Group 2.**

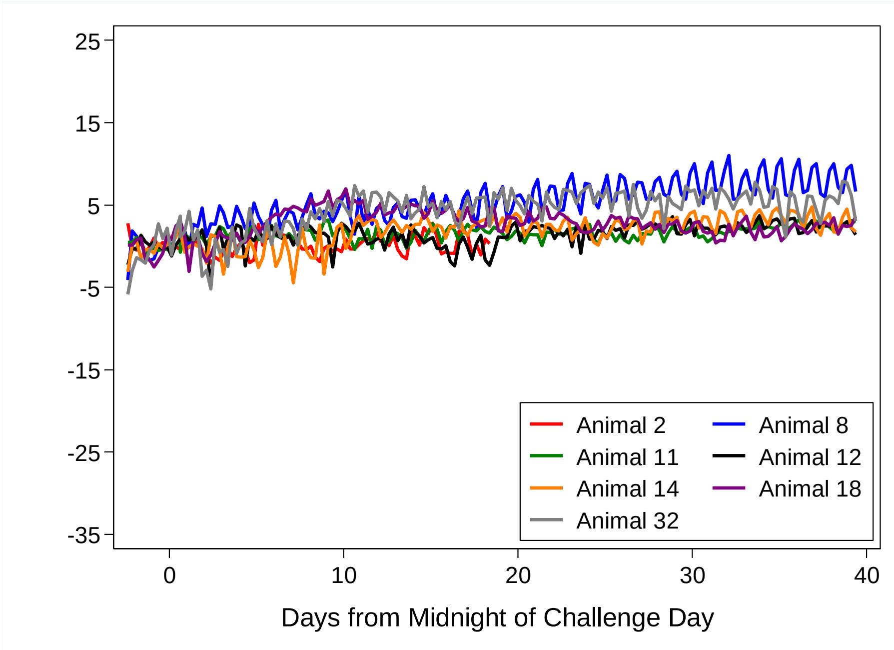


Figure 5c. **Plot of baseline adjusted RP Integral (mmHg-seconds) for each animal in Group 3.**

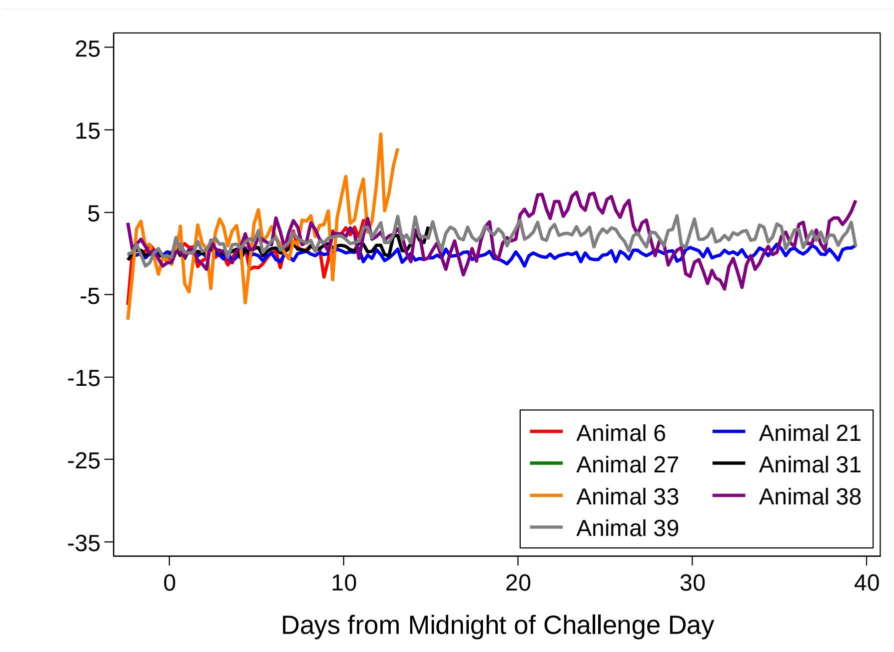


Figure 5d. **Plot of baseline adjusted RP Integral (mmHg-seconds) for each animal in Group 4.**

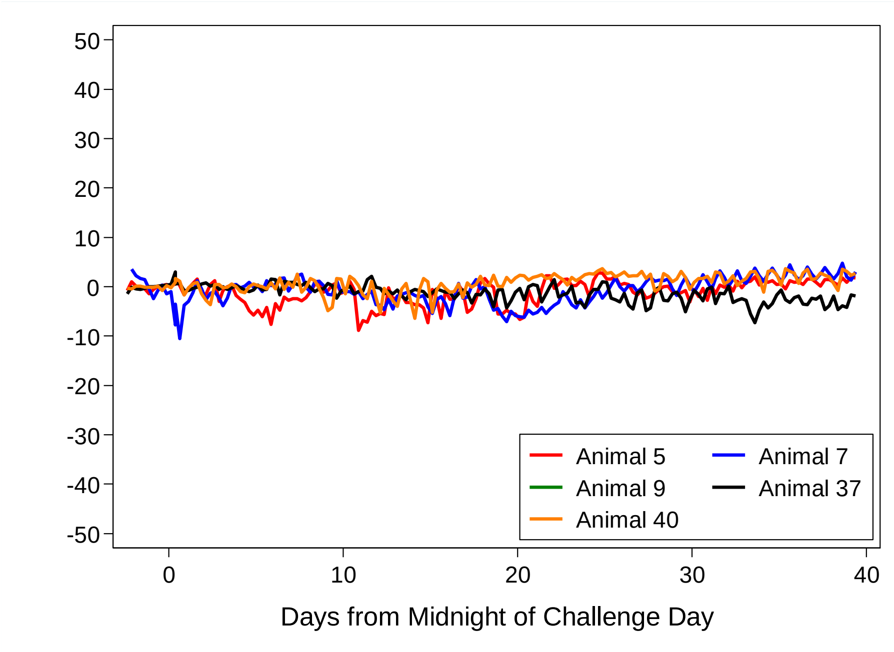


Figure 6a. Plot of baseline adjusted RP Peak Amplitude (mmHg) for each animal in Group 1.

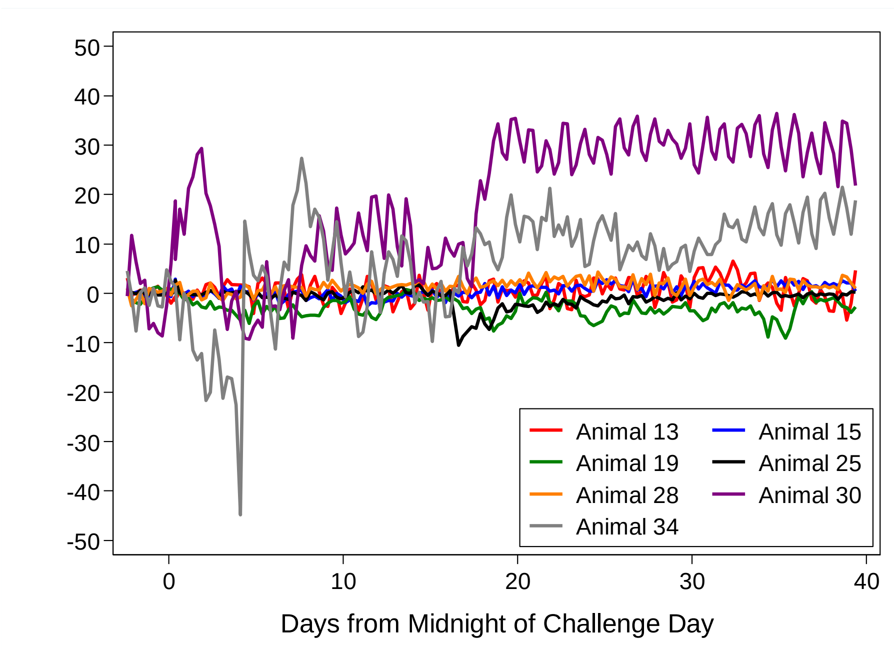


Figure 6b. Plot of baseline adjusted RP Peak Amplitude (mmHg) for each animal in Group 2.

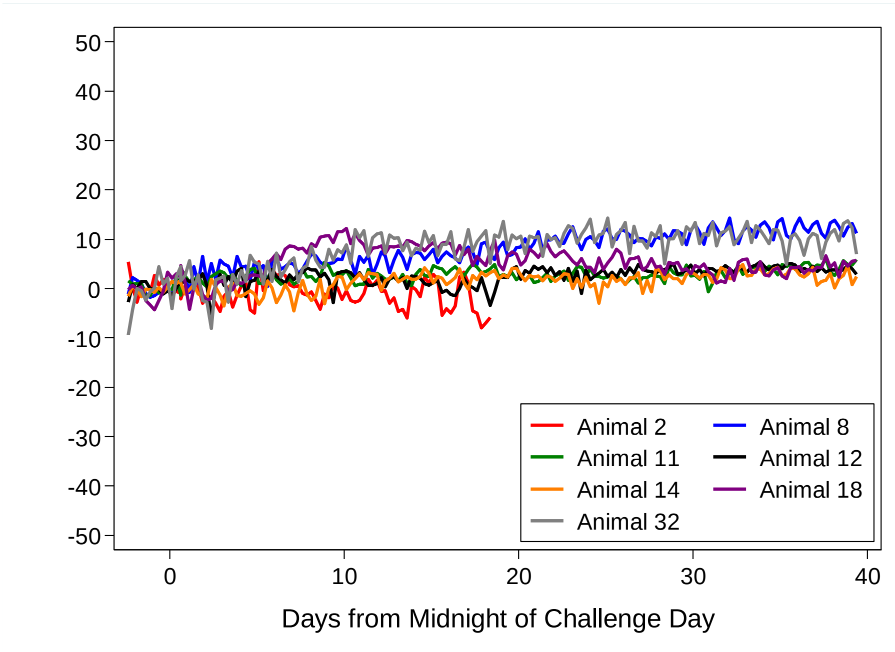


Figure 6c. **Plot of baseline adjusted RP Peak Amplitude (mmHg) for each animal in Group 3.**

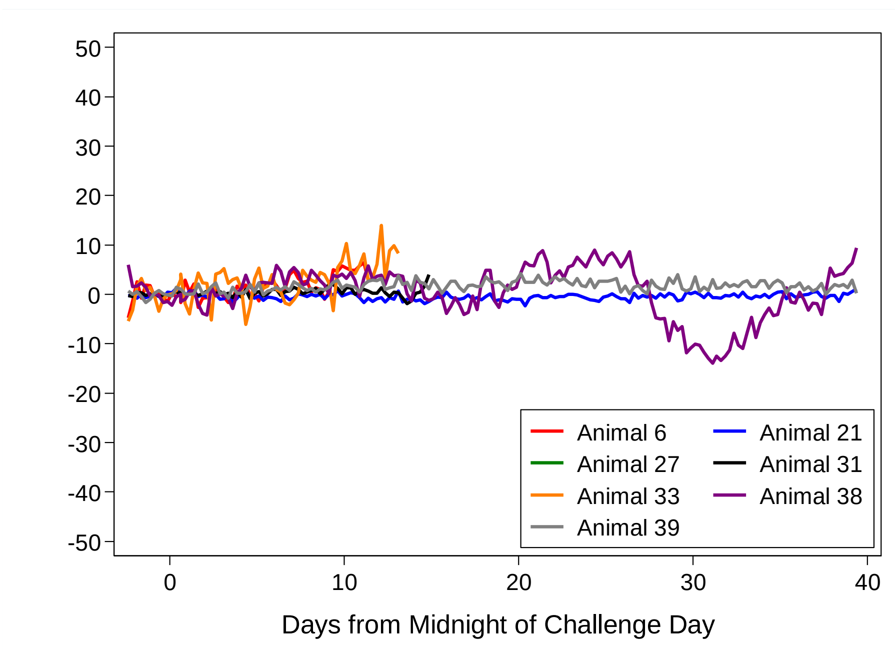


Figure 6d. **Plot of baseline adjusted RP Peak Amplitude (mmHg) for each animal in Group 4.**

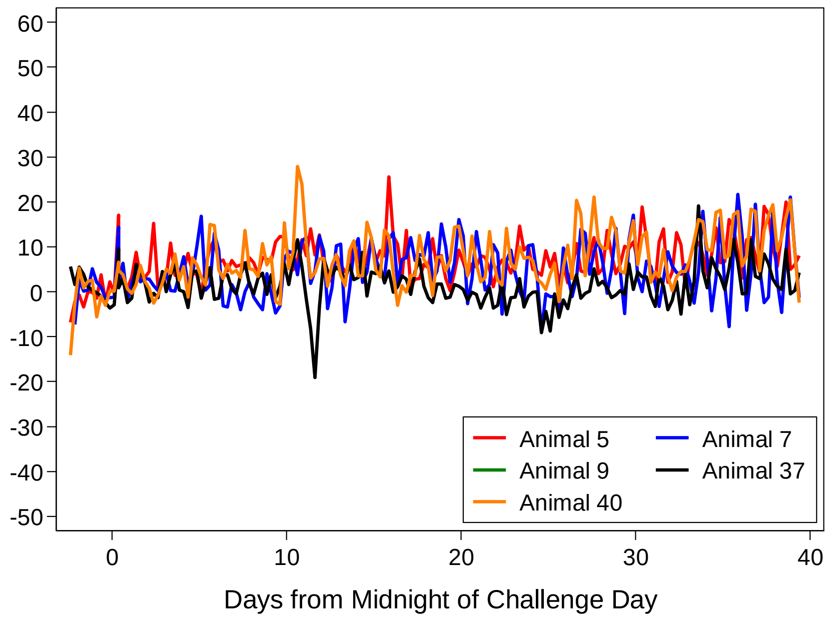


Figure 7a. **Plot of baseline adjusted RP Respiratory Rate (RCPM) for each animal in Group 1.**

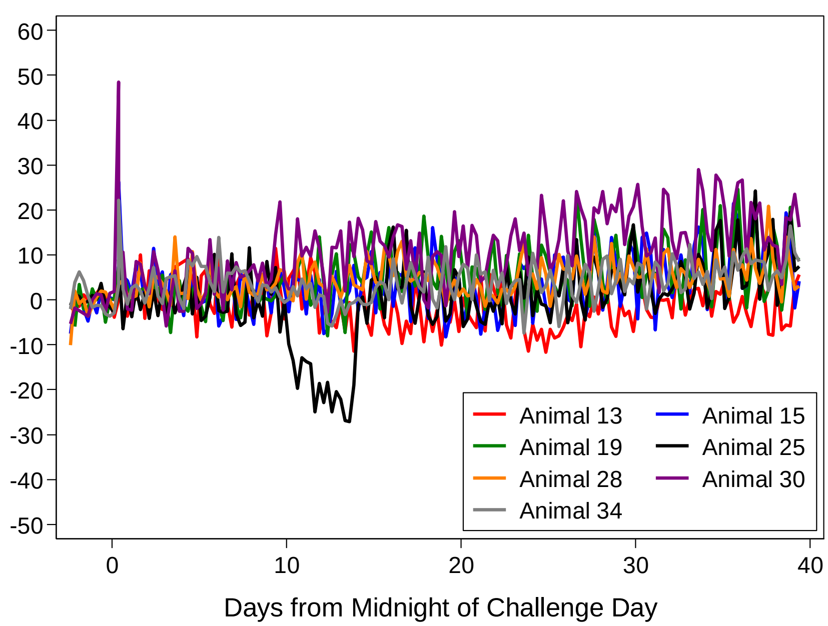


Figure 7b. **Plot of baseline adjusted RP Respiratory Rate (RCPM) for each animal in Group 2.**

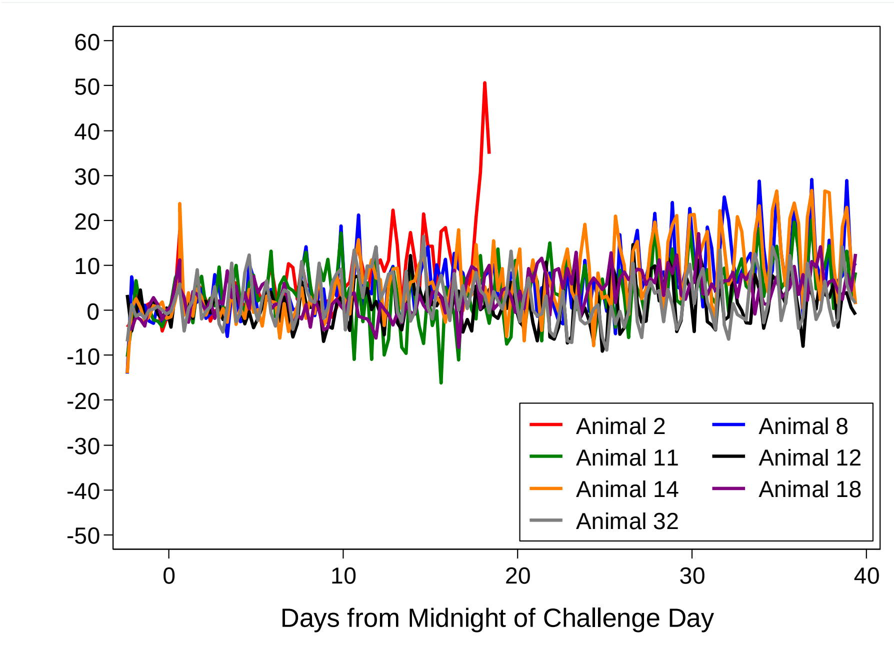


Figure 7c. **Plot of baseline adjusted RP Respiratory Rate (RCPM) for each animal in Group 3.**

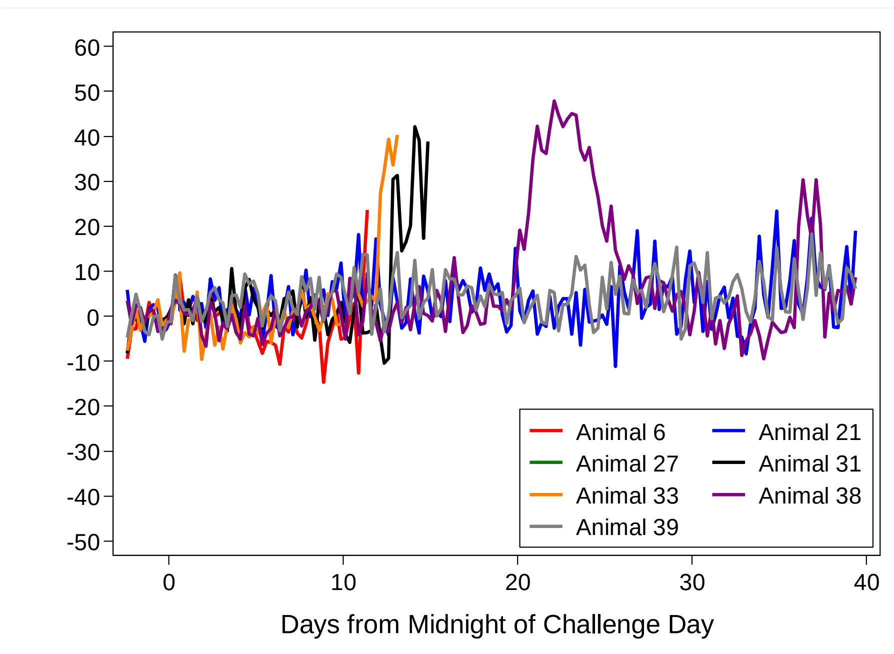


Figure 7d. **Plot of baseline adjusted RP Respiratory Rate (RCPM) for each animal in Group 4.**

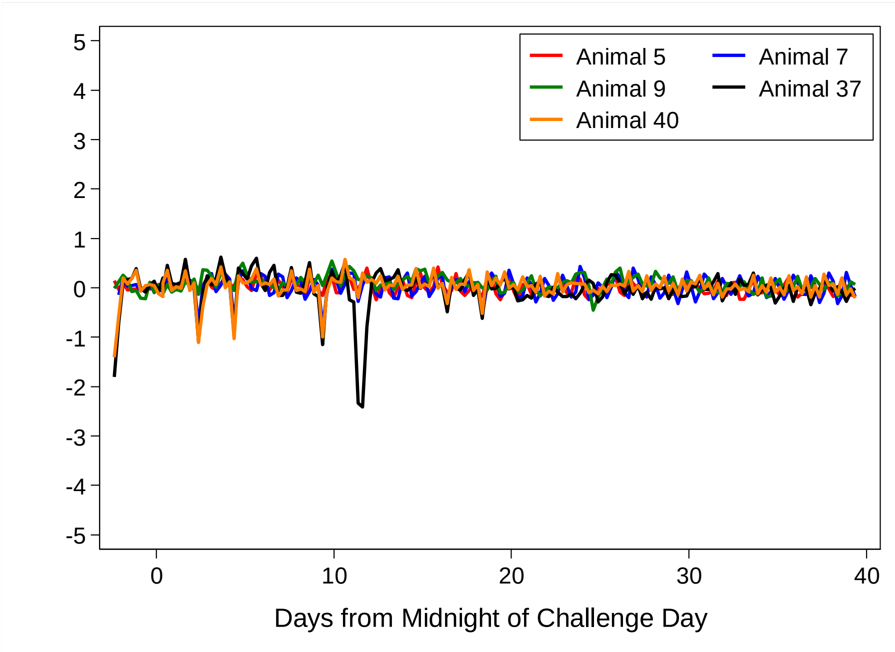


Figure 8a. Plot of baseline adjusted Temperature (Celsius) for each animal in Group 1.

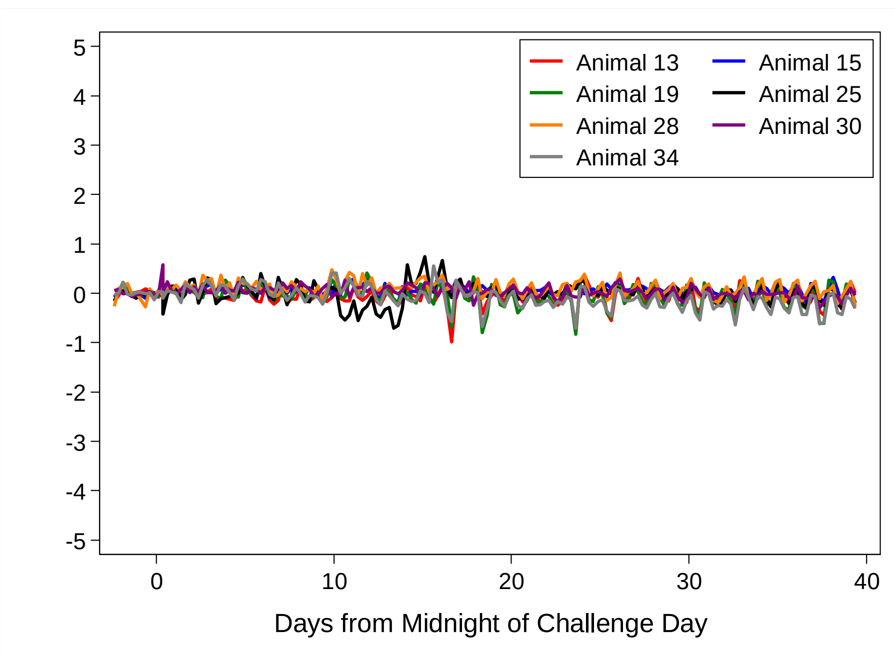


Figure 8b. Plot of baseline adjusted Temperature (Celsius) for each animal in Group 2.

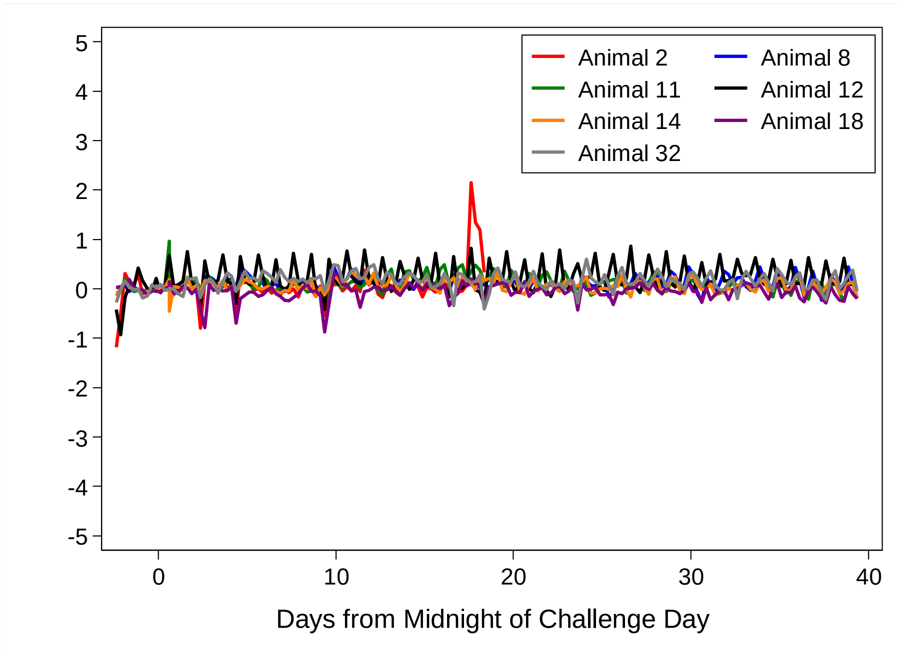


Figure 8c. Plot of baseline adjusted Temperature (Celsius) for each animal in Group 3.

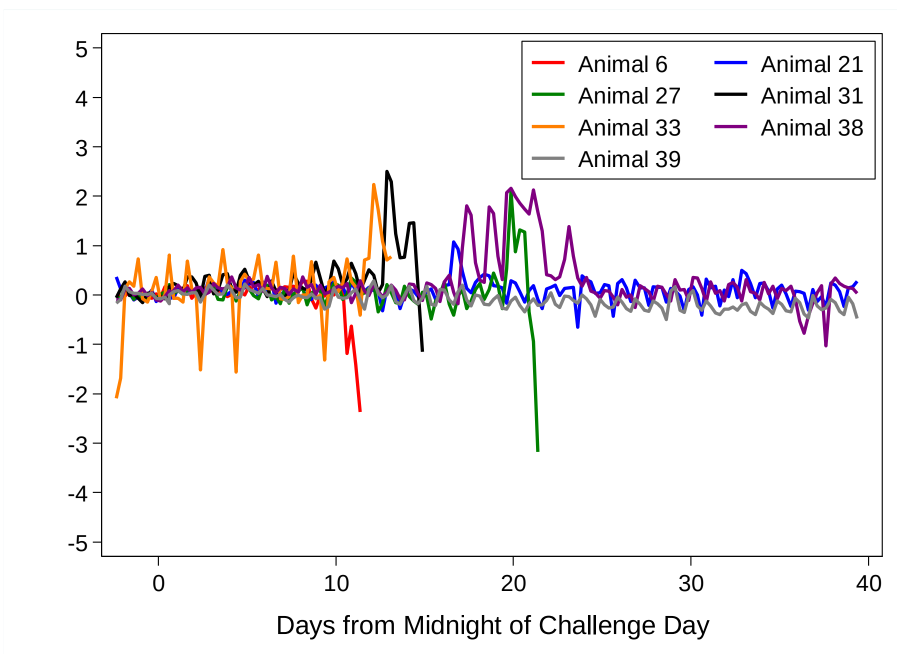


Figure 8d. Plot of baseline adjusted Temperature (Celsius) for each animal in Group 4.

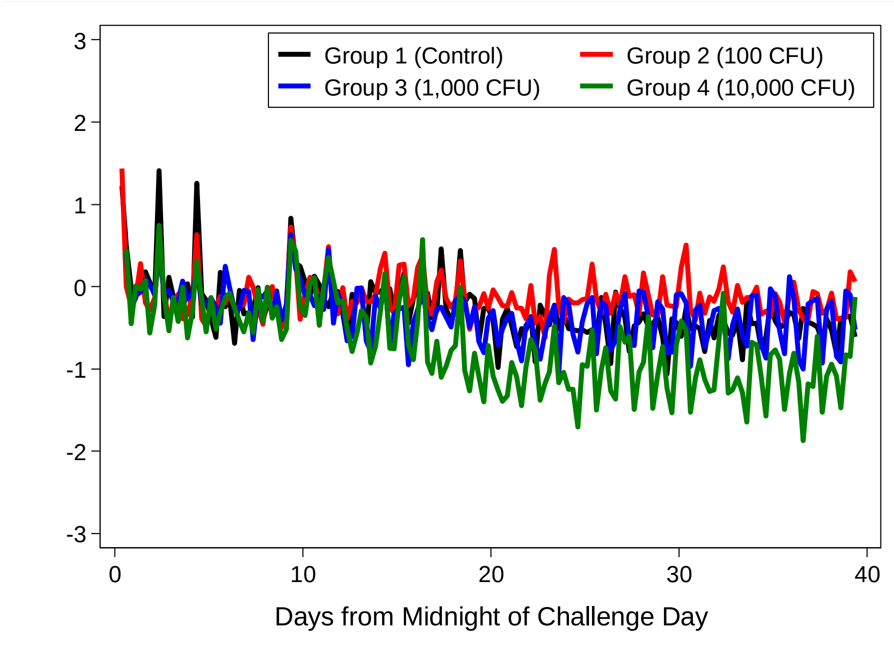


Figure 9. **Plot of mean baseline adjusted Activity (counts/minute) for each group.**

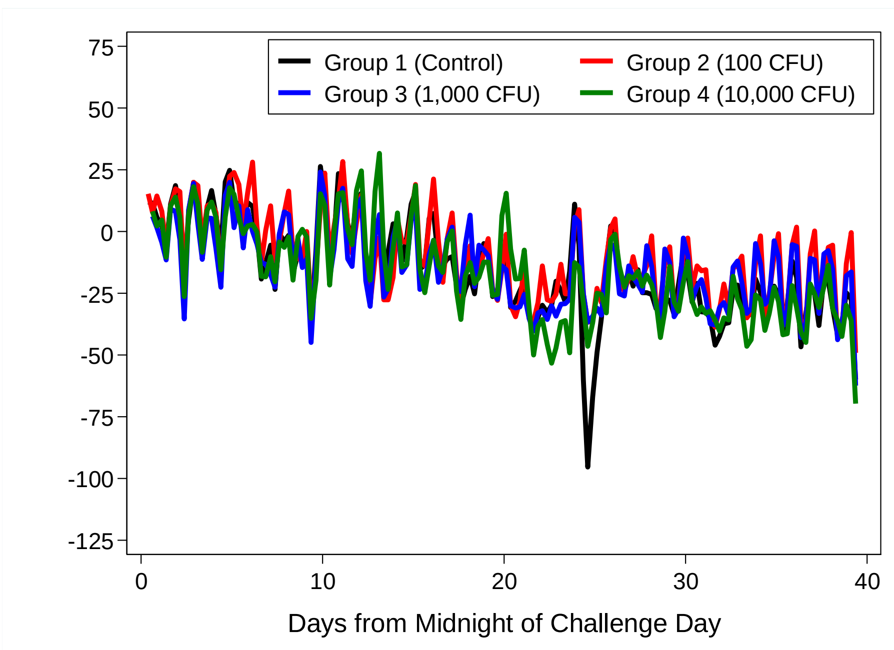


Figure 10. **Plot of mean baseline adjusted Heart Rate (BPM) for each group.**

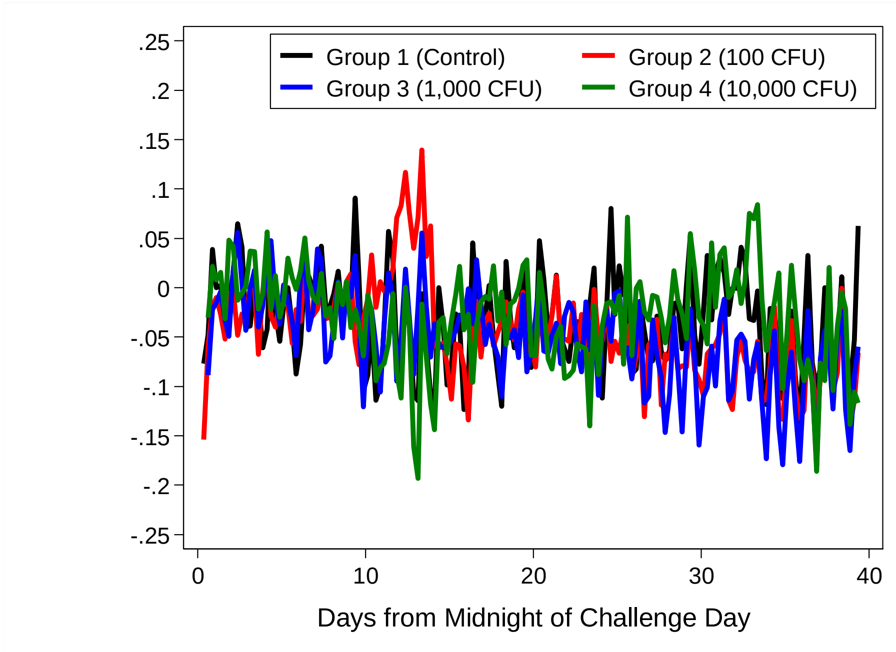


Figure 11. **Plot of mean baseline adjusted RP Expiratory Time (seconds) for each group.**

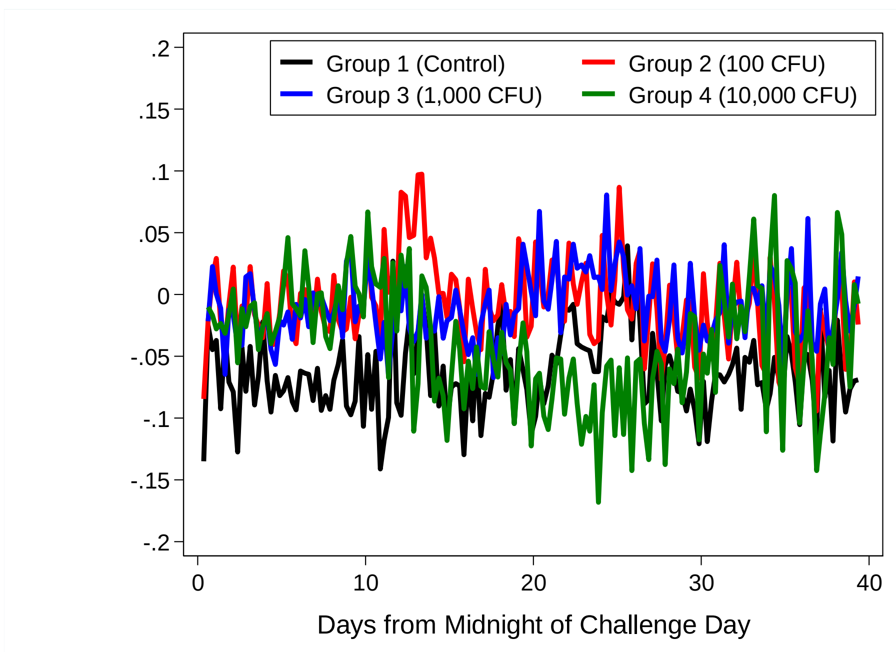


Figure 12. **Plot of mean baseline adjusted RP Inspiratory Time (seconds) for each group.**

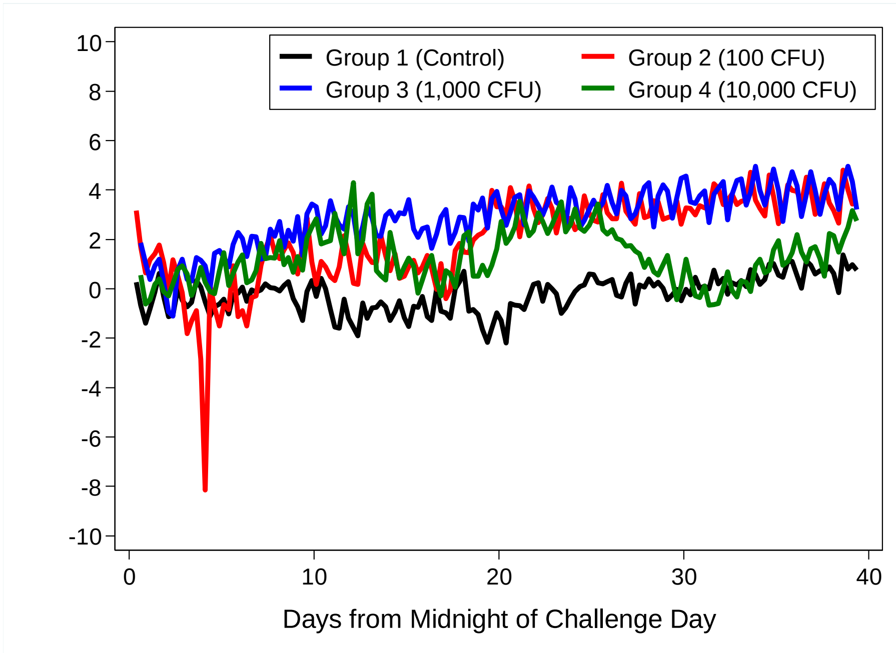


Figure 13. **Plot of mean baseline adjusted RP Integral (mmHg-seconds) for each group.**

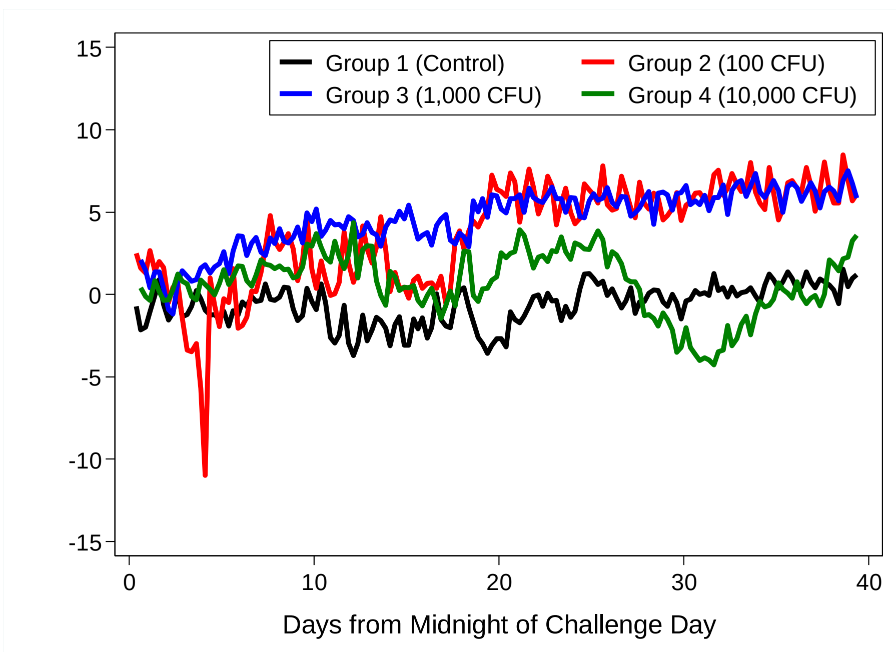


Figure 14. **Plot of mean baseline adjusted RP Peak Amplitude (mmHg) for each group.**

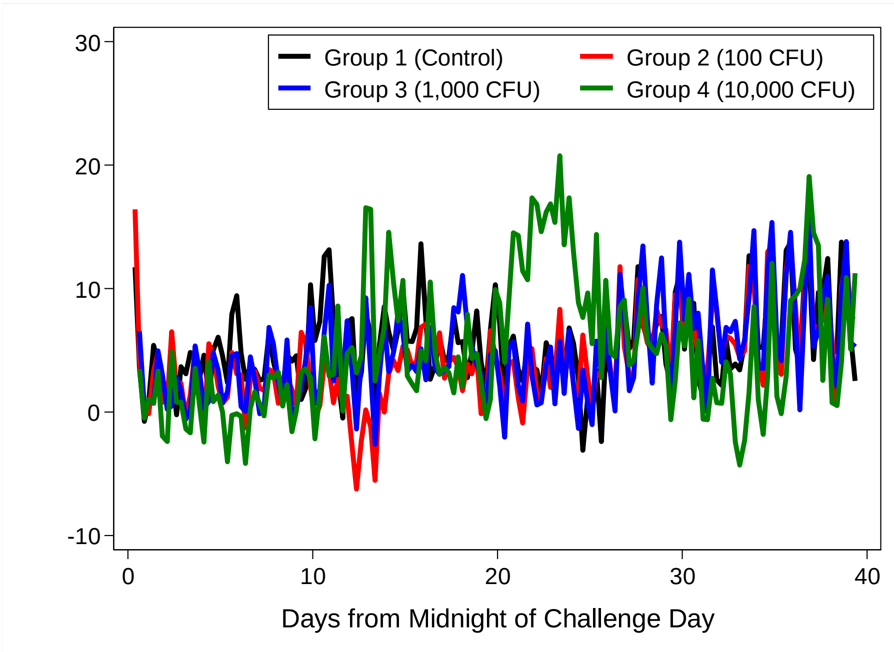


Figure 15. Plot of mean baseline adjusted RP Respiratory Rate (RCPM) for each group.

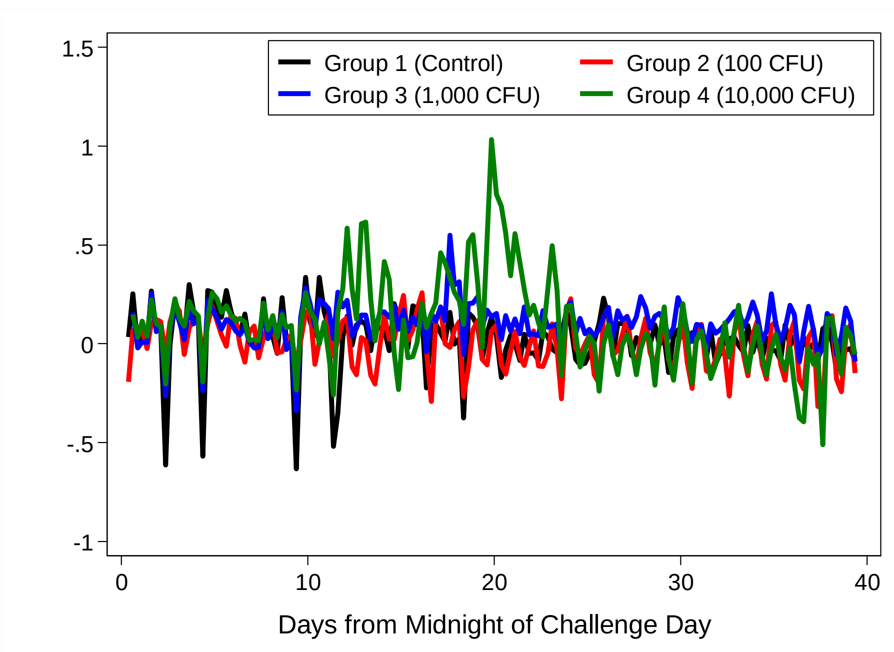


Figure 16. Plot of mean baseline adjusted Temperature (Celsius) for each group.

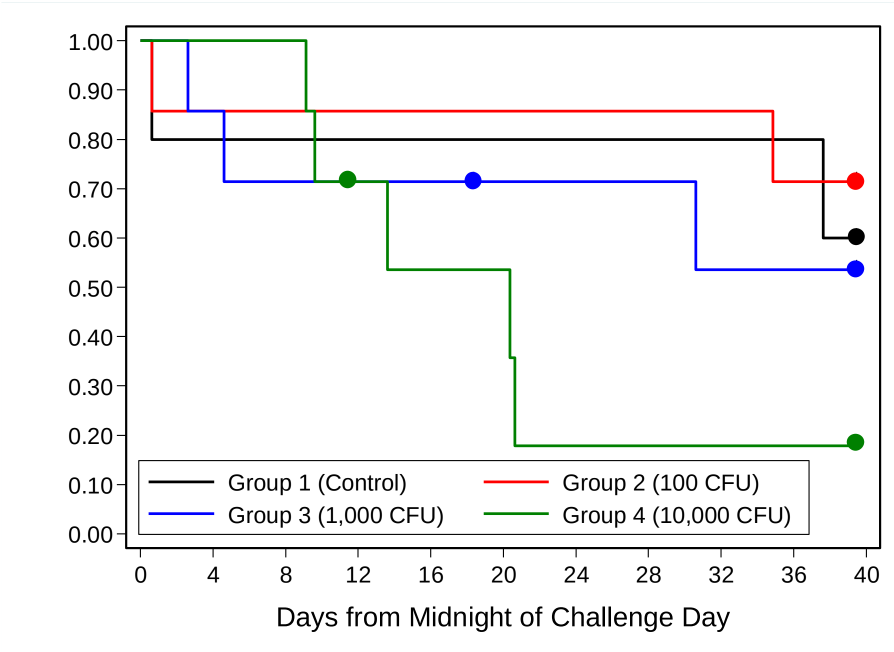


Figure 17. Kaplan-Meier curves for time to abnormality based on Activity.

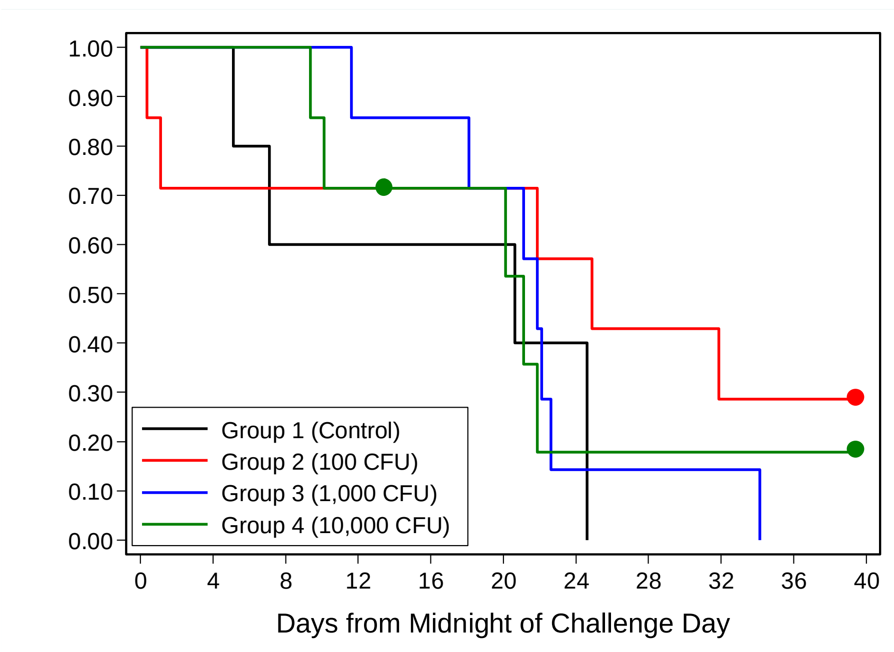


Figure 18. Kaplan-Meier curves for time to abnormality based on Heart Rate.

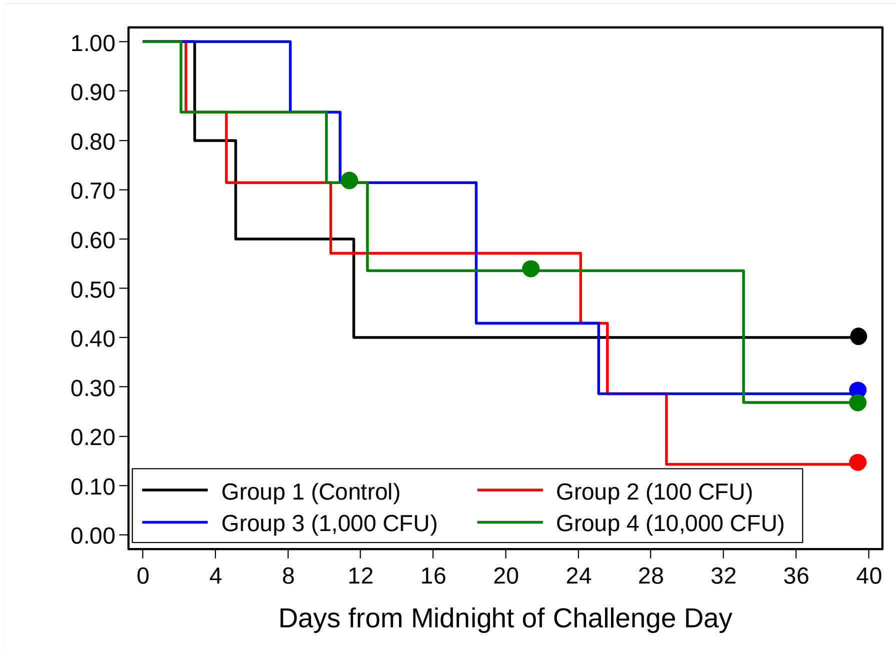


Figure 19. Kaplan-Meier curves for time to abnormality based on RP Expiratory Time.

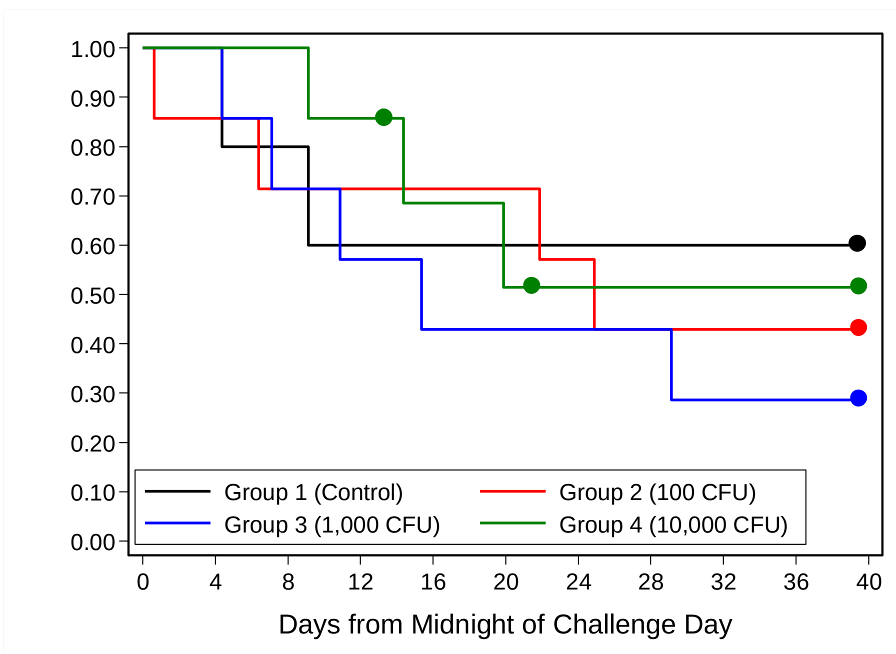


Figure 20. Kaplan-Meier curves for time to abnormality based on RP Inspiratory Time.

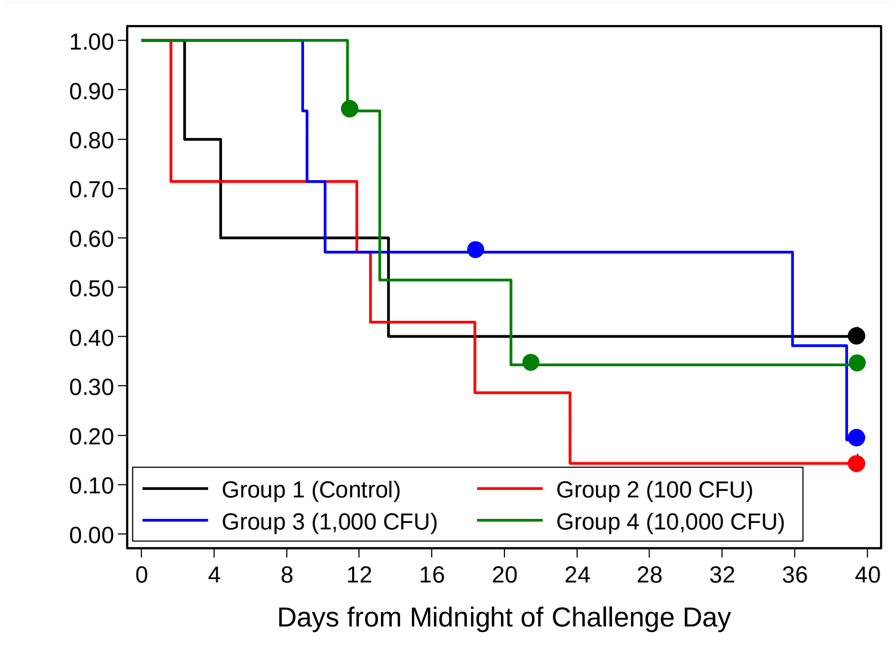


Figure 21. **Kaplan-Meier curves for time to abnormality based on RP Integral.**

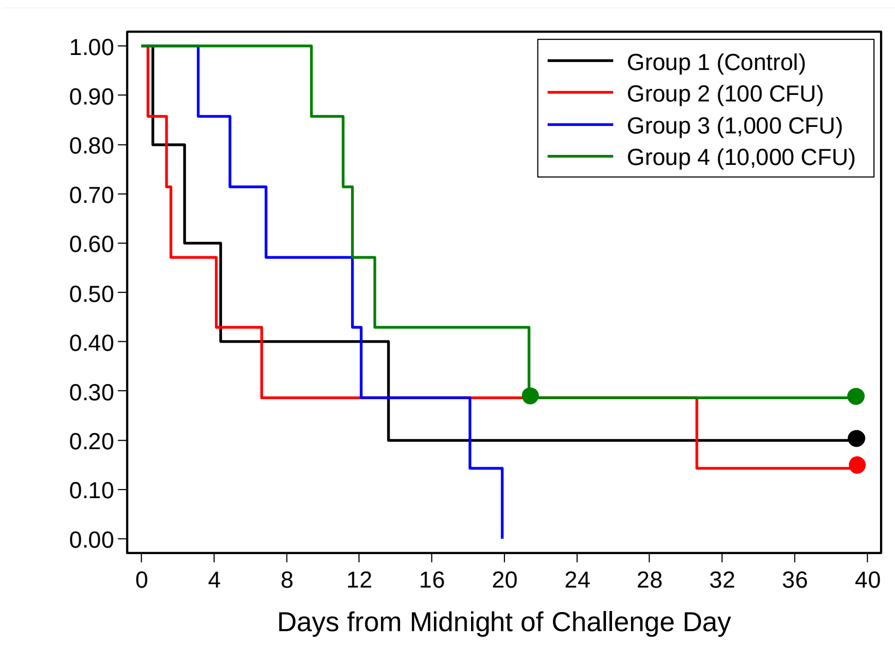


Figure 22. **Kaplan-Meier curves for time to abnormality based on RP Peak Amplitude.**

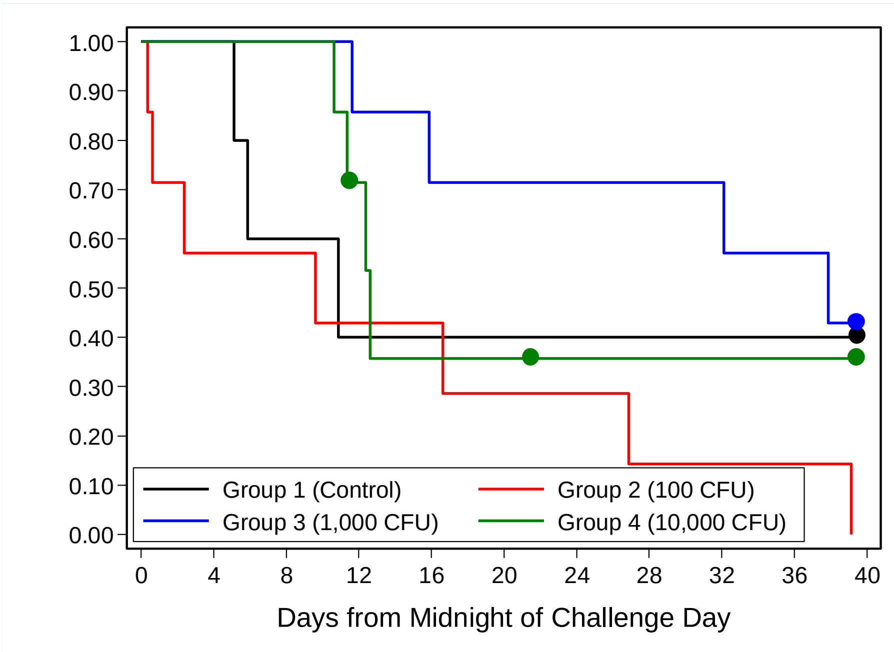


Figure 23. Kaplan-Meier curves for time to abnormality based on RP Respiratory Rate.

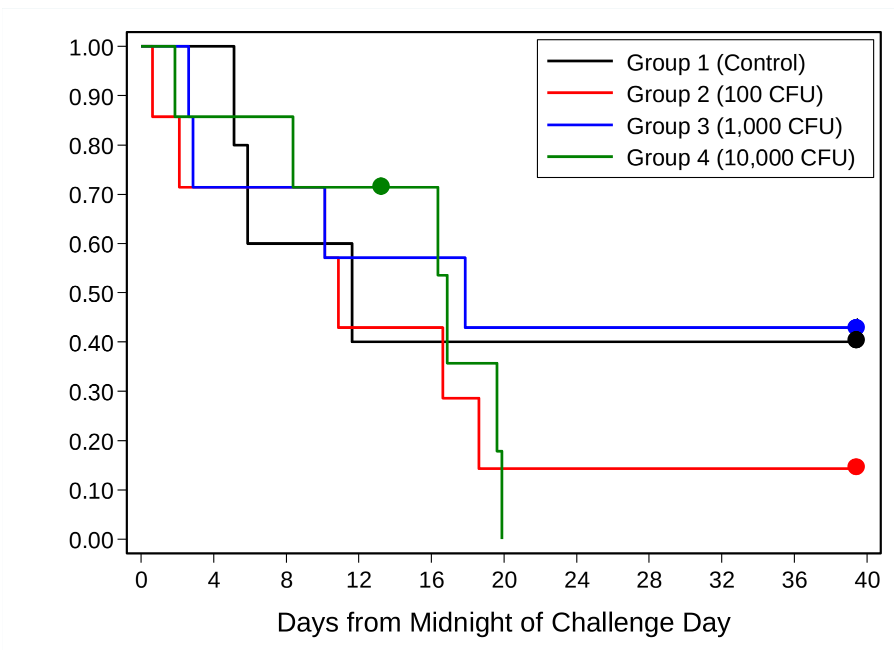


Figure 24. Kaplan-Meier curves for time to abnormality based on Temperature.

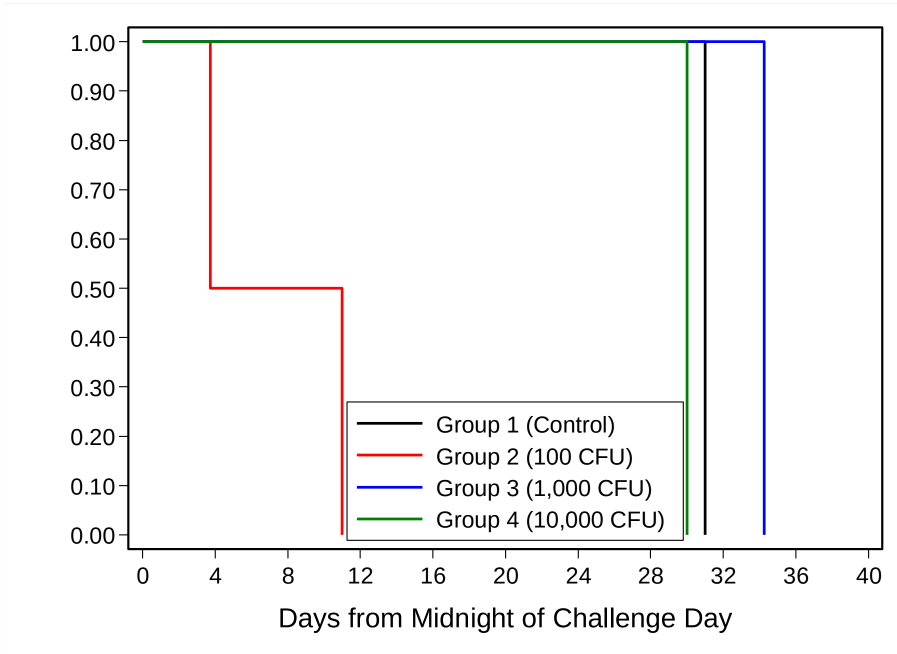


Figure 25. Kaplan-Meier curves for duration of abnormality based on Activity.

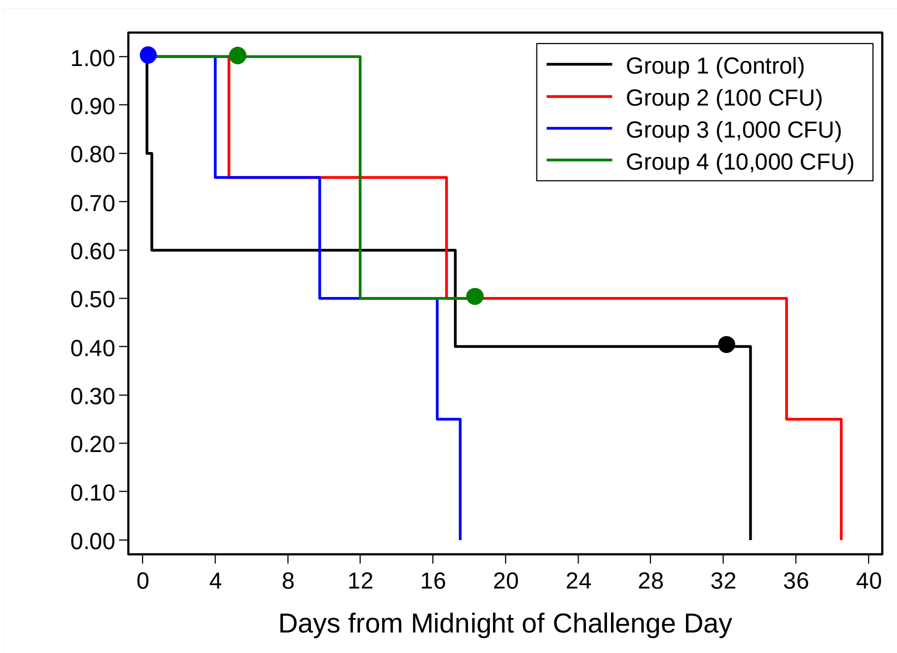


Figure 26. Kaplan-Meier curves for duration of abnormality based on Heart Rate.

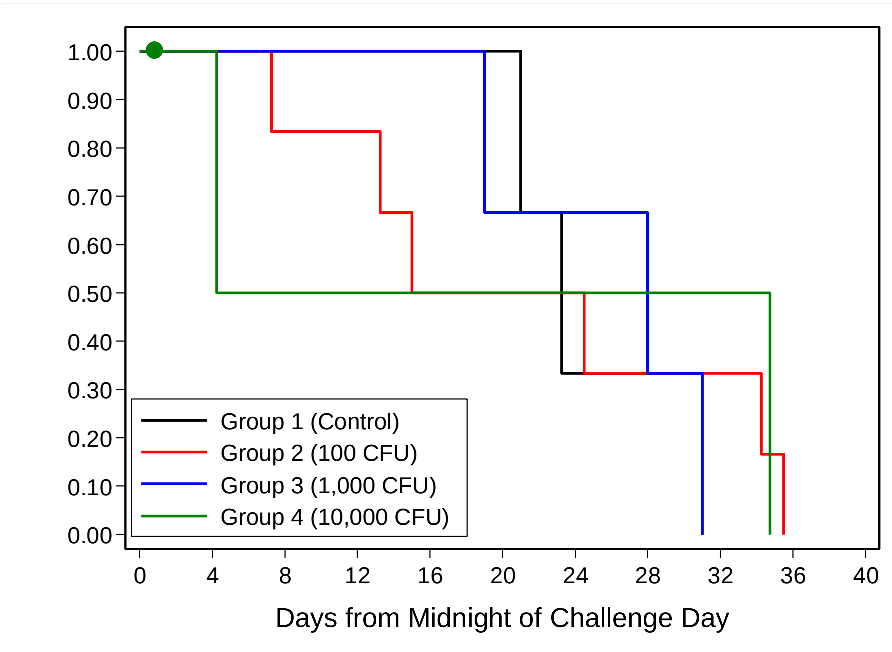


Figure 27. Kaplan-Meier Curves for duration of abnormality based on RP Expiratory Time.

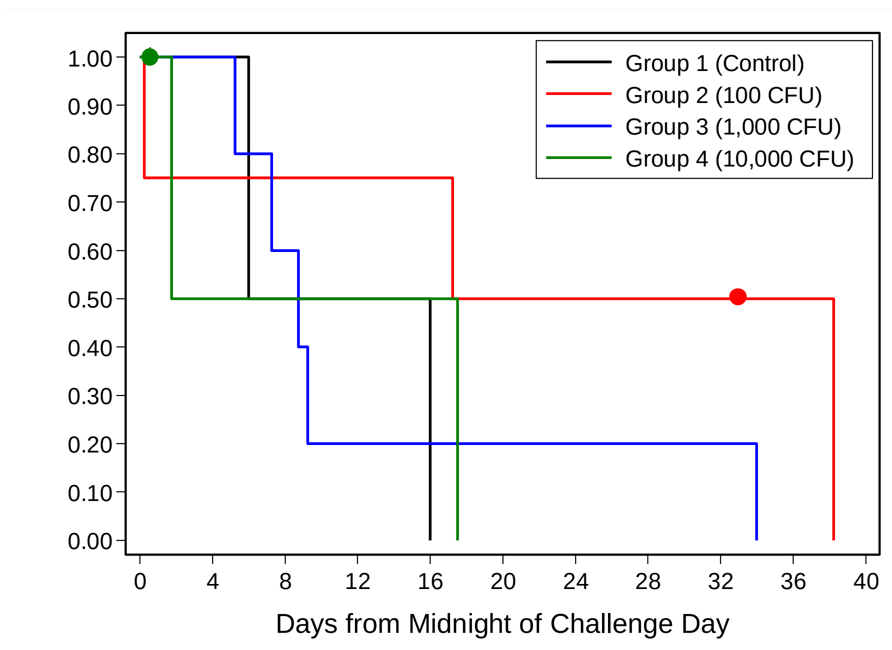


Figure 28. Kaplan-Meier curves for duration of abnormality based on RP Inspiratory Time.

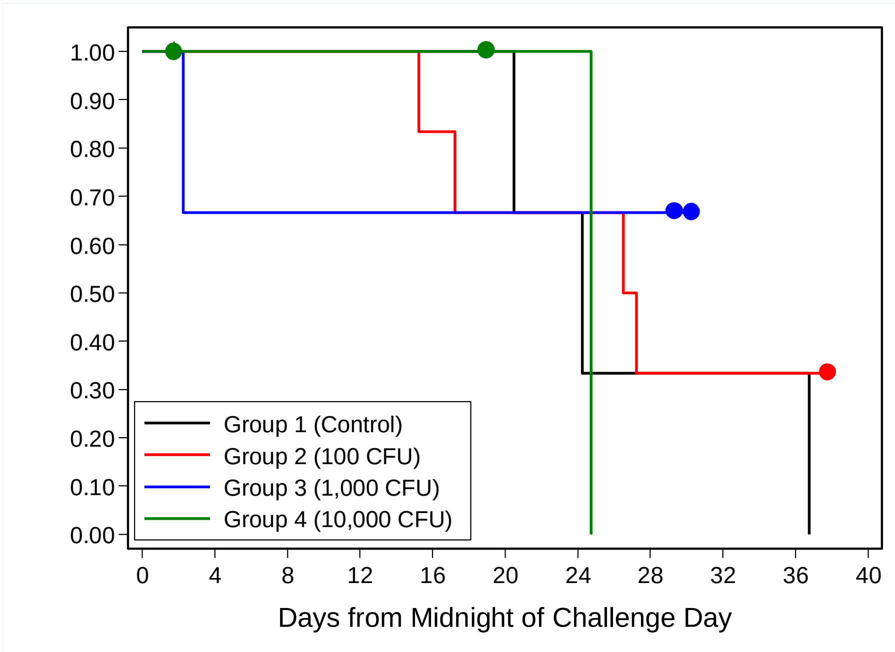


Figure 29. Kaplan-Meier curves for duration to abnormality based on RP Integral.

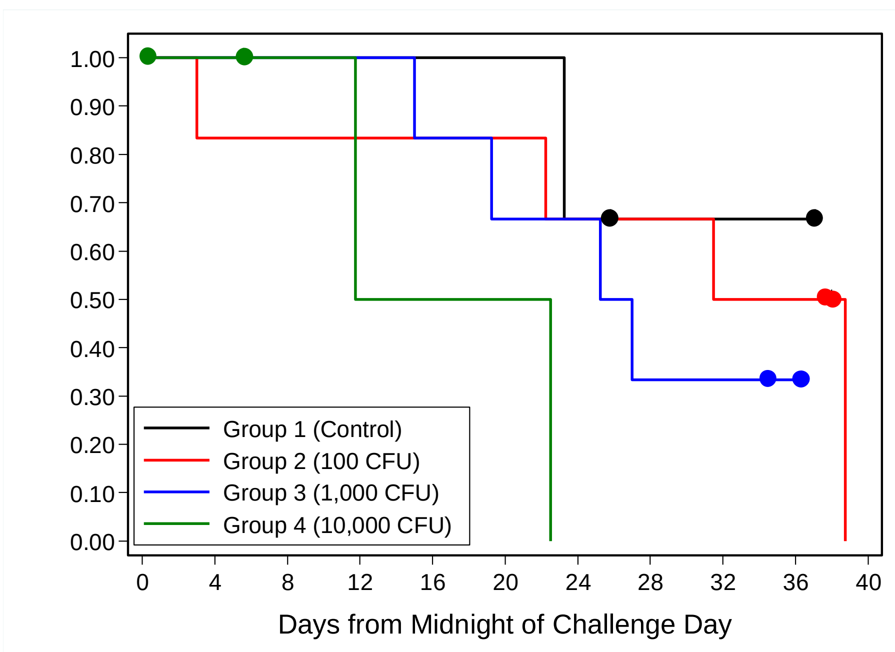


Figure 30. Kaplan-Meier curves for duration to abnormality based on RP Amplitude.

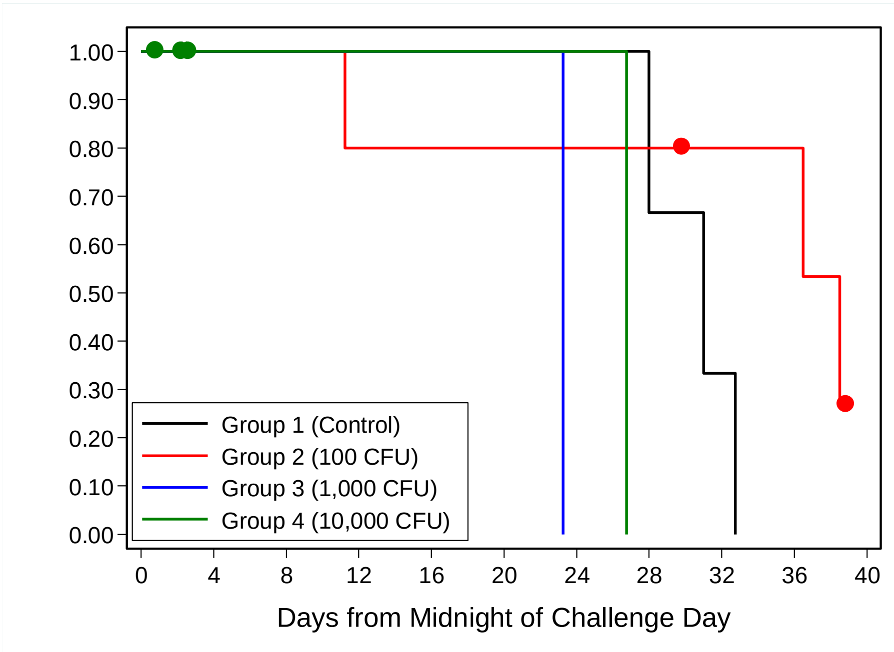


Figure 31. **Kaplan-Meier curves for duration of abnormality based on RP Respiratory Rate.**

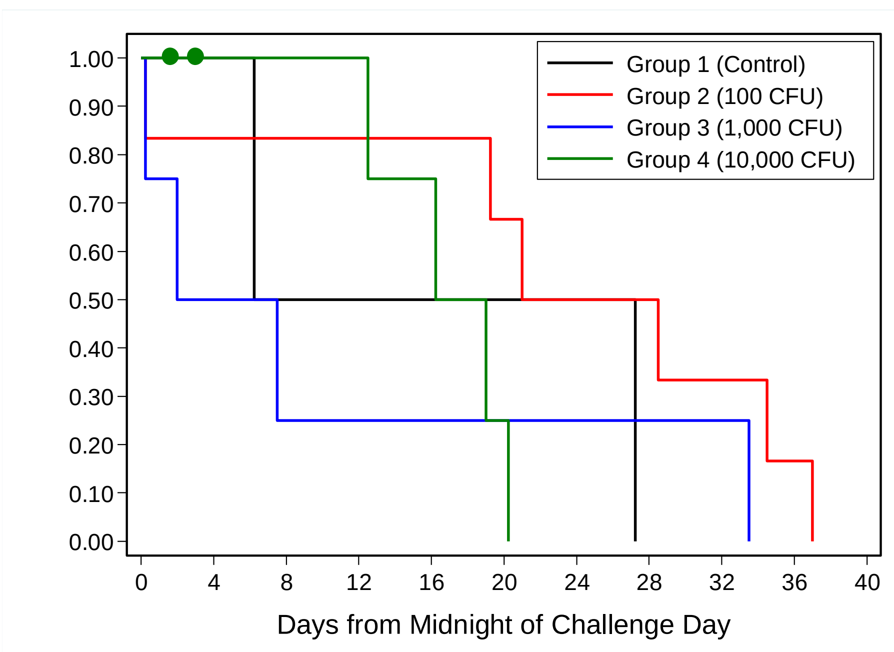


Figure 32. **Kaplan-Meier curves for duration of abnormality based on Temperature.**

APPENDIX G
STATISTICAL REPORT - MORTALITY

Table of Contents

List of Tables

List of Figures

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. 1078-CG920794. Twenty-six (26) pathogen free New Zealand White rabbits were randomly assigned to one of four groups of animals as shown in Table 1. Beginning on Study Day 0, animals were exposed to *Bacillus anthracis* (Ames strain) spores once a day for five straight working days each week for three straight weeks, at targeted doses shown in Table 1. The control group (Group 1) was exposed to gamma-irradiated spores.

Table 1. Study Design

Group	Number of Animals per Group	Target Spore Dose (CFU)	Number of Spore Challenges
1	5	10,000*	15
2	7	100	
3	7	1,000	
4	7	10,000	

*These spores were inactivated by radiation.

2. Statistical Methods

Estimates with exact 95% binomial confidence intervals for the proportion of surviving animals within each group were calculated. 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. A Bonferroni-Holm adjustment was made to maintain an overall 0.05 level of significance for the multiple pairwise comparisons.

For each animal, the geometric mean inhaled dose from the 15 spore challenges was calculated and used in the statistical analysis. Excluding the control group (Group 1), a logistic regression model was fitted to the survival data as a function of the base -10 log transformed geometric mean 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 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. Kaplan-Meier curves were plotted for each group 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.

All statistical analyses were performed using Stata (StataCorp LP; College Station, TX; version 11.1). All results are reported at the 0.05 level of significance.

3. Results

Table 2 contains the estimated proportion of surviving animals within each group, along with exact binomial 95% confidence intervals. All animals in Groups 1 and 2 survived the length of the study. Six of the seven animals in Group 3 survived, and three of the seven animals in Group 4 survived.

The overall Fisher's exact test was significant (p-value=0.0425). 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. Regardless of the adjustment for 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 -1.30 (p-value=0.0288). The estimated LD_{50} was 8,094 colony forming units (CFU) with a 95% Fieller confidence interval ranging from 2,276 CFU to 36,135,187 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=0.0135), 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. Prior to adjusting for multiple comparisons, the time to death in Group 2 was significantly greater than that in Group 4. However, this relationship was no longer significant after adjusting for the multiple pairwise comparisons. Figure 2 displays the Kaplan-Meier curves for each of the four dose groups. Since all animals in Groups 1 and 2 survived the length of the study, Group 2 was plotted with a slight offset so that the 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 targeted spore doses. All animals in the control group and the targeted 100 CFU dose group (Groups 1 and 2, respectively) survived the length of the study. Six of seven animals in the targeted 1,000 CFU dose group (Group 3) survived, while only three of seven animals in the targeted 10,000 CFU dose group (Group 4) survived. There were no significant differences in survival rates between any pair of groups according to a Fisher's exact test.

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 8,094 CFU per challenge day with a 95% Fieller confidence interval ranging from 2,276 CFU to 36,135,187 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 comparisons, the time to death in the targeted 100 CFU dose group (Group 2) was significantly greater than that in the targeted 10,000 CFU dose group (Group 4). However, this relationship was no longer significant after adjusting for the multiple pairwise comparisons. 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 (Exact 95% Confidence Interval)
1	5/5	1.00 (0.48, 1.00)
2	7/7	1.00 (0.59, 1.00)
3	6/7	0.86 (0.42, 1.00)
4	3/7	0.43 (0.10, 0.82)

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	2	3	4
1	1.0000[a]	1.0000	0.0808	1.0000[a]	1.0000	0.3497
2		1.0000	0.0699		1.0000	0.3497
3			0.2657			0.7972
4						

[a]A p-value of 1.0000 was substituted since all animals in both groups survived.

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	2	3	4
1	1.0000[a]	0.3980	0.0526	1.0000[a]	0.6346	0.2102
2		0.3173	0.0221*		0.6346	0.1103
3			0.0916			0.2747
4						

[a]A p-value of 1.0000 was substituted since all animals in both groups survived.
*Comparison was significant at the 0.05 level.

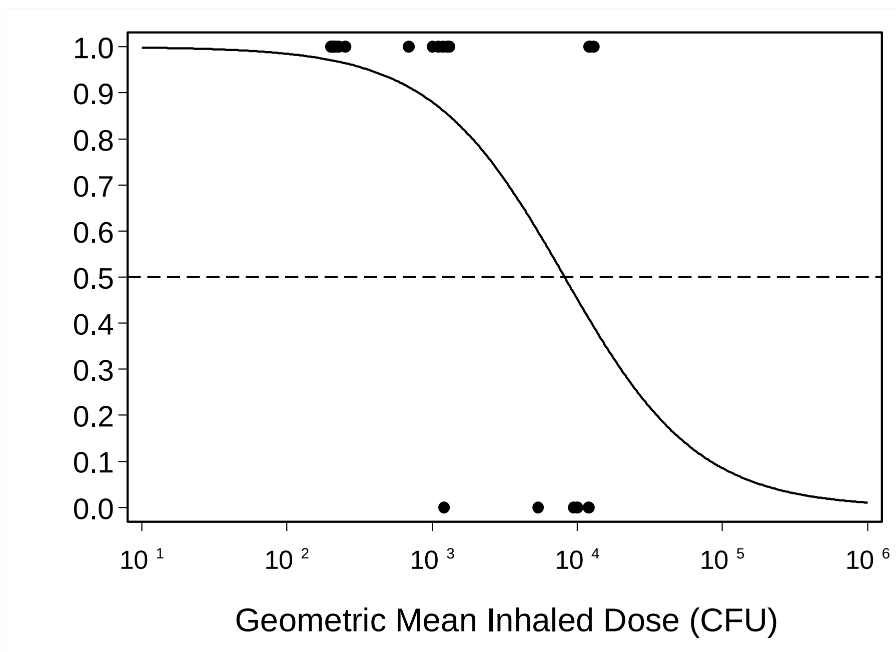
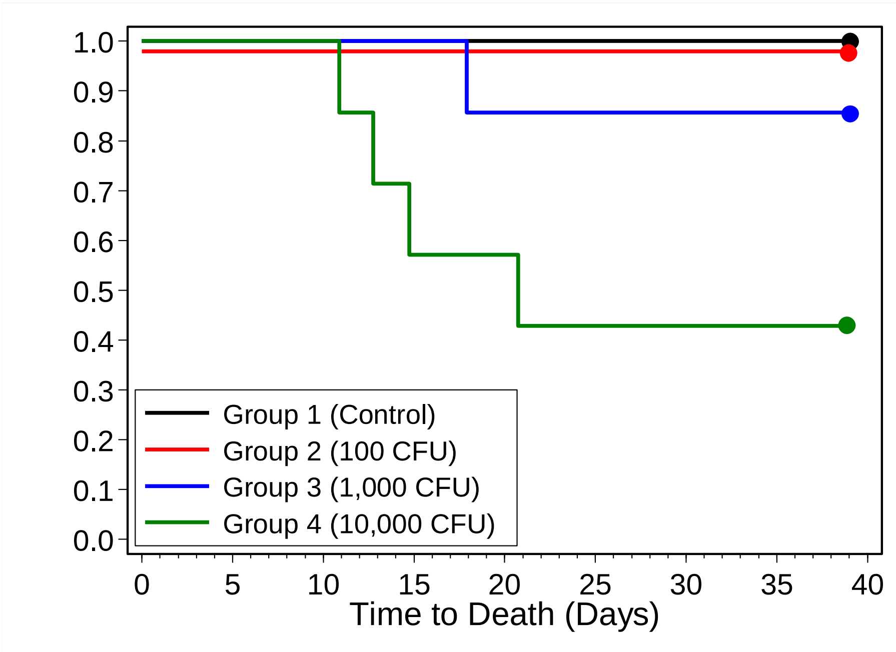


Figure 1. Estimated logistic regression curve and observed survival or mortality.



All animals in Groups 1 and 2 survived the length of the study.

Figure 2. Kaplan-Meier curves representing time to death and survival data for each group.

APPENDIX H
STASTICIAL REPORT – BODY WEIGHTS

Table of Contents

List of Tables

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List of Acronyms

ANOVA ....analysis of variance

BBRC .... Battelle Biomedical Research Center

CFU .... colony forming unit

N .... number of animals

1. Introduction

This report summarizes the statistical analysis of body weight data collected under Battelle Biomedical Research Center (BBRC) Study No. 1078-CG920794. Twenty-six (26) pathogen-free New Zealand White rabbits were randomly assigned to one of four groups of animals as shown in Table 1. Beginning on Study Day 0, animals were exposed to *Bacillus anthracis* (Ames strain) spores once a day for five straight working days each week for three straight weeks, at targeted doses shown in Table 1. The control group (Group 1) was exposed to gamma-irradiated spores. Animals were weighed on Study Days 2, 9, 16, 23, 30 and 37.

Table 1. Study Design

Group	Number of Animals per Group	Target Spore Dose (CFU)	Number of Spore Challenges	Body Weight Measurements
1	5	10,000*	15	Study days 2, 9, 16, 23, 30, and 37
2	7	100		
3	7	1,000		
4	7	10,000		

CFU Colony forming units.
* These spores were inactivated by radiation.

2. Statistical Methods

An analysis of variance (ANOVA) model fitted to the body weight data with effects for group, study day, and the interaction between group and study day was used to assess the model assumption of normality and to identify potential outliers. Standardized residuals from this ANOVA model were obtained and a hypothesis test was performed to assess the model assumption of normality for the untransformed data. Weight was then transformed by taking the base-10 logarithm of the original values, and the ANOVA model was refitted. A hypothesis test was again performed 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 data were used throughout the analysis. Deleted studentized residuals, which are the standardized residuals from the model fitted to all data except the current observation, 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 statistical analysis was performed both with and without these observations to evaluate their effect on the results.

On each study day, the following ANOVA model was fitted to the body weight data to determine if there were significant differences between the groups:

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

where Y_{dij} is the observed weight for the *j*th animal in Group *i* (*i*=1, 2, 3, 4) on Study Day *d* (*d*=2, 9, 16, 23, 30, and 37), μ is an overall constant, Group $_i$ is the effect of Group *i*, and ε_{ij} is the random error left unexplained by the model. Tukey's multiple comparisons procedure was also performed on each study day to determine which pairs of groups had mean body weights that were significantly different from each other.

All statistical analyses were performed using Stata (StataCorp LP; College Station, TX; Version 11.1). All results are reported at the 0.05 level of significance.

3. Results

The model assumption of normality was not more reasonable when the models were fitted to the log-transformed data; therefore, all models were fitted to the untransformed values. Additionally, no potential outliers were identified.

Table 2 contains descriptive statistics (including means with 95% confidence intervals) for the weights within each group on each study day. All animals in Groups 1 and 2 survived the length of the study. One of seven animals in Group 3 died prior to Study Day 23. In Group 4, three of seven animals died prior to Study Day 16 and one additional animal died prior to Study Day 23. The analyses performed on each study day included only surviving animals and, thus, are based on smaller sample sizes for these groups on later study days. Figure 1 displays the group mean weights with 95% confidence intervals for Study Days 2 through 37.

Table 3 contains the results obtained from fitting ANOVA models with a group effect to the body weight data on each study day. There were no significant differences between the groups on any study day.

4. Conclusions

All animals in the control group and the targeted 100 colony forming units (CFU) dose group (Groups 1 and 2, respectively) survived the length of the study. One of seven animals in the targeted 1,000 CFU dose group (Group 3) died prior to Study Day 23. In the targeted 10,000 CFU dose group (Group 4), three of seven animals died prior to Study Day 16 and one additional animal died prior to Study Day 23. The analyses performed on each study day include only surviving animals and, thus, are based on smaller sample sizes for these groups on later study days. Body weights were not significantly different between the groups on any study day.

Table 2. Means with 95% Confidence Intervals for Weight (kilograms) by Group and Study Day

Group	Study Day	N	Mean (95% Confidence Interval)
1	2	5	2.82 (2.50, 3.13)
	9	5	2.87 (2.55, 3.20)
	16	5	2.96 (2.66, 3.26)
	23	5	2.96 (2.67, 3.26)
	30	5	3.00 (2.74, 3.25)
	37	5	3.04 (2.79, 3.29)
2	2	7	2.76 (2.66, 2.87)
	9	7	2.84 (2.74, 2.94)
	16	7	2.92 (2.83, 3.01)
	23	7	2.95 (2.85, 3.04)
	30	7	2.99 (2.91, 3.07)
	37	7	3.02 (2.92, 3.12)
3	2	7	2.78 (2.59, 2.97)
	9	7	2.84 (2.66, 3.02)
	16	7	2.91 (2.70, 3.11)
	23	6	2.98 (2.76, 3.19)
	30	6	3.03 (2.83, 3.23)
	37	6	3.06 (2.85, 3.26)
4	2	7	2.77 (2.66, 2.89)
	9	7	2.81 (2.71, 2.92)
	16	4	2.94 (2.75, 3.14)
	23	3	2.94 (2.47, 3.40)
	30	3	2.97 (2.43, 3.51)
	37	3	2.96 (2.39, 3.53)

N Number of animals.

Table 3. Summary of Test Results for Group Comparisons of Body Weight by Study Day

Study Day	Mean Body Weight (Kilograms), by Group				Group Effect P-Value
	1	2	3	4	
2	2.82	2.76	2.78	2.77	0.9632
9	2.87	2.84	2.84	2.81	0.9481
16	2.96	2.92	2.91	2.94	0.9606
23	2.96	2.95	2.98	2.94	0.9881
30	3.00	2.99	3.03	2.97	0.9521
37	3.04	3.02	3.06	2.96	0.8865

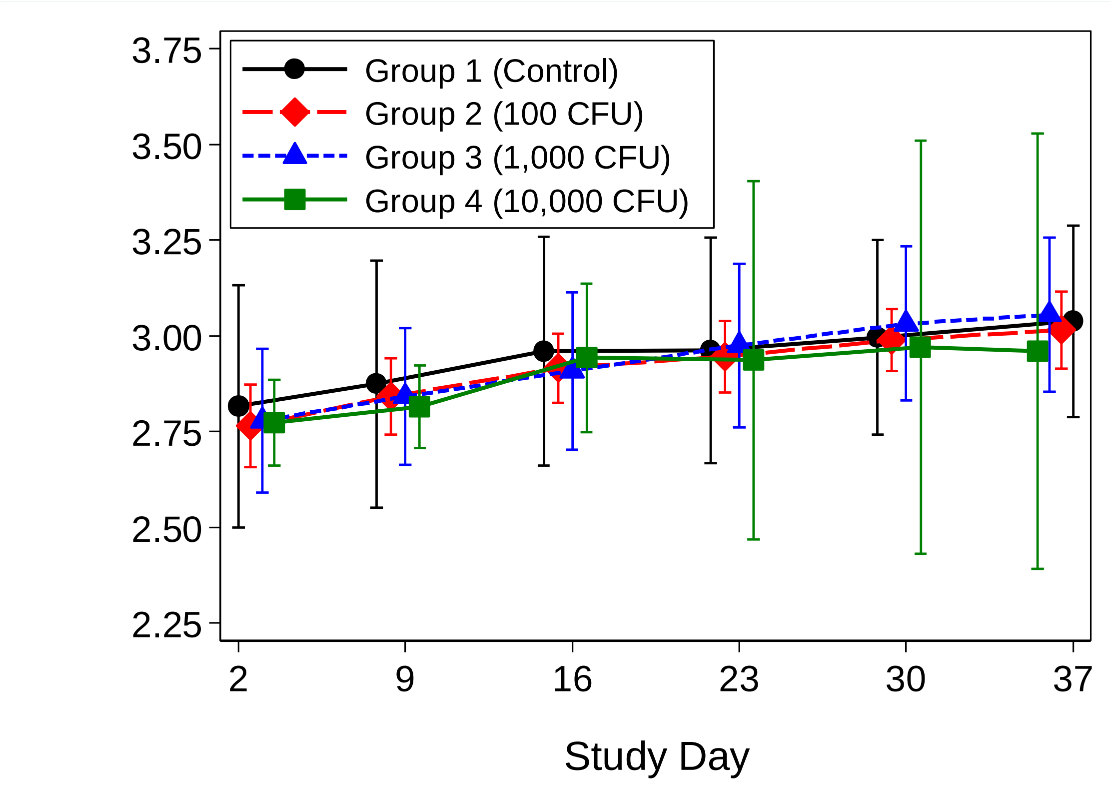

Figure 1. Group mean weights with 95% confidence intervals over time.

APPENDIX I
BLOOD DRAW TIMES

Challenge Order	Animal ID	Sex	Group	Study Day (-3)		Study Day 2		Study Day 4		Study Day 9		Study Day 11		Study Day 16		Study Day 18	
				Date	Time	Date	Time	Date	Time	Date	Time	Date	Time	Date	Time	Date	Time
1	40	M	1	7/23/2010	0937 C	7/28/2010	0830 C	7/30/2010	0801 C	8/4/2010	0825 C	8/6/2010	0836 C	8/11/2010	0849 C	8/13/2010	0840 C
2	7	M	1	7/23/2010	0948 C	7/28/2010	0836 C	7/30/2010	0821 C	8/4/2010	0830 C	8/6/2010	0839 C	8/11/2010	0854 C	8/13/2010	0845 C
3	5	M	1	7/23/2010	0900	7/28/2010	0823	7/30/2010	0747	8/4/2010	0800	8/6/2010	0849	8/11/2010	0932	8/13/2010	0830
4	9	M	1	7/23/2010	0901	7/28/2010	0827	7/30/2010	0754	8/4/2010	0805	8/6/2010	0850	8/11/2010	0935	8/13/2010	0835
5	37	M	1	7/23/2010	0951 C	7/28/2010	0844 C	7/30/2010	0829 C	8/4/2010	0838 C	8/6/2010	0842 C	8/11/2010	0900 C	8/13/2010	0855 C
1	13	M	2	7/23/2010	0908	7/28/2010	0923	7/30/2010	0903	8/4/2010	0811	8/6/2010	0855	8/11/2010	1020 C	8/13/2010	0901 C
2	34	M	2	7/23/2010	0915	7/28/2010	0929	7/30/2010	0846	8/4/2010	0857	8/6/2010	0928	8/11/2010	1023 C	8/13/2010	0914 C
3	25	M	2	7/23/2010	0915	7/28/2010	0933	7/30/2010	0853	8/4/2010	0906	8/6/2010	0929	8/11/2010	0956	8/13/2010	0840
4	15	M	2	7/23/2010	0921	7/28/2010	0937	7/30/2010	0859	8/4/2010	0908	8/6/2010	0934	8/11/2010	1000	8/13/2010	0850
5	30	M	2	7/23/2010	0925	7/28/2010	0948	7/30/2010	0906	8/4/2010	0912	8/6/2010	0934	8/11/2010	1003	8/13/2010	0857
6	28	M	2	7/23/2010	1003	7/28/2010	0957	7/30/2010	0911	8/4/2010	0913	8/6/2010	0939	8/11/2010	1031	8/13/2010	0911
7	19	M	2	7/23/2010	0957	7/28/2010	1000	7/30/2010	0917	8/4/2010	0917	8/6/2010	0939	8/11/2010	1102 C	8/13/2010	0946 C
1	14	M	3	7/23/2010	1002	7/28/2010	1003	7/30/2010	0920	8/4/2010	0916	8/6/2010	0944	8/11/2010	1045	8/13/2010	0923
2	11	M	3	7/23/2010	1007	7/28/2010	1008	7/30/2010	0923	8/4/2010	0918	8/6/2010	0945	8/11/2010	1047	8/13/2010	0928
3	2	M	3	7/23/2010	1039 C	7/28/2010	0847 C	7/30/2010	0842 C	8/4/2010	0844 C	8/6/2010	0900 C	8/11/2010	0905 C		
4	8	M	3	7/23/2010	1011	7/28/2010	1011	7/30/2010	0929	8/4/2010	0920	8/6/2010	0950	8/11/2010	1052	8/13/2010	0939
5	12	M	3	7/23/2010	1048 C	7/28/2010	0854 C	7/30/2010	0847 C	8/4/2010	0850 C	8/6/2010	0910 C	8/11/2010	0916 C	8/13/2010	1019 C
6	18	M	3	7/23/2010	1019	7/28/2010	1047 C	7/30/2010	0852 C	8/4/2010	0857 C	8/6/2010	0916 C	8/11/2010	0924 C	8/13/2010	1027 C
7	32	M	3	7/23/2010	1024	7/28/2010	1027	7/30/2010	0935	8/4/2010	0920	8/6/2010	0950	8/11/2010	1131 C	8/13/2010	1032 C
1	6	M	4	7/23/2010	1027	7/28/2010	1030	7/30/2010	0940	8/4/2010	0925	8/6/2010	1004				
2	33	M	4	7/23/2010	1101 C	7/28/2010	0903 C	7/30/2010	0857 C	8/4/2010	0901 C	8/6/2010	0919 C				
3	27	M	4	7/23/2010	1031	7/28/2010	1034	7/30/2010	0950	8/4/2010	0930	8/6/2010	0957	8/11/2010	1059	8/13/2010	0950
4	31	M	4	7/23/2010	1035	7/28/2010	1039	7/30/2010	0953	8/4/2010	0935	8/6/2010	1009				
5	39	M	4	7/23/2010	1054	7/28/2010	1042	7/30/2010	0957	8/4/2010	0936	8/6/2010	1009	8/11/2010	1107	8/13/2010	1000
6	21	M	4	7/23/2010	1054	7/28/2010	1052	7/30/2010	1002	8/4/2010	0940	8/6/2010	1014	8/11/2010	1118	8/13/2010	1023
7	38	M	4	7/23/2010	1058	7/28/2010	1055	7/30/2010	1005	8/4/2010	0940	8/6/2010	1014	8/11/2010	1121	8/13/2010	1034

Animal Deceased

A) Initial sample drawn at 1003 unable to obtain full sample, more blood drawn at 1039 after animal found deac
B) Only able to obtain ~ 0.25 ml on blood draw, drew ~ 0.5 ml at 1140
C) Acepromazine (0.7 ml) was administered prior to blood collect

QA AUDIT COMPLETED
BY/DATE: [signature] 1/20/11

Printed By: DW 12-15-10
QC Reviewed By: JSM 12-15-10

I-3

Challenge Order	Animal ID	Sex	Group	Study Day 23		Study Day 25		Study Day 30		Study Day 32		Study Day 37		Day 39-Terminal	
				Date	Time	Date	Time	Date	Time	Date	Time	Date	Time	Date	Time
1	40	M	1	8/18/2010	1104 C	8/20/2010	0958 C	8/25/2010	1006 C	8/27/2010	1020 C	9/1/2010	1014 C	9/3/2010	0906
2	7	M	1	8/18/2010	1112/1140 B,C	8/20/2010	1005 C	8/25/2010	1012 C	8/27/2010	1027 C	9/1/2010	1016 C	9/3/2010	0923
3	5	M	1	8/18/2010	1107	8/20/2010	0949	8/25/2010	0953	8/27/2010	1012	9/1/2010	1012	9/3/2010	0934
4	9	M	1	8/18/2010	1114	8/20/2010	0958	8/25/2010	0959	8/27/2010	1018	9/1/2010	1039	9/3/2010	0955
5	37	M	1	8/18/2010	1117 C	8/20/2010	1007 C	8/25/2010	1016 C	8/27/2010	1032 C	9/1/2010	1021 C	9/3/2010	1015
1	13	M	2	8/18/2010	1131 C	8/20/2010	1012 C	8/25/2010	1027 C	8/27/2010	1038 C	9/1/2010	1027 C	9/3/2010	1030
2	34	M	2	8/18/2010	1145 C	8/20/2010	1016 C	8/25/2010	1031 C	8/27/2010	1044 C	9/1/2010	1031 C	9/3/2010	1045
3	25	M	2	8/18/2010	1123	8/20/2010	1005	8/25/2010	1006	8/27/2010	1026	9/1/2010	1046	9/3/2010	1055
4	15	M	2	8/18/2010	1128	8/20/2010	1011	8/25/2010	1011	8/27/2010	1037	9/1/2010	1050	9/3/2010	1109
5	30	M	2	8/18/2010	1205 C	8/20/2010	1028 C	8/25/2010	1040 C	8/27/2010	1101 C	9/1/2010	1042 C	9/3/2010	1122
6	28	M	2	8/18/2010	1148	8/20/2010	1019	8/25/2010	1023	8/27/2010	1049	9/1/2010	1055	9/3/2010	1130
7	19	M	2	8/18/2010	1149 C	8/20/2010	1036 C	8/25/2010	1047 C	8/27/2010	1106 C	9/1/2010	1049 C	9/3/2010	1219
1	14	M	3	8/18/2010	1153	8/20/2010	1024	8/25/2010	1031	8/27/2010	1110	9/1/2010	1102	9/3/2010	1232
2	11	M	3	8/18/2010	1202	8/20/2010	1029	8/25/2010	1036	8/27/2010	1118	9/1/2010	1109	9/3/2010	1210
3	2	M	3											8/13/2010	1003/1039 A
4	8	M	3	8/18/2010	1226 C	8/20/2010	1040 C	8/25/2010	1053 C	8/27/2010	1111 C	9/1/2010	1053 C	9/3/2010	1236
5	12	M	3	8/18/2010	1121 C	8/20/2010	1046 C	8/25/2010	1100 C	8/27/2010	1115 C	9/1/2010	1057 C	9/3/2010	1249
6	18	M	3	8/18/2010	1127 C	8/20/2010	1050 C	8/25/2010	1105 C	8/27/2010	1119 C	9/1/2010	1102 C	9/3/2010	1300
7	32	M	3	8/18/2010	1152 C	8/20/2010	1053 C	8/25/2010	1113 C	8/27/2010	1122 C	9/1/2010	1106 C	9/3/2010	1307
1	6	M	4											8/6/2010	1135
2	33	M	4											8/8/2010	0814
3	27	M	4											8/16/2010	0835
4	31	M	4											8/10/2010	0831
5	39	M	4	8/18/2010	1211	8/20/2010	1035	8/25/2010	1043	8/27/2010	1125	9/1/2010	1114	9/3/2010	1315
6	21	M	4	8/18/2010	1230 C	8/20/2010	1056 C	8/25/2010	1126 C	8/27/2010	1128 C	9/1/2010	1115 C	9/3/2010	1323
7	38	M	4	8/18/2010	1221	8/20/2010	1041	8/25/2010	1048	8/27/2010	1132	9/1/2010	1149 C	9/3/2010	1330

Animal Deceased

A) Initial sample drawn at 1003 unable to obtain full sample, more blood drawn at 1039 after animal found deac
B) Only able to obtain ~ 0.25 ml on blood draw, drew ~ 0.5 ml at 1140
C) Acepromazine (0.7 ml) was administered prior to blood collect

QA AUDIT COMPLETED
BY/DATE: [signature] 1/20/11

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QC Reviewed By: JM 12-15-10

APPENDIX J
STATISTICAL REPORT - HEMATOLOGY AND C-REACTIVE PROTEIN

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List of Acronyms

ANOVA .... Analysis of Variance
BBRC .... Battelle Biomedical Research Center
CFU .... Colony Forming Units
CRP .... C-Reactive Protein
dL .... deciliter
fL .... femtoliter
g .... gram
HCT .... Hematocrit
HGB .... Hemoglobin
MCH .... Mean Corpuscular Hemoglobin
MCHC .... Mean Corpuscular Hemoglobin Concentration
MCV .... Mean Corpuscular Volume
MPV .... Mean Platelet Volume
N .... Number of Animals
N/L Ratio .... Neutrophil Count/Lymphocyte Count Ratio
PLT .... Platelet Count
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. 1078-CG920794. Twenty-six (26) pathogen-free New Zealand White rabbits were randomly assigned to one of four groups of animals as shown in Table 1. Beginning on Study Day 0, animals were exposed to *Bacillus anthracis* (Ames strain) spores once a day for five straight working days each week for three straight weeks, at targeted doses shown in Table 1. The control group (Group 1) was exposed to gamma irradiated spores.

Table 1. Study Design

Group	Number of Animals per Group	Target Spore Dose (CFU)	Hematology and CRP Blood Collection Study Days
1	5	10,000*	-3, 2, 4, 9, 11, 16, 18, 23, 25, 30, 32, 37, and 39[a]
2	7	100	
3	7	1,000	
4	7	10,000	

* Spores are gamma-irradiated (negative control)
[a] Terminal blood draw

Blood samples were collected for hematology and C-reactive protein (CRP) analysis as indicated in Table 1. Blood collection on Study Day -3 served as a pre-challenge baseline for each animal. The hematology parameters that were included in this analysis are:

Red Blood Cell Parameters

- 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 and Volume

- Platelet count (PLT, 10^3 cells/µL)
- Mean platelet volume (MPV, fL)

Total and Differential White Blood Cell Parameters

- White blood cell count (WBC, 10^3 cells/μL)
- Neutrophil count (10^3 cells/μL)
- Lymphocyte count (10^3 cells/μL)
- Neutrophil count/lymphocyte count ratio (N/L ratio)
- Monocyte count (10^3 cells/μL)
- Eosinophil count (10^3 cells/μL)
- Basophil count (10^3 cells/μL)

Hematology and CRP analysis was performed at four levels:

1. Descriptive statistics (including 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 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.

2. Statistical Methods

Analysis of variance (ANOVA) models fitted separately to each hematology parameter and CRP 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 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 with 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 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 to evaluate their effect on the results.

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

$$Y_{ij} = \mu + \text{group}_i + \varepsilon_{ij} \tag{1}$$

where Y_{ij} is the observed hematology result for the jth animal in group i (i=1 to 4) 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 to determine which pairs of groups had baseline means that were significantly different from each other; however, the results are only presented if significant differences were identified. If the parameter was log-transformed for analysis, then the same model was used with Y_{ij} replaced by $\text{Log}(Y_{ij})$, the base-10 log-transformed parameter value for the jth animal in group i (i=1 to 4).

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 and CRP on each post-challenge Study Day:

$$Y_{dij} - Y_{bij} = \mu + \text{group}_i + \varepsilon_{ij} \qquad (2)$$

where Y_{dij} is the observed hematology result for the jth animal in group i (i=1 to 4) on Study Day d (d=2, 4, 9, 11, 16, 18, 23, 25, 30, 32, and 39), Y_{bij} is the observed hematology result for the jth animal in Group i at baseline (Study Day -3), 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 with their base-10 log-transformed counterparts $\text{Log}(Y_{dij})$ and $\text{Log}(Y_{bij})$, respectively. 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 between baseline and each post-challenge Study Day. For untransformed data, this tests whether the difference of 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.

All statistical analyses were conducted using Stata (Version 11.1) and R software that has been performance tested by Battelle staff. All results are reported at the 0.05 level of significance.

3. Results

The model assumption of normality was more reasonable for 9 of 16 hematology parameters and CRP data when models were fitted to the base-10 log-transformed data. These parameters were: MCV, MCH, RDW, MPV, WBC, neutrophil count, N/L ratio, monocyte count, and eosinophil count. Therefore, models were fitted to base-10 log-transformed values in the final analysis for these parameters. Table 2 contains a list of eleven 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 to evaluate their effect on the results. Attachment I contain 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 significant at the baseline for RDW and MPV. Tukey's multiple comparisons procedure indicated that the baseline mean in Group 2 was significantly greater than those in Groups 3 and 4 for MPV; however, no significant pairwise differences were identified for RDW. 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. Considering that the random probability of measuring a significant difference when none truly exists is 0.05, two significant differences out of 17 is not enough evidence to say there was an *a priori* difference between the groups.

Descriptive statistics and group comparisons for each parameter are presented in pairs of tables, where the table numbers 4 through 20 are associated with the parameter of interest. For each parameter, Table "a" contains the descriptive statistics and Table "b" contains the test results for comparing the mean shifts from baseline within each group at each post-challenge Study Day.

Tables 4a through 19a contain descriptive statistics (including 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 on each Study

Day. Table 20a presents similar information for CRP. 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 means with 95% confidence intervals for untransformed data, or geometric means with 95% confidence intervals for base-10 log-transformed data, over the course of the study for each hematology parameter, respectively. Figure 17 displays similar information for CRP. For plotting purposes, confidence intervals were not displayed for a particular Study Day on which a group had only two animals.

Tables 4b through 19b contain test results for the mean shift from baseline within each group and post-challenge Study Day for each hematology parameter, respectively. Table 20b contains similar information for CRP. In each cell, an estimate of the shift (difference or ratio) from baseline is shown for that parameter, group, and post-challenge 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 test results for significant differences between the group shifts from baseline at each post-challenge Study Day. The results from Tables 4b through 20b are discussed below in groups of related parameters.

<u>Red Blood Cell Parameters</u>

- **RBC (Tables 4a-b, Figure 1)**: There were significant decreases from baseline in Group 1 on Study Day 4, in Group 2 on Study Day 16, and in Group 4 on Study Day 11. There were significant group effects on Study Days 23 and 25. On Study Day 23, the mean decrease from baseline in Group 4 was significantly different than the mean increase from baseline in Group 3. On Study Day 25, the mean decrease from baseline in Group 4 was significantly different than the mean increases from baseline in Groups 1 and 2.

- **HGB (Tables 5a-b, Figure 2)**: There was a significant decrease from baseline in Group 1 on Study Day 4. There were significant group effects on Study Days 23 and 25. On Study Day 23, the mean decrease from baseline in Group 4 was significantly different than the mean increases from baseline in Groups 1, 2, and 3. On Study Day 25, the mean decrease from baseline in Group 4 was significantly different than the mean changes from baseline in Groups 1, 2, and 3.

- **HCT (Tables 6a-b, Figure 3)**: There was a significant decrease from baseline in Group 1 on Study Day 4. There was a significant group effect on Study Day 25. On Study Day 25, the mean decrease from baseline in Group 4 was significantly different than the mean increase from baseline in Group 1.

- **MCV (Tables 7a-b, Figure 4)**: There were significant decreases as a proportion of baseline in Group 1 on Study Day 39, in Group 3 on Study Day 32, and in Group 4 on Study Days 18, 32, 37, and 39. There were significant group effects on Study Days 32 and 39. On Study Day 32, the mean decrease as a proportion of baseline in Group 4 was significantly different than the mean increase as a proportion of baseline in Group 1. On Study Day 39, the mean decrease as a proportion of baseline in Group 4 was significantly greater than that in Group 2.

- **MCH (Tables 8a-b, Figure 5)**: There were no significant shifts as a proportion of baseline and no significant differences between the groups on any post-challenge Study Day.

- **MCHC (Tables 9a-b, Figure 6)**: There were significant increases from baseline in Group 2 on Study Days 18 and 23, and in Group 3 on Study Day 18. On Study Day 25, the mean decrease from baseline in Group 4 was significantly different than the mean increase from baseline in Group 3.

- **RDW (Tables 10a-b, Figure 7)**: There was a significant increase as a proportion of baseline in Group 2 on Study Day 18. There was a significant Group effect on Study Day 18. On Study Day 18, the mean increase as a proportion of baseline in Group 2 was significantly different than the mean decrease as a proportion of baseline in Group 4. On Study Day 25, the mean decrease as a proportion of baseline in Group 1 was significantly different than the mean increase as a proportion of baseline in Group 4.

Platelet Count and Volume

- **PLT (Tables 11a-b, Figure 8)**: There were significant decreases from baseline in Groups 1 and 2 on Study Day 39, in Group 3 on Study Days 4 and 39, and in Group 4 on Study Day 11. There were no significant differences between the groups on any post-challenge Study Day.

- **MPV (Tables 12a-b, Figure 9)**: There were significant increases as a proportion of baseline in Group 1 on Study Days 4 and 9, in Group 2 on Study Day 9, in Group 3 on Study Days 9 and 39, and in Group 4 on Study Day 9. There were significant decreases as a proportion of baseline in Group 1 on Study Day 37, in Group 2 on Study Days 2, 23, 30, and 37, and in Group 3 on Study Day 37. There were significant group effects on Study Days 4, 9, and 16; however, no significant pairwise group comparisons were identified on Study Day 9. On Study Day 4, the mean increase as a proportion of baseline in Group 1 was significantly different than the mean changes as a proportion of baseline in Groups 2, 3, and 4. On Study Day 16, the mean decrease as a proportion of baseline in Group 2 was significantly different than the mean increase as a proportion of baseline in Group 1.

Total and Differential White Blood Cell Parameters

- **WBC (Tables 13a-b, Figure 10)**: There were no significant shifts as a proportion of baseline and no significant differences between the groups on any post-challenge Study Day.

- **Neutrophil Count (Tables 14a-b, Figure 11)**: There were no significant shifts as a proportion of baseline and no significant differences between the groups on any post-challenge Study Day.

- **Lymphocyte Count (Tables 15a-b, Figure 12)**: There was a significant decrease from baseline in Group 3 on Study Day 39. There were no significant differences between the groups on any post-challenge Study Day.

- **N/L Ratio (Tables 16a-b, Figure 13)**: There was a significant decrease as a proportion of baseline in Group 1 on Study Day 18. There were no significant differences between the groups on any post-challenge Study Day.

- **Monocyte Count (Tables 17a-b, Figure 14)**: There was a significant decrease as a proportion of baseline in Group 2 on Study Day 39. On Study Day 9, the mean decrease as a proportion of baseline in Group 1 was significantly different than the mean increase as a proportion of baseline in Group 3.

- **Eosinophil Count (Tables 18a-b, Figure 15)**: There were no significant shifts as a proportion of baseline on any post-challenge Study Day. There was a significant group effect on Study Day 37. On Study Day 37, the mean increase as a proportion of baseline in Group 1 was significantly different than the mean decrease as a proportion of baseline in Group 3.

- **Basophil Count (Tables 19a-b, Figure 16)**: There was a significant increase from baseline in Group 2 on Study Day 23. There were no significant differences between the groups on any post-challenge Study Day.

C-Reactive Protein

- **CRP (Tables 20a-b, Figure 17)**: There was a significant increase as a proportion of baseline in Group 4 on Study Day 2. There were no significant differences between the groups on any post-challenge Study Day.

4. Conclusions

Among the red blood cell parameters, all significant differences between the group mean shifts from baseline involved the targeted 10,000 CFU dose group (Group 4). On Study Day 25, the mean change from baseline in the targeted 10,000 CFU dose group (Group 4) was significantly different than that in at least one of the other groups (Groups 1, 2, and 3) for RBC, HGB, HCT, MCHC, and RDW.

Among the platelet counts and volume, the mean shifts from baseline were not significantly different between the groups on any post-challenge Study Day for PLT. For MPV, the mean increase as a proportion of baseline in the control group (Group 1) was significantly different than the mean changes as a proportion of baseline in the challenged groups (Groups 2, 3, and 4) on Study Day 4, and the mean decrease as a proportion of baseline in the targeted 100 CFU dose group (Group 2) was significantly different than the mean increase as a proportion of baseline in the control group (Group 1) on Study Day 16.

Among the white blood cell parameters, the mean shifts from baseline were significantly different between the groups only for monocyte count and eosinophil count. For monocyte count, the mean decrease as a proportion of baseline in the control group (Group 1) was significantly different than the mean increase as a proportion of baseline in the targeted 1,000 CFU dose group (Group 3) on Study Day 9. For eosinophil count, the mean increase as a proportion of baseline in the control group (Group 1) was significantly different than the mean decrease as a proportion of baseline in the targeted 1,000 CFU dose group (Group 3) on Study Day 37.

Table 2. Potential Hematology and CRP Outliers

Parameter	Animal	Group	Study Day	Parameter Value	Deleted Studentized Residual
Hemoglobin	6	4	9	15.2	4.03
Red Cell Distribution Width†	38	4	25	17.9	5.09
			30	16.8	5.08
	39	4	25	12.3	-5.09
Platelet Count	38	4	25	2044	5.59
			37	1408	4.03
	39	4	25	547	-5.59
White Blood Cell Count†	7	1	4	1.60	-4.30
	11	3	39	1.23	-4.36
Neutrophil Count†	40	1	23	0.24	-4.10
C-Reactive Protein†	38	4	23	7.42	4.70

† 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 (Study Day -3) Data

Parameter	Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
Red Blood Cell Count	0.5079	
Hemoglobin	0.6553	
Hematocrit	0.5376	
Mean Corpuscular Volume†	0.6306	
Mean Corpuscular Hemoglobin†	0.7228	
Mean Corpuscular Hemoglobin Concentration	0.1048	
Red Cell Distribution Width†	0.0392*	
Platelet Count	0.3253	
Mean Platelet Volume†	0.0255*	0.04 (3<2) 0.0339 0.04 (4<2) 0.0425
White Blood Cell Count†	0.3865	
Neutrophil Count†	0.2256	
Lymphocyte Count	0.2257	
Neutrophil Count/Lymphocyte Count Ratio†	0.1454	
Monocyte Count†	0.2461	
Eosinophil Count†	0.3489	
Basophil Count	0.5573	
C-Reactive Protein†	0.3295	

† Indicates that values for this parameter were log-transformed for the analysis.
* The overall group effect was significant at the 0.05 level.
# Cells contain all pairwise comparisons that were significant at the 0.05 level. The format within each cell is: (1) the ratio of group geometric means at baseline, (2) the relationship between the corresponding pair of group geometric means at baseline shown in parentheses, and (3) the Tukey-adjusted p-value.

Table 4a. Descriptive Statistics for Red Blood Cell Count (RBC, 106 cells/µL), by Group and Study Day

Group	Study Day	N	Mean (95% Confidence Interval)
1	-3	5	5.60 (4.87, 6.33)
	2	5	5.52 (4.90, 6.14)
	4	4	5.12 (4.04, 6.19)
	9	5	5.45 (4.76, 6.14)
	11	5	5.57 (4.91, 6.23)
	16	4	5.54 (4.45, 6.63)
	18	5	5.46 (4.74, 6.19)
	23	4	5.91 (5.22, 6.60)
	25	3	6.57 (5.32, 7.81)
	30	5	5.61 (4.88, 6.34)
	32	1	5.45 (--)
	37	5	5.87 (5.11, 6.63)
	39	5	5.02 (3.55, 6.49)
2	-3	7	5.99 (5.72, 6.26)
	2	7	5.98 (5.66, 6.30)
	4	7	5.83 (5.53, 6.14)
	9	7	5.86 (5.61, 6.11)
	11	7	5.93 (5.43, 6.43)
	16	7	5.71 (5.53, 5.89)
	18	7	5.78 (5.49, 6.07)
	23	6	6.04 (5.58, 6.51)
	25	6	6.06 (5.65, 6.46)
	30	7	5.96 (5.60, 6.32)
	32	3	5.76 (4.93, 6.58)
	37	7	6.11 (5.86, 6.36)
	39	7	5.70 (5.32, 6.08)
3	-3	7	5.86 (5.54, 6.19)
	2	7	5.73 (5.45, 6.00)
	4	7	5.59 (5.28, 5.91)
	9	6	5.66 (5.40, 5.92)
	11	6	5.53 (5.14, 5.92)
	16	5	6.04 (5.48, 6.59)
	18	6	5.86 (5.39, 6.34)
	23	3	6.03 (5.56, 6.51)
	25	6	5.85 (5.53, 6.17)
	30	3	5.86 (4.69, 7.03)
	32	5	5.75 (5.22, 6.28)
	37	4	5.92 (5.39, 6.46)
	39	6	5.48 (4.56, 6.39)

Table 4a. (Continued)

Group	Study Day	N	Mean (95% Confidence Interval)
4	-3	7	5.85 (5.40, 6.30)
	2	7	5.66 (5.27, 6.06)
	4	4	5.75 (5.38, 6.11)
	9	7	5.78 (5.09, 6.47)
	11	6	5.46 (5.07, 5.84)
	16	4	5.88 (5.73, 6.04)
	18	4	5.84 (5.44, 6.24)
	23	2	5.45 (0.00[a], 15.04)
	25	2	5.44 (0.00[a], 13.57)
	30	3	5.78 (4.55, 7.01)
	32	3	5.45 (4.64, 6.25)
	37	3	5.82 (4.79, 6.85)
	39	3	5.90 (5.15, 6.64)

-- Confidence interval could not be calculated since only one observation was available for this group on this Study Day.

a Negative lower confidence limit was set to 0 since negative values are not possible.

Table 4b. Test Results for Red Blood Cell Count (RBC, 106 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		
2	-0.08	-0.01	-0.14	-0.18	0.7544	
4	-0.64↓	-0.16	-0.27	-0.36	0.2161	
9	-0.15	-0.13	-0.27	-0.07	0.7885	
11	-0.03	-0.06	-0.29	-0.34↓	0.2088	
16	-0.10	-0.28↓	0.17	-0.09	0.1074	
18	-0.14	-0.21	-0.07	-0.13	0.9154	
23	0.16	0.05	0.23	-0.98	0.0432*	1.21 (4<3) 0.0489
25	0.63	0.06	-0.08	-0.98	0.0040*	1.62 (4<1) 0.0023 1.05 (4<2) 0.0229
30	0.01	-0.03	0.14	-0.32	0.5839	
32	0.25	-0.08	-0.20	-0.65	0.2839	
37	0.27	0.12	0.00	-0.28	0.2678	
39	-0.58	-0.29	-0.46	-0.20	0.8059	

Cells contain all pairwise comparisons that were significant at the 0.05 level. The format within each cell is: (1) the difference of group mean shifts, (2) the relationship between the corresponding pair of group mean shifts shown in parentheses, and (3) the 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 5a. Descriptive Statistics for Hemoglobin (HGB, g/dL) by Group and Study Day

Group	Study Day	N	Mean (95% Confidence Interval)
1	-3	5	11.6 (10.2, 13.0)
	2	5	11.5 (10.4, 12.6)
	4	4	10.5 (8.5, 12.6)
	9	5	11.4 (10.2, 12.5)
	11	5	11.7 (10.3, 13.0)
	16	4	11.5 (9.6, 13.3)
	18	5	11.3 (10.1, 12.6)
	23	4	12.2 (11.1, 13.3)
	25	3	13.3 (10.8, 15.7)
	30	5	11.6 (10.2, 13.1)
	32	1	11.5 (--)
	37	5	12.0 (10.8, 13.3)
	39	5	10.3 (7.4, 13.2)
2	-3	7	12.2 (11.5, 12.8)
	2	7	12.2 (11.6, 12.9)
	4	7	11.9 (11.2, 12.5)
	9	7	11.9 (11.4, 12.5)
	11	7	12.1 (11.0, 13.3)
	16	7	11.7 (11.3, 12.2)
	18	7	11.8 (11.1, 12.5)
	23	6	12.3 (11.4, 13.2)
	25	6	12.2 (11.5, 13.0)
	30	7	12.0 (11.4, 12.7)
	32	3	11.6 (10.4, 12.7)
	37	7	12.3 (11.8, 12.8)
	39	7	11.6 (10.9, 12.3)
3	-3	7	12.1 (11.7, 12.5)
	2	7	11.9 (11.4, 12.4)
	4	7	11.6 (11.1, 12.1)
	9	6	11.8 (11.5, 12.2)
	11	6	11.6 (10.9, 12.2)
	16	5	12.6 (11.7, 13.5)
	18	6	12.1 (11.1, 13.1)
	23	3	12.7 (12.5, 12.9)
	25	6	12.1 (11.4, 12.8)
	30	3	12.3 (9.1, 15.5)
	32	5	11.8 (11.3, 12.3)
	37	4	12.2 (11.3, 13.0)
	39	6	11.3 (9.8, 12.7)

Table 5a. (Continued)

Group	Study Day	N	Mean (95% Confidence Interval)
4	-3	7	12.0 (11.1, 12.9)
	2	7	11.7 (10.8, 12.5)
	4	4	11.8 (10.8, 12.7)
	9	7	11.9 (10.5, 13.3)
	11	6	11.3 (10.5, 12.0)
	16	4	12.2 (11.7, 12.6)
	18	4	11.9 (11.6, 12.2)
	23	2	10.7 (0.0[a], 31.0)
	25	2	10.7 (0.0[a], 28.5)
	30	3	11.6 (8.9, 14.3)
	32	3	10.9 (8.4, 13.4)
	37	3	11.4 (8.6, 14.2)
	39	3	11.7 (9.0, 14.5)

-- Confidence interval could not be calculated since only one observation was available for this group on this Study Day.

a Negative lower confidence limit was set to 0 since negative values are not possible.

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		
2	-0.12	0.04	-0.17	-0.33	0.7542	
4	-1.35↓	-0.31	-0.49	-0.77	0.1264	
9	-0.22	-0.24	-0.38	-0.09	0.9395	
11	0.06	-0.04	-0.50	-0.67	0.2609	
16	-0.13	-0.44	0.48	-0.20	0.1157	
18	-0.26	-0.39	-0.13	-0.47	0.8869	
23	0.35	0.18	0.50	-2.40	0.0351*	2.75 (4<1) 0.0430 2.58 (4<2) 0.0438 2.90 (4<3) 0.0429
25	1.03	0.12	-0.10	-2.40	0.0088*	3.43 (4<1) 0.0056 2.52 (4<2) 0.0218 2.30 (4<3) 0.0370
30	0.04	-0.14	0.17	-1.03	0.4223	
32	0.70	-0.10	-0.46	-1.70	0.2362	
37	0.44	0.13	-0.05	-1.23	0.1090	
39	-1.30	-0.56	-0.95	-0.90	0.8199	

Cells contain all pairwise comparisons that were significant at the 0.05 level. The format within each cell is: (1) the difference of group mean shifts, (2) the relationship between the corresponding pair of group mean shifts shown in parentheses, and (3) the 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 6a. Descriptive Statistics for Hematocrit (HCT, %) by Group and Study Day

Group	Study Day	N	Mean (95% Confidence Interval)
1	-3	5	35.5 (30.5, 40.5)
	2	5	35.0 (31.6, 38.5)
	4	4	32.1 (25.0, 39.2)
	9	5	34.8 (30.6, 39.0)
	11	5	35.7 (31.2, 40.2)
	16	4	35.4 (29.0, 41.7)
	18	5	34.5 (30.7, 38.3)
	23	4	36.5 (33.0, 40.1)
	25	3	40.6 (32.6, 48.6)
	30	5	35.1 (30.9, 39.3)
	32	1	34.5 (--)
	37	5	36.3 (31.9, 40.7)
	39	5	31.1 (21.7, 40.4)
2	-3	7	37.8 (36.0, 39.5)
	2	7	37.7 (35.6, 39.8)
	4	7	36.9 (34.7, 39.1)
	9	7	37.3 (36.1, 38.5)
	11	7	38.0 (34.6, 41.4)
	16	7	36.3 (34.7, 37.8)
	18	7	36.1 (34.0, 38.2)
	23	6	37.4 (34.4, 40.4)
	25	6	37.7 (34.6, 40.8)
	30	7	37.2 (34.8, 39.5)
	32	3	34.8 (31.3, 38.3)
	37	7	37.8 (36.1, 39.5)
	39	7	35.5 (33.1, 37.9)
3	-3	7	37.2 (35.9, 38.6)
	2	7	36.5 (34.6, 38.4)
	4	7	35.3 (33.0, 37.5)
	9	6	36.1 (34.7, 37.5)
	11	6	36.0 (33.4, 38.5)
	16	5	38.9 (35.9, 42.0)
	18	6	36.7 (33.6, 39.8)
	23	3	38.6 (36.6, 40.6)
	25	6	36.6 (34.1, 39.1)
	30	3	38.5 (26.6, 50.5)
	32	5	35.8 (34.2, 37.3)
	37	4	36.8 (33.2, 40.4)
	39	6	34.0 (29.4, 38.7)

Table 6a. (Continued)

Group	Study Day	N	Mean (95% Confidence Interval)
4	-3	7	37.8 (34.3, 41.4)
	2	7	36.7 (34.1, 39.3)
	4	4	36.9 (34.2, 39.6)
	9	7	37.7 (33.2, 42.3)
	11	6	35.8 (32.8, 38.7)
	16	4	38.5 (37.6, 39.3)
	18	4	37.5 (35.5, 39.4)
	23	2	34.2 (0.0[a], 101.5)
	25	2	36.3 (0.0[a], 87.1)
	30	3	37.3 (29.9, 44.8)
	32	3	34.3 (29.5, 39.2)
	37	3	36.4 (28.3, 44.5)
	39	3	37.1 (31.0, 43.2)

-- Confidence interval could not be calculated since only one observation was available for this group on this Study Day.

a Negative lower confidence limit was set to 0 since negative values are not possible.

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		
2	-0.48	-0.10	-0.73	-1.13	0.8645	
4	-4.25↓	-0.89	-1.94	-3.05	0.2157	
9	-0.68	-0.51	-1.55	-0.07	0.8047	
11	0.18	0.21	-1.23	-2.00	0.2519	
16	-0.45	-1.51	1.50	-1.13	0.1121	
18	-1.02	-1.70	-0.97	-2.13	0.8544	
23	0.23	-0.30	0.67	-8.35	0.0579	
25	2.93	0.00	-1.07	-6.25	0.0284*	9.18 (4<1) 0.0188
30	-0.42	-0.61	0.80	-3.23	0.4166	
32	2.20	-1.47	-2.06	-6.23	0.1439	
37	0.82	0.01	-0.65	-4.20	0.1505	
39	-4.46	-2.29	-3.63	-3.43	0.8440	

Cells contain all pairwise comparisons that were significant at the 0.05 level. The format within each cell is: (1) the difference of group mean shifts, (2) the relationship between the corresponding pair of group mean shifts shown in parentheses, and (3) the 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 7a. Descriptive Statistics for Mean Corpuscular Volume (MCV, fL) by Group and Study Day

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
1	-3	5	63.4 (61.6, 65.2)
	2	5	63.6 (62.2, 64.9)
	4	4	62.6 (60.3, 65.0)
	9	5	64.0 (62.7, 65.3)
	11	5	64.1 (62.4, 65.8)
	16	4	63.9 (62.0, 65.8)
	18	5	63.2 (61.6, 64.9)
	23	4	62.0 (59.3, 64.7)
	25	3	61.9 (60.2, 63.5)
	30	5	62.7 (61.3, 64.1)
	32	1	63.2 (--)
	37	5	61.9 (60.6, 63.2)
	39	5	61.8 (60.6, 63.1)
2	-3	7	63.1 (61.9, 64.3)
	2	7	63.0 (61.8, 64.3)
	4	7	63.2 (61.4, 65.1)
	9	7	63.6 (62.5, 64.7)
	11	7	64.0 (62.8, 65.2)
	16	7	63.5 (62.1, 65.0)
	18	7	62.3 (61.0, 63.6)
	23	6	61.8 (60.5, 63.2)
	25	6	62.2 (60.4, 64.1)
	30	7	62.2 (61.2, 63.3)
	32	3	60.6 (57.1, 64.2)
	37	7	61.8 (60.7, 63.0)
	39	7	62.3 (61.0, 63.6)
3	-3	7	63.6 (60.9, 66.4)
	2	7	63.7 (61.0, 66.6)
	4	7	63.0 (60.1, 66.1)
	9	6	63.8 (60.2, 67.7)
	11	6	65.0 (61.7, 68.4)
	16	5	64.6 (60.7, 68.7)
	18	6	62.6 (59.7, 65.7)
	23	3	64.0 (56.6, 72.5)
	25	6	62.5 (59.0, 66.3)
	30	3	65.6 (56.9, 75.6)
	32	5	62.3 (58.3, 66.6)
	37	4	62.0 (55.9, 68.9)
	39	6	62.4 (59.6, 65.3)

Table 7a. (Continued)

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
4	-3	7	64.6 (62.4, 66.8)
	2	7	64.7 (62.9, 66.7)
	4	4	64.3 (60.6, 68.2)
	9	7	65.3 (63.8, 66.8)
	11	6	65.4 (63.7, 67.2)
	16	4	65.3 (63.6, 67.2)
	18	4	64.2 (62.8, 65.6)
	23	2	62.7 (50.7, 77.6)
	25	2	66.7 (60.1, 74.1)
	30	3	64.7 (62.6, 66.8)
	32	3	63.0 (60.8, 65.3)
	37	3	62.5 (59.7, 65.4)
	39	3	62.9 (60.3, 65.7)

-- Confidence interval could not be calculated since only one observation was available for this group on this Study Day.

Table 7b. Test Results for Mean Corpuscular Volume (MCV, fL)

Mean Corpuscular Volume†						
Study Day	Mean Shift as a Proportion from Baseline, by Group				Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value #
	1	2	3	4		
2	1.00	1.00	1.00	1.00	0.9591	
4	0.99	1.00	0.99	0.98	0.2406	
9	1.01	1.01	1.00	1.01	0.8678	
11	1.01	1.01	1.02	1.01	0.8177	
16	1.01	1.01	1.01	0.99	0.1814	
18	1.00	0.99	0.99	0.97↓	0.1402	
23	0.98	0.98	0.98	0.95	0.4505	
25	0.98	0.99	0.98	1.01	0.2851	
30	0.99	0.99	0.99	0.97	0.5572	
32	1.02	0.97	0.98↓	0.95↓	0.0123*	1.07 (4<1) 0.0110
37	0.98	0.98	0.98	0.94↓	0.0868	
39	0.98↓	0.99	0.98	0.95↓	0.0431*	1.04 (4<2) 0.0296

† Indicates that values for this parameter were log-transformed for the analysis.
# Cells contain all pairwise comparisons that were significant at the 0.05 level. The format within each cell is: (1) the ratio of group mean shifts, (2) the relationship between the corresponding pair of group mean shifts shown in parentheses, and (3) the 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.

Table 8a. Descriptive Statistics for Mean Corpuscular Hemoglobin (MCH, pg) by Group and Study Day

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
1	-3	5	20.7 (20.1, 21.4)
	2	5	20.8 (20.2, 21.3)
	4	4	20.5 (19.7, 21.4)
	9	5	20.9 (20.1, 21.6)
	11	5	20.9 (20.3, 21.5)
	16	4	20.8 (19.9, 21.6)
	18	5	20.8 (20.2, 21.4)
	23	4	20.7 (20.0, 21.4)
	25	3	20.2 (19.0, 21.5)
	30	5	20.8 (20.3, 21.3)
	32	1	21.2 (--)
	37	5	20.5 (19.8, 21.3)
	39	5	20.7 (20.1, 21.2)
2	-3	7	20.3 (19.7, 21.0)
	2	7	20.5 (19.8, 21.1)
	4	7	20.3 (19.7, 21.0)
	9	7	20.4 (19.7, 21.0)
	11	7	20.4 (20.0, 20.9)
	16	7	20.5 (20.0, 21.1)
	18	7	20.4 (19.8, 21.1)
	23	6	20.3 (19.7, 20.9)
	25	6	20.2 (19.8, 20.7)
	30	7	20.2 (19.6, 20.9)
	32	3	20.1 (18.4, 22.0)
	37	7	20.2 (19.6, 20.8)
	39	7	20.4 (19.8, 21.0)
3	-3	7	20.7 (19.8, 21.5)
	2	7	20.8 (20.2, 21.5)
	4	7	20.7 (20.1, 21.4)
	9	6	20.9 (20.1, 21.7)
	11	6	20.9 (20.1, 21.8)
	16	5	20.9 (19.8, 22.0)
	18	6	20.6 (19.7, 21.6)
	23	3	21.1 (19.1, 23.2)
	25	6	20.7 (19.9, 21.5)
	30	3	21.0 (19.3, 22.8)
	32	5	20.5 (19.3, 21.9)
	37	4	20.6 (19.0, 22.2)
	39	6	20.6 (19.6, 21.6)

Table 8a. (Continued)

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
4	-3	7	20.4 (19.9, 21.0)
	2	7	20.6 (20.0, 21.2)
	4	4	20.5 (19.3, 21.8)
	9	7	20.6 (20.2, 21.0)
	11	6	20.6 (20.0, 21.3)
	16	4	20.6 (19.4, 21.9)
	18	4	20.4 (19.3, 21.7)
	23	2	19.6 (16.1, 23.8)
	25	2	19.5 (16.6, 23.0)
	30	3	20.0 (17.6, 22.7)
	32	3	20.0 (18.0, 22.3)
	37	3	19.6 (17.5, 22.0)
	39	3	19.9 (17.8, 22.1)

-- Confidence interval could not be calculated since only one observation was available for this group on this Study Day.

Table 8b. Test Results for Mean Corpuscular Hemoglobin (MCH, pg)

Mean Corpuscular Hemoglobin†						
Study Day	Mean Shift as a Proportion from Baseline, by Group				Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value #
	1	2	3	4		
2	1.00	1.01	1.01	1.01	0.6843	
4	0.99	1.00	1.00	1.00	0.7866	
9	1.01	1.00	1.01	1.01	0.5964	
11	1.01	1.01	1.01	1.00	0.9808	
16	1.01	1.01	1.01	1.00	0.4839	
18	1.00	1.00	1.00	0.99	0.3509	
23	1.00	1.01	1.00	0.96	0.2630	
25	0.98	1.00	1.00	0.96	0.0886	
30	1.00	1.00	0.98	0.97	0.1597	
32	1.02	1.01	0.99	0.97	0.2488	
37	0.99	0.99	1.00	0.95	0.0534	
39	1.00	1.00	1.00	0.96	0.0825	

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

Cells contain all pairwise comparisons that were significant at the 0.05 level. The format within each cell is: (1) the ratio of group mean shifts, (2) the relationship between the corresponding pair of group mean shifts shown in parentheses, and (3) the Tukey-adjusted p-value.

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)
1	-3	5	32.7 (31.5, 33.8)
	2	5	32.7 (32.4, 32.9)
	4	4	32.8 (31.7, 34.0)
	9	5	32.6 (31.7, 33.6)
	11	5	32.7 (31.9, 33.5)
	16	4	32.5 (31.9, 33.1)
	18	5	32.9 (32.5, 33.3)
	23	4	33.4 (32.8, 34.0)
	25	3	32.7 (31.4, 34.1)
	30	5	33.2 (32.3, 34.1)
	32	1	33.5 (--)
	37	5	33.1 (32.5, 33.7)
	39	5	33.4 (32.5, 34.3)
2	-3	7	32.2 (31.5, 32.9)
	2	7	32.5 (31.8, 33.1)
	4	7	32.2 (31.6, 32.8)
	9	7	32.0 (31.4, 32.6)
	11	7	31.9 (31.7, 32.2)
	16	7	32.4 (31.7, 33.0)
	18	7	32.7 (32.1, 33.3)
	23	6	32.8 (32.3, 33.3)
	25	6	32.5 (31.7, 33.2)
	30	7	32.5 (31.8, 33.1)
	32	3	33.3 (32.0, 34.5)
	37	7	32.6 (32.0, 33.2)
	39	7	32.8 (32.0, 33.5)
3	-3	7	32.5 (32.1, 32.9)
	2	7	32.7 (32.1, 33.2)
	4	7	32.9 (32.1, 33.7)
	9	6	32.7 (31.8, 33.6)
	11	6	32.2 (31.5, 32.9)
	16	5	32.4 (31.9, 32.9)
	18	6	32.9 (32.4, 33.4)
	23	3	32.9 (31.9, 34.0)
	25	6	33.1 (32.3, 33.9)
	30	3	32.0 (30.2, 33.8)
	32	5	32.9 (32.6, 33.3)
	37	4	33.1 (32.1, 34.1)
	39	6	33.0 (32.3, 33.8)

Table 9a. (Continued)

Group	Study Day	N	Mean (95% Confidence Interval)
4	-3	7	31.7 (31.1, 32.4)
	2	7	31.8 (31.1, 32.5)
	4	4	31.9 (31.3, 32.5)
	9	7	31.5 (30.8, 32.2)
	11	6	31.5 (30.9, 32.1)
	16	4	31.6 (30.4, 32.8)
	18	4	31.9 (30.6, 33.1)
	23	2	31.2 (29.9, 32.5)
	25	2	29.4 (21.8, 37.0)
	30	3	31.0 (26.2, 35.8)
	32	3	31.8 (28.8, 34.8)
	37	3	31.4 (28.6, 34.2)
	39	3	31.6 (29.2, 33.9)

-- Confidence interval could not be calculated since only one observation was available for this group on 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		
2	0.00	0.29	0.17	0.06	0.7912	
4	0.08	0.01	0.43	0.48	0.5351	
9	-0.06	-0.19	0.30	-0.20	0.2699	
11	-0.02	-0.26	-0.33	-0.13	0.6484	
16	0.10	0.16	-0.10	0.33	0.6689	
18	0.18	0.51↑	0.45↑	0.58	0.4966	
23	0.67	0.77↑	0.70	0.45	0.9464	
25	0.23	0.42	0.65	-1.35	0.0618	2.00 (4<3) 0.0438
30	0.52	0.27	-0.23	-0.13	0.4258	
32	0.00	1.10	0.48	0.63	0.3221	
37	0.44	0.41	0.50	0.27	0.9659	
39	0.70	0.57	0.62	0.43	0.9672	

Cells contain all pairwise comparisons that were significant at the 0.05 level. The format within each cell is: (1) the difference of group mean shifts, (2) the relationship between the corresponding pair of group mean shifts shown in parentheses, and (3) the 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 10a. Descriptive Statistics for Red Cell Distribution Width (RDW, %) by Group and Study Day

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
1	-3	5	12.7 (11.8, 13.8)
	2	5	12.4 (11.7, 13.3)
	4	4	12.5 (12.0, 13.1)
	9	5	12.7 (11.8, 13.7)
	11	5	12.4 (11.8, 13.1)
	16	4	12.5 (11.4, 13.6)
	18	5	12.4 (11.7, 13.1)
	23	4	12.0 (11.2, 12.8)
	25	3	11.6 (10.7, 12.5)
	30	5	12.2 (11.8, 12.5)
	32	1	12.1 (--)
	37	5	12.2 (11.9, 12.6)
	39	5	12.3 (11.9, 12.6)
2	-3	7	11.9 (11.5, 12.4)
	2	7	11.7 (11.3, 12.2)
	4	7	12.0 (11.4, 12.6)
	9	7	12.1 (11.5, 12.7)
	11	7	12.0 (11.6, 12.5)
	16	7	12.0 (11.5, 12.5)
	18	7	12.5 (11.9, 13.1)
	23	6	11.7 (11.2, 12.2)
	25	6	11.6 (11.2, 12.2)
	30	7	11.8 (11.5, 12.1)
	32	3	12.1 (11.6, 12.6)
	37	7	11.8 (11.5, 12.2)
	39	7	11.8 (11.6, 12.1)
3	-3	7	12.0 (11.6, 12.5)
	2	7	11.9 (11.4, 12.4)
	4	7	11.9 (11.4, 12.3)
	9	6	12.0 (11.4, 12.6)
	11	6	12.1 (11.3, 13.0)
	16	5	12.0 (11.2, 13.0)
	18	6	11.9 (11.5, 12.3)
	23	3	11.6 (11.0, 12.3)
	25	6	11.6 (11.2, 12.0)
	30	3	11.8 (10.6, 13.2)
	32	5	11.8 (11.2, 12.5)
	37	4	11.6 (10.9, 12.4)
	39	6	11.7 (11.2, 12.2)

Table 10a. (Continued)

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
4	-3	7	12.7 (12.2, 13.2)
	2	7	12.4 (11.8, 12.9)
	4	4	12.6 (11.5, 13.8)
	9	7	12.7 (12.1, 13.3)
	11	6	12.4 (11.6, 13.3)
	16	4	12.5 (11.7, 13.3)
	18	4	12.1 (11.3, 13.0)
	23	2	13.6 (4.2, 43.7)
	25	2	14.8 (1.4, 160.9)
	30	3	13.5 (8.5, 21.6)
	32	3	13.6 (9.4, 19.6)
	37	3	12.9 (9.7, 17.2)
	39	3	12.9 (10.0, 16.7)

-- Confidence interval could not be calculated since only one observation was available for this group on this Study Day.

Table 10b. Test Results for Red Cell Distribution Width (RDW, %)

Red Cell Distribution Width†						
Study Day	Mean Shift as a Proportion from Baseline, by Group				Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value #
	1	2	3	4		
2	0.98	0.98	0.99	0.98	0.7815	
4	0.99	1.00	0.98	1.00	0.7898	
9	1.00	1.01	1.01	1.00	0.9052	
11	0.98	1.01	1.00	0.97	0.3128	
16	1.00	1.01	0.99	0.98	0.7196	
18	0.97	1.05↑	1.00	0.95	0.0204*	1.10 (4<2) 0.0278
23	0.95	0.98	0.98	1.04	0.2958	
25	0.92	0.97	0.97	1.14	0.0544	1.24 (1<4) 0.0389
30	0.96	0.99	1.00	1.05	0.4365	
32	0.95	1.00	0.99	1.05	0.5851	
37	0.96	0.99	0.97	1.00	0.6306	
39	0.96	0.99	0.98	1.00	0.6035	

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

Cells contain all pairwise comparisons that were significant at the 0.05 level. The format within each cell is: (1) the ratio of group mean shifts, (2) the relationship between the corresponding pair of group mean shifts shown in parentheses, and (3) the 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.

Table 11a. Descriptive Statistics for Platelet Count (PLT, 103 cells/µL) by Group and Study Day

Group	Study Day	N	Mean (95% Confidence Interval)
1	-3	5	627 (387, 868)
	2	5	634 (386, 882)
	4	4	364 (0[a], 746)
	9	5	518 (179, 858)
	11	5	597 (325, 869)
	16	4	446 (171, 722)
	18	5	527 (368, 687)
	23	4	368 (0[a], 805)
	25	3	503 (168, 839)
	30	5	430 (148, 712)
	32	1	447 (--)
	37	5	446 (306, 585)
	39	5	236 (0[a], 493)
2	-3	7	547 (378, 716)
	2	7	520 (370, 670)
	4	7	548 (468, 627)
	9	7	598 (500, 695)
	11	7	576 (478, 674)
	16	7	492 (437, 548)
	18	7	471 (359, 583)
	23	6	483 (365, 601)
	25	6	386 (268, 503)
	30	7	408 (332, 485)
	32	3	400 (176, 624)
	37	7	429 (347, 511)
	39	7	250 (143, 358)
3	-3	7	565 (348, 783)
	2	7	516 (413, 618)
	4	7	413 (222, 605)
	9	6	586 (457, 715)
	11	6	438 (309, 566)
	16	5	461 (385, 536)
	18	6	537 (353, 721)
	23	3	401 (110, 692)
	25	6	395(166, 625)
	30	3	498 (0[a], 1019)
	32	5	385 (262, 509)
	37	4	498 (387, 609)
	39	6	210 (23, 396)

Table 11a. (Continued)

Group	Study Day	N	Mean (95% Confidence Interval)
4	-3	7	744 (533, 955)
	2	7	680 (483, 878)
	4	4	649 (238, 1060)
	9	7	670 (532, 808)
	11	6	489 (225, 752)
	16	4	594 (260, 928)
	18	4	630 (396, 863)
	23	2	1020 (0[a], 6750)
	25	2	1296 (0[a], 10806)
	30	3	732 (0[a], 1974)
	32	3	615 (0[a], 1448)
	37	3	765 (0[a], 2149)
	39	3	716 (0[a], 1440)

-- Confidence interval could not be calculated since only one observation was available for this group on this Study Day.

a Negative lower confidence limit was set to 0 since negative values are not possible.

Table 11b. Test Results for Platelet Count (PLT, 103 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		
2	7.00	-27.00	-49.71	-63.57	0.8491	
4	-209.25	0.57	-152.14↓	-49.75	0.1120	
9	-109.20	50.57	30.00	-74.29	0.4877	
11	-30.60	28.71	-188.67	-303.50↓	0.1052	
16	-202.00	-54.57	5.40	-119.25	0.4353	
18	-100.00	-76.14	-18.83	-83.75	0.8636	
23	-204.75	-55.17	4.67	179.50	0.3292	
25	-129.67	-152.17	-160.67	455.00	0.1110	
30	-197.40	-138.57	-92.00	-48.33	0.7641	
32	55.00	-113.67	-126.00	-164.67	0.7869	
37	-181.80	-118.00	-184.25	-15.00	0.6654	
39	-391.60↓	-296.71↓	-346.50↓	-63.67	0.4431	

Cells contain all pairwise comparisons that were significant at the 0.05 level. The format within each cell is (1) the difference of group mean shifts, (2) the relationship between the corresponding pair of group mean shifts shown in parentheses, and (3) the 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 12a. Descriptive Statistics for Mean Platelet Volume (MPV, fL) by Group and Study Day

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
1	-3	5	6.9 (6.2, 7.8)
	2	5	6.2 (5.8, 6.7)
	4	4	9.4 (5.9, 14.8)
	9	5	8.6 (8.0, 9.3)
	11	5	8.0 (6.3, 10.2)
	16	4	7.9 (7.1, 8.9)
	18	5	7.8 (7.3, 8.4)
	23	4	7.4 (5.2, 10.4)
	25	3	6.8 (5.3, 8.8)
	30	5	6.8 (5.8, 8.0)
	32	1	6.2 (--)
	37	5	6.4 (5.6, 7.3)
	39	5	9.4 (6.6, 13.3)
2	-3	7	7.4 (7.3, 7.6)
	2	7	6.6 (6.2, 7.1)
	4	7	7.3 (6.8, 7.7)
	9	7	8.5 (8.3, 8.6)
	11	7	7.0 (6.6, 7.5)
	16	7	6.9 (6.2, 7.7)
	18	7	7.9 (6.6, 9.4)
	23	6	6.3 (5.9, 6.7)
	25	6	6.6 (5.9, 7.5)
	30	7	6.8 (6.4, 7.3)
	32	3	6.7 (4.1, 10.9)
	37	7	6.3 (5.9, 6.6)
	39	7	8.5 (6.5, 11.1)
3	-3	7	6.7 (6.3, 7.1)
	2	7	6.4 (5.7, 7.2)
	4	7	6.9 (6.4, 7.5)
	9	6	7.6 (7.2, 8.1)
	11	6	7.8 (6.4, 9.4)
	16	5	6.7 (5.8, 7.8)
	18	6	6.3 (5.6, 7.1)
	23	3	6.6 (5.9, 7.4)
	25	6	6.5 (5.4, 7.9)
	30	3	6.6 (4.6, 9.4)
	32	5	6.7 (4.9, 9.1)
	37	4	5.8 (5.1, 6.6)
	39	6	9.0 (6.9, 11.8)

Table 12a. (Continued)

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
4	-3	7	6.7 (6.3, 7.2)
	2	7	6.6 (6.0, 7.2)
	4	4	6.5 (6.0, 7.0)
	9	7	8.3 (7.8, 8.7)
	11	6	6.9 (6.0, 7.8)
	16	4	6.4 (6.1, 6.7)
	18	4	6.5 (6.0, 7.1)
	23	2	7.0 (3.7, 13.2)
	25	2	7.9 (1.4, 46.0)
	30	3	6.7 (4.8, 9.2)
	32	3	7.0 (4.1, 12.1)
	37	3	6.2 (5.1, 7.5)
	39	3	6.9 (3.5, 13.5)

-- Confidence interval could not be calculated since only one observation was available for this group on this Study Day.

Table 12b. Test Results for Mean Platelet Volume (MPV, fL)

Mean Platelet Volume†						
Study Day	Mean Shift as a Proportion from Baseline, by Group				Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value #
	1	2	3	4		
2	0.90	0.89↓	0.96	0.97	0.2070	
4	1.33↑	0.98	1.03	0.96	0.0064*	1.37 (2<1) 0.0071 1.30 (3<1) 0.0258 1.39 (4<1) 0.0130
9	1.24↑	1.14↑	1.13↑	1.22↑	0.0376*	
11	1.16	0.95	1.16	1.01	0.0583	
16	1.15	0.93	0.99	0.93	0.0362*	1.23 (2<1) 0.0343
18	1.13	1.06	0.93	0.95	0.1046	
23	1.05	0.85↓	0.94	0.97	0.1236	
25	0.97	0.90	0.97	1.09	0.4534	
30	0.98	0.92↓	0.94	0.98	0.4191	
32	0.87	0.91	0.99	1.03	0.7925	
37	0.92↓	0.84↓	0.89↓	0.91	0.1247	
39	1.35	1.14	1.33↑	1.01	0.3745	

† Indicates that values for this parameter were log-transformed for the analysis.
# Cells contain all pairwise comparisons that were significant at the 0.05 level. The format within each cell is: (1) the ratio of group mean shifts, (2) the relationship between the corresponding pair of group mean shifts shown in parentheses, and (3) the 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.

Table 13a. Descriptive Statistics for White Blood Cell Count (WBC, 103 cells/µL) by Group and Study Day

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
1	-3	5	5.76 (3.92, 8.48)
	2	5	6.09 (4.80, 7.74)
	4	4	4.41 (1.46, 13.31)
	9	5	5.78 (3.88, 8.63)
	11	5	5.88 (4.63, 7.46)
	16	4	6.21 (4.55, 8.49)
	18	5	6.04 (4.49, 8.13)
	23	4	5.67 (2.84, 11.31)
	25	3	6.02 (3.06, 11.85)
	30	5	6.06 (4.16, 8.82)
	32	1	6.51 (--)
	37	5	6.60 (5.22, 8.34)
	39	5	3.21 (1.25, 8.22)
2	-3	7	6.91 (5.77, 8.27)
	2	7	7.12 (5.74, 8.82)
	4	7	7.26 (6.06, 8.71)
	9	7	7.35 (6.24, 8.67)
	11	7	7.20 (6.23, 8.32)
	16	7	6.93 (5.90, 8.14)
	18	7	6.99 (5.74, 8.50)
	23	6	6.56 (5.65, 7.61)
	25	6	6.09 (5.02, 7.40)
	30	7	6.66 (5.86, 7.58)
	32	3	5.41 (4.88, 6.01)
	37	7	6.38 (5.14, 7.92)
	39	7	4.38 (3.02, 6.35)
3	-3	7	6.18 (5.16, 7.40)
	2	7	5.32 (4.55, 6.23)
	4	7	5.37 (4.37, 6.62)
	9	6	5.86 (4.89, 7.02)
	11	6	6.34 (5.16, 7.79)
	16	5	5.85 (4.65, 7.37)
	18	6	5.87 (4.41, 7.82)
	23	3	7.33 (4.10, 13.08)
	25	6	6.01 (4.68, 7.73)
	30	3	6.93 (3.53, 13.59)
	32	5	5.92 (4.81, 7.29)
	37	4	5.63 (4.68, 6.76)
	39	6	3.63 (1.97, 6.68)

Table 13a. (Continued)

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
4	-3	7	7.16 (5.62, 9.13)
	2	7	7.00 (5.38, 9.10)
	4	4	8.23 (5.54, 12.22)
	9	7	6.79 (5.74, 8.03)
	11	6	6.58 (4.21, 10.29)
	16	4	7.28 (5.35, 9.92)
	18	4	9.36 (8.17, 10.72)
	23	2	13.49 (0.07, 2475.61)
	25	2	13.27 (0.26, 675.42)
	30	3	9.12 (2.37, 35.15)
	32	3	8.85 (5.51, 14.20)
	37	3	6.81 (3.81, 12.17)
	39	3	6.54 (4.77, 8.97)

-- Confidence interval could not be calculated since only one observation was available for this group on this Study Day.

Table 13b. Test Results for White Blood Cell Count (WBC, 103 cells/µL)

White Blood Cell Count†						
Study Day	Mean Shift as a Proportion from Baseline, by Group				Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value #
	1	2	3	4		
2	1.06	1.03	0.86	0.98	0.2954	
4	0.84	1.05	0.87	0.99	0.5770	
9	1.00	1.06	0.92	0.95	0.7112	
11	1.02	1.04	0.98	0.94	0.8349	
16	1.06	1.00	1.00	0.89	0.8006	
18	1.05	1.01	0.92	1.14	0.7318	
23	1.08	0.97	1.24	1.60	0.4020	
25	1.09	0.90	0.95	1.57	0.1273	
30	1.05	0.96	0.96	1.08	0.9490	
32	1.45	0.75	0.98	1.05	0.1716	
37	1.14	0.92	0.81	0.81	0.1815	
39	0.56	0.63	0.57	0.78	0.8894	

† Indicates that values for this parameter were log-transformed for the analysis.
# Cells contain all pairwise comparisons that were significant at the 0.05 level. The format within each cell is: (1) the ratio of group mean shifts, (2) the relationship between the corresponding pair of group mean shifts shown in parentheses, and (3) the Tukey-adjusted p-value.

Table 14a. Descriptive Statistics for Neutrophil Count (103 cells/µL) by Group and Study Day

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
1	-3	5	1.69 (0.77, 3.74)
	2	5	1.55 (1.03, 2.33)
	4	4	1.14 (0.28, 4.56)
	9	5	1.25 (0.94, 1.67)
	11	5	1.36 (0.76, 2.45)
	16	4	1.40 (1.15, 1.70)
	18	5	1.16 (0.87, 1.55)
	23	4	1.00 (0.22, 4.52)
	25	3	1.33 (0.54, 3.25)
	30	5	1.21 (0.72, 2.03)
	32	1	1.39 (--)
	37	5	1.53 (1.40, 1.66)
	39	5	0.52 (0.16, 1.66)
2	-3	7	1.61 (1.16, 2.23)
	2	7	1.42 (1.02, 1.96)
	4	7	1.55 (1.26, 1.91)
	9	7	1.41 (1.19, 1.69)
	11	7	1.51 (1.21, 1.88)
	16	7	1.40 (1.19, 1.64)
	18	7	1.29 (1.14, 1.46)
	23	6	1.57 (1.26, 1.97)
	25	6	1.39 (1.14, 1.69)
	30	7	1.39 (1.14, 1.70)
	32	3	1.25 (0.89, 1.74)
	37	7	1.39 (1.10, 1.77)
	39	7	0.75 (0.50, 1.12)
3	-3	7	1.61 (1.22, 2.14)
	2	7	1.20 (1.04, 1.40)
	4	7	1.29 (0.95, 1.76)
	9	6	1.49 (1.23, 1.81)
	11	6	1.54 (1.24, 1.92)
	16	5	1.33 (1.06, 1.68)
	18	6	1.49 (1.06, 2.10)
	23	3	2.56 (0.85, 7.67)
	25	6	1.59 (1.03, 2.45)
	30	3	1.74 (1.16, 2.62)
	32	5	1.68 (1.08, 2.63)
	37	4	1.40 (1.13, 1.72)
	39	6	0.88 (0.36, 2.13)

Table 14a. (Continued)

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
4	-3	7	2.35 (1.90, 2.92)
	2	7	2.06 (1.60, 2.65)
	4	4	2.41 (0.89, 6.55)
	9	7	1.99 (1.52, 2.60)
	11	6	1.81 (1.13, 2.88)
	16	4	1.41 (0.69, 2.89)
	18	4	2.02 (0.99, 4.12)
	23	2	4.04 (0.00, 70945.34)
	25	2	3.00 (0.00, 1133098.47)
	30	3	2.31 (0.19, 27.74)
	32	3	3.02 (0.58, 15.81)
	37	3	1.31 (1.20, 1.44)
	39	3	1.39 (0.36, 5.32)

-- Confidence interval could not be calculated since only one observation was available for this group on this Study Day.

Table 14b. Test Results for Neutrophil Count (103 cells/µL)

Neutrophil Count†						
Study Day	Mean Shift as a Proportion from Baseline, by Group				Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value #
	1	2	3	4		
2	0.92	0.88	0.75	0.87	0.7568	
4	0.86	0.96	0.80	0.96	0.9257	
9	0.74	0.88	0.86	0.84	0.8840	
11	0.81	0.93	0.90	0.75	0.7220	
16	0.82	0.87	0.91	0.55	0.4450	
18	0.69	0.80	0.86	0.79	0.8898	
23	0.75	0.98	1.58	1.78	0.4806	
25	0.97	0.87	0.92	1.32	0.7763	
30	0.71	0.87	0.83	0.87	0.9419	
32	1.19	0.71	1.04	1.13	0.6774	
37	0.90	0.86	0.75	0.49	0.2981	
39	0.31	0.46	0.51	0.52	0.8322	

† Indicates that values for this parameter were log-transformed for the analysis.
# Cells contain all pairwise comparisons that were significant at the 0.05 level. The format within each cell is: (1) the ratio of group mean shifts, (2) the relationship between the corresponding pair of group mean shifts shown in parentheses, and (3) the Tukey-adjusted p-value.

Table 15a. Descriptive Statistics for Lymphocyte Count (103 cells/µL) by Group and Study Day

Group	Study Day	N	Mean (95% Confidence Interval)
1	-3	5	3.50 (2.20, 4.80)
	2	5	4.02 (2.68, 5.37)
	4	4	3.37 (0.44, 6.29)
	9	5	4.35 (1.75, 6.95)
	11	5	4.00 (2.41, 5.60)
	16	4	4.38 (2.55, 6.21)
	18	5	4.45 (2.51, 6.39)
	23	4	4.32 (1.44, 7.20)
	25	3	4.27 (0.76, 7.77)
	30	5	4.35 (2.56, 6.13)
	32	1	4.49 (--)
	37	5	4.57 (2.74, 6.40)
	39	5	2.87 (0.71, 5.02)
2	-3	7	4.78 (4.01, 5.55)
	2	7	5.17 (4.23, 6.11)
	4	7	5.19 (4.01, 6.37)
	9	7	5.45 (4.44, 6.45)
	11	7	5.17 (4.41, 5.92)
	16	7	5.06 (3.98, 6.14)
	18	7	5.24 (3.91, 6.56)
	23	6	4.39 (3.51, 5.27)
	25	6	4.23 (3.15, 5.31)
	30	7	4.74 (3.95, 5.52)
	32	3	3.57 (2.92, 4.22)
	37	7	4.50 (3.39, 5.61)
	39	7	3.53 (2.17, 4.90)
3	-3	7	4.10 (3.42, 4.77)
	2	7	3.73 (3.02, 4.44)
	4	7	3.65 (2.65, 4.64)
	9	6	3.83 (2.79, 4.88)
	11	6	4.37 (3.36, 5.38)
	16	5	4.15 (3.22, 5.08)
	18	6	4.05 (2.66, 5.44)
	23	3	4.20 (0.75, 7.64)
	25	6	3.89 (2.84, 4.95)
	30	3	4.57 (1.26, 7.88)
	32	5	3.58 (2.61, 4.54)
	37	4	3.69 (3.06, 4.31)
	39	6	2.52 (1.07, 3.98)

Table 15a. (Continued)

Group	Study Day	N	Mean (95% Confidence Interval)
4	-3	7	4.47 (3.11, 5.83)
	2	7	4.60 (2.70, 6.50)
	4	4	4.99 (3.19, 6.79)
	9	7	4.36 (3.17, 5.56)
	11	6	4.53 (2.60, 6.46)
	16	4	5.28 (3.94, 6.62)
	18	4	6.55 (5.94, 7.17)
	23	2	7.79 (0.00[a], 24.38)
	25	2	7.78 (3.14, 12.41)
	30	3	5.77 (0.02, 11.53)
	32	3	4.63 (1.26, 8.01)
	37	3	4.85 (0.52, 9.19)
	39	3	4.42 (2.56, 6.27)

-- Confidence interval could not be calculated since only one observation was available for this group on this Study Day.

a Negative lower confidence limit was set to 0 since negative values are not possible.

Table 15b. Test Results for Lymphocyte Count (103 cells/µL)

Lymphocyte Count						
Study Day	Mean Shift from Baseline, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
	1	2	3	4		
2	0.53	0.39	-0.37	0.13	0.2704	
4	-0.25	0.41	-0.45	-0.22	0.5563	
9	0.85	0.67	-0.35	-0.11	0.1985	
11	0.51	0.39	0.11	0.24	0.8899	
16	0.83	0.28	0.24	0.23	0.8293	
18	0.95	0.46	-0.13	1.51	0.2451	
23	0.70	-0.33	0.37	2.18	0.3101	
25	0.38	-0.49	-0.29	2.16	0.1161	
30	0.85	-0.04	-0.00	0.67	0.6610	
32	1.68	-1.33	-0.37	-0.47	0.1112	
37	1.07	-0.28	-0.81	-0.25	0.0704	
39	-0.63	-1.25	-1.66↓	-0.69	0.6146	

\# Cells contain all pairwise comparisons that were significant at the 0.05 level. The format within each cell is: (1) the difference of group mean shifts, (2) the relationship between the corresponding pair of group mean shifts shown in parentheses, and (3) the 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 16a. Descriptive Statistics for Neutrophil Count/Lymphocyte Count Ratio by Group and Study Day

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
1	-3	5	0.498 (0.221, 1.124)
	2	5	0.396 (0.227, 0.691)
	4	4	0.390 (0.225, 0.677)
	9	5	0.312 (0.181, 0.536)
	11	5	0.356 (0.148, 0.855)
	16	4	0.327 (0.252, 0.423)
	18	5	0.273 (0.172, 0.433)
	23	4	0.245 (0.074, 0.810)
	25	3	0.322 (0.154, 0.673)
	30	5	0.289 (0.200, 0.419)
	32	1	0.310 (--)
	37	5	0.347 (0.237, 0.506)
	39	5	0.222 (0.136, 0.362)
2	-3	7	0.341 (0.256, 0.454)
	2	7	0.279 (0.214, 0.362)
	4	7	0.306 (0.229, 0.409)
	9	7	0.264 (0.210, 0.332)
	11	7	0.295(0.230, 0.378)
	16	7	0.281 (0.229, 0.346)
	18	7	0.255 (0.207, 0.314)
	23	6	0.364 (0.285, 0.465)
	25	6	0.336 (0.275, 0.411)
	30	7	0.298 (0.235, 0.379)
	32	3	0.350 (0.221, 0.552)
	37	7	0.318 (0.267, 0.380)
	39	7	0.227 (0.192, 0.270)
3	-3	7	0.399 (0.334, 0.476)
	2	7	0.329 (0.283, 0.381)
	4	7	0.368 (0.243, 0.560)
	9	6	0.402 (0.271, 0.597)
	11	6	0.360 (0.292, 0.443)
	16	5	0.325 (0.271, 0.390)
	18	6	0.386 (0.246, 0.605)
	23	3	0.631 (0.126, 3.159)
	25	6	0.418 (0.280, 0.625)
	30	3	0.393 (0.270, 0.571)
	32	5	0.479 (0.272, 0.842)
	37	4	0.381 (0.333, 0.437)
	39	6	0.402 (0.176, 0.920)

Table 16a. (Continued)

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
4	-3	7	0.558 (0.373, 0.835)
	2	7	0.492 (0.265, 0.914)
	4	4	0.491 (0.168, 1.435)
	9	7	0.477 (0.292, 0.780)
	11	6	0.434 (0.275, 0.683)
	16	4	0.270 (0.160, 0.454)
	18	4	0.308 (0.142, 0.670)
	23	2	0.525 (0.000, 1078.118)
	25	2	0.386 (0.000, 265028.326)
	30	3	0.427 (0.040, 4.586)
	32	3	0.671 (0.072, 6.265)
	37	3	0.281 (0.123, 0.642)
	39	3	0.317 (0.060, 1.669)

-- Confidence interval could not be calculated since only one observation was available for this group on this Study Day.

Table 16b. Test Results for Neutrophil Count/Lymphocyte Count Ratio

Neutrophil Count/Lymphocyte Count Ratio†						
Study Day	Mean Shift as a Proportion from Baseline, by Group				Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value #
	1	2	3	4		
2	0.79	0.82	0.82	0.88	0.9518	
4	1.03	0.90	0.92	1.00	0.9726	
9	0.63	0.77	0.96	0.86	0.3502	
11	0.72	0.87	0.89	0.73	0.6277	
16	0.66	0.83	0.86	0.52	0.3631	
18	0.55↓	0.75	0.92	0.60	0.2191	
23	0.65	1.06	1.47	1.28	0.3969	
25	0.88	0.98	1.00	0.94	0.9857	
30	0.58	0.87	0.85	0.80	0.4913	
32	0.74	0.95	1.16	1.25	0.7897	
37	0.70	0.93	0.91	0.53	0.2121	
39	0.45	0.67	0.96	0.59	0.3655	

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

Cells contain all pairwise comparisons that were significant at the 0.05 level. The format within each cell is: (1) the ratio of group mean shifts, (2) the relationship between the corresponding pair of group mean shifts shown in parentheses, and (3) the 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).

Table 17a. Descriptive Statistics for Monocyte Count (103 cells/µL) by Group and Study Day

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
1	-3	5	0.09 (0.04, 0.21)
	2	5	0.09 (0.04, 0.18)
	4	4	0.05 (0.01, 0.26)
	9	5	0.05 (0.03, 0.09)
	11	5	0.09 (0.05, 0.18)
	16	4	0.08 (0.04, 0.16)
	18	5	0.07 (0.05, 0.09)
	23	4	0.06 (0.02, 0.20)
	25	3	0.10 (0.01, 0.71)
	30	5	0.11 (0.07, 0.17)
	32	1	0.06 (--)
	37	5	0.08 (0.03, 0.20)
	39	4	0.04 (0.01, 0.24)
2	-3	7	0.10 (0.06, 0.15)
	2	7	0.12 (0.08, 0.20)
	4	7	0.10 (0.06, 0.17)
	9	7	0.10 (0.06, 0.16)
	11	7	0.10 (0.07, 0.14)
	16	7	0.12 (0.08, 0.18)
	18	7	0.11 (0.07, 0.18)
	23	6	0.10 (0.05, 0.18)
	25	6	0.09 (0.06, 0.13)
	30	7	0.09 (0.07, 0.11)
	32	3	0.07 (0.02, 0.20)
	37	7	0.10 (0.05, 0.23)
	39	7	0.03 (0.02, 0.06)
3	-3	7	0.09 (0.06, 0.13)
	2	7	0.09 (0.06, 0.12)
	4	7	0.08 (0.04, 0.16)
	9	6	0.13 (0.08, 0.22)
	11	6	0.08 (0.05, 0.12)
	16	5	0.07 (0.04, 0.15)
	18	6	0.10 (0.06, 0.16)
	23	3	0.10 (0.02, 0.44)
	25	6	0.12 (0.06, 0.25)
	30	3	0.16 (0.01, 3.26)
	32	5	0.13 (0.07, 0.26)
	37	4	0.12 (0.03, 0.41)
	39	6	0.06 (0.02, 0.16)

Table 17a. (Continued)

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
4	-3	7	0.14 (0.11, 0.20)
	2	7	0.14 (0.08, 0.26)
	4	4	0.15 (0.04, 0.60)
	9	7	0.12 (0.08, 0.18)
	11	6	0.12 (0.05, 0.28)
	16	4	0.15 (0.05, 0.42)
	18	4	0.18 (0.06, 0.53)
	23	2	0.48 (0.00, 3207918.13)
	25	2	0.50 (0.00, 514082.32)
	30	3	0.25 (0.02, 2.57)
	32	3	0.22 (0.02, 2.82)
	37	3	0.15 (0.04, 0.58)
	39	3	0.11 (0.03, 0.38)

-- Confidence interval could not be calculated since only one observation was available for this group on this Study Day.

Table 17b. Test Results for Monocyte Count (103 cells/µL)

Monocyte Count†						
Study Day	Mean Shift as a Proportion from Baseline, by Group				Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value #
	1	2	3	4		
2	0.93	1.28	0.94	0.99	0.7183	
4	0.64	1.02	0.85	0.90	0.7599	
9	0.55	1.05	1.36	0.84	0.0537	2.46 (1<3) 0.0409
11	0.96	1.00	0.75	0.80	0.8501	
16	0.80	1.20	0.84	0.94	0.6687	
18	0.73	1.18	0.98	1.12	0.6203	
23	0.76	1.08	1.00	3.24	0.2750	
25	1.04	0.93	1.24	3.40	0.2561	
30	1.14	0.91	1.34	1.35	0.7953	
32	1.50	0.51	1.32	1.21	0.3380	
37	0.83	1.06	1.12	0.84	0.8672	
39	0.54	0.31↓	0.61	0.59	0.5866	

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

Cells contain all pairwise comparisons that were significant at the 0.05 level. The format within each cell is: (1) the ratio of group mean shifts, (2) the relationship between the corresponding pair of group mean shifts shown in parentheses, and (3) the 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).

Table 18a. Descriptive Statistics for Eosinophil Count (103 cells/µL) by Group and Study Day

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
1	-3	5	0.14 (0.10, 0.18)
	2	5	0.14 (0.08, 0.24)
	4	4	0.11 (0.05, 0.23)
	9	5	0.13 (0.09, 0.19)
	11	5	0.14 (0.10, 0.21)
	16	4	0.19 (0.11, 0.33)
	18	5	0.19 (0.14, 0.25)
	23	4	0.16 (0.08, 0.32)
	25	3	0.16 (0.08, 0.34)
	30	5	0.16 (0.13, 0.20)
	32	1	0.21 (--)
	37	5	0.19 (0.18, 0.20)
	39	5	0.14 (0.11, 0.19)
2	-3	7	0.16 (0.12, 0.21)
	2	7	0.18 (0.12, 0.26)
	4	7	0.17 (0.13, 0.23)
	9	7	0.15 (0.11, 0.19)
	11	7	0.16 (0.13, 0.18)
	16	7	0.15 (0.13, 0.18)
	18	7	0.14 (0.12, 0.18)
	23	6	0.15 (0.11, 0.22)
	25	6	0.17 (0.13, 0.22)
	30	7	0.15 (0.11, 0.19)
	32	3	0.14 (0.12, 0.17)
	37	7	0.15 (0.13, 0.17)
	39	7	0.12 (0.09, 0.16)
3	-3	7	0.18 (0.14, 0.23)
	2	7	0.15 (0.11, 0.20)
	4	7	0.17 (0.13, 0.22)
	9	6	0.16 (0.12, 0.21)
	11	6	0.17 (0.16, 0.19)
	16	5	0.15 (0.12, 0.19)
	18	6	0.13 (0.09, 0.19)
	23	3	0.15 (0.10, 0.21)
	25	6	0.16 (0.12, 0.21)
	30	3	0.24 (0.10, 0.56)
	32	5	0.16 (0.09, 0.27)
	37	4	0.14 (0.12, 0.16)
	39	6	0.13 (0.09, 0.21)

Table 18a. (Continued)

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
4	-3	7	0.14 (0.12, 0.17)
	2	7	0.13 (0.09, 0.20)
	4	4	0.19 (0.14, 0.25)
	9	7	0.14 (0.11, 0.18)
	11	6	0.16 (0.11, 0.23)
	16	4	0.14 (0.08, 0.23)
	18	4	0.15 (0.08, 0.29)
	23	2	0.15 (0.15, 0.15)
	25	2	0.13 (0.00, 3.80)
	30	3	0.16 (0.08, 0.33)
	32	3	0.16 (0.11, 0.26)
	37	3	0.17 (0.14, 0.21)
	39	3	0.16 (0.03, 0.83)

-- Confidence interval could not be calculated since only one observation was available for this group on this Study Day.

Table 18b. Test Results for Eosinophil Count (103 cells/µL)

Eosinophil Count†						
Study Day	Mean Shift as a Proportion from Baseline, by Group				Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value #
	1	2	3	4		
2	1.03	1.13	0.84	0.94	0.5673	
4	0.76	1.08	0.95	1.30	0.1536	
9	0.95	0.95	0.95	0.98	0.9929	
11	1.03	1.00	0.97	1.15	0.8155	
16	1.40	0.96	0.85	0.90	0.2067	
18	1.37	0.92	0.78	0.98	0.0911	
23	1.09	1.06	0.80	1.12	0.6402	
25	1.14	1.15	0.93	0.97	0.7508	
30	1.20	0.94	1.22	1.14	0.6631	
32	1.24	0.90	0.93	1.16	0.7732	
37	1.37	0.96	0.76	1.22	0.0249*	1.80 (3<1) 0.0227
39	1.04	0.77	0.79	1.13	0.4833	

† Indicates that values for this parameter were log-transformed for the analysis.
# Cells contain all pairwise comparisons that were significant at the 0.05 level. The format within each cell is: (1) the ratio of group mean shifts, (2) the relationship between the corresponding pair of group mean shifts shown in parentheses, and (3) the Tukey-adjusted p-value.
* The overall group effect was significant at the 0.05 level.

Table 19a. Descriptive Statistics for Basophil Count (103 cells/µL) by Group and Study Day

Group	Study Day	N	Mean (95% Confidence Interval)
1	-3	5	0.22 (0.03, 0.41)
	2	5	0.27 (0.05, 0.50)
	4	4	0.14 (0.00[a], 0.32)
	9	5	0.22 (0.06, 0.38)
	11	5	0.22 (0.07, 0.38)
	16	4	0.23 (0.01, 0.44)
	18	5	0.29 (0.12, 0.45)
	23	4	0.22 (0.03, 0.40)
	25	3	0.22 (0.00[a], 0.48)
	30	5	0.35 (0.05, 0.65)
	32	1	0.36 (--)
	37	5	0.30 (0.17, 0.44)
	39	5	0.13 (0.00[a], 0.28)
2	-3	7	0.26 (0.09, 0.43)
	2	7	0.27 (0.11, 0.43)
	4	7	0.31 (0.12, 0.50)
	9	7	0.30 (0.13, 0.46)
	11	7	0.29 (0.15, 0.43)
	16	7	0.26 (0.15, 0.38)
	18	7	0.30 (0.15, 0.45)
	23	6	0.33 (0.12, 0.53)
	25	6	0.28 (0.09, 0.47)
	30	7	0.32 (0.15, 0.49)
	32	3	0.38 (0.00[a], 0.79)
	37	7	0.30 (0.15, 0.44)
	39	7	0.18 (0.05, 0.30)
3	-3	7	0.23 (0.13, 0.32)
	2	7	0.20 (0.10, 0.30)
	4	7	0.20 (0.09, 0.32)
	9	6	0.27 (0.17, 0.36)
	11	6	0.23 (0.13, 0.33)
	16	5	0.20 (0.06, 0.33)
	18	6	0.19 (0.10, 0.29)
	23	3	0.24 (0.00[a], 0.65)
	25	6	0.26 (0.15, 0.37)
	30	3	0.28 (0.01, 0.56)
	32	5	0.30 (0.14, 0.47)
	37	4	0.27 (0.14, 0.40)
	39	6	0.14 (0.05, 0.23)

Table 19a. (Continued)

Group	Study Day	N	Mean (95% Confidence Interval)
4	-3	7	0.16 (0.08, 0.24)
	2	7	0.19 (0.06, 0.32)
	4	4	0.23 (0.01, 0.44)
	9	7	0.18 (0.09, 0.27)
	11	6	0.22 (0.09, 0.34)
	16	4	0.26 (0.11, 0.41)
	18	4	0.27 (0.09, 0.44)
	23	2	0.40 (0.00[a], 2.05)
	25	2	0.42 (0.00[a], 3.15)
	30	3	0.40 (0.00[a], 0.90)
	32	3	0.40 (0.04, 0.75)
	37	3	0.42 (0.00[a], 0.90)
	39	3	0.28 (0.00[a], 0.71)

-- Confidence interval could not be calculated since only one observation was available for this group on this Study Day.

a Negative lower confidence limit was set to 0 since negative values are not possible.

Table 19b. Test Results for Basophil Count (103 cells/µL)

Study Day	Basophil Count: Mean Shift from Baseline, by Group 1	2	3	4	Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
2	0.06	0.01	-0.03	0.03	0.1120	
4	-0.03	0.05	-0.02	0.04	0.3090	
9	0.00	0.03	0.05	0.02	0.7242	
11	0.00	0.03	-0.01	0.04	0.8349	
16	0.01	0.00	0.01	0.06	0.7620	
18	0.07	0.04	-0.03	0.06	0.5612	
23	0.05	0.10↑	0.04	0.13	0.6075	
25	0.09	0.05	0.04	0.15	0.6998	
30	0.13	0.06	0.03	0.18	0.5113	
32	0.08	0.09	0.10	0.17	0.8650	
37	0.09	0.04	0.01	0.19	0.2922	
39	-0.08	-0.09	-0.08	0.06	0.7349	

Cells contain all pairwise comparisons that were significant at the 0.05 level. The format within each cell is: (1) the difference of group mean shifts, (2) the relationship between the corresponding pair of group mean shifts shown in parentheses, and (3) the 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 20a. Descriptive Statistics for C-Reactive Protein (103 cells/µL) by Group and Study Day

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
1	-3	5	0.47 (0.16, 1.39)
	2	5	0.63 (0.31, 1.30)
	4	2	1.04 (0.01, 105.17)
	9	4	0.39 (0.17, 0.87)
	11	5	0.35 (0.14, 0.85)
	16	3	0.33 (0.10, 1.07)
	18	5	0.36 (0.13, 1.00)
	23	4	0.25 (0.25, 0.25)
	25	4	0.25 (0.25, 0.25)
	30	4	0.25 (0.25, 0.25)
	32	5	0.25 (0.25, 0.25)
	37	5	0.25 (0.25, 0.25)
	39	4	0.25 (0.25, 0.25)
2	-3	7	0.37 (0.19, 0.69)
	2	6	0.44 (0.22, 0.88)
	4	7	0.38 (0.23, 0.62)
	9	7	0.25 (0.25, 0.25)
	11	6	0.29 (0.20, 0.44)
	16	5	0.34 (0.14, 0.82)
	18	7	0.25 (0.25, 0.25)
	23	5	0.25 (0.25, 0.25)
	25	6	0.25 (0.25, 0.25)
	30	6	0.25 (0.25, 0.25)
	32	5	0.30 (0.18, 0.53)
	37	6	0.25 (0.25, 0.25)
	39	7	0.34 (0.16, 0.73)
3	-3	7	0.25 (0.25, 0.25)
	2	7	0.28 (0.21, 0.39)
	4	6	0.30 (0.19, 0.47)
	9	5	0.25 (0.25, 0.25)
	11	6	0.39 (0.17, 0.90)
	16	6	0.39 (0.18, 0.83)
	18	6	0.29 (0.19, 0.45)
	23	2	0.25 (0.25, 0.25)
	25	6	0.28 (0.21, 0.38)
	30	2	0.25 (0.25, 0.25)
	32	6	0.47 (0.16, 1.35)
	37	4	0.25 (0.25, 0.25)
	39	6	0.29 (0.20, 0.43)

Table 20a. (Continued)

Group	Study Day	N	Geometric Mean (95% Confidence Interval)
4	-3	7	0.43 (0.22, 0.84)
	2	7	0.80 (0.34, 1.88)
	4	4	0.46 (0.15, 1.42)
	9	6	0.42 (0.18, 1.00)
	11	5	0.32 (0.16, 0.61)
	16	3	0.43 (0.04, 4.18)
	18	4	1.03 (0.15, 7.26)
	23	3	0.77 (0.01, 100.14)
	25	3	0.88 (0.02, 48.84)
	30	2	0.69 (0.00, 281753.42)
	32	3	0.43 (0.04, 4.24)
	37	2	1.20 (0.00, 5246.62)
	39	3	0.44 (0.13, 1.53)

Table 20b. Test Results for C-Reactive Protein (10^3 cells/µL)

C-Reactive Protein†						
Study Day	Mean Shift as a Proportion from Baseline, by Group				Group Effect P-Value	Estimated Ratio (Relationship) Tukey's P-Value #
	1	2	3	4		
2	1.35	1.12	1.14	1.86↑	0.4243	
4	2.09	1.03	1.19	1.04	0.7210	
9	0.70	0.68	1.00	1.16	0.6774	
11	0.74	0.75	1.56	0.60	0.1996	
16	0.73	0.80	1.54	1.35	0.4062	
18	0.77	0.68	1.18	2.35	0.2266	
23	0.71	0.59	1.00	1.46	0.7314	
25	0.64	0.64	1.12	1.66	0.3604	
30	0.64	0.75	1.00	1.95	0.3995	
32	0.53	0.71	1.88	0.80	0.1860	
37	0.53	0.64	1.00	1.55	0.3728	
39	0.46	0.93	1.16	0.83	0.4306	

† Indicates that values for this parameter were log-transformed for the analysis.
# Cells contain all pairwise comparisons that were significant at the 0.05 level. The format within each cell is: (1) the ratio of group mean shifts, (2) the relationship between the corresponding pair of group mean shifts shown in parentheses, and (3) the 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).

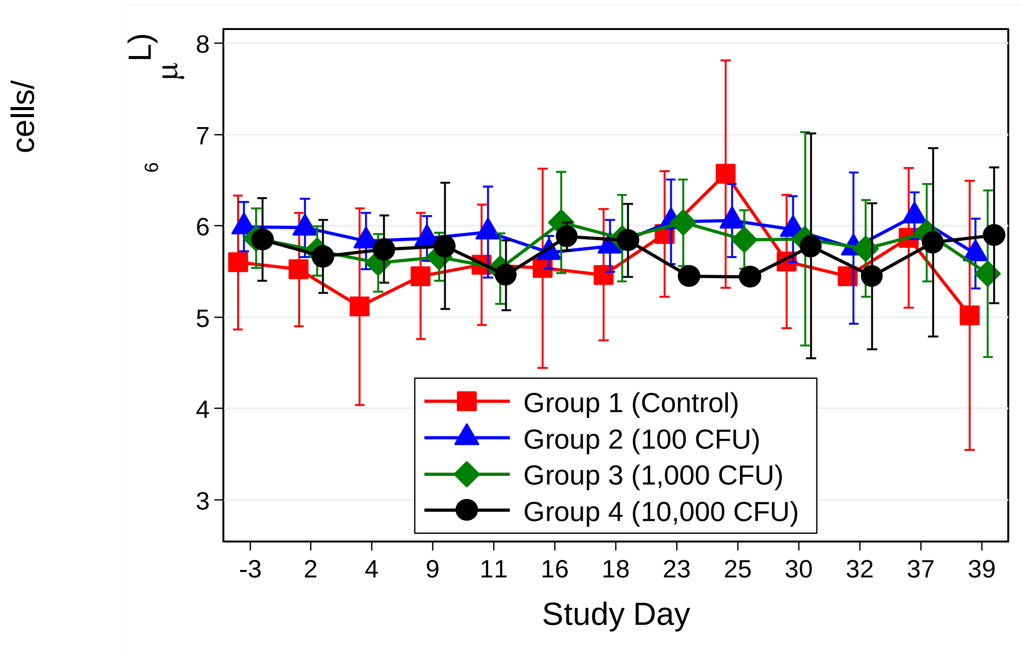

Figure 1. Plot of Red Blood Cell Count over time.

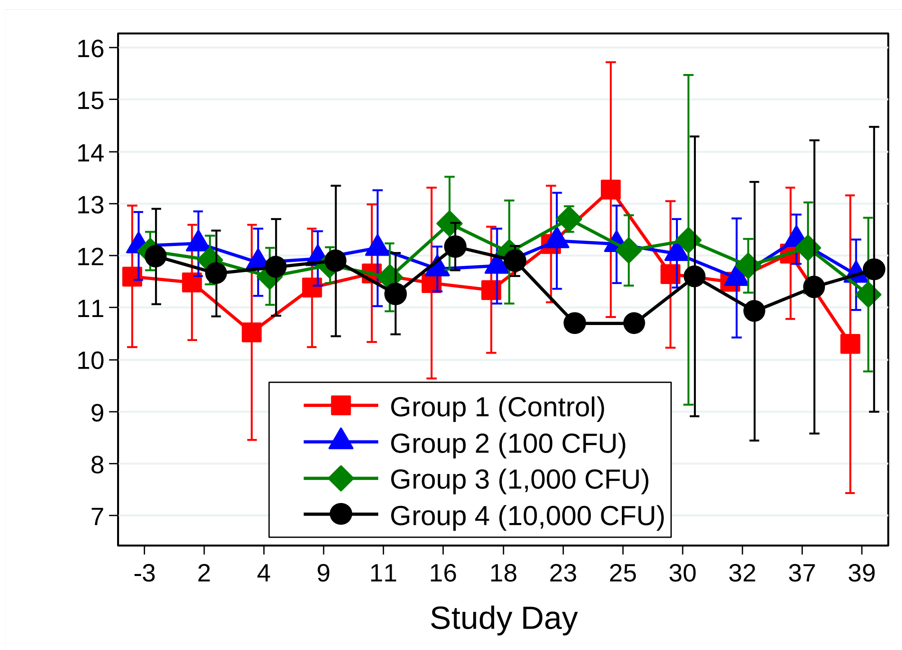

Figure 2. Plot of Hemoglobin over time.

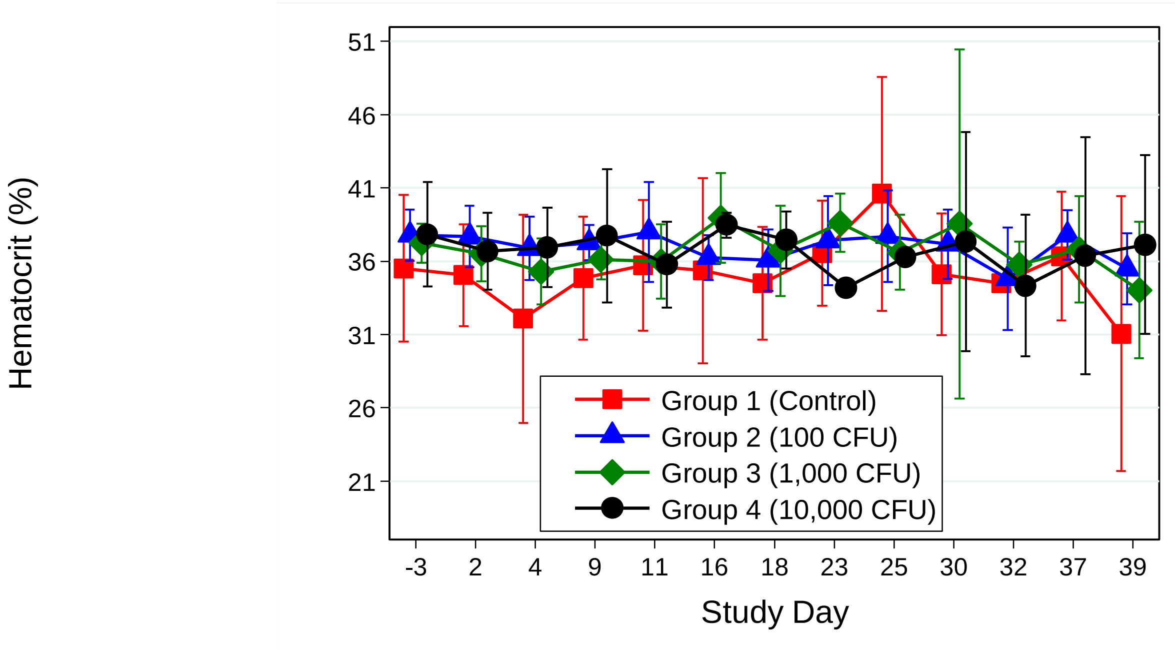

Figure 3. Plot of Hematocrit over time.

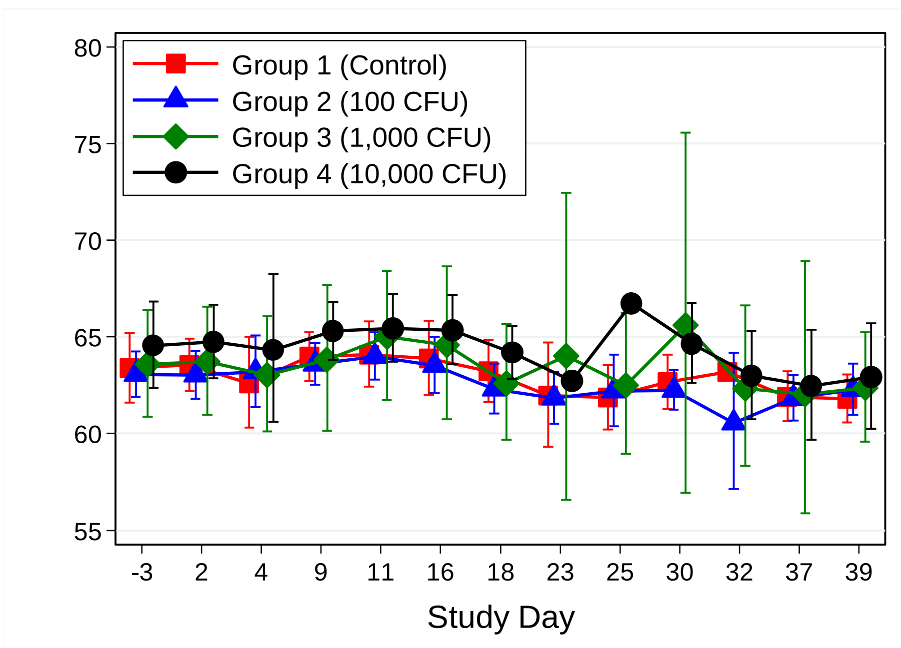

Figure 4. Plot of Mean Corpuscular Volume (MCV) over time.

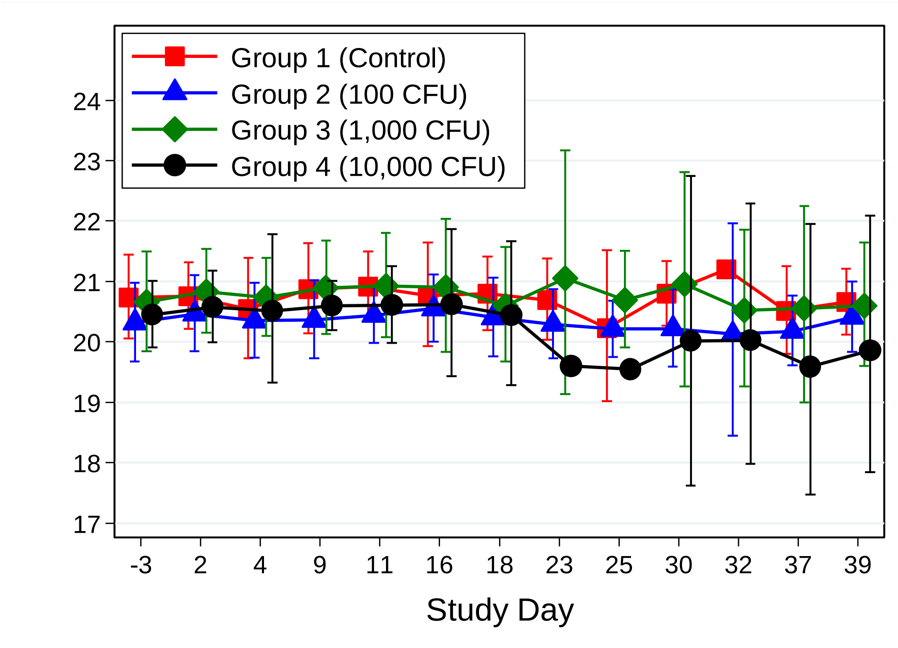

Figure 5. Plot of Mean Corpuscular Hemoglobin (MCH) over time.

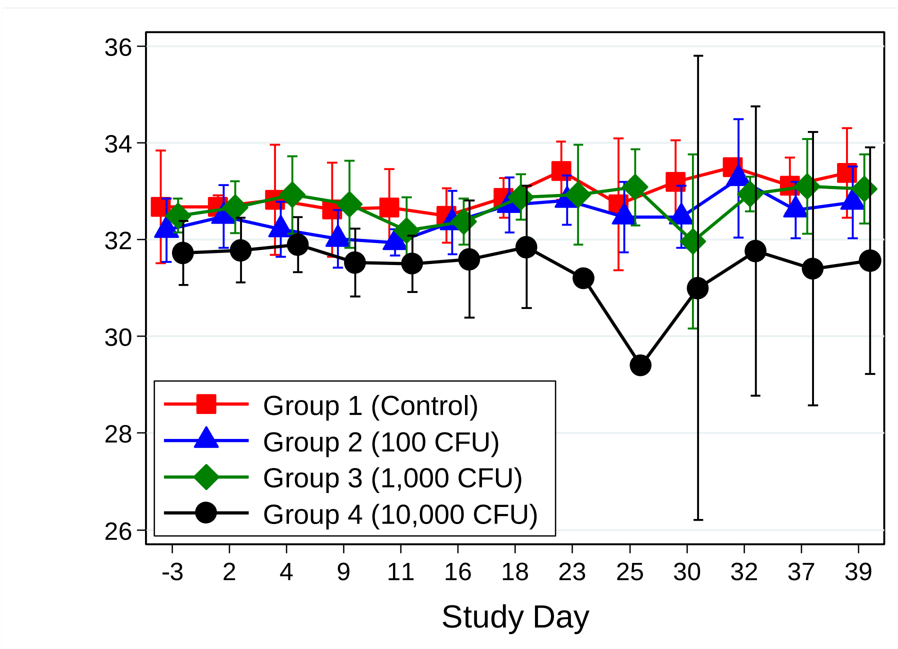

Figure 6. Plot of Mean Corpuscular Hemoglobin Concentration (MCHC) over time.

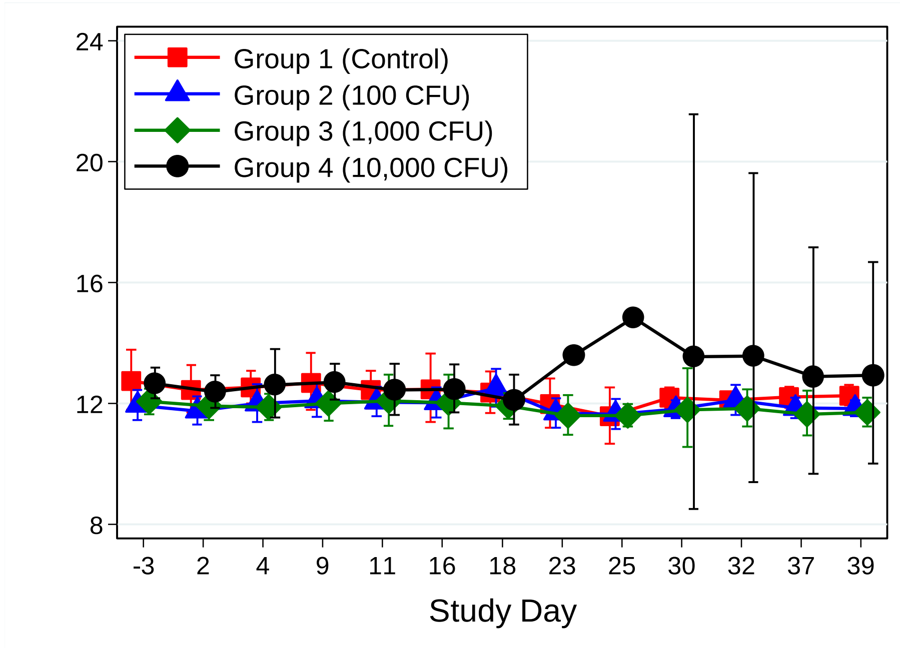

Figure 7. Plot of Red Cell Distribution Width (RDW) over time.

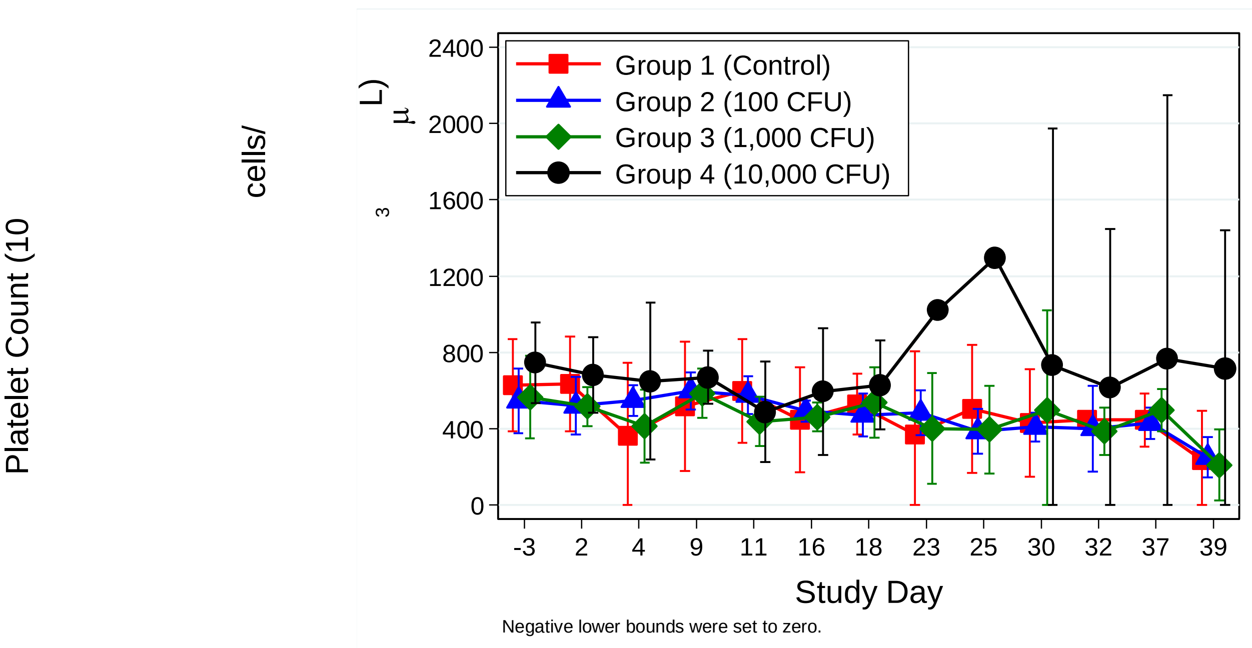

Figure 8. Plot of Platelet Count (PLT) over time.

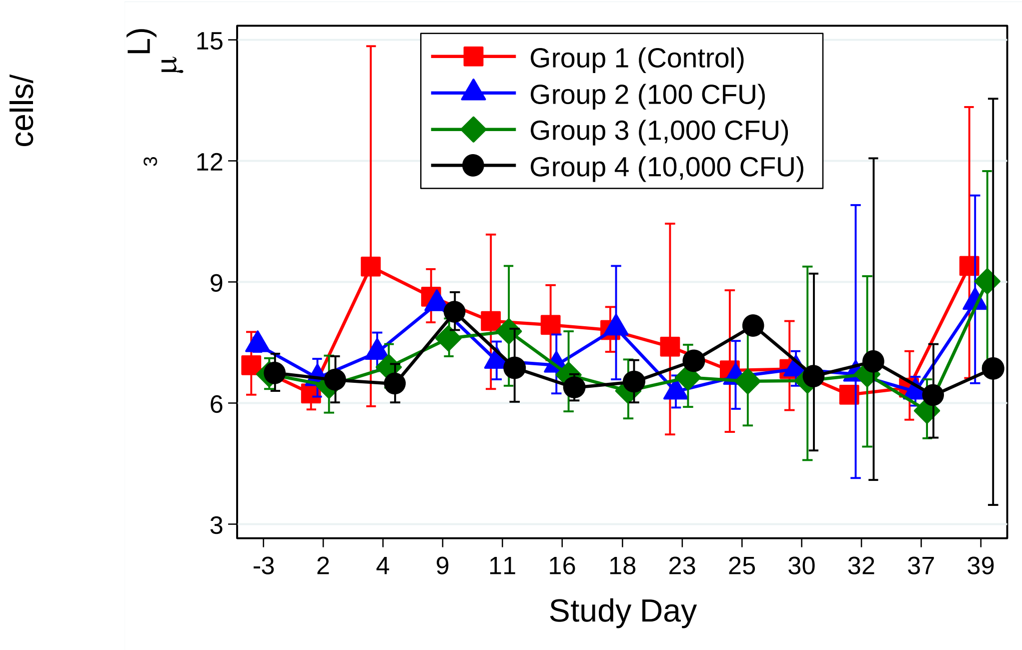

Figure 9. Plot of Mean Platelet Volume (MPV) over time.

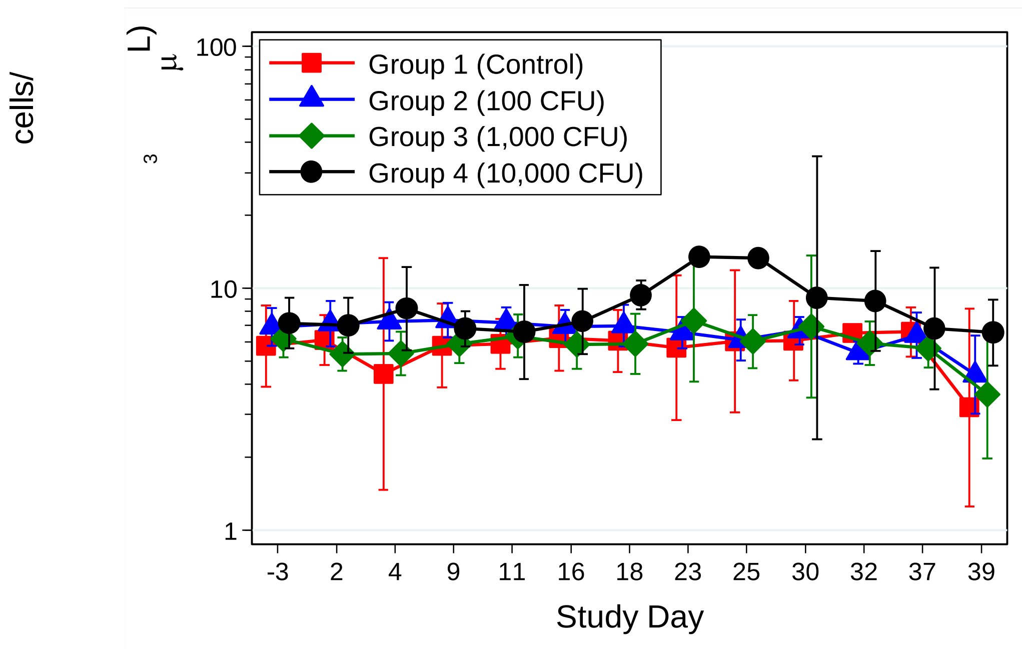

Figure 10. Plot of White Blood Cell Count over time.

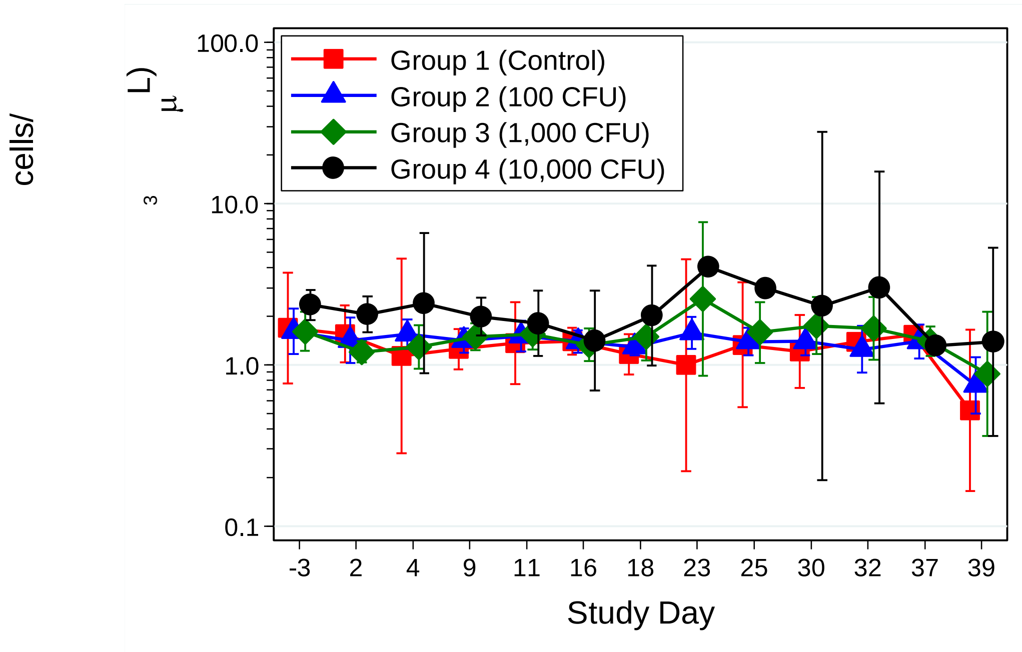

Figure 11. Plot of Neutrophil Count over time.

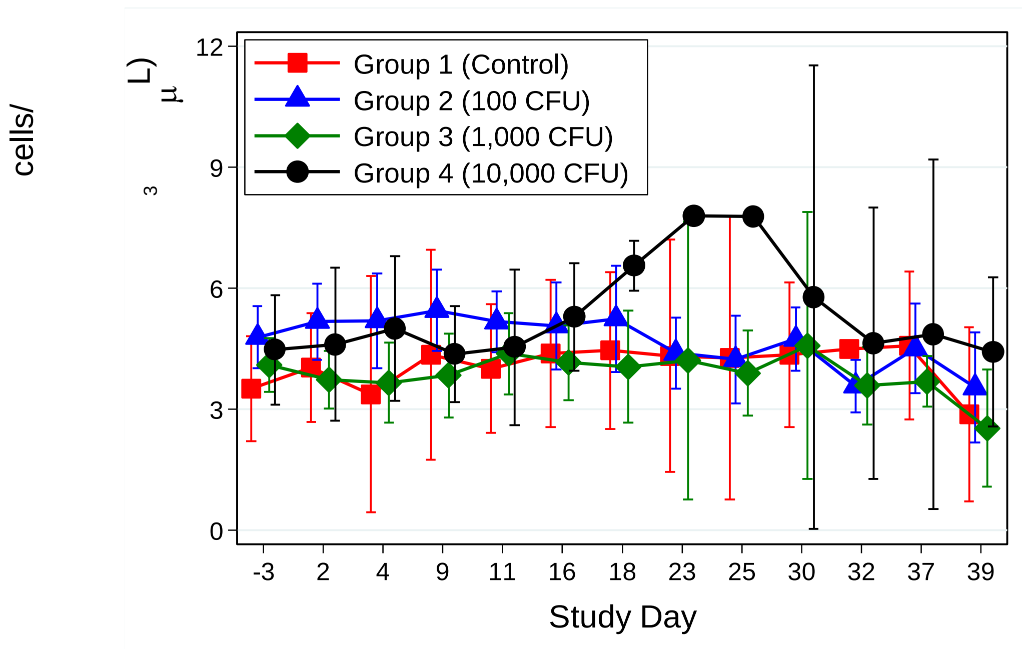

Figure 12. Plot of Lymphocyte Count over time.

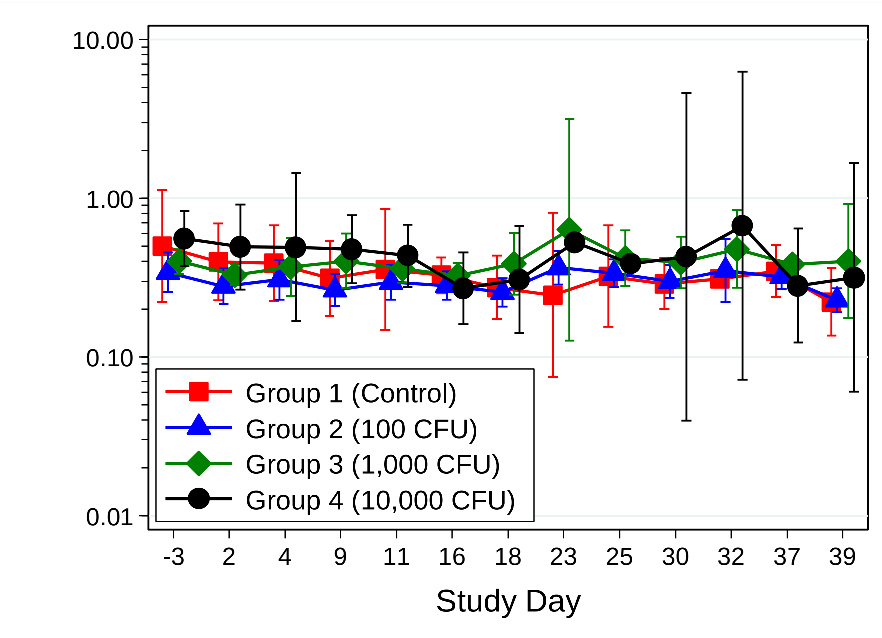

Figure 13. Plot of Neutrophil Count/Lymphocyte Count Ratio over time.

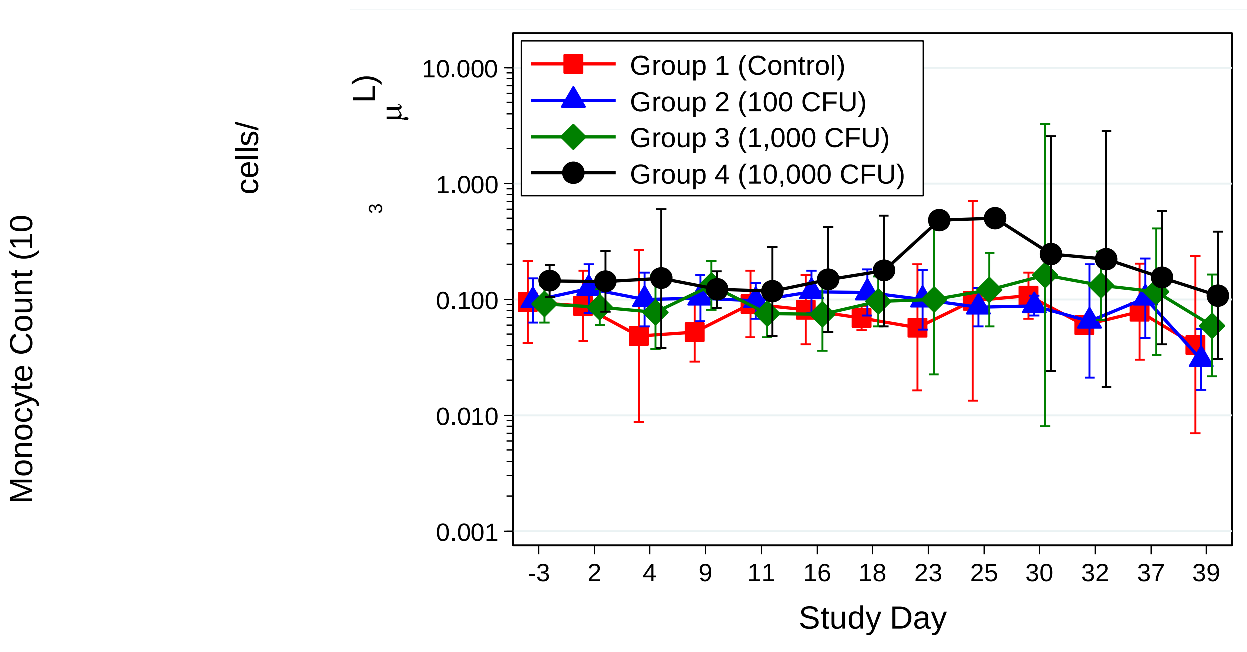

Figure 14. Plot of Monocyte Count over time.

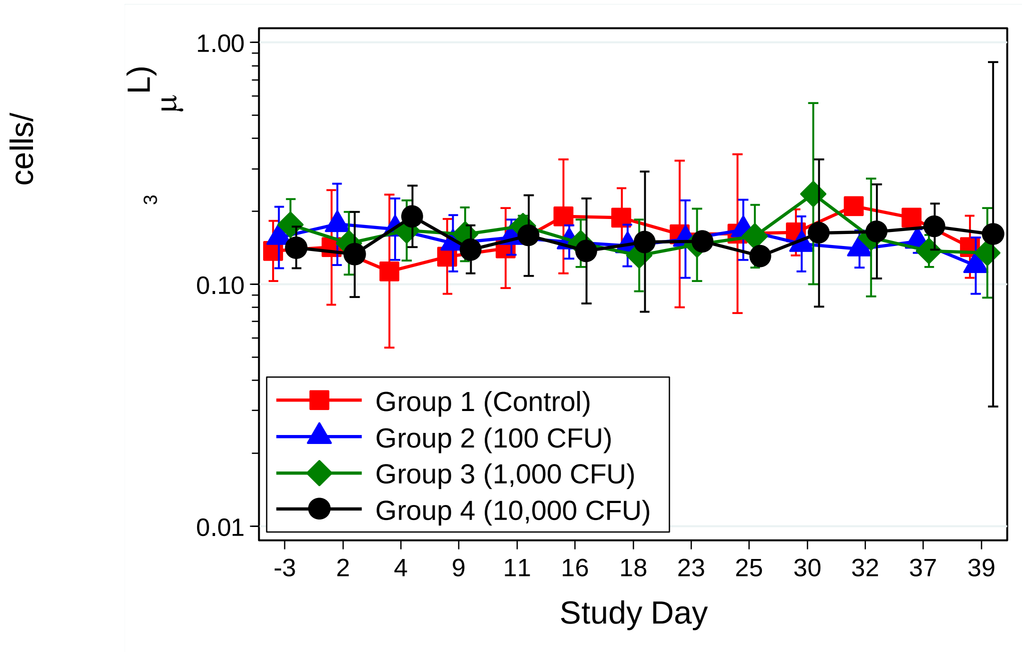

Figure 15. Plot of Eosinophil Count over time.

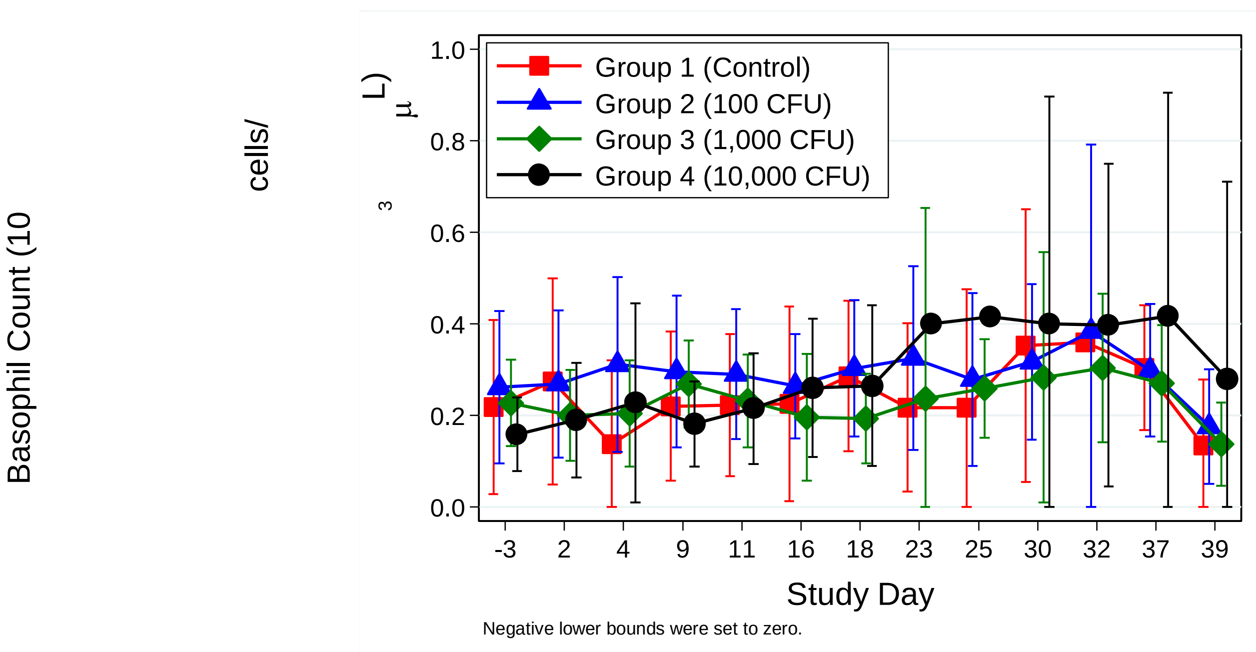

Figure 16. Plot of Basophil Count over time.

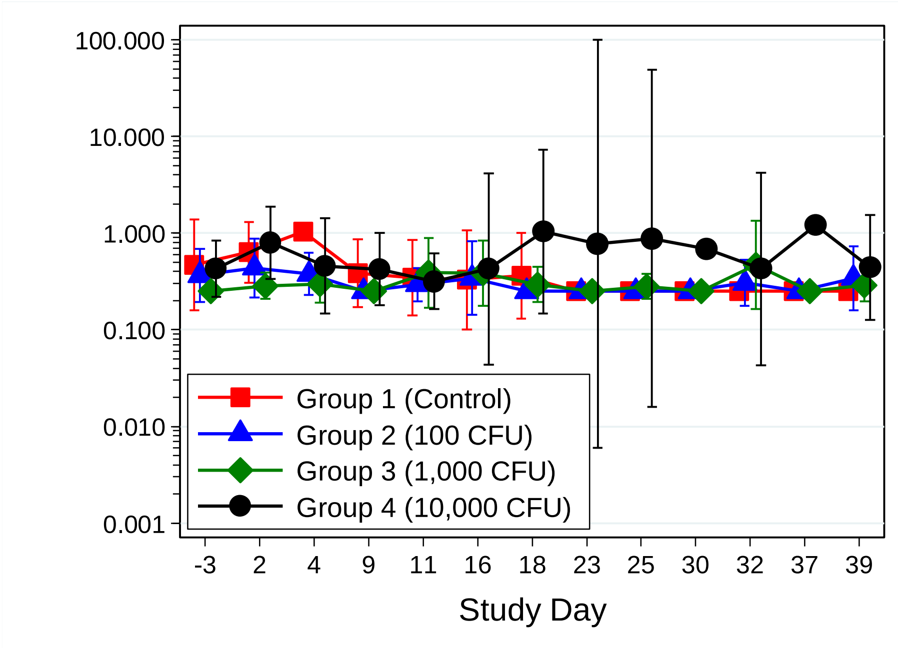

Figure 17. Plot of C-Reactive Protein over time.

ATTACHMENT I

RESULTS OF ANALYSIS REPEATED WITH POTENTIAL OUTLIERS EXCLUDED

ATTACHMENT I: RESULTS OF ANALYSIS REPEATED WITH POTENTIAL OUTLIERS EXCLUDED

Eleven (11) 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 A-1 and A-2 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:

- **HGB (Table A-1)**: There was a significant decrease from baseline in group 4 on Study Day 9.
- **RDW (Table A-2)**: There was a significant decrease as a proportion of baseline in group 1 on Study Day 25. On Study Day 25, there was no longer a significant difference between mean changes as a proportion of baseline in Groups 1 and 4.

Table I-1. Test Results for Hemoglobin (HGB, g/dL) with Potential Outliers Excluded

Hemoglobin						
Study Day	Mean Shift from Baseline, by Group				Group Effect P-Value	Estimated Difference (Relationship) Tukey's P-Value #
	1	2	3	4		
9	-0.22	-0.24	-0.38	**-0.58↓**	0.5691	

\# 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 I-2. Test Results for Red Cell Distribution Width (RDW, %) with Potential Outliers Excluded

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**		
25	**0.92**↓	0.97	0.97	NA	0.0613	**Groups 1 and 4 were no longer significantly different.**

† 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).
NA There were no measurements available for this group on this Study Day.

APPENDIX K
INDIVIDUAL CLINICAL OBSERVATIONS

K-2

Challenge Order	Animal ID	Sex	Group	7/20/2010		7/21/2010		7/22/2010		7/23/2010		7/24/2010		7/25/2010		7/26/2010	
				AM *	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PMα
1	40	M	1	*	N	N	N	N	N	N	N	N	N	N	N	N	α
2	7	M	1	*	N	N	N	N	N	N	N	N	N	N	N	N	α
3	5	M	1	*	N	N	N	N	N	N	N	N	N	N	N	N	α
4	9	M	1	*	N	N	N	N	N	N	N	N	N	N	N	N	α
5	37	M	1	*	N	N	N	N	N	N	N	N	N	N	N	N	α
1	13	M	2	*	N	N	N	N	N	N	N	N	N	N	N	N	α
2	34	M	2	*	N	NE	N	NE	N	N	N	N	N	N	N	N	α
3	25	M	2	*	N	N	N	N	N	N	N	N	N	N	N	N	α
4	15	M	2	*	N	N	N	N	N	N	N	N	N	N	N	N	α
5	30	M	2	*	N	N	N	N	N	N	N	N	N	N	N	N	α
6	28	M	2	*	N	N	N	N	N	N	N	N	N	N	N	N	α
7	19	M	2	*	N	N	N	N	N	N	N	N	N	N	N	N	α
1	14	M	3	*	N	N	N	N	N	N	N	N	N	N	N	N	α
2	11	M	3	*	N	N	N	N	N	N	N	N	N	N	N	N	α
3	2	M	3	*	N	NE	N	N	N	N	N	N	N	N	N	N	α
4	8	M	3	*	N	N	N	N	N	N	N	N	N	N	N	N	α
5	12	M	3	*	N	N	N	N	N	N	N	N	N	N	N	N	α
6	18	M	3	*	N	N	N	N	N	N	N	N	N	N	N	N	α
7	32	M	3	*	N	N	N	N	N	N	N	N	N	N	N	N	α
1	6	M	4	*	N	N	N	N	N	N	N	N	N	N	N	N	α
2	33	M	4	*	N	N	N	N	N	N	N	N	N	N	N	N	α
3	27	M	4	*	N	N	N	N	N	N	N	N	N	N	N	N	α
4	31	M	4	*	N	N	N	N	N	N	N	N	N	N	N	N	α
5	39	M	4	*	N	N	N	N	N	N	N	N	N	N	N	N	α
6	21	M	4	*	N	N	N	N	N	N	N	N	N	N	N	N	α
7	38	M	4	*	N	NE	N	N	N	N	N	N	N	N	N	N	α

N = Normal
NE = Not Eating

* Animals received from LAR, all appear N
α Animals challenged after am observations, refer to Clinical Observations

QA AUDIT COMPLETED
BY/DATE: [signature] 1/20/11

Printed By: JJM 10/20/10
QC Reviewed By: EEJ 10/20/10

Challenge Order	Animal ID	Sex	Group	7/26/2010		7/27/2010		7/28/2010		7/29/2010		7/30/2010		7/31/2010		8/1/2010		8/2/2010		8/3/2010	
				AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
1	40	M	1		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	7	M	1		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	5	M	1		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4	9	M	1		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5	37	M	1		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
1	13	M	2		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	34	M	2		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	25	M	2		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4	15	M	2		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5	30	M	2		N	N	N	N	N	N	N	N	N	N	N	DI	N	N	N	N	N
6	28	M	2		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7	19	M	2		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
1	14	M	3		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	11	M	3		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	2	M	3		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4	8	M	3		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5	12	M	3		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6	18	M	3		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7	32	M	3		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
1	6	M	4		N	N	N	N	N	N	N	NE	N	NE,DI	N	N	N	N	N	N	N
2	33	M	4		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	27	M	4		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4	31	M	4		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5	39	M	4		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	DI	SS,DI
6	21	M	4		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7	38	M	4		N	N	N	N	N	NE	N	N	N	N	N	NE	N	N	N	SS	N

Not Apllicable

Animal Deceased

N = Normal
NE = Not Eating
DI = Diarrhea
SS = Soft Stool
NS = No Stool
RA = Respiratory Abnormalities
L = Lethargic
FD = Found Dead
O = Other (requires comment)

Comments:
[1]Small amount of stool
[2]Animal found prostrate, unresponsive to touch, and gasping at 1055, died at 1113 prior to administration of B-euthanasia
[3]Animal found dead after am observations
[4]Vet notified about NE, vet ordered weight to be taken and yogurt to be added to food
[5]Fed yogurt, appears animal did not eat previous yogurt feeding, but no pellets in feeder, still dehydrated
[6]Impaired use of right front limb. N unless condition worsens

QA AUDIT COMPLETED
BY/DATE: [signature] 1/20/11

Printed By: [initials] 10/20/10
QC Reviewed By: [initials] 10/20/10

Challenge Order	Animal ID	Sex	Group	8/4/2010		8/5/2010		8/6/2010		8/7/2010		8/8/2010		8/9/2010		8/10/2010		8/11/2010		8/12/2010		8/13/2010	
				AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
1	40	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	7	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	5	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4	9	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5	37	M	1	N	N	N	N	N	N	NE	N	N	N	N	N	N	N	N	N	N	N	N	N
1	13	M	2	N	NS	N	N	N	N	NE	N	N	N	N	N	N	N	N	N	N	N	N	N
2	34	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	NE	N	N	N
3	25	M	2	N	N	SS	N	NE	N	NE	N	NE,O[1]	O[2]	N	N	N	N	N	N	N	N	N	N
4	15	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5	30	M	2	N	NS	N	N	NE,SS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6	28	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7	19	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
1	14	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	11	M	3	N	N	N	N	NE,SS	N	N	N	N	N	N	N	N	N	NE	N	N	N	N	N
3	2	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	NE	N	N	N	N	N
4	8	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	NE,L[3]	
5	12	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6	18	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7	32	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
1	6	M	4	NE	N	N	N	NE,L[2]															
2	33	M	4	N	N	N	N	N	N	N	N	FD											
3	27	M	4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	NE	N
4	31	M	4	SS	NS	N	N	N	N	N	N	N	N	NE	NS	FD							
5	39	M	4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6	21	M	4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7	38	M	4	N	N	N	N	NE	N	N	N	N	N	N	N	N	N	NE	N	N	N	N	N

Not Apllicable
Animal Deceased

N = Normal
NE = Not Eating
DI = Diarrhea
SS = Soft Stool
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RA = Respiratory Abnormalities
L = Lethargic
FD = Found Dead
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Comments:
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QA AUDIT COMPLETED
BY/DATE: [signature] 1/20/11

Printed By: SSM 10/20/10
QC Reviewed By: EGS 10/20/10

Challenge Order	Animal ID	Sex	Group	8/14/2010		8/15/2010		8/16/2010		8/17/2010		8/18/2010		8/19/2010		8/20/2010		8/21/2010		8/22/2010		8/23/2010	
				AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
1	40	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	7	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	5	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4	9	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5	37	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
1	13	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	34	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	25	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4	15	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5	30	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6	28	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7	19	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
1	14	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	11	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	2	M	3																				
4	8	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5	12	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6	18	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7	32	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
1	6	M	4																				
2	33	M	4																				
3	27	M	4	N	N	NE	L	FD															
4	31	M	4																				
5	39	M	4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6	21	M	4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7	38	M	4	N	N	NE	L	NE	N	NE,L,RA	L,RA	NE,L,RA[4]	RA	RA,O[1]	RA	RA	RA	NE,RA,O[5]	RA	NE,RA	SS,RA	N	N

Not Apllicable
Animal Deceased

N = Normal
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QA AUDIT COMPLETED
BY/DATE: [signature] 1/20/11

Printed By: JSM 10/20/10
QC Reviewed By: FEJ 10/20/10

K-6

Challenge Order	Animal ID	Sex	Group	8/24/2010		8/25/2010		8/26/2010		8/27/2010		8/28/2010		8/29/2010		8/30/2010		8/31/2010		9/1/2010		9/2/2010		9/3/2010	
				AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
1	40	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
2	7	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
3	5	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
4	9	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
5	37	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
1	13	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
2	34	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
3	25	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
4	15	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
5	30	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
6	28	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
7	19	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
1	14	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
2	11	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
3	2	M	3																						
4	8	M	3	N	N	N	N	N	N	O[6]	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
5	12	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
6	18	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
7	32	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
1	6	M	4																						
2	33	M	4																						
3	27	M	4																						
4	31	M	4																						
5	39	M	4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
6	21	M	4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
7	38	M	4	N	N	NE	N	N	N	N	N	N	N	N	N	N	N	N	N	NE	N	N	N	N	

[shaded box] Not Apllicable
[slash] Animal Deceased

N = Normal
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FD = Found Dead
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QA AUDIT COMPLETED
BY/DATE: [signature] 1/20/11

Printed By: [initials] 10/20/10
QC Reviewed By: EEJ 10/20/10

10/20/2010

K-7

Challenge Order	Animal ID	Sex	Group	7/20/2010		7/21/2010		7/22/2010		7/23/2010		7/24/2010		7/25/2010		7/26/2010	
				AM *	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PMα
1	40	M	1	*	N	N	N	N	N	N	N	N	N	N	N	N	α
2	7	M	1	*	N	N	N	N	N	N	N	N	N	N	N	N	α
3	5	M	1	*	N	N	N	N	N	N	N	N	N	N	N	N	α
4	9	M	1	*	N	N	N	N	N	N	N	N	N	N	N	N	α
5	37	M	1	*	N	N	N	N	N	N	N	N	N	N	N	N	α
1	13	M	2	*	N	N	N	N	N	N	N	N	N	N	N	N	α
2	34	M	2	*	N	NE	N	NE	N	N	N	N	N	N	N	N	α
3	25	M	2	*	N	N	N	N	N	N	N	N	N	N	N	N	α
4	15	M	2	*	N	N	N	N	N	N	N	N	N	N	N	N	α
5	30	M	2	*	N	N	N	N	N	N	N	N	N	N	N	N	α
6	28	M	2	*	N	N	N	N	N	N	N	N	N	N	N	N	α
7	19	M	2	*	N	N	N	N	N	N	N	N	N	N	N	N	α
1	14	M	3	*	N	N	N	N	N	N	N	N	N	N	N	N	α
2	11	M	3	*	N	N	N	N	N	N	N	N	N	N	N	N	α
3	2	M	3	*	N	NE	N	N	N	N	N	N	N	N	N	N	α
4	8	M	3	*	N	N	N	N	N	N	N	N	N	N	N	N	α
5	12	M	3	*	N	N	N	N	N	N	N	N	N	N	N	N	α
6	18	M	3	*	N	N	N	N	N	N	N	N	N	N	N	N	α
7	32	M	3	*	N	N	N	N	N	N	N	N	N	N	N	N	α
1	6	M	4	*	N	N	N	N	N	N	N	N	N	N	N	N	α
2	33	M	4	*	N	N	N	N	N	N	N	N	N	N	N	N	α
3	27	M	4	*	N	N	N	N	N	N	N	N	N	N	N	N	α
4	31	M	4	*	N	N	N	N	N	N	N	N	N	N	N	N	α
5	39	M	4	*	N	N	N	N	N	N	N	N	N	N	N	N	α
6	21	M	4	*	N	N	N	N	N	N	N	N	N	N	N	N	α
7	38	M	4	*	N	NE	N	N	N	N	N	N	N	N	N	N	α

N = Normal
NE = Not Eating

* Animals received from LAR, all appear N
α Animals challenged after am observations, refer to Clinical Observations

QA AUDIT COMPLETED
BY/DATE: [signature] 1/20/11

Printed By: [signature] 10/20/10
QC Reviewed By: EEJ 10/20/10

Challenge Order	Animal ID	Sex	Group	7/26/2010		7/27/2010		7/28/2010		7/29/2010		7/30/2010		7/31/2010		8/1/2010		8/2/2010		8/3/2010	
				AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
1	40	M	1		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	7	M	1		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	5	M	1		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4	9	M	1		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5	37	M	1		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
1	13	M	2		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	34	M	2		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	25	M	2		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4	15	M	2		N	N	N	N	N	N	N	N	N	N	N	DI	N	N	N	N	N
5	30	M	2		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6	28	M	2		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7	19	M	2		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
1	14	M	3		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	11	M	3		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	2	M	3		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4	8	M	3		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5	12	M	3		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6	18	M	3		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7	32	M	3		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
1	6	M	4		N	N	N	N	N	N	N	NE	N	NE,DI	N	N	N	N	N	N	N
2	33	M	4		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	27	M	4		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4	31	M	4		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	DI	SS,DI
5	39	M	4		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6	21	M	4		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	SS	N
7	38	M	4		N	N	N	N	N	NE	N	N	N	N	N	NE	N	N	N	N	N

Not Apllicable
Animal Deceased

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QA AUDIT COMPLETED
BY/DATE: [signature] 1/20/11

Printed By: [initials] 10/20/10
QC Reviewed By: [initials] 10/20/10

Challenge Order	Animal ID	Sex	Group	8/4/2010		8/5/2010		8/6/2010		8/7/2010		8/8/2010		8/9/2010		8/10/2010		8/11/2010		8/12/2010		8/13/2010	
				AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
1	40	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	7	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	5	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4	9	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5	37	M	1	N	N	N	N	N	N	NE	N	N	N	N	N	N	N	N	N	N	N	N	N
1	13	M	2	N	NS	N	N	N	N	NE	N	N	N	N	N	N	N	N	N	NE	N	N	N
2	34	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	25	M	2	N	N	SS	N	NE	N	NE	N	NE,O[1]	O[2]	N	N	N	N	N	N	N	N	N	N
4	15	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5	30	M	2	N	NS	N	N	NE,SS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6	28	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7	19	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
1	14	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	NE	N	N	N	N	N
2	11	M	3	N	N	N	N	NE,SS	N	N	N	N	N	N	N	N	N	NE	N	N	N	N	N
3	2	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	NE,L[3]	
4	8	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5	12	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6	18	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7	32	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
1	6	M	4	NE	N	N	N	NE,L[2]															
2	33	M	4	N	N	N	N	N	N	N	N	FD											
3	27	M	4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	NE	N
4	31	M	4	SS	NS	N	N	N	N	N	N	N	N	NE	NS	FD							
5	39	M	4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6	21	M	4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7	38	M	4	N	N	N	N	NE	N	N	N	N	N	N	N	N	N	NE	N	N	N	N	N

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QA AUDIT COMPLETED
BY/DATE: [signature] 1/20/11

Printed By: [initials] 10/20/10
QC Reviewed By: [initials] 10/20/10

K-10

Challenge Order	Animal ID	Sex	Group	8/14/2010		8/15/2010		8/16/2010		8/17/2010		8/18/2010		8/19/2010		8/20/2010		8/21/2010		8/22/2010		8/23/2010	
				AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
1	40	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	7	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	5	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4	9	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5	37	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
1	13	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	34	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	25	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4	15	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5	30	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6	28	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7	19	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
1	14	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	11	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	2	M	3	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
4	8	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5	12	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6	18	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7	32	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
1	6	M	4	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
2	33	M	4	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
3	27	M	4	N	N	NE	L	FD	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
4	31	M	4	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
5	39	M	4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6	21	M	4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7	38	M	4	N	N	NE	L	NE	N	NE,L,RA	L,RA	NE,L,RA[4]	RA	RA,O[1]	RA	RA	RA	NE,RA,O[5]	RA	NE,RA	SS,RA	N	N

[shaded box] Not Apllicable
/ Animal Deceased

N = Normal
NE = Not Eating
DI = Diarrhea
SS = Soft Stool
NS = No Stool
RA = Respiratory Abnormalities
L = Lethargic
FD = Found Dead
O = Other (requires comment)

Comments:
[1]Small amount of stool
[2]Animal found prostrate, unresponsive to touch, and gasping at 1055, died at 1113 prior to administration of B-euthanasia
[3]Animal found dead after am observations
[4]Vet notified about NE, vet ordered weight to be taken and yogurt to be added to food
[5]Fed yogurt, appears animal did not eat previous yogurt feeding, but no pellets in feeder, still dehydrated
[6]Impaired use of right front limb. N unless condition worsens

QA AUDIT COMPLETED
BY/DATE: [signature] 1/20/11

Printed By: JSM 10/20/10
QC Reviewed By: FEJ 10/20/10

Challenge Order	Animal ID	Sex	Group	8/24/2010		8/25/2010		8/26/2010		8/27/2010		8/28/2010		8/29/2010		8/30/2010		8/31/2010		9/1/2010		9/2/2010		9/3/2010	
				AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
1	40	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
2	7	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
3	5	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
4	9	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
5	37	M	1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
1	13	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
2	34	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
3	25	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
4	15	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
5	30	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
6	28	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
7	19	M	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
1	14	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
2	11	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
3	2	M	3																						
4	8	M	3	N	N	N	N	N	N	O[6]	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
5	12	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
6	18	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
7	32	M	3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
1	6	M	4																						
2	33	M	4																						
3	27	M	4																						
4	31	M	4																						
5	39	M	4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
6	21	M	4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
7	38	M	4	N	N	NE	N	N	N	N	N	N	N	N	N	N	N	N	N	NE	N	N	N	N	

[shaded box] Not Apllicable
[diagonal line] Animal Deceased

N = Normal
NE = Not Eating
DI = Diarrhea
SS = Soft Stool
NS = No Stool
RA = Respiratory Abnormalities
L = Lethargic
FD = Found Dead
O = Other (requires comment)

Comments:
[1]Small amount of stool
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[5]Fed yogurt, appears animal did not eat previous yogurt feeding, but no pellets in feeder, still dehydrated
[6]Impaired use of right front limb. N unless condition worsens

QA AUDIT COMPLETED
BY/DATE: [signature] 1/20/11

Printed By: [initials] 10/20/10
QC Reviewed By: EEJ 10/20/10

APPENDIX L
INDIVIDUAL BODY WEIGHTS

Challenge Order	Animal ID	Sex	Group	Study Day 2		Study Day 9		Study Day 16		Study Day 23		Study Day 30		Study Day 37	
				Date	Weight (kg)	Date	Weight (kg)	Date	Weight (kg)	Date	Weight (kg)	Date	Weight (kg)	Date	Weight (kg)
1	40	M	1	7/28/2010	3.13	8/4/2010	3.16	8/11/2010	3.22	8/18/2010	3.23	8/25/2010	3.25	9/1/2010	3.30
2	7	M	1	7/28/2010	2.89	8/4/2010	2.94	8/11/2010	3.05	8/18/2010	2.98	8/25/2010	3.00	9/1/2010	3.05
3	5	M	1	7/28/2010	2.68	8/4/2010	2.80	8/11/2010	2.85	8/18/2010	2.90	8/25/2010	2.99	9/1/2010	3.01
4	9	M	1	7/28/2010	2.92	8/4/2010	3.00	8/11/2010	3.08	8/18/2010	3.10	8/25/2010	3.06	9/1/2010	3.09
5	37	M	1	7/28/2010	2.46	8/4/2010	2.47	8/11/2010	2.60	8/18/2010	2.60	8/25/2010	2.68	9/1/2010	2.74
1	13	M	2	7/28/2010	2.68	8/4/2010	2.75	8/11/2010	2.77	8/18/2010	2.80	8/25/2010	2.86	9/1/2010	2.87
2	34	M	2	7/28/2010	2.75	8/4/2010	2.81	8/11/2010	2.91	8/18/2010	2.98	8/25/2010	3.03	9/1/2010	3.04
3	25	M	2	7/28/2010	2.89	8/4/2010	2.95	8/11/2010	2.98	8/18/2010	2.95	8/25/2010	3.01	9/1/2010	3.06
4	15	M	2	7/28/2010	2.85	8/4/2010	2.91	8/11/2010	3.02	8/18/2010	3.07	8/25/2010	3.09	9/1/2010	3.18
5	30	M	2	7/28/2010	2.81	8/4/2010	2.87	8/11/2010	2.93	8/18/2010	2.94	8/25/2010	2.99	9/1/2010	2.96
6	28	M	2	7/28/2010	2.82	8/4/2010	2.94	8/11/2010	3.00	8/18/2010	3.05	8/25/2010	3.06	9/1/2010	3.09
7	19	M	2	7/28/2010	2.55	8/4/2010	2.66	8/11/2010	2.80	8/18/2010	2.83	8/25/2010	2.88	9/1/2010	2.91
1	14	M	3	7/28/2010	2.79	8/4/2010	2.83	8/11/2010	2.90	8/18/2010	2.98	8/25/2010	3.06	9/1/2010	3.08
2	11	M	3	7/28/2010	2.55	8/4/2010	2.65	8/11/2010	2.56	8/18/2010	2.70	8/25/2010	2.76	9/1/2010	2.79
3	2	M	3	7/28/2010	2.55	8/4/2010	2.63	8/11/2010	2.79						
4	8	M	3	7/28/2010	2.93	8/4/2010	2.96	8/11/2010	3.06	8/18/2010	3.05	8/25/2010	3.09	9/1/2010	3.11
5	12	M	3	7/28/2010	2.79	8/4/2010	2.86	8/11/2010	2.94	8/18/2010	2.95	8/25/2010	3.01	9/1/2010	3.03
6	18	M	3	7/28/2010	2.72	8/4/2010	2.77	8/11/2010	2.84	8/18/2010	2.86	8/25/2010	2.93	9/1/2010	2.95
7	32	M	3	7/28/2010	3.12	8/4/2010	3.19	8/11/2010	3.27	8/18/2010	3.31	8/25/2010	3.34	9/1/2010	3.37
1	6	M	4	7/28/2010	2.86	8/4/2010	2.77								
2	33	M	4	7/28/2010	2.63	8/4/2010	2.66								
3	27	M	4	7/28/2010	2.59	8/4/2010	2.71	8/11/2010	2.76						
4	31	M	4	7/28/2010	2.75	8/4/2010	2.77								
5	39	M	4	7/28/2010	2.86	8/4/2010	2.95	8/11/2010	2.99	8/18/2010	3.06	8/25/2010	3.11	9/1/2010	3.13
6	21	M	4	7/28/2010	2.82	8/4/2010	2.89	8/11/2010	3.00	8/18/2010	3.03	8/25/2010	3.08	9/1/2010	3.05
7	38	M	4	7/28/2010	2.90	8/4/2010	2.95	8/11/2010	3.02	8/18/2010	2.72	8/25/2010	2.72	9/1/2010	2.70

/ Animal Deceased

QA AUDIT COMPLETED
BY/DATE: [signature] 1/20/11

L-2

Printed By: JSM 10/20/10
QC Reviewed BY: EEJ 10/20/10

APPENDIX M
INDIVIVUAL MORTALITY RESULTS

Animal ID	Group ID	Challenge Date & Time	Date & Time of Death	Time to Death (days)	Found Dead or Moribund/Euthanized/ Survived
40	1	7/26/10 9:32	9/3/10 9:09	39.0	Survived
7	1	7/26/10 9:46	9/3/10 9:27	39.0	Survived
5	1	7/26/10 10:00	9/3/10 9:36	39.0	Survived
9	1	7/26/10 10:11	9/3/10 9:58	39.0	Survived
37	1	7/26/10 10:22	9/3/10 10:17	39.0	Survived
13	2	7/26/10 10:55	9/3/10 10:32	39.0	Survived
34	2	7/26/10 11:11	9/3/10 10:50	39.0	Survived
25	2	7/26/10 11:22	9/3/10 10:57	39.0	Survived
15	2	7/26/10 11:33	9/3/10 11:10	39.0	Survived
30	2	7/26/10 11:48	9/3/10 11:24	39.0	Survived
28	2	7/26/10 11:58	9/3/10 11:33	39.0	Survived
19	2	7/26/10 12:09	9/3/10 12:21	39.0	Survived
14	3	7/26/10 12:25	9/3/10 12:34	39.0	Survived
11	3	7/26/10 12:36	9/3/10 12:12	39.0	Survived
2	3	7/26/10 12:49	8/13/10 10:39	17.9	Found Dead
8	3	7/26/10 12:59	9/3/10 12:37	39.0	Survived
12	3	7/26/10 13:12	9/3/10 12:51	39.0	Survived
18	3	7/26/10 13:28	9/3/10 13:01	39.0	Survived
32	3	7/26/10 13:39	9/3/10 13:08	39.0	Survived
6	4	7/26/10 13:53	8/6/10 11:13	10.9	Found Dead
33	4	7/26/10 14:05	8/8/10 7:57	12.7	Found Dead
27	4	7/26/10 14:16	8/16/10 8:26	20.8	Found Dead
31	4	7/26/10 14:27	8/10/10 8:16	14.7	Found Dead
39	4	7/26/10 14:36	9/3/10 13:16	38.9	Survived
21	4	7/26/10 14:47	9/3/10 13:24	38.9	Survived
38	4	7/26/10 14:57	9/3/10 13:31	38.9	Survived

QA AUDIT COMPLETED
BY/DATE: [signature] 1/20/11

Printed By: DW 12-15-10
QC Reviewed By: [illegible]

APPENDIX N
INDIVIDUAL CIRCULATING PA ELIZA RESULTS

1078-CG920794 PA Results Summary (ng/mL)															
Animal ID	Group	Day -3	Day 2	Day 4	Day 9	Day 11	Day 16	Day 18	Day 23	Day 25	Day 30	Day 32	Day 37	Day 39	Terminal
40	1	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
7[a]	1	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
5[a]	1	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
9[a]	1	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
37	1	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
13[a]	2	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
34	2	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
25	2	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
15	2	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
30[a]	2	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
28	2	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
19	2	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
14	3	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
11	3	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
2	3	BD	BD	BD	BD	BD	BD								158.668
8	3	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
12	3	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
18	3	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
32	3	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
6	4	BD	BD	BD	BD										BD
33	4	BD	BD	BD	BD	BD									
27	4	BD	BD	BD	BD	BD	BD	BD							65330.895
31	4	BD	BD	BD	BD	BD									
39[a]	4	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
21	4	BD	BD	BD	BD	BD	BD	BD	BD	4.967	BD	BD	BD	BD	
38	4	BD	BD	BD	BD	BD	BD	7.669	6.280	BD	BD	BD	BD	BD	

BD- below limit of detection (LLOQ 4.9 ng/mL per QD-186)

[a]Sample results from failing plates and will be accepted per Study Director's discretion. Refer to memo titled Acceptance of failing PA ELISA results.

Lineout means no sample was obtained at that timepoint.

JKC 11-30-10

QA AUDIT COMPLETED
BY/DATE: [signature] 1/20/11

QC/Tech Rev. By/Date: KAC 120110

ALS 11-30-10

1078-CG920794 PA Results Comparison for Samples From Failing Plates (ng/mL)

Animal ID	Group	Plate ID	Day -3	Day 2	Day 4	Day 9	Day 11	Day 16	Day 18	Day 23	Day 25	Day 30	Day 32	Day 37	Day 39	Terminal
7[a]	1	093010-001	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
7	1	110910-001	*	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
5[a]	1	093010-002	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
5	1	100710-001	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
5	1	101110-001	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
5	1	110910-004	*	*	*	BD	*	*	BD	*	*	*	*	*	BD	
9[a]	1	093010-003	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
9	1	110910-002	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
13	2	100410-003	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
13	2	100710-003	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
13[a]	2	101110-003	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
13	2	110910-004	*	*	BD	BD	BD	*	BD	*	*	*	*	*	BD	
30[a]	2	100410-005	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
30	2	101010-002	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
30	2	110910-003	BD	BD	BD	BD	BD	BD	BD	BD	BD	*	BD	BD	BD	
39	4	100710-002	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
39[a]	4	100310-008	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
39	4	101110-002	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	BD	
39	4	110910-004	*	*	*	*	*	*	*	BD	*	*	*	*	BD	

BD - below assay detection (LLOQ 4.9 ng/mL per QD-186)
[a]Failing results accepted per Study Director's discretion. Refer to memo titled Acceptance of Failing PA ELISA results.
* Insufficient volume for analysis.
Lineout means no sample was obtained at that timepoint.

JEC 11.30.10

QA AUDIT COMPLETED
BY/DATE: Hnitz 1/20/11

QC/Tech Rev. By/Date: KAL 120110

ALS 11-30-10

APPENDIX O
INDIVIDUAL BACTEREMIA CULTURE RESULTS

1078-CG920794 Quantitative Bacteremia (CFU/mL)[a]

Animal ID	Group	Day -3	Day 2	Day 4	Day 9	Day 11	Day 16	Day 18	Day 23	Day 25	Day 30	Day 32	Day 37	Day 39	Terminal
40	1	0	0	0	0	0	0	0	0	0	0	0	0	0	
7	1	0	0	0	0	0	0	0	0	0	0	0	0	0	
5	1	0	0	0	0	0	0, C	0	0	0	0	0	0	0	
9	1	0	0	0	0	0	0	0	0	0	0	0	0	0	
37	1	0	0	0	0	0	0	0	0	0	0	0	0	0	
13	2	0	0	0	0	0	0	0	0	0, C	0	0	0	0	
34	2	0	0	0	0	0	0	0	0	0	0	0	0	0	
25	2	0	0	0	0	0	0	0	0	0	0	0	0	0	
15	2	0	0	0	0	0	0	0	0	0	0	0	0	0	
30	2	0	0, C	0	0	0	0	0	0	0	0	0	0	0	
28	2	0	0, C	0	0	0	0	0	0	0	0	0	0	0	
19	2	0	0	0	0	0	0	0, C	0	0	0	0	0	0	
14	3	0	0	0	0, C	0	0	0	0	0	0	0	0	0	
11	3	0	0	0	0	0	0	0	0	0	0	0	0	0	
2	3	0	0	0	0	0	0								3.87E+05
8	3	0	0	0, C	0, C	0, C	0, C	0, C	0	0	0	0	0	0	
12	3	0	0	0	0	0	0	0	0	0	0	0	0	0	
18	3	0	0	0	0	0	0	0	0	0	0	0	0	0	
32	3	0	0	0	0	0	0	0	0	0	0	0	0	0	
6	4	0	0	0	0										0
33	4	0, C	0	0	0	0									4.13E+05
27	4	0	0	0, C	0	0	0	0							2.60E+03
31	4	0, C	0	0	0	0									4.00E+01
39	4	0	0	0	0	0	0	0	0	0	0	0	0	0	
21	4	0	0	0	0, C	0, C	0, C	0, C	0	0	0	0	0	0	
38	4	0	0	0	0, C	0	0	{1.80E+02}	0	0	0	0	0	0	

[a] Values in brackets indicate results derived from mean colony counts outside of the countable range (25-250 colonies)

0 = Negative for *B. Anthracis*

C = Indicates presence of organism other than *B. anthracis*

QA AUDIT COMPLETED
BY/DATE: [signature] 2/1/11

Printed By/Date: DW 1-25-11

QC/Tech Review By/Date: DW 1-25-11

APPENDIX P
INDIVIDUAL BACTEREMIA QPCR RESULTS

1078-CG920794
PCR Result Table *

Group	Animal ID	Day -3	Day 2	Day 4	Day 9	Day 11	Day 16	Day 18	Day 23	Day 25	Day 30	Day 32	Day 37	Day 39	Terminal
1	5	<LOQ	<LOQ	<LOQ	<LOQ	**	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
	7	<LOQ	<LOQ	<LOQ	<LOQ	**	<LOQ	<LOQ	<LOQ	NS	<LOQ	<LOQ	<LOQ	<LOQ	
	9	<LOQ	<LOQ	<LOQ	<LOQ	**	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
	37	<LOQ	<LOQ	<LOQ	<LOQ	**	<LOQ	<LOQ	NS	NS	<LOQ	<LOQ	<LOQ	<LOQ	
	40	<LOQ	<LOQ	<LOQ	<LOQ	**	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	NS	
2	13	<LOQ	<LOQ	<LOQ	<LOQ	**	<LOQ	<LOQ	<LOQ	NS	<LOQ	<LOQ	<LOQ	<LOQ	
	15	<LOQ	<LOQ	<LOQ	<LOQ	**	<LOQ	<LOQ	<LOQ	NS	<LOQ	<LOQ	<LOQ	<LOQ	
	19	<LOQ	<LOQ	<LOQ	<LOQ	**	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
	25	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
	28	<LOQ	<LOQ	<LOQ	<LOQ	**	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
	30	<LOQ	<LOQ	<LOQ	<LOQ	**	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	NS	<LOQ	<LOQ	
	34	<LOQ	<LOQ	<LOQ	<LOQ	**	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
3	2	<LOQ	<LOQ	<LOQ	<LOQ	**	<LOQ								3188
	8	<LOQ	<LOQ	<LOQ	<LOQ	**	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
	11	<LOQ	<LOQ	<LOQ	<LOQ	**	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
	12	<LOQ	<LOQ	<LOQ	<LOQ	**	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
	14	<LOQ	<LOQ	<LOQ	<LOQ	**	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
	18	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
	32	<LOQ	<LOQ	<LOQ	<LOQ	**	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
4	6	<LOQ	<LOQ	<LOQ	<LOQ	**									<LOQ
	21	<LOQ	<LOQ	<LOQ	<LOQ	**	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
	27	<LOQ	<LOQ	<LOQ	<LOQ	**	<LOQ	<LOQ							2522
	31	<LOQ	<LOQ	<LOQ	<LOQ	**									6.42E+05
	33	<LOQ	<LOQ	<LOQ	<LOQ	**									7766
	38	<LOQ	<LOQ	<LOQ	<LOQ	**	<LOQ	<LOQ	3	<LOQ	5	<LOQ	<LOQ	<LOQ	
	39	<LOQ	<LOQ	<LOQ	<LOQ	**	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	

*All results are in copies/μL
**See memo dated 8/25/10
NS=No sample
LOQ=Limits of quantification
[slashed box] Not applicable

QA AUDIT COMPLETED
BY/DATE: 1/20/11

Printed by: 1/13/11
QC/TR by: 1-13-11

APPENDIX Q
INDIVIDUAL TNA RESULTS

1078-CG920794 TNA RESULTS

Animal ID Information			Plate ID Information								ED50								
Animal ID	Time Point	TNA Sample ID#	Test 1 Analyst	Test 1 Plate ID	Test 2 Analyst	Test 2 Plate ID	Test 3 Analyst	Test 3 Plate ID	Test 4 Analyst	Test 4 Plate ID	Test 1 ED50	Test 2 ED50	Test 3 ED50	Test 4 ED50	Mean ED50	Std Dev ED50	%CV ED50	Median ED50	Reportable Value
2	Day 11	2 Day 11	AGN	082310-725	JA	082510-756	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
2	Day -3	2 Day -3	AGN	082310-720	JA	082510-751	AGN	090810-890	JA	091310-905	0	0	0	0	0	0	0%	0	<LOD
2	Day 4	2 Day 4	AGN	082310-723	JA	082510-754	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
2	Terminal	2 Terminal	KN	092710-038	JA	092710-044	KN	092810-116	NA	NA	24.849761	0	0	NA	8	14	173%	0	<LOD
5	Day 11	5 Day 11	AGN	082310-724	JA	082510-755	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
5	Day 18	5 Day 18	AGN	091310-913	JA	092710-044	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
5	Day 25	5 Day 25	AGN	090810-894	JA	091310-909	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
5	Day -3	5 Day -3	AGN	090810-889	JA	091310-904	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
5	Day 32	5 Day 32	KN	092710-033	JA	092710-039	AGN	092810-077	KN	092810-111	0	0	0	0	0	0	0%	0	<LOD
5	Day 39	5 Day 39	KN	092710-036	JA	092710-042	KN	092810-114	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
5	Day 4	5 Day 4	AGN	082310-721	JA	082510-752	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
6	Day -3	6 Day -3	AGN	082310-721	JA	082510-752	JA	091310-906	AGN	091310-912	0	0	0	0	0	0	0%	0	<LOD
6	Day 4	6 Day 4	AGN	082310-723	JA	082510-754	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
7	Day 11	7 Day 11	AGN	082310-724	JA	082510-755	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
7	Day 18	7 Day 18	JA	091310-906	AGN	091310-912	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
7	Day 25	7 Day 25	AGN	090810-894	JA	091310-909	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
7	Day -3	7 Day -3	AGN	090810-889	JA	091310-904	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
7	Day 32	7 Day 32	KN	092710-033	JA	092710-039	AGN	092810-077	KN	092810-111	0	0	0	0	0	0	0%	0	<LOD
7	Day 39	7 Day 39	KN	092710-035	JA	092710-041	KN	092810-113	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
8	Day 11	8 Day 11	AGN	082310-725	JA	082510-756	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
8	Day 18	8 Day 18	AGN	090810-893	JA	092710-045	AGN	092810-083	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
8	Day 25	8 Day 25	JA	091310-910	AGN	091310-914	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
8	Day -3	8 Day -3	AGN	082310-720	JA	082510-751	AGN	090810-890	JA	091310-905	0	0	0	0	0	0	0%	0	<LOD
8	Day 32	8 Day 32	KN	092710-035	JA	092710-041	KN	092810-113	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
8	Day 39	8 Day 39	KN	092710-037	JA	092710-043	KN	092810-115	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
8	Day 4	8 Day 4	AGN	082310-723	JA	082510-754	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
9	Day 18	9 Day 18	AGN	091310-913	JA	092710-044	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
9	Day 25	9 Day 25	AGN	090810-894	JA	091310-909	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
9	Day -3	9 Day -3	AGN	090810-889	JA	091310-904	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
9	Day 32	9 Day 32	KN	092710-033	JA	092710-039	AGN	092810-077	KN	092810-111	0	0	0	0	0	0	0%	0	<LOD
9	Day 39	9 Day 39	KN	092710-036	JA	092710-042	KN	092810-114	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
9	Day 4	9 Day 4	AGN	082310-722	JA	082510-753	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
11	Day 11	11 Day 11	AGN	082310-725	JA	082510-756	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
11	Day 18	11 Day 18	AGN	090810-893	JA	092710-045	AGN	092810-083	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
11	Day 25	11 Day 25	JA	091310-910	AGN	091310-914	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
11	Day -3	11 Day -3	AGN	082310-720	JA	082510-751	AGN	090810-890	JA	091310-905	0	0	0	0	0	0	0%	0	<LOD
11	Day 32	11 Day 32	KN	092710-034	JA	092710-040	KN	092810-112	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
11	Day 39	11 Day 39	KN	092710-037	JA	092710-043	KN	092810-115	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
11	Day 4	11 Day 4	AGN	082310-723	JA	082510-754	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
12	Day 11	12 Day 11	AGN	082310-725	JA	082510-756	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
12	Day 18	12 Day 18	AGN	090810-893	JA	092710-045	AGN	092810-083	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
12	Day 25	12 Day 25	JA	091310-910	AGN	091310-914	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
12	Day -3	12 Day -3	AGN	082310-720	JA	082510-751	AGN	090810-890	JA	091310-905	0	0	0	0	0	0	0%	0	<LOD
12	Day 32	12 Day 32	KN	092710-035	JA	092710-041	KN	092810-113	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
12	Day 39	12 Day 39	KN	092710-037	JA	092710-043	KN	092810-115	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
13	Day 11	13 Day 11	AGN	082310-724	JA	082510-755	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
13	Day 18	13 Day 18	AGN	091310-913	JA	092710-044	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
13	Day 25	13 Day 25	AGN	090810-894	JA	091310-909	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
13	Day -3	13 Day -3	AGN	090810-889	JA	091310-904	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
13	Day 32	13 Day 32	KN	092710-034	JA	092710-040	KN	092810-112	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
13	Day 39	13 Day 39	KN	092710-036	JA	092710-042	KN	092810-114	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
13	Day 4	13 Day 4	AGN	082310-722	JA	082510-753	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
14	Day 11	14 Day 11	AGN	082310-725	JA	082510-756	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
14	Day 18	14 Day 18	AGN	090810-893	JA	092710-045	AGN	092810-083	NA	NA	13.148790	0	0	NA	4	8	173%	0	<LOD
14	Day 25	14 Day 25	JA	091310-910	AGN	091310-914	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
14	Day -3	14 Day -3	AGN	082310-720	JA	082510-751	AGN	090810-890	JA	091310-905	0	0	0	0	0	0	0%	0	<LOD
14	Day 32	14 Day 32	KN	092710-034	JA	092710-040	KN	092810-112	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
14	Day 39	14 Day 39	KN	092710-037	JA	092710-043	KN	092810-115	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
14	Day 4	14 Day 4	AGN	082310-723	JA	082510-754	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD

QD50 LOD=23

QA AUDIT COMPLETED
BY/DATE: [signature] 2/14/11

Printed By: DW 2-11-11

QC/Tech Review by: DW 2-11-11

Animal ID Information			NF50							
Animal ID	Time Point	TNA Sample ID#	Test 1 NF50	Test 2 NF50	Test 3 NF50	Test 4 NF50	Mean NF50	Std Dev NF50	%CV NF50	Median NF50
2	Day 11	2 Day 11	0	0	NA	NA	0.000	0.000	0%	0.000
2	Day -3	2 Day -3	0	0	0	0	0.000	0.000	0%	0.000
2	Day 4	2 Day 4	0	0	NA	NA	0.000	0.000	0%	0.000
2	Terminal	2 Terminal	0.048	0	0	NA	0.016	0.028	173%	0.000
5	Day 11	5 Day 11	0	0	NA	NA	0.000	0.000	0%	0.000
5	Day 18	5 Day 18	0	0	NA	NA	0.000	0.000	0%	0.000
5	Day 25	5 Day 25	0	0	NA	NA	0.000	0.000	0%	0.000
5	Day -3	5 Day -3	0	0	NA	NA	0.000	0.000	0%	0.000
5	Day 32	5 Day 32	0	0	0	0	0.000	0.000	0%	0.000
5	Day 39	5 Day 39	0	0	0	NA	0.000	0.000	0%	0.000
5	Day 4	5 Day 4	0	0	NA	NA	0.000	0.000	0%	0.000
6	Day -3	6 Day -3	0	0	0	0	0.000	0.000	0%	0.000
6	Day 4	6 Day 4	0	0	NA	NA	0.000	0.000	0%	0.000
7	Day 11	7 Day 11	0	0	NA	NA	0.000	0.000	0%	0.000
7	Day 18	7 Day 18	0	0	NA	NA	0.000	0.000	0%	0.000
7	Day 25	7 Day 25	0	0	NA	NA	0.000	0.000	0%	0.000
7	Day -3	7 Day -3	0	0	NA	NA	0.000	0.000	0%	0.000
7	Day 32	7 Day 32	0	0	0	0	0.000	0.000	0%	0.000
7	Day 39	7 Day 39	0	0	0	NA	0.000	0.000	0%	0.000
8	Day 11	8 Day 11	0	0	NA	NA	0.000	0.000	0%	0.000
8	Day 18	8 Day 18	0	0	0	NA	0.000	0.000	0%	0.000
8	Day 25	8 Day 25	0	0	NA	NA	0.000	0.000	0%	0.000
8	Day -3	8 Day -3	0	0	0	0	0.000	0.000	0%	0.000
8	Day 32	8 Day 32	0	0	0	NA	0.000	0.000	0%	0.000
8	Day 39	8 Day 39	0	0	0	NA	0.000	0.000	0%	0.000
8	Day 4	8 Day 4	0	0	NA	NA	0.000	0.000	0%	0.000
9	Day 18	9 Day 18	0	0	NA	NA	0.000	0.000	0%	0.000
9	Day 25	9 Day 25	0	0	NA	NA	0.000	0.000	0%	0.000
9	Day -3	9 Day -3	0	0	NA	NA	0.000	0.000	0%	0.000
9	Day 32	9 Day 32	0	0	0	0	0.000	0.000	0%	0.000
9	Day 39	9 Day 39	0	0	0	NA	0.000	0.000	0%	0.000
9	Day 4	9 Day 4	0	0	NA	NA	0.000	0.000	0%	0.000
11	Day 11	11 Day 11	0	0	NA	NA	0.000	0.000	0%	0.000
11	Day 18	11 Day 18	0	0	0	NA	0.000	0.000	0%	0.000
11	Day 25	11 Day 25	0	0	NA	NA	0.000	0.000	0%	0.000
11	Day -3	11 Day -3	0	0	0	0	0.000	0.000	0%	0.000
11	Day 32	11 Day 32	0	0	0	NA	0.000	0.000	0%	0.000
11	Day 39	11 Day 39	0	0	0	NA	0.000	0.000	0%	0.000
11	Day 4	11 Day 4	0	0	NA	NA	0.000	0.000	0%	0.000
12	Day 11	12 Day 11	0	0	NA	NA	0.000	0.000	0%	0.000
12	Day 18	12 Day 18	0	0	0	NA	0.000	0.000	0%	0.000
12	Day 25	12 Day 25	0	0	NA	NA	0.000	0.000	0%	0.000
12	Day -3	12 Day -3	0	0	0	0	0.000	0.000	0%	0.000
12	Day 32	12 Day 32	0	0	0	NA	0.000	0.000	0%	0.000
12	Day 39	12 Day 39	0	0	0	NA	0.000	0.000	0%	0.000
13	Day 11	13 Day 11	0	0	NA	NA	0.000	0.000	0%	0.000
13	Day 18	13 Day 18	0	0	NA	NA	0.000	0.000	0%	0.000
13	Day 25	13 Day 25	0	0	NA	NA	0.000	0.000	0%	0.000
13	Day -3	13 Day -3	0	0	NA	NA	0.000	0.000	0%	0.000
13	Day 32	13 Day 32	0	0	0	NA	0.000	0.000	0%	0.000
13	Day 39	13 Day 39	0	0	0	NA	0.000	0.000	0%	0.000
13	Day 4	13 Day 4	0	0	NA	NA	0.000	0.000	0%	0.000
14	Day 11	14 Day 11	0	0	NA	NA	0.000	0.000	0%	0.000
14	Day 18	14 Day 18	0.022	0	0	NA	0.007	0.013	173%	0.000
14	Day 25	14 Day 25	0	0	NA	NA	0.000	0.000	0%	0.000
14	Day -3	14 Day -3	0	0	0	0	0.000	0.000	0%	0.000
14	Day 32	14 Day 32	0	0	0	NA	0.000	0.000	0%	0.000
14	Day 39	14 Day 39	0	0	0	NA	0.000	0.000	0%	0.000
14	Day 4	14 Day 4	0	0	NA	NA	0.000	0.000	0%	0.000

QD50 LOD=23

QA AUDIT COMPLETED
BY/DATE: [signature] 2/14/11

Printed By: DW 2-11-11

QC/Tech Review by: DW 2-11-11

Animal ID Information			Plate ID Information								ED50								
			Test 1		Test 2		Test 3		Test 4		Test 1	Test 2	Test 3	Test 4					
Animal ID	Time Point	TNA Sample ID#	Analyst	Plate ID	Analyst	Plate ID	Analyst	Plate ID	Analyst	Plate ID	ED50	ED50	ED50	ED50	Mean ED50	Std Dev ED50	%CV ED50	Median ED50	Reportable Value
15	Day 11	15 Day 11	AGN	082310-725	JA	082510-756	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
15	Day 18	15 Day 18	AGN	091310-913	JA	092710-044	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
15	Day 25	15 Day 25	JA	091310-910	AGN	091310-914	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
15	Day 32	15 Day 32	KN	092710-034	JA	092710-040	KN	092810-112	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
15	Day 39	15 Day 39	KN	092710-036	JA	092710-042	KN	092810-114	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
15	Day 4	15 Day 4	AGN	082310-722	JA	082510-753	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
18	Day 18	18 Day 18	AGN	090810-893	JA	092710-045	AGN	092810-083	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
18	Day 25	18 Day 25	JA	091310-910	AGN	091310-914	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
18	Day -3	18 Day -3	AGN	082310-720	JA	082510-751	AGN	090810-890	JA	091310-905	0	0	0	0	0	0	0%	0	<LOD
18	Day 32	18 Day 32	KN	092710-035	JA	092710-041	KN	092810-113	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
18	Day 39	18 Day 39	KN	092710-037	JA	092710-043	KN	092810-115	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
18	Day 4	18 Day 4	AGN	082310-723	JA	082510-754	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
19	Day 11	19 Day 11	AGN	082310-725	JA	082510-756	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
19	Day 18	19 Day 18	AGN	090810-893	JA	092710-045	AGN	092810-083	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
19	Day 25	19 Day 25	JA	091310-910	AGN	091310-914	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
19	Day -3	19 Day -3	AGN	082310-720	JA	082510-751	AGN	090810-890	JA	091310-905	0	0	0	0	0	0	0%	0	<LOD
19	Day 32	19 Day 32	KN	092710-034	JA	092710-040	KN	092810-112	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
19	Day 39	19 Day 39	KN	092710-037	JA	092710-043	KN	092810-115	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
19	Day 4	19 Day 4	AGN	082310-722	JA	082510-753	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
21	Day 11	21 Day 11	JA	082510-757	AGN	082510-760	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
21	Day 18	21 Day 18	AGN	090810-894	JA	091310-909	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
21	Day 25	21 Day 25	KN	092710-033	JA	092710-039	AGN	092710-077	KN	092810-111	0	0	0	0	0	0	0%	0	<LOD
21	Day -3	21 Day -3	AGN	082310-721	JA	082510-752	JA	091310-906	AGN	091310-912	0	0	0	0	0	0	0%	0	<LOD
21	Day 32	21 Day 32	KN	092710-035	JA	092710-041	KN	092810-113	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
21	Day 39	21 Day 39	KN	092710-037	JA	092710-043	KN	092810-115	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
21	Day 4	21 Day 4	AGN	082310-724	JA	082510-755	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
25	Day 18	25 Day 18	AGN	091310-913	JA	092710-044	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
25	Day 25	25 Day 25	AGN	090810-894	JA	091310-909	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
25	Day -3	25 Day -3	AGN	090810-889	JA	091310-904	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
25	Day 32	25 Day 32	KN	092710-034	JA	092710-040	KN	092810-112	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
25	Day 39	25 Day 39	KN	092710-036	JA	092710-042	KN	092810-114	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
25	Day 4	25 Day 4	AGN	082310-722	JA	082510-753	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
27	Day 11	27 Day 11	JA	082510-757	AGN	082510-760	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
27	Day 18	27 Day 18	AGN	090810-893	KN	092710-038	KN	092810-116	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
27	Day -3	27 Day -3	AGN	082310-721	JA	082510-752	JA	091310-906	AGN	091310-912	0	0	0	0	0	0	0%	0	<LOD
27	Day 4	27 Day 4	AGN	082310-723	JA	082510-754	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
28	Day 11	28 Day 11	AGN	082310-725	JA	082510-756	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
28	Day 18	28 Day 18	AGN	091310-913	JA	092710-045	AGN	092810-083	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
28	Day 25	28 Day 25	JA	091310-910	AGN	091310-914	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
28	Day -3	28 Day -3	AGN	082310-720	JA	082510-751	AGN	090810-890	JA	091310-905	0	0	0	0	0	0	0%	0	<LOD
28	Day 32	28 Day 32	KN	092710-034	JA	092710-040	KN	092810-112	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
28	Day 39	28 Day 39	KN	092710-036	JA	092710-042	KN	092810-114	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
28	Day 4	28 Day 4	AGN	082310-722	JA	082510-753	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
30	Day 18	30 Day 18	AGN	091310-913	NA	NA	NA	NA	NA	NA	0	NA	NA	NA	0	NA	NA	0	<LOD
30	Day 25	30 Day 25	JA	091310-910	AGN	091310-914	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
30	Day -3	30 Day -3	AGN	090810-889	JA	091310-904	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
30	Day 32	30 Day 32	KN	092710-034	JA	092710-040	KN	092810-112	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
30	Day 39	30 Day 39	KN	092710-036	JA	092710-042	KN	092810-114	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
30	Day 4	30 Day 4	AGN	082310-722	JA	082510-753	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
31	Day 11	31 Day 11	JA	082510-757	AGN	082510-760	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
31	Day -3	31 Day -3	AGN	082310-721	JA	082510-752	JA	091310-906	AGN	091310-912	0	0	0	0	0	0	0%	0	<LOD
31	Day 4	31 Day 4	AGN	082310-723	JA	082510-754	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
32	Day 11	32 Day 11	JA	082510-757	AGN	082510-760	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
32	Day 18	32 Day 18	AGN	090810-893	JA	092710-045	AGN	092810-083	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
32	Day 25	32 Day 25	KN	092710-033	JA	092710-039	AGN	092810-077	KN	092810-111	0	0	0	0	0	0	0%	0	<LOD
32	Day -3	32 Day -3	AGN	082310-720	JA	082510-751	AGN	090810-890	JA	091310-905	0	0	0	0	0	0	0%	0	<LOD
32	Day 32	32 Day 32	KN	092710-035	JA	092710-041	KN	092810-113	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
32	Day 39	32 Day 39	KN	092710-037	JA	092710-043	KN	092810-115	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
32	Day 4	32 Day 4	AGN	082310-723	JA	082510-754	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD

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Animal ID Information			NF50							
Animal ID	Time Point	TNA Sample ID#	Test 1 NF50	Test 2 NF50	Test 3 NF50	Test 4 NF50	Mean NF50	Std Dev NF50	%CV NF50	Median NF50
15	Day 11	15 Day 11	0	0	NA	NA	0.000	0.000	0%	0.000
15	Day 18	15 Day 18	0	0	NA	NA	0.000	0.000	0%	0.000
15	Day 25	15 Day 25	0	0	NA	NA	0.000	0.000	0%	0.000
15	Day 32	15 Day 32	0	0	0	NA	0.000	0.000	0%	0.000
15	Day 39	15 Day 39	0	0	0	NA	0.000	0.000	0%	0.000
15	Day 4	15 Day 4	0	0	NA	NA	0.000	0.000	0%	0.000
18	Day 18	18 Day 18	0	0	0	NA	0.000	0.000	0%	0.000
18	Day 25	18 Day 25	0	0	NA	NA	0.000	0.000	0%	0.000
18	Day -3	18 Day -3	0	0	0	0	0.000	0.000	0%	0.000
18	Day 32	18 Day 32	0	0	0	NA	0.000	0.000	0%	0.000
18	Day 39	18 Day 39	0	0	0	NA	0.000	0.000	0%	0.000
18	Day 4	18 Day 4	0	0	NA	NA	0.000	0.000	0%	0.000
19	Day 11	19 Day 11	0	0	NA	NA	0.000	0.000	0%	0.000
19	Day 18	19 Day 18	0	0	0	NA	0.000	0.000	0%	0.000
19	Day 25	19 Day 25	0	0	NA	NA	0.000	0.000	0%	0.000
19	Day -3	19 Day -3	0	0	0	0	0.000	0.000	0%	0.000
19	Day 32	19 Day 32	0	0	0	NA	0.000	0.000	0%	0.000
19	Day 39	19 Day 39	0	0	0	NA	0.000	0.000	0%	0.000
19	Day 4	19 Day 4	0	0	NA	NA	0.000	0.000	0%	0.000
21	Day 11	21 Day 11	0	0	NA	NA	0.000	0.000	0%	0.000
21	Day 18	21 Day 18	0	0	NA	NA	0.000	0.000	0%	0.000
21	Day 25	21 Day 25	0	0	0	0	0.000	0.000	0%	0.000
21	Day -3	21 Day -3	0	0	0	0	0.000	0.000	0%	0.000
21	Day 32	21 Day 32	0	0	0	NA	0.000	0.000	0%	0.000
21	Day 39	21 Day 39	0	0	0	NA	0.000	0.000	0%	0.000
21	Day 4	21 Day 4	0	0	NA	NA	0.000	0.000	0%	0.000
25	Day 18	25 Day 18	0	0	NA	NA	0.000	0.000	0%	0.000
25	Day 25	25 Day 25	0	0	NA	NA	0.000	0.000	0%	0.000
25	Day -3	25 Day -3	0	0	NA	NA	0.000	0.000	0%	0.000
25	Day 32	25 Day 32	0	0	0	NA	0.000	0.000	0%	0.000
25	Day 39	25 Day 39	0	0	0	NA	0.000	0.000	0%	0.000
25	Day 4	25 Day 4	0	0	NA	NA	0.000	0.000	0%	0.000
27	Day 11	27 Day 11	0	0	NA	NA	0.000	0.000	0%	0.000
27	Day 18	27 Day 18	0	0	0	NA	0.000	0.000	0%	0.000
27	Day -3	27 Day -3	0	0	0	0	0.000	0.000	0%	0.000
27	Day 4	27 Day 4	0	0	NA	NA	0.000	0.000	0%	0.000
28	Day 11	28 Day 11	0	0	NA	NA	0.000	0.000	0%	0.000
28	Day 18	28 Day 18	0	0	0	NA	0.000	0.000	0%	0.000
28	Day 25	28 Day 25	0	0	NA	NA	0.000	0.000	0%	0.000
28	Day -3	28 Day -3	0	0	0	0	0.000	0.000	0%	0.000
28	Day 32	28 Day 32	0	0	0	NA	0.000	0.000	0%	0.000
28	Day 39	28 Day 39	0	0	0	NA	0.000	0.000	0%	0.000
28	Day 4	28 Day 4	0	0	NA	NA	0.000	0.000	0%	0.000
30	Day 18	30 Day 18	0	NA	NA	NA	0.000	NA	0%	0.000
30	Day 25	30 Day 25	0	0	NA	NA	0.000	0.000	0%	0.000
30	Day -3	30 Day -3	0	0	NA	NA	0.000	0.000	0%	0.000
30	Day 32	30 Day 32	0	0	0	NA	0.000	0.000	0%	0.000
30	Day 39	30 Day 39	0	0	0	NA	0.000	0.000	0%	0.000
30	Day 4	30 Day 4	0	0	NA	NA	0.000	0.000	0%	0.000
31	Day 11	31 Day 11	0	0	NA	NA	0.000	0.000	0%	0.000
31	Day -3	31 Day -3	0	0	0	0	0.000	0.000	0%	0.000
31	Day 4	31 Day 4	0	0	NA	NA	0.000	0.000	0%	0.000
32	Day 11	32 Day 11	0	0	NA	NA	0.000	0.000	0%	0.000
32	Day 18	32 Day 18	0	0	0	NA	0.000	0.000	0%	0.000
32	Day 25	32 Day 25	0	0	0	0	0.000	0.000	0%	0.000
32	Day -3	32 Day -3	0	0	0	0	0.000	0.000	0%	0.000
32	Day 32	32 Day 32	0	0	0	NA	0.000	0.000	0%	0.000
32	Day 39	32 Day 39	0	0	0	NA	0.000	0.000	0%	0.000
32	Day 4	32 Day 4	0	0	NA	NA	0.000	0.000	0%	0.000

QD50 LOD=23

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QC/Tech Review by: DN 2-11-11

Animal ID Information			Plate ID Information								ED50								
			Test 1		Test 2		Test 3		Test 4		Test 1	Test 2	Test 3	Test 4					
Animal ID	Time Point	TNA Sample ID#	Analyst	Plate ID	Analyst	Plate ID	Analyst	Plate ID	Analyst	Plate ID	ED50	ED50	ED50	ED50	Mean ED50	Std Dev ED50	%CV ED50	Median ED50	Reportable Value
33	Day 11	33 Day 11	JA	082510-757	AGN	082510-760	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
33	Day -3	33 Day -3	AGN	082310-721	JA	082510-752	JA	091310-906	AGN	091310-912	0	0	0	0	0	0	0%	0	<LOD
34	Day 11	34 Day 11	AGN	082310-724	JA	082510-755	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
34	Day 18	34 Day 18	AGN	091310-913	JA	092710-044	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
34	Day 25	34 Day 25	AGN	090810-894	JA	091310-909	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
34	Day -3	34 Day -3	AGN	090810-889	JA	091310-904	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
34	Day 32	34 Day 32	KN	092710-034	JA	092710-040	KN	092810-112	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
34	Day 39	34 Day 39	KN	092710-036	JA	092710-042	KN	092810-114	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
34	Day 4	34 Day 4	AGN	082310-722	JA	082510-753	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
37	Day 11	37 Day 11	AGN	082310-724	JA	082510-755	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
37	Day 18	37 Day 18	AGN	091310-913	JA	092710-044	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
37	Day 25	37 Day 25	AGN	090810-894	JA	091310-909	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
37	Day -3	37 Day -3	AGN	090810-889	JA	091310-904	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
37	Day 32	37 Day 32	KN	092710-033	JA	092710-039	AGN	092810-077	KN	092810-111	0	0	0	0	0	0	0%	0	<LOD
37	Day 39	37 Day 39	KN	092710-036	JA	092710-042	KN	092810-114	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
37	Day 4	37 Day 4	AGN	082310-722	JA	082510-753	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
38	Day 11	38 Day 11	JA	082510-757	AGN	082510-760	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
38	Day 25	38 Day 25	KN	092710-033	JA	092710-039	AGN	092810-077	KN	092810-111	8440.374079	4508.651414	5195.658816	6521.202436	6166	1731	28%	5858	5858
38	Day -3	38 Day -3	AGN	082310-721	JA	082510-752	JA	091310-906	AGN	091310-912	0	0	0	0	0	0	0%	0	<LOD
38	Day 32	38 Day 32	KN	092710-035	JA	092710-041	KN	092810-113	NA	NA	12789.078381	15258.337052	11269.603444	NA	13106	2013	15%	12789	12789
38	Day 39	38 Day 39	KN	092710-038	JA	092710-044	KN	092810-116	NA	NA	7230.515022	7249.765087	10040.50374	NA	8174	1617	20%	7250	7250
38	Day 4	38 Day 4	AGN	082310-724	JA	082510-755	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
39	Day 11	39 Day 11	JA	082510-757	AGN	082510-760	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
39	Day 18	39 Day 18	AGN	090810-893	KN	092710-038	KN	092810-116	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
39	Day 25	39 Day 25	KN	092710-033	JA	092710-039	AGN	092810-077	KN	092810-111	0	0	0	0	0	0	0%	0	<LOD
39	Day -3	39 Day -3	AGN	082310-721	JA	082510-752	JA	091310-906	AGN	091310-912	0	0	0	0	0	0	0%	0	<LOD
39	Day 32	39 Day 32	KN	092710-035	JA	092710-041	KN	092810-113	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
39	Day 39	39 Day 39	JA	092710-043	KN	092810-115	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
39	Day 4	39 Day 4	AGN	082310-724	JA	082510-755	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
40	Day 11	40 Day 11	AGN	082310-724	JA	082510-755	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
40	Day 18	40 Day 18	JA	091310-906	AGN	091310-912	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
40	Day 25	40 Day 25	AGN	090810-894	JA	091310-909	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
40	Day -3	40 Day -3	AGN	090810-889	JA	091310-904	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD
40	Day 32	40 Day 32	KN	092710-033	JA	092710-039	AGN	092810-077	KN	092810-111	0	0	0	0	0	0	0%	0	<LOD
40	Day 39	40 Day 39	KN	092710-035	JA	092710-041	KN	092810-113	NA	NA	0	0	0	NA	0	0	0%	0	<LOD
40	Day 4	40 Day 4	AGN	082310-721	JA	082510-752	NA	NA	NA	NA	0	0	NA	NA	0	0	0%	0	<LOD

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Q-7

Animal ID Information			NF50							
Animal ID	Time Point	TNA Sample ID#	Test 1 NF50	Test 2 NF50	Test 3 NF50	Test 4 NF50	Mean NF50	Std Dev NF50	%CV NF50	Median NF50
33	Day 11	33 Day 11	0	0	NA	NA	0.000	0.000	0%	0.000
33	Day -3	33 Day -3	0	0	0	0	0.000	0.000	0%	0.000
34	Day 11	34 Day 11	0	0	NA	NA	0.000	0.000	0%	0.000
34	Day 18	34 Day 18	0	0	NA	NA	0.000	0.000	0%	0.000
34	Day 25	34 Day 25	0	0	NA	NA	0.000	0.000	0%	0.000
34	Day -3	34 Day -3	0	0	NA	NA	0.000	0.000	0%	0.000
34	Day 32	34 Day 32	0	0	0	NA	0.000	0.000	0%	0.000
34	Day 39	34 Day 39	0	0	0	NA	0.000	0.000	0%	0.000
34	Day 4	34 Day 4	0	0	NA	NA	0.000	0.000	0%	0.000
37	Day 11	37 Day 11	0	0	NA	NA	0.000	0.000	0%	0.000
37	Day 18	37 Day 18	0	0	NA	NA	0.000	0.000	0%	0.000
37	Day 25	37 Day 25	0	0	NA	NA	0.000	0.000	0%	0.000
37	Day -3	37 Day -3	0	0	NA	NA	0.000	0.000	0%	0.000
37	Day 32	37 Day 32	0	0	0	0	0.000	0.000	0%	0.000
37	Day 39	37 Day 39	0	0	0	NA	0.000	0.000	0%	0.000
37	Day 4	37 Day 4	0	0	NA	NA	0.000	0.000	0%	0.000
38	Day 11	38 Day 11	0	0	NA	NA	0.000	0.000	0%	0.000
38	Day 25	38 Day 25	16.863	10.734	7.916	14.679	12.548	3.996	32%	12.706
38	Day -3	38 Day -3	0	0	0	0	0.000	0.000	0%	0.000
38	Day 32	38 Day 32	20.853	32.880	26.444	NA	26.726	6.018	23%	26.444
38	Day 39	38 Day 39	13.896	14.819	22.235	NA	16.983	4.571	27%	14.819
38	Day 4	38 Day 4	0	0	NA	NA	0.000	0.000	0%	0.000
39	Day 11	39 Day 11	0	0	NA	NA	0.000	0.000	0%	0.000
39	Day 18	39 Day 18	0	0	0	NA	0.000	0.000	0%	0.000
39	Day 25	39 Day 25	0	0	0	0	0.000	0.000	0%	0.000
39	Day -3	39 Day -3	0	0	0	0	0.000	0.000	0%	0.000
39	Day 32	39 Day 32	0	0	0	NA	0.000	0.000	0%	0.000
39	Day 39	39 Day 39	0	0	NA	NA	0.000	0.000	0%	0.000
39	Day 4	39 Day 4	0	0	NA	NA	0.000	0.000	0%	0.000
40	Day 11	40 Day 11	0	0	NA	NA	0.000	0.000	0%	0.000
40	Day 18	40 Day 18	0	0	NA	NA	0.000	0.000	0%	0.000
40	Day 25	40 Day 25	0	0	NA	NA	0.000	0.000	0%	0.000
40	Day -3	40 Day -3	0	0	NA	NA	0.000	0.000	0%	0.000
40	Day 32	40 Day 32	0	0	0	0	0.000	0.000	0%	0.000
40	Day 39	40 Day 39	0	0	0	NA	0.000	0.000	0%	0.000
40	Day 4	40 Day 4	0	0	NA	NA	0.000	0.000	0%	0.000

QA AUDIT COMPLETED
BY/DATE: [signature] 2/14/11

QD50 LOD=23

Printed By: DW 2-11-11

QC/Tech Review by: DW 2-11-11

APPENDIX R
INDIVIDUAL ANTI-PA IGG ELISA RESULTS

1078-CG920794 IgG ELISA Reportable Values (µg/mL)										
Animal ID	Group	Spore Dose (CFU)	Day -3 ①	Day 4	Day 11	Day 18	Day 25	Day 32	Day 39	Terminal
40	1*	10,000*	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	
7	1*	10,000*	<LOD		<LOD	<LOD	<LOD	<LOD	<LOD	
5	1*	10,000*	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	
9	1*	10,000*	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	
37	1*	10,000*	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	
13	2	100	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	
34	2	100	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	
25	2	100	<LOD	<LOD		<LOD	<LOD	<LOD	<LOD	
15	2	100		<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	
30	2	100	<LOD	<LOD		<LOD	<LOD	<LOD	<LOD	
28	2	100	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	
19	2	100	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	
14	3	1,000	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	
11	3	1,000	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	
2	3	1,000	<LOD	<LOD	<LOD					<LOD
8	3	1,000	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	
12	3	1,000	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	
18	3	1,000	<LOD	<LOD		<LOD	<LOD	<LOD	<LOD	
32	3	1,000	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	
6	4	10,000	<LOD	<LOD						
33	4	10,000	<LOD		<LOD					
27	4	10,000	<LOD	<LOD	<LOD	<LOD				<LOD
31	4	10,000	<LOD	<LOD	<LOD					
39	4	10,000	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	
21	4	10,000	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	
38	4	10,000	<LOD	<LOD	<LOD	<LOD	1636.019	2190.848	1728.468	

* = Negative Control; Challenged with inactivated, irradiated spores

① = Day -3 results from prescreen analysis

QA Audit Completed By/Date: [handwritten initials] 5/20/11

LOD = 1.0ug/mL
LOQ = 5.0ug/mL
(As per form ELISA-008)

QC Tech Review By/Date: JEC 5-20-11

APPENDIX S
INDIVIDUAL HEMATOLOGY RESULTS

Parameter	Group	Animal ID	Day -3	Day 2	Day 4	Day 9	Day 11	Day 16	Day 18	Day 23	Day 25	Day 30	Day 32	Day 37	Day 39
White Blood Cell Count	1	37	**8.35**	6.78	QNS	4.54 *	5.45	5.17 *	4.74	QNS	QNS	6.93	QNS	6.29	1.30 *
(2.9 - 8.1 ×10³/uL)	1	40	5.39	5.01	5.27	4.73 *	5.72	QNS	5.29 *	3.12	4.56	3.84 *	QNS	5.68	4.79
	1	5	7.59	7.49	7.63	**9.64**	7.46	7.80	**8.59**	**8.73**	7.86	**8.49**	QNS	**8.97**	6.14
	1	7	4.48	6.71	1.60 *	6.57	6.66	6.86	6.89	6.59	QNS	6.72	6.51	6.85	1.53 *
	1	9	4.16	4.91	5.89	4.76	4.53	5.39	5.43	5.74	6.09	5.38	QNS	5.69	5.81
		Average	5.99	6.18	5.10	6.05	5.96	6.31	6.19	6.05	6.17	6.27	6.51	6.70	3.91
		Std Dev	1.88	1.16	2.54	2.17	1.13	1.25	1.56	2.32	1.65	1.75	0.00	1.36	2.34
	2	13	7.66	**8.34**	**9.70**	**9.39**	7.53	**8.66**	7.69	QNS	QNS	6.68	QNS	7.77	2.93 *
	2	15	6.75	**8.11**	**8.27**	7.58	7.90	7.00	7.75	7.47	6.20	**8.37**	QNS	7.99	6.95
	2	19	7.92	7.63	7.03	7.40	6.69	6.54	4.97	6.18	6.68	6.15 *	5.16	5.36	2.63 *
	2	25	6.16	8.00	7.70	8.00	**8.75**	**8.89**	**9.85**	**8.16**	**8.37**	7.79	QNS	**8.67**	7.50
	2	28	5.14	4.97	5.53	5.37	5.41	5.64	6.71	6.25	5.36	6.12	5.58	5.28	4.67
	2	30	6.24	5.22	5.81	6.47	6.76	6.36	6.24	5.85	5.16	5.81	QNS	4.94	4.55
	2	34	**9.26**	**8.62**	7.64	7.95	7.86	6.08	6.65	5.76	5.34	6.12	5.51	5.71	3.62
		Average	7.02	7.27	7.38	7.45	7.27	7.02	7.12	6.61	6.19	6.72	5.42	6.53	4.69
		Std Dev	1.37	1.52	1.43	1.27	1.08	1.27	1.53	0.98	1.22	0.98	0.23	1.55	1.89
	3	11	7.09	6.41	6.78	6.32	5.78	6.56	6.88	**8.72**	6.70	7.04	6.80	6.24	1.23 *
	3	12	4.62	4.66	3.66 *	4.66	QNS	4.95	4.21	5.62	3.96	QNS	5.61	QNS	5.07
	3	14	6.28	6.58	7.27	7.33	7.65	7.00	7.93	8.02	7.94	**9.01**	7.20	QNS	3.53 *
	3	18	**8.40**	4.44	5.07	5.01	6.67	QNS	6.08	QNS	6.79	5.24	QNS	5.95	6.26 *
	3	2	5.22	4.73	5.19	QNS	4.82	4.64	--	--	--	--	--	--	--
	3	32	6.07	4.80	5.00	5.69	5.65	QNS	4.19	QNS	5.50	QNS	4.76	4.79	3.34 *
	3	8	6.30	6.12	5.46	6.59	8.08	6.50	7.02	QNS	5.99	QNS	5.55	5.63	4.97
		Average	6.28	5.39	5.49	5.93	6.44	5.93	6.05	7.45	6.15	7.10	5.98	5.65	4.07
		Std Dev	1.23	0.93	1.20	1.01	1.26	1.06	1.55	1.63	1.35	1.89	1.00	0.63	1.76
	4	21	**8.37**	5.72	5.98	6.46	6.69	5.62	**8.62**	QNS	QNS	5.13	7.78	5.91	5.83
	4	27	7.54	7.65	QNS	**8.76**	**10.00**	**8.60**	**10.25**	--	--	--	--	--	--
	4	31	6.44	7.54	QNS	5.59	6.43	--	--	--	--	--	--	--	--
	4	33	4.17	4.29	QNS	5.28	2.94 *	--	--	--	--	--	--	--	--
	4	38	7.81	6.91	7.84	6.99	7.85	**8.30**	**9.88**	**20.33**	**18.08**	**15.09**	**11.01**	5.99	7.50 *
	4	39	**9.15**	**10.63**	**9.12**	7.09	**8.20**	7.02	**8.79**	**8.95**	**9.74**	**9.80**	8.08	**8.92**	6.40
	4	6	8.00	7.90	**10.73**	8.01	--	--	--	--	--	--	--	--	--
		Average	7.35	7.23	8.42	6.88	7.02	7.39	9.39	14.64	13.91	10.01	8.96	6.94	6.58
		Std Dev	1.63	1.97	2.01	1.24	2.37	1.36	0.80	8.05	5.90	4.98	1.78	1.72	0.85

ATC 10.4.10

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* Clot Removed
QNS - Sample Volume not Sufficient for analysis

QA AUDIT COMPLETED
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QC/Tech Rev. By/Date: ATC 10.4.10

Parameter	Group	Animal ID	Day -3	Day 2	Day 4	Day 9	Day 11	Day 16	Day 18	Day 23	Day 25	Day 30	Day 32	Day 37	Day 39
Red Blood Cell Count	1	37	4.99	4.80	QNS	4.79 *	5.29	4.77 *	4.63	QNS	QNS	4.71	QNS	4.90	3.63 *
(4.2 - 6.7 ×10^6/uL)	1	40	5.44	5.57	5.33	5.55 *	5.58	QNS	5.55 *	5.78	6.08	5.91 *	QNS	5.88	5.93
	1	5	5.91	5.81	5.47	5.94	5.66	6.00	6.02	6.11	6.54	5.75	QNS	6.22	5.74
	1	7	5.20	5.32	4.11 *	4.96	4.95	5.16	5.16	5.37	QNS	5.42	5.45	5.81	3.82 *
	1	9	6.46	6.11	5.55	6.01	6.38	6.22	5.96	6.37	**7.08**	6.26	QNS	6.54	5.98
		Average	5.60	5.52	5.12	5.45	5.57	5.54	5.46	5.91	6.57	5.61	5.45	5.87	5.02
		Std Dev	0.59	0.50	0.68	0.56	0.53	0.69	0.58	0.43	0.50	0.59	0.00	0.62	1.19
	2	13	5.96	5.81	5.67	5.73	5.74	5.48	5.48	QNS	QNS	5.61	QNS	5.93	4.95 *
	2	15	6.26	6.34	5.96	6.01	5.93	5.87	6.19	6.28	6.34	6.42	QNS	6.22	6.00
	2	19	5.58	5.83	5.50	5.66	5.55	5.55	5.53	5.68	5.79	5.79 *	5.81	6.08	5.42 *
	2	25	6.33	6.52	6.43	6.31	**7.12**	5.97	6.08	**6.83**	**6.71**	6.61	QNS	6.65	6.04
	2	28	5.71	5.65	5.46	5.75	5.58	5.77	5.93	5.89	5.90	5.83	5.40	5.84	5.59
	2	30	5.85	5.63	5.90	5.54	5.78	5.80	5.84	5.88	5.79	5.69	QNS	6.14	5.79
	2	34	6.23	6.06	5.91	6.02	5.82	5.51	5.41	5.70	5.81	5.79	6.06	5.91	6.08
		Average	5.99	5.98	5.83	5.86	5.93	5.71	5.78	6.04	6.06	5.96	5.76	6.11	5.70
		Std Dev	0.29	0.34	0.33	0.27	0.54	0.19	0.31	0.44	0.38	0.39	0.33	0.27	0.41
	3	11	5.51	5.67	5.75	5.48	5.43	5.87	5.80	5.89	5.84	5.83	5.32	5.85	4.04 *
	3	12	6.12	5.66	5.30 *	5.80	QNS	6.27	5.97	6.25	6.25	QNS	6.20	QNS	5.47
	3	14	5.79	5.69	5.36	5.49	5.78	6.23	6.17	5.96	5.86	6.34	5.50	QNS	5.94 *
	3	18	5.85	5.39	5.29	5.56	5.34	QNS	5.24	QNS	5.46	5.40	QNS	5.78	5.33 *
	3	2	5.43	5.54	5.56	QNS	5.08	5.34	--	--	--	--	--	--	--
	3	32	5.87	5.81	5.65	5.52	5.43	QNS	5.51	QNS	5.57	QNS	5.52	5.65	5.39 *
	3	8	6.47	6.32	6.25	6.11	6.13	6.47	6.49	QNS	6.12	QNS	6.22	6.41	6.69
		Average	5.86	5.73	5.59	5.66	5.53	6.04	5.86	6.03	5.85	5.86	5.75	5.92	5.48
		Std Dev	0.35	0.29	0.34	0.25	0.37	0.45	0.45	0.19	0.30	0.47	0.43	0.34	0.87
	4	21	5.45	5.40	5.62	5.48	5.39	5.76	5.51	QNS	QNS	5.60	5.57	5.83	6.19
	4	27	5.59	5.58	QNS	5.42	5.54	5.86	5.83	--	--	--	--	--	--
	4	31	5.74	6.21	QNS	5.73	5.57	--	--	--	--	--	--	--	--
	4	33	5.18	5.10	QNS	4.94	4.77 *	--	--	--	--	--	--	--	--
	4	38	6.51	5.95	5.88	5.75	5.83	5.98	5.90	4.69	4.80	5.40	5.08	5.40	5.59 *
	4	39	6.34	5.30	5.49	5.79	5.65	5.94	6.12	6.20	6.08	6.34	5.69	6.23	5.91
	4	6	6.13	6.11	5.99	**7.34**	--	--	--	--	--	--	--	--	--
		Average	5.85	5.66	5.75	5.78	5.46	5.89	5.84	5.45	5.44	5.78	5.45	5.82	5.90
		Std Dev	0.49	0.43	0.23	0.75	0.37	0.10	0.25	1.07	0.91	0.50	0.32	0.42	0.30

ATC 10·4·10

Bold: > Normal Range
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-- No Value
* Clot Removed
QNS - Sample Volume not Sufficient for analysis

QA AUDIT COMPLETED
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QC/Tech Rev. By/Date: ATC 10·4·10

S-4

Parameter	Group	Animal ID	Day -3	Day 2	Day 4	Day 9	Day 11	Day 16	Day 18	Day 23	Day 25	Day 30	Day 32	Day 37	Day 39
Hemoglobin	1	37	10.5	10.2	QNS	10.3 *	11.3	10.2 *	9.9	QNS	QNS	9.9	QNS	10.4	7.6 *
(9.5 - 14.5 g/dL)	1	40	11.6	11.8	11.3	11.8 *	11.9	QNS	11.7 *	12.2	12.6	12.5 *	QNS	12.2	12.3
	1	5	11.8	11.7	10.9	12.0	11.4	12.0	12.1	12.4	12.8	11.6	QNS	12.3	11.5
	1	7	10.8	11.1	8.6 *	10.5	10.4	10.9	10.8	11.3	QNS	11.4	11.5	12.1	8.0 *
	1	9	13.3	12.6	11.3	12.3	13.3	12.8	12.2	13.0	14.4	12.8	QNS	13.2	12.1
		Average	11.6	11.5	10.5	11.4	11.7	11.5	11.3	12.2	13.3	11.6	11.5	12.0	10.3
		Std Dev	1.1	0.9	1.3	0.9	1.1	1.2	1.0	0.7	1.0	1.1	0.0	1.0	2.3
	2	13	12.7	12.3	12.1	12.2	12.1	11.9	11.7	QNS	QNS	12.0	QNS	12.5	10.6 *
	2	15	12.9	13.0	12.2	12.4	12.1	12.0	12.7	12.8	12.8	13.1	QNS	12.7	12.3
	2	19	10.8	11.3	10.6	11.0	10.9	10.9	10.7	11.1	11.4	11.2 *	11.3	11.7	10.6 *
	2	25	12.7	13.2	12.8	12.6	**14.7**	12.1	12.2	13.6	13.3	12.9	QNS	13.1	12.0
	2	28	12.0	12.0	11.4	12.0	11.6	12.1	12.4	12.4	12.2	12.0	11.3	12.0	11.7
	2	30	12.0	11.7	12.2	11.4	12.0	11.9	12.1	12.2	11.9	11.6	QNS	12.4	11.9
	2	34	12.2	12.1	11.8	12.0	11.6	11.3	10.8	11.6	11.7	11.5	12.1	11.8	12.3
		Average	12.2	12.2	11.9	11.9	12.1	11.7	11.8	12.3	12.2	12.0	11.6	12.3	11.6
		Std Dev	0.7	0.7	0.7	0.6	1.2	0.5	0.8	0.9	0.7	0.7	0.5	0.5	0.7
	3	11	12.2	12.5	12.4	12.1	12.1	12.9	12.8	12.8	12.8	12.6	11.7	12.8	8.9 *
	3	12	12.1	11.6	11.0 *	12.0	QNS	12.7	12.1	12.6	12.7	QNS	12.4	QNS	11.0
	3	14	12.3	12.1	11.4	11.7	12.2	13.3	13.0	12.7	12.3	13.4	11.6	QNS	12.6 *
	3	18	11.9	11.1	10.9	11.5	11.1	QNS	10.6	QNS	11.2	10.9	QNS	11.8	10.9 *
	3	2	11.4	11.7	11.8	QNS	10.8	11.4	--	--	--	--	--	--	--
	3	32	12.0	11.9	11.4	11.4	11.2	QNS	11.3	QNS	11.5	QNS	11.3	11.6	11.3 *
	3	8	12.7	12.5	12.3	12.2	12.1	12.8	12.6	QNS	12.1	QNS	12.0	12.4	12.8
		Average	12.1	11.9	11.6	11.8	11.6	12.6	12.1	12.7	12.1	12.3	11.8	12.2	11.3
		Std Dev	0.4	0.5	0.6	0.3	0.6	0.7	0.9	0.1	0.6	1.3	0.4	0.6	1.4
	4	21	11.7	11.8	12.1	11.7	11.7	12.5	11.7	QNS	QNS	11.9	11.7	11.9	12.8
	4	27	11.6	11.6	QNS	11.3	11.6	12.2	12.1	--	--	--	--	--	--
	4	31	11.6	12.6	QNS	11.7	11.4	--	--	--	--	--	--	--	--
	4	33	10.5	10.5	QNS	10.1	9.8 *	--	--	--	--	--	--	--	--
	4	38	13.7	12.3	12.1	11.7	11.8	12.2	11.8	9.1	9.3	10.4	9.8	10.1	10.6 *
	4	39	12.5	10.4	10.9	11.6	11.3	11.8	12.0	12.3	12.1	12.5	11.3	12.2	11.8
	4	6	12.3	12.4	12.0	**15.2**	--	--	--	--	--	--	--	--	--
		Average	12.0	11.7	11.8	11.9	11.3	12.2	11.9	10.7	10.7	11.6	10.9	11.4	11.7
		Std Dev	1.0	0.9	0.6	1.6	0.7	0.3	0.2	2.3	2.0	1.1	1.0	1.1	1.1

PAC 10.4.10

Bold: > Normal Range
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-- No Value
* Clot Removed
QNS - Sample Volume not Sufficient for analysis

QA AUDIT COMPLETED
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Tech Rev. By/Date: PAC 10.4.10

Parameter	Group	Animal ID	Day -3	Day 2	Day 4	Day 9	Day 11	Day 16	Day 18	Day 23	Day 25	Day 30	Day 32	Day 37	Day 39
Hematocrit	1	37	32.3	31.1	QNS	31.3 *	34.8	31.1 *	30.1	QNS	QNS	30.0	QNS	30.8	22.3 *
(27.2 - 45.9 %)	1	40	34.4	35.8	34.1	35.3 *	35.3	QNS	35.0 *	36.3	37.8	36.4 *	QNS	36.3	37.5
	1	5	36.6	36.0	33.4	37.3	35.3	37.4	37.0	36.4	39.9	35.3	QNS	37.5	34.8
	1	7	32.3	33.7	25.5 *	31.5	31.6	33.1	32.8	34.0	QNS	34.6	34.5	36.5	23.5 *
	1	9	42.0	38.6	35.3	38.8	41.5	39.8	37.6	39.5	44.1	39.2	QNS	40.6	37.2
		Average	35.5	35.0	32.1	34.8	35.7	35.4	34.5	36.6	40.6	35.1	34.5	36.3	31.1
		Std Dev	4.0	2.8	4.5	3.4	3.6	4.0	3.1	2.3	3.2	3.3	0.0	3.5	7.5
	2	13	38.2	37.5	36.8	37.2	37.4	35.4	34.9	QNS	QNS	35.7	QNS	37.3	31.0 *
	2	15	39.6	40.7	37.6	38.2	37.6	37.5	38.7	38.8	41.0	40.3	QNS	38.7	37.6
	2	19	34.6	36.1	33.5	35.6	34.6	34.1	33.5	34.3	34.8	35.1 *	34.6	36.4	32.7 *
	2	25	40.1	41.0	40.2	39.4	45.9	37.2	37.5	42.1	41.7	41.3	QNS	41.2	37.8
	2	28	36.3	35.8	34.9	36.9	36.1	37.4	37.4	37.2	37.3	36.6	33.5	37.0	35.6
	2	30	37.7	35.8	39.2	36.0	37.9	38.0	37.6	37.4	36.3	35.8	QNS	38.2	36.9
	2	34	37.9	36.8	36.0	37.5	36.4	34.2	32.9	34.6	35.1	35.3	36.3	35.7	36.8
		Average	37.8	37.7	36.9	37.3	38.0	36.3	36.1	37.4	37.7	37.2	34.8	37.8	35.5
		Std Dev	1.9	2.3	2.3	1.3	3.7	1.7	2.3	2.9	3.0	2.5	1.4	1.8	2.6
	3	11	37.6	38.7	39.1	38.0	37.6	39.9	38.7	39.4	39.9	39.9	35.7	39.8	27.0 *
	3	12	37.4	35.0	31.9 *	35.7	QNS	38.6	36.1	37.8	37.4	QNS	37.2	QNS	33.4
	3	14	38.8	38.4	35.6	36.9	39.2	41.6	40.3	38.6	38.3	42.5	35.5	QNS	38.0 *
	3	18	36.8	33.2	32.7	34.4	33.5	QNS	32.6	QNS	33.5	33.2	QNS	35.4	33.4 *
	3	2	34.6	35.8	35.6	QNS	33.5	35.0	--	--	--	--	--	--	--
	3	32	36.6	36.3	35.2	35.1	34.6	QNS	34.2	QNS	34.3	QNS	33.9	34.7	32.8 *
	3	8	38.8	38.1	36.9	36.6	37.4	39.6	38.3	QNS	36.2	QNS	36.6	37.3	39.6
		Average	37.2	36.5	35.3	36.1	36.0	38.9	36.7	38.6	36.6	38.5	35.8	36.8	34.0
		Std Dev	1.4	2.0	2.4	1.3	2.4	2.5	2.9	0.8	2.4	4.8	1.3	2.3	4.4
	4	21	36.6	36.8	37.7	37.2	36.6	38.5	36.0	QNS	QNS	35.8	35.6	36.6	39.7
	4	27	36.6	36.4	QNS	35.3	37.1	38.0	37.5	--	--	--	--	--	--
	4	31	35.8	39.6	QNS	36.8	36.0	--	--	--	--	--	--	--	--
	4	33	32.4	32.1	QNS	31.1	30.3 *	--	--	--	--	--	--	--	--
	4	38	44.5	39.7	38.4	38.3	38.2	39.2	37.3	28.9	32.3	35.4	32.1	33.0	34.8 *
	4	39	40.6	34.0	34.5	38.1	36.3	38.1	39.0	39.5	40.3	40.8	35.3	39.5	36.9
	4	6	38.2	38.2	37.1	**47.4**	--	--	--	--	--	--	--	--	--
		Average	37.8	36.7	36.9	37.7	35.8	38.5	37.5	34.2	36.3	37.3	34.3	36.4	37.1
		Std Dev	3.8	2.8	1.7	4.9	2.8	0.5	1.2	7.5	5.7	3.0	1.9	3.3	2.5

PTC 10·4·10

Bold: > Normal Range
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QNS - Sample Volume not Sufficient for analysis

QA AUDIT COMPLETED
BY/DATE: [signature] 11/4/10

Tech Rev. By/Date: PTC 10·4·10

Parameter	Group	Animal ID	Day -3	Day 2	Day 4	Day 9	Day 11	Day 16	Day 18	Day 23	Day 25	Day 30	Day 32	Day 37	Day 39
Mean Corpuscular Volume	1	37	64.8	64.9	QNS	65.4 *	65.8	65.2 *	65.1	QNS	QNS	63.8	QNS	62.8	61.5 *
(59.1 - 75.4 fL)	1	40	63.2	64.2	64.0	63.6 *	63.3	QNS	63.0 *	62.8	62.2	61.7 *	QNS	61.7	63.2
	1	5	61.9	62.0	60.9	62.8	62.4	62.3	61.5	59.6	61.1	61.4	QNS	60.3	60.5
	1	7	62.1	63.4	61.9 *	63.5	63.9	64.2	63.6	63.4	QNS	63.8	63.2	62.8	61.6 *
	1	9	65.0	63.3	63.7	64.6	65.1	63.9	63.0	62.1	62.3	62.6	QNS	62.0	62.3
		Average	63.4	63.6	62.6	64.0	64.1	63.9	63.2	62.0	61.9	62.7	63.2	61.9	61.8
		Std Dev	1.5	1.1	1.5	1.0	1.4	1.2	1.3	1.7	0.7	1.1	0.0	1.0	1.0
	2	13	64.2	64.5	64.8	65.1	65.2	64.7	63.5	QNS	QNS	63.6	QNS	62.9	62.6 *
	2	15	63.2	64.2	63.0	63.5	63.4	63.9	62.5	61.7	64.8	62.7	QNS	62.2	62.6
	2	19	62.1	61.9	61.0	62.9	62.3	61.5	60.6	60.4	60.1	60.6 *	59.6	60.0	60.2 *
	2	25	63.4	62.8	62.5	62.4	64.4	62.3	61.7	61.6	62.1	62.4	QNS	62.0	62.6
	2	28	63.5	63.4	64.0	64.1	64.8	64.8	63.0	63.1	63.2	62.7	62.2	63.4	63.8
	2	30	64.4	63.7	66.4	65.0	65.6	65.6	64.3	63.6	62.8	62.9	QNS	62.1	63.7
	2	34	60.8	60.8	60.9	62.3	62.4	62.1	60.7	60.7	60.4	60.9	59.9	60.3	60.5
		Average	63.1	63.0	63.2	63.6	64.0	63.6	62.3	61.9	62.2	62.3	60.6	61.8	62.3
		Std Dev	1.3	1.3	2.0	1.2	1.3	1.6	1.4	1.3	1.8	1.1	1.4	1.3	1.4
	3	11	68.2	68.3	68.0	69.3	69.3	68.1	66.7	66.8	68.3	68.5	67.0	68.0	66.9 *
	3	12	61.2	61.9	60.3 *	61.6	QNS	61.5	60.5	60.6	59.8	QNS	60.0	QNS	61.0
	3	14	67.0	67.4	66.4	67.2	67.8	66.9	65.3	64.8	65.3	67.0	64.7	QNS	63.9 *
	3	18	63.0	61.6	61.7	61.8	62.6	QNS	62.3	QNS	61.4	61.5	QNS	61.2	62.6 *
	3	2	63.8	64.5	63.9	QNS	66.0	65.6	--	--	--	--	--	--	--
	3	32	62.4	62.5	62.3	63.6	63.7	QNS	62.1	QNS	61.6	QNS	61.5	61.3	60.8 *
	3	8	59.9	60.3	59.0	59.9	61.0	61.1	59.1	QNS	59.1	QNS	58.9	58.1	59.2
		Average	63.6	63.8	63.1	63.9	65.1	64.6	62.7	64.1	62.6	65.7	62.4	62.2	62.4
		Std Dev	3.0	3.1	3.2	3.6	3.2	3.2	2.9	3.2	3.5	3.7	3.4	4.2	2.7
	4	21	67.1	68.2	67.2	67.9	67.9	66.8	65.3	QNS	QNS	64.0	63.8	62.8	64.2
	4	27	65.5	65.2	QNS	65.3	66.9	64.8	64.4	--	--	--	--	--	--
	4	31	62.3	63.8	QNS	64.2	64.6	--	--	--	--	--	--	--	--
	4	33	62.6	62.9	QNS	63.0	63.4 *	--	--	--	--	--	--	--	--
	4	38	68.3	66.7	65.4	66.6	65.6	65.6	63.3	61.7	67.3	65.6	63.2	61.2	62.2 *
	4	39	64.1	64.1	62.9	65.7	64.4	64.2	63.8	63.8	66.2	64.4	62.0	63.4	62.4
	4	6	62.3	62.5	61.9	64.5	--	--	--	--	--	--	--	--	--
		Average	64.6	64.8	64.4	65.3	65.5	65.4	64.2	62.8	66.8	64.7	63.0	62.5	62.9
		Std Dev	2.4	2.1	2.4	1.6	1.7	1.1	0.9	1.5	0.8	0.8	0.9	1.1	1.1

PAC 10·4·10

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QNS - Sample Volume not Sufficient for analysis

QA AUDIT COMPLETED
BY/DATE: Km 11/4/10

QC/Tech Rev. By/Date: PAC 10·4·10

Parameter	Group	Animal ID	Day -3	Day 2	Day 4	Day 9	Day 11	Day 16	Day 18	Day 23	Day 25	Day 30	Day 32	Day 37	Day 39
Mean Corpuscular Hemoglobin	1	37	21.0	21.2	QNS	21.5 *	21.4	21.3 *	21.4	QNS	QNS	21.1	QNS	21.2	21.0 *
(19.7 - 24.6 pg)	1	40	21.4	21.1	21.1	21.2 *	21.2	QNS	21.0 *	21.0	20.7	21.2 *	QNS	20.7	20.8
	1	5	19.9	20.1	19.9	20.1	20.2	20.1	20.2	20.2	19.7	20.2	QNS	19.7	20.1
	1	7	20.8	20.8	20.8 *	21.2	21.0	21.1	21.0	21.1	QNS	21.0	21.2	20.8	21.1 *
	1	9	20.6	20.6	20.4	20.4	20.8	20.6	20.4	20.5	20.3	20.5	QNS	20.2	20.3
		Average	20.7	20.8	20.6	20.9	20.9	20.8	20.8	20.7	20.2	20.8	21.2	20.5	20.7
		Std Dev	0.6	0.4	0.5	0.6	0.5	0.5	0.5	0.4	0.5	0.4	0.0	0.6	0.4
	2	13	21.3	21.2	21.3	21.3	21.0	21.6	21.4	QNS	QNS	21.3	QNS	21.2	21.4 *
	2	15	20.5	20.6	20.5	20.6	20.4	20.5	20.5	20.4	20.3	20.5	QNS	20.4	20.5
	2	19	19.4	19.4	19.3	19.3	19.6	19.7	19.3	19.5	19.6	19.4 *	19.5	19.3	19.5 *
	2	25	20.0	20.2	19.9	19.9	20.6	20.2	20.0	19.9	19.9	19.5	QNS	19.6	19.9
	2	28	21.0	21.2	20.8	20.9	20.8	21.0	20.9	21.0	20.8	20.7	20.9	20.5	20.9
	2	30	20.5	20.8	20.7	20.7	20.7	20.4	20.8	20.7	20.6	20.4	QNS	20.3	20.5
	2	34	19.6	19.9	20.0	19.9	20.0	20.5	20.0	20.3	20.1	19.8	20.0	20.0	20.2
		Average	20.3	20.5	20.4	20.4	20.4	20.6	20.4	20.3	20.2	20.2	20.1	20.2	20.4
		Std Dev	0.7	0.7	0.7	0.7	0.5	0.6	0.7	0.5	0.4	0.7	0.7	0.6	0.6
	3	11	22.2	22.1	21.6	22.1	22.2	21.9	22.1	21.8	22.0	21.6	21.9	21.9	21.9 *
	3	12	19.8	20.5	20.7 *	20.7	QNS	20.2	20.3	20.2	20.3	QNS	20.0	QNS	20.1
	3	14	21.3	21.3	21.2	21.3	21.0	21.3	21.1	21.2	21.0	21.1	21.2	QNS	21.2 *
	3	18	20.4	20.6	20.6	20.7	20.7	QNS	20.3	QNS	20.6	20.2	QNS	20.4	20.5 *
	3	2	21.0	21.2	21.3	QNS	21.3	21.4	--	--	--	--	--	--	--
	3	32	20.4	20.4	20.2	20.7	20.7	QNS	20.5	QNS	20.6	QNS	20.4	20.6	20.9 *
	3	8	19.6	19.8	19.6	19.9	19.7	19.8	19.4	QNS	19.7	QNS	19.2	19.4	19.1
		Average	20.7	20.8	20.7	20.9	20.9	20.9	20.6	21.1	20.7	21.0	20.5	20.6	20.6
		Std Dev	0.9	0.8	0.7	0.7	0.8	0.9	0.9	0.8	0.8	0.7	1.0	1.0	1.0
	4	21	21.4	21.8	21.6	21.3	21.7	21.6	21.3	QNS	QNS	21.2	21.0	20.5	20.7
	4	27	20.7	20.8	QNS	21.0	20.9	20.8	20.8	--	--	--	--	--	--
	4	31	20.1	20.2	QNS	20.5	20.5	--	--	--	--	--	--	--	--
	4	33	20.2	20.6	QNS	20.3	20.4 *	--	--	--	--	--	--	--	--
	4	38	21.0	20.7	20.6	20.4	20.2	20.3	20.1	19.3	19.3	19.2	19.3	18.7	19.0 *
	4	39	19.7	19.7	19.9	20.0	20.0	19.8	19.6	19.9	19.8	19.7	19.8	19.6	19.9
	4	6	20.1	20.3	20.0	20.7	--	--	--	--	--	--	--	--	--
		Average	20.5	20.6	20.5	20.6	20.6	20.6	20.5	19.6	19.6	20.0	20.0	19.6	19.9
		Std Dev	0.6	0.7	0.8	0.4	0.6	0.8	0.8	0.4	0.4	1.0	0.9	0.9	0.9

ATC 10·4·10

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QNS - Sample Volume not Sufficient for analysis

QA AUDIT COMPLETED
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Tech Rev. By/Date: ATC 10·4·10

Parameter	Group	Animal ID	Day -3	Day 2	Day 4	Day 9	Day 11	Day 16	Day 18	Day 23	Day 25	Day 30	Day 32	Day 37	Day 39
Mean Corpuscular Hemoglobin Concentration	1	37	32.4	32.7	QNS	32.9 *	32.6	32.7 *	32.8	QNS	QNS	33.1	QNS	33.7	34.1 *
(31 - 34.9 g/dL)	1	40	33.8	32.9	33.0	33.3 *	33.6	QNS	33.3 *	33.5	33.3	34.4 *	QNS	33.5	32.9
	1	5	32.1	32.4	32.6	32.0	32.3	32.2	32.8	33.9	32.2	32.9	QNS	32.8	33.2
	1	7	33.5	32.8	33.7 *	33.3	32.9	32.9	33.0	33.3	QNS	32.9	33.5	33.0	34.2 *
	1	9	31.6	32.6	32.0	31.6	31.9	32.2	32.4	33.0	32.7	32.7	QNS	32.6	32.5
		Average	32.7	32.7	32.8	32.6	32.7	32.5	32.9	33.4	32.7	33.2	33.5	33.1	33.4
		Std Dev	0.9	0.2	0.7	0.8	0.6	0.4	0.3	0.4	0.6	0.7	0.0	0.5	0.7
	2	13	33.1	32.9	32.8	32.7	32.2	33.4	33.7	QNS	QNS	33.5	QNS	33.6	34.2 *
	2	15	32.5	32.1	32.5	32.4	32.2	32.1	32.8	33.0	31.3	32.6	QNS	32.8	32.7
	2	19	31.3	31.3	31.6	30.8	31.5	32.0	31.8	32.3	32.7	32.0 *	32.7	32.1	32.4 *
	2	25	31.5	32.2	31.9	31.9	31.9	32.4	32.4	32.3	32.0	31.3	QNS	31.7	31.8
	2	28	33.0	33.5	32.5	32.6	32.2	32.4	33.1	33.3	32.8	32.9	33.6	32.4	32.7
	2	30	31.8	32.6	31.3	31.8	31.6	31.2	32.3	32.6	32.8	32.4	QNS	32.6	32.2
	2	34	32.2	32.8	32.9	31.9	32.0	33.0	32.9	33.4	33.2	32.6	33.5	33.1	33.4
		Average	32.2	32.5	32.2	32.0	31.9	32.4	32.7	32.8	32.5	32.5	33.3	32.6	32.8
		Std Dev	0.7	0.7	0.6	0.6	0.3	0.7	0.6	0.5	0.7	0.7	0.5	0.6	0.8
	3	11	32.5	32.3	31.9	31.8	32.1	32.2	33.1	32.6	32.2	31.6	32.7	32.2	32.8 *
	3	12	32.4	33.1	34.3 *	33.6	QNS	32.9	33.5	33.4	33.9	QNS	33.3	QNS	32.9
	3	14	31.8	31.6	32.0	31.7	31.0	31.9	32.2	32.8	32.1	31.5	32.8	QNS	33.2 *
	3	18	32.3	33.4	33.4	33.6	33.0	QNS	32.6	QNS	33.5	32.8	QNS	33.3	32.8 *
	3	2	32.9	32.8	33.3	QNS	32.3	32.6	--	--	--	--	--	--	--
	3	32	32.8	32.7	32.4	32.5	32.4	QNS	33.1	QNS	33.4	QNS	33.2	33.6	34.3 *
	3	8	32.8	32.8	33.2	33.2	32.3	32.3	32.8	QNS	33.4	QNS	32.7	33.3	32.3
		Average	32.5	32.7	32.9	32.7	32.2	32.4	32.9	32.9	33.1	32.0	32.9	33.1	33.1
		Std Dev	0.4	0.6	0.9	0.9	0.7	0.4	0.5	0.4	0.7	0.7	0.3	0.6	0.7
	4	21	31.9	32.0	32.2	31.4	31.9	32.4	32.6	QNS	QNS	33.1	32.9	32.7	32.3
	4	27	31.7	31.8	QNS	32.1	31.3	32.1	32.3	--	--	--	--	--	--
	4	31	32.4	31.7	QNS	31.9	31.8	--	--	--	--	--	--	--	--
	4	33	32.3	32.8	QNS	32.3	32.2 *	--	--	--	--	--	--	--	--
	4	38	30.7	31.1	31.5	30.6	30.8	31.0	31.7	31.3	28.8	29.3	30.5	30.6	30.5 *
	4	39	30.8	30.7	31.7	30.4	31.0	30.9	30.8	31.1	30.0	30.6	31.9	30.9	31.9
	4	6	32.3	32.4	32.2	32.0	--	--	--	--	--	--	--	--	--
		Average	31.7	31.8	31.9	31.5	31.5	31.6	31.9	31.2	29.4	31.0	31.8	31.4	31.6
		Std Dev	0.7	0.7	0.4	0.8	0.6	0.8	0.8	0.1	0.8	1.9	1.2	1.1	0.9

ATC 10·4·10

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QNS - Sample Volume not Sufficient for analysis

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S-9

Parameter	Group	Animal ID	Day -3	Day 2	Day 4	Day 9	Day 11	Day 16	Day 18	Day 23	Day 25	Day 30	Day 32	Day 37	Day 39
Red Cell Distribution Width	1	37	13.1	13.0	QNS	12.9 *	12.6	12.6 *	12.5	QNS	QNS	12.3	QNS	12.6	12.5 *
(10.3 - 14.4 %)	1	40	13.9	13.2	13.0	13.0 *	12.6	QNS	12.3 *	12.0	12.0	12.0 *	QNS	12.2	12.4
	1	5	12.1	11.9	12.2	12.1	12.1	12.2	12.1	11.6	11.3	12.5	QNS	12.2	12.3
	1	7	12.8	12.4	12.5 *	13.7	13.1	13.4	13.2	12.7	QNS	12.3	12.1	12.2	12.3 *
	1	9	11.9	11.8	12.4	11.8	11.8	11.7	11.7	11.6	11.4	11.8	QNS	11.8	11.8
		Average	12.8	12.5	12.5	12.7	12.4	12.5	12.4	12.0	11.6	12.2	12.1	12.2	12.3
		Std Dev	0.8	0.6	0.3	0.8	0.5	0.7	0.6	0.5	0.4	0.3	0.0	0.3	0.3
	2	13	11.7	11.8	12.2	12.5	12.3	12.4	12.6	QNS	QNS	12.1	QNS	12.0	11.9 *
	2	15	11.7	11.6	11.9	12.2	11.9	12.5	12.7	11.9	11.7	12.0	QNS	12.2	12.0
	2	19	12.9	12.2	12.3	12.2	12.5	12.3	12.7	12.1	12.2	12.2 *	12.3	12.2	12.2 *
	2	25	11.8	11.2	11.3	11.9	11.7	11.4	12.7	11.6	11.5	11.6	QNS	11.7	11.8
	2	28	11.2	11.2	11.5	11.1	11.4	11.5	11.7	11.2	11.2	11.6	12.1	11.6	11.7
	2	30	12.0	11.7	11.5	11.8	11.7	11.5	11.6	11.1	11.1	11.3	QNS	11.2	11.3
	2	34	12.3	12.6	13.3	13.0	12.9	12.6	13.6	12.2	12.2	11.8	11.9	12.0	11.9
		Average	11.9	11.8	12.0	12.1	12.1	12.0	12.5	11.7	11.7	11.8	12.1	11.8	11.8
		Std Dev	0.5	0.5	0.7	0.6	0.5	0.5	0.7	0.5	0.5	0.3	0.2	0.4	0.3
	3	11	11.9	11.3	11.4	11.9	11.6	11.5	12.1	11.9	12.1	12.4	12.5	12.1	12.1 *
	3	12	11.8	12.1	11.8 *	12.1	QNS	11.8	11.7	11.5	11.3	QNS	11.6	QNS	12.0
	3	14	11.9	11.6	11.6	11.6	11.5	11.4	11.3	11.4	11.4	11.6	11.8	QNS	11.5 *
	3	18	11.5	11.5	11.5	11.3	11.1	QNS	11.9	QNS	11.3	11.4	QNS	11.3	11.2 *
	3	2	12.9	12.9	12.8	QNS	13.1	13.2	--	--	--	--	--	--	--
	3	32	12.0	12.0	11.9	12.2	12.5	QNS	12.0	QNS	11.5	QNS	11.2	11.2	11.2 *
	3	8	12.4	12.1	12.1	12.9	12.8	12.3	12.5	QNS	12.0	QNS	12.1	12.0	12.2
		Average	12.1	11.9	11.9	12.0	12.1	12.0	11.9	11.6	11.6	11.8	11.8	11.7	11.7
		Std Dev	0.5	0.5	0.5	0.6	0.8	0.7	0.4	0.3	0.4	0.5	0.5	0.5	0.5
	4	21	12.5	12.0	12.2	12.7	12.3	12.1	11.9	QNS	QNS	12.0	12.4	11.9	11.9
	4	27	12.1	11.8	QNS	12.0	11.7	12.0	11.8	--	--	--	--	--	--
	4	31	13.5	12.8	QNS	13.0	12.7	--	--	--	--	--	--	--	--
	4	33	12.5	12.1	QNS	11.8	11.4 *	--	--	--	--	--	--	--	--
	4	38	13.2	13.0	12.9	13.2	13.2	12.8	11.8	**14.9**	**17.9**	**16.8**	**16.1**	**14.7**	**14.5** *
	4	39	12.9	13.2	13.5	13.6	13.4	13.0	12.9	12.4	12.3	12.3	12.5	12.2	12.5
	4	6	12.0	11.8	11.9	12.7	--	--	--	--	--	--	--	--	--
		Average	12.7	12.4	12.6	12.7	12.5	12.5	12.1	13.7	15.1	13.7	13.7	12.9	13.0
		Std Dev	0.6	0.6	0.7	0.6	0.8	0.5	0.5	1.8	4.0	2.7	2.1	1.5	1.4

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QNS - Sample Volume not Sufficient for analysis

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Parameter	Group	Animal ID	Day -3	Day 2	Day 4	Day 9	Day 11	Day 16	Day 18	Day 23	Day 25	Day 30	Day 32	Day 37	Day 39
Platelet Count	1	37	**846**	**962**	QNS	235 *	**837**	255 *	**618**	QNS	QNS	**674**	QNS	**585**	32 *
(137 - 558 ×10³/uL)	1	40	544	**622**	540	326 *	**586**	QNS	390 *	30	479	98 *	QNS	396	362
	1	5	545	445	413	555	280	351	385	262	382	319	QNS	287	247
	1	7	392	507	12 *	532	518	550	**603**	555	QNS	482	447	460	33 *
	1	9	**810**	**636**	489	**943**	**763**	**629**	**641**	**625**	**649**	**577**	QNS	500	505
		Average	627	634	364	518	597	446	527	368	503	430	447	446	236
		Std Dev	194	200	240	273	219	173	128	275	135	227	0	112	207
	2	13	**602**	**763**	**667**	**780**	**704**	**604**	**568**	QNS	QNS	382	QNS	**579**	137 *
	2	15	**665**	**624**	**646**	**686**	**617**	529	**642**	**594**	212	526	QNS	478	143
	2	19	**674**	**604**	540	485	459	492	522	429	304	303 *	412	431	170 *
	2	25	**571**	509	550	**617**	**696**	489	295	**585**	529	413	QNS	466	357
	2	28	176	331	502	539	436	454	498	445	424	337	304	352	425
	2	30	451	308	511	508	**579**	460	367	302	399	509	QNS	315	197
	2	34	**690**	501	417	**568**	539	419	404	541	446	389	483	382	323
		Average	547	520	548	598	576	492	471	483	386	408	400	429	250
		Std Dev	183	162	86	105	106	60	121	112	112	83	90	89	116
	3	11	**583**	548	539	**575**	499	521	**561**	530	**579**	534	451	436	30 *
	3	12	200	433	86 *	442	QNS	389	364	301	37	QNS	315	QNS	258
	3	14	407	417	389	438	438	401	413	373	309	272	257	QNS	134 *
	3	18	**779**	**714**	**687**	**727**	**626**	QNS	**787**	QNS	**631**	**687**	QNS	**595**	314 *
	3	2	**622**	412	330	QNS	454	491	--	--	--	--	--	--	--
	3	32	**903**	**590**	**591**	**670**	318	QNS	**699**	QNS	490	QNS	502	498	36 *
	3	8	464	496	271	**664**	291	501	399	QNS	326	QNS	402	463	485
		Average	565	516	413	586	438	461	537	401	395	498	385	498	210
		Std Dev	235	111	207	123	123	61	175	117	219	210	100	69	177
	4	21	**659**	**641**	**597**	502	376	350	441	QNS	QNS	384	406	424	**622**
	4	27	513	**654**	QNS	**697**	**673**	504	**615**	--	--	--	--	--	--
	4	31	**842**	**688**	QNS	**600**	513	--	--	--	--	--	--	--	--
	4	33	**1058**	**880**	QNS	**775**	55 *	--	--	--	--	--	--	--	--
	4	38	**986**	**1008**	**1000**	**931**	**763**	**826**	**796**	**1471**	**2044**	**1305**	**1002**	**1408**	**1043** *
	4	39	**695**	532	**622**	**657**	552	**696**	**666**	**569**	547	506	438	463	484
	4	6	455	360	377	526	--	--	--	--	--	--	--	--	--
		Average	744	680	649	670	489	594	630	1020	1296	732	615	765	716
		Std Dev	228	214	259	149	251	210	147	638	1059	500	335	557	291

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Parameter	Group	Animal ID	Day -3	Day 2	Day 4	Day 9	Day 11	Day 16	Day 18	Day 23	Day 25	Day 30	Day 32	Day 37	Day 39
Mean Platelet Volume	1	37	6.6	6.5	QNS	**8.3** *	7.0	**8.0** *	**7.8**	QNS	QNS	6.5	QNS	6.1	**12.4** *
(5.2 - 7.7 fL)	1	40	7.0	5.9	**7.9**	**8.6** *	7.6	QNS	7.7 *	**10.0**	6.2	6.6 *	QNS	6.3	**7.9**
	1	5	**7.9**	6.6	**8.6**	**9.6**	**11.2**	**8.6**	**8.6**	7.5	7.6	**8.6**	QNS	7.5	**8.2**
	1	7	7.1	6.3	**14.4** *	**8.3**	**7.8**	**8.0**	7.4	6.3	QNS	6.4	6.2	6.5	**13.0** *
	1	9	6.2	5.9	**7.9**	**8.4**	7.2	7.2	7.6	6.3	6.7	6.3	QNS	5.6	7.0
		Average	7.0	6.2	9.7	8.6	8.2	8.0	7.8	7.5	6.8	6.9	6.2	6.4	9.7
		Std Dev	0.6	0.3	3.2	0.6	1.7	0.6	0.5	1.7	0.7	1.0	0.0	0.7	2.8
	2	13	**7.8**	6.6	**8.3**	**8.4**	6.7	6.0	6.8	QNS	QNS	7.0	QNS	6.1	**10.8** *
	2	15	7.4	6.3	6.9	**8.6**	7.0	7.6	**8.1**	6.3	**8.2**	7.7	QNS	6.3	**13.6**
	2	19	7.4	6.6	6.8	**8.4**	6.5	6.1	6.3	5.9	6.7	6.6 *	5.9	5.9	7.6 *
	2	25	7.3	6.3	7.4	**8.6**	7.3	**7.9**	**10.2**	6.2	6.3	7.2	QNS	6.4	**8.1**
	2	28	7.5	7.1	7.3	**8.2**	**8.1**	7.4	**7.8**	6.5	5.8	6.7	**8.4**	6.0	6.0
	2	30	7.5	7.5	7.4	**8.6**	7.0	7.4	**10.0**	6.9	6.9	6.4	QNS	7.1	**9.4**
	2	34	7.2	6.0	6.8	**8.4**	6.8	6.4	6.7	5.9	6.2	6.4	6.1	6.2	6.3
		Average	7.4	6.6	7.3	8.5	7.1	7.0	8.0	6.3	6.7	6.9	6.8	6.3	8.8
		Std Dev	0.2	0.5	0.5	0.2	0.5	0.8	1.6	0.4	0.8	0.5	1.4	0.4	2.7
	3	11	6.7	6.1	6.5	**7.8**	6.5	6.3	7.3	6.9	6.3	6.5	**8.0**	6.5	**13.0** *
	3	12	6.8	6.7	**8.2** *	7.3	QNS	6.8	6.0	6.3	**9.0**	QNS	5.9	QNS	**9.6**
	3	14	7.6	7.4	7.0	**8.1**	7.0	7.1	7.2	6.7	7.1	7.6	**9.5**	QNS	**8.9** *
	3	18	6.7	5.8	6.5	6.9	6.8	QNS	6.0	QNS	5.7	5.7	QNS	5.7	**8.2** *
	3	2	6.5	7.6	7.1	QNS	**8.3**	**7.8**	--	--	--	--	--	--	--
	3	32	6.6	5.7	6.6	**7.8**	**10.6**	QNS	5.8	QNS	5.7	QNS	5.3	5.4	**9.8** *
	3	8	6.2	5.9	6.5	**7.8**	**8.1**	5.7	5.7	QNS	6.0	QNS	5.7	5.7	6.0
		Average	6.7	6.5	6.9	7.6	7.9	6.7	6.3	6.6	6.6	6.6	6.9	5.8	9.3
		Std Dev	0.4	0.8	0.6	0.4	1.5	0.8	0.7	0.3	1.3	1.0	1.8	0.5	2.3
	4	21	6.0	6.2	6.1	**8.1**	7.1	6.6	6.3	QNS	QNS	5.8	5.7	5.7	5.8
	4	27	7.0	6.7	QNS	**8.3**	7.3	6.4	6.7	--	--	--	--	--	--
	4	31	6.4	6.3	QNS	7.5	6.0	--	--	--	--	--	--	--	--
	4	33	6.9	**8.0**	QNS	**8.7**	**8.4** *	--	--	--	--	--	--	--	--
	4	38	7.0	6.0	6.6	**8.8**	6.1	6.1	6.9	7.4	**9.1**	7.5	**8.8**	6.6	**9.4** *
	4	39	7.5	6.5	6.8	**7.8**	6.6	6.4	6.2	6.7	6.9	6.8	6.9	6.3	5.9
	4	6	6.5	6.4	6.4	**8.7**	--	--	--	--	--	--	--	--	--
		Average	6.8	6.6	6.5	8.3	6.9	6.4	6.5	7.1	8.0	6.7	7.1	6.2	7.0
		Std Dev	0.5	0.7	0.3	0.5	0.9	0.2	0.3	0.5	1.6	0.9	1.6	0.5	2.1

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Parameter	Group	Animal ID	Day -3	Day 2	Day 4	Day 9	Day 11	Day 16	Day 18	Day 23	Day 25	Day 30	Day 32	Day 37	Day 39
Neutrophils Percentage	1	37	**54.4**	39.8	QNS	34.2 *	**48.0**	23.9 *	26.9	QNS	QNS	25.9	QNS	26.0	12.8 *
(17.2 - 40 %)	1	40	30.6	25.2	28.5	18.5 *	22.1	QNS	16.3 *	7.6	19.4	15.7 *	QNS	24.1	14.2
	1	5	25.1	18.4	23.1	15.0	16.8	19.4	13.8	16.6	19.8	17.2	QNS	16.7	14.1
	1	7	26.2	23.6	19.4 *	20.8	23.9	23.2	23.1	24.9	QNS	21.0	21.3	22.7	15.6 *
	1	9	19.9	24.5	34.5	24.3	15.6	23.8	18.7	29.9	28.2	21.3	QNS	28.0	27.9
		Average	31.2	26.3	26.4	22.6	25.3	22.6	19.8	19.8	22.5	20.2	21.3	23.5	16.9
		Std Dev	13.5	8.0	6.6	7.3	13.2	2.1	5.3	9.8	5.0	4.0	0.0	4.3	6.2
	2	13	21.5	14.7	13.3	12.9	17.4	15.3	17.5	QNS	QNS	20.5	QNS	23.3	14.7 *
	2	15	23.1	19.2	20.3	17.3	18.7	23.3	16.4	25.4	20.3	22.3	QNS	24.6	16.0
	2	19	36.3	26.5	25.8	23.6	24.7	23.3	24.1	28.1	25.2	27.3 *	26.5	25.2	18.1 *
	2	25	21.5	19.7	23.3	21.8	28.4	19.1	16.3	23.1	21.2	17.5	QNS	18.6	16.2
	2	28	18.0	18.5	21.9	20.8	24.7	18.1	21.1	25.3	21.8	19.6	23.6	22.9	22.3
	2	30	24.1	17.6	20.6	19.2	18.8	19.8	19.7	18.5	22.1	16.9	QNS	19.4	18.2
	2	34	22.3	25.7	27.7	21.0	16.5	23.5	15.9	24.9	27.0	24.3	19.5	20.0	15.1
		Average	23.8	20.3	21.8	19.5	21.3	20.3	18.7	24.2	22.9	21.2	23.2	22.0	17.2
		Std Dev	5.8	4.3	4.6	3.5	4.6	3.2	3.1	3.2	2.6	3.7	3.5	2.6	2.6
	3	11	27.8	21.8	22.4	21.5	23.8	21.4	20.8	**48.3**	25.6	23.3	34.4	23.5	18.9 *
	3	12	24.5	28.7	26.2 *	27.4	QNS	24.9	33.7	39.1	17.9	QNS	**41.6**	QNS	**50.6**
	3	14	30.6	22.4	21.8	24.3	23.1	25.6	22.7	22.5	22.5	23.2	20.5	QNS	38.2 *
	3	18	29.1	21.0	**40.8**	39.6	31.5	QNS	**40.8**	QNS	35.4	29.5	QNS	25.4	22.4 *
	3	2	20.4	23.1	29.0	QNS	26.0	23.7	--	--	--	--	--	--	--
	3	32	27.9	22.9	20.6	22.3	22.5	QNS	22.6	QNS	31.5	QNS	20.8	24.1	17.3 *
	3	8	23.8	19.5	14.8	21.6	20.5	18.9	18.2	QNS	29.3	QNS	30.7	26.9	14.2
		Average	26.3	22.8	25.1	26.1	24.6	22.9	26.5	36.6	27.0	25.3	29.6	25.0	26.9
		Std Dev	3.5	2.9	8.2	7.0	3.8	2.7	8.8	13.1	6.3	3.6	9.1	1.5	14.3
	4	21	**44.5**	23.9	19.7	21.6	21.4	13.8	17.2	QNS	QNS	23.7	**44.9**	21.3	21.0
	4	27	29.8	24.8	QNS	28.9	27.3	22.0	31.9	--	--	--	--	--	--
	4	31	37.6	**43.0**	QNS	38.7	**42.6**	--	--	--	--	--	--	--	--
	4	33	**44.9**	**59.0**	QNS	**51.7**	29.8 *	--	--	--	--	--	--	--	--
	4	38	33.4	30.6	35.6	28.0	29.5	25.0	26.8	**42.8**	**45.5**	**48.5**	**49.0**	22.6	33.5 *
	4	39	21.5	16.3	21.4	18.2	19.4	18.7	14.6	20.9	11.2	14.3	18.1	15.0	13.7
	4	6	25.9	25.3	**49.0**	28.9	--	--	--	--	--	--	--	--	--
		Average	33.9	31.8	31.4	30.9	28.3	19.9	22.6	31.9	28.4	28.8	37.3	19.6	22.7
		Std Dev	9.0	14.5	13.7	11.2	8.2	4.8	8.1	15.5	24.3	17.7	16.8	4.1	10.0

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Parameter	Group	Animal ID	Day -3	Day 2	Day 4	Day 9	Day 11	Day 16	Day 18	Day 23	Day 25	Day 30	Day 32	Day 37	Day 39
Lymphocytes Percentage	1	37	36.2	48.5	QNS	55.9 *	41.3	64.6 *	59.3	QNS	QNS	57.8	QNS	62.1	75.5 *
(57.5 - 81.1 %)	1	40	61.0	64.2	60.6	69.6 *	67.6	QNS	71.8 *	**84.8**	69.6	72.3 *	QNS	65.1	76.5
	1	5	70.5	76.5	73.7	**81.3**	76.9	75.0	81.0	78.1	74.6	77.6	QNS	78.4	80.0
	1	7	62.8	67.0	70.5 *	71.2	68.9	69.0	68.8	66.2	QNS	70.5	69.0	70.1	73.2 *
	1	9	72.7	69.1	59.7	71.5	78.8	66.9	72.7	60.1	62.0	67.3	QNS	59.8	63.0
		Average	60.6	65.1	66.1	69.9	66.7	68.9	70.7	72.3	68.7	69.1	69.0	67.1	73.6
		Std Dev	14.5	10.3	7.0	9.1	15.0	4.5	7.8	11.2	6.3	7.3	0.0	7.4	6.4
	2	13	67.1	76.0	78.5	78.5	74.3	76.7	74.2	QNS	QNS	72.0	QNS	67.2	77.5 *
	2	15	67.3	70.2	69.3	73.2	70.9	67.4	72.5	63.6	68.9	68.3	QNS	63.8	74.2
	2	19	55.7	63.3	62.5	66.5	67.2	66.7	61.5	59.5	66.4	64.4 *	63.3	62.4	73.0 *
	2	25	75.0	75.7	73.1	74.5	65.5	76.4	79.1	71.5	72.7	77.4	QNS	76.7	78.6
	2	28	74.3	73.1	68.7	71.5	67.2	73.8	72.3	64.5	70.7	70.4	67.2	68.7	68.5
	2	30	71.5	78.3	75.4	77.1	76.8	75.3	74.6	76.3	72.3	78.3	QNS	74.1	76.4
	2	34	69.8	64.6	62.5	69.2	75.1	65.3	74.6	62.2	57.2	61.7	67.0	68.3	77.6
		Average	68.7	71.6	70.0	72.9	71.0	71.7	72.7	66.3	68.0	70.4	65.8	68.7	75.1
		Std Dev	6.5	5.8	6.1	4.2	4.5	5.0	5.4	6.3	5.8	6.2	2.2	5.2	3.5
	3	11	62.0	67.1	68.4	68.9	66.3	68.9	71.0	42.7	65.6	63.2	52.0	64.2	69.7 *
	3	12	67.7	65.0	67.1 *	65.4	QNS	67.7	60.0	55.2	75.8	QNS	52.0	QNS	39.4
	3	14	63.0	71.8	72.2	68.0	70.9	68.6	71.1	71.8	71.8	66.1	68.2	QNS	42.4 *
	3	18	63.6	67.8	47.3	47.0	61.1	QNS	54.0	QNS	53.1	62.9	QNS	67.4	71.2 *
	3	2	69.1	70.8	65.7	QNS	68.4	71.8	--	--	--	--	--	--	--
	3	32	61.8	64.7	65.5	64.5	69.3	QNS	67.4	QNS	59.0	QNS	69.9	67.1	74.1 *
	3	8	71.2	74.5	75.1	69.3	70.5	72.9	73.2	QNS	56.7	QNS	57.7	62.2	77.3
		Average	65.5	68.8	65.9	63.9	67.8	70.0	66.1	56.6	63.7	64.1	60.0	65.2	62.4
		Std Dev	3.8	3.7	8.9	8.5	3.7	2.2	7.5	14.6	8.9	1.8	8.6	2.5	16.8
	4	21	48.6	66.8	71.7	70.2	69.3	76.0	74.1	QNS	QNS	64.0	44.2	67.9	68.1
	4	27	64.5	70.0	QNS	65.9	68.4	73.6	63.2	--	--	--	--	--	--
	4	31	55.4	49.1	QNS	52.3	46.9	--	--	--	--	--	--	--	--
	4	33	48.0	37.9	QNS	43.6	64.5 *	--	--	--	--	--	--	--	--
	4	38	59.2	57.8	55.1	63.3	60.4	63.8	63.2	44.7	41.0	41.0	39.5	61.4	53.3 *
	4	39	72.4	77.6	73.0	75.7	73.8	74.5	81.0	72.5	**83.6**	80.2	75.6	76.9	**82.5**
	4	6	69.2	69.2	43.7	64.7	--	--	--	--	--	--	--	--	--
		Average	59.6	61.2	60.9	62.2	63.9	72.0	70.4	58.6	62.3	61.7	53.1	68.7	68.0
		Std Dev	9.6	13.8	14.1	10.9	9.5	5.5	8.8	19.7	30.1	19.7	19.6	7.8	14.6

PAC 10.4.10

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QNS - Sample Volume not Sufficient for analysis

QA AUDIT COMPLETED
BY/DATE: Jm 11/4/10

PAC 10.4.10

Parameter	Group	Animal ID	Day -3	Day 2	Day 4	Day 9	Day 11	Day 16	Day 18	Day 23	Day 25	Day 30	Day 32	Day 37	Day 39
Monocytes Percentage	1	37	2.7	1.3	QNS	1.0 *	3.0	1.1 *	1.2	QNS	QNS	2.3	QNS	2.8	0.2 *
(0 - 6.1 %)	1	40	1.3	1.2	1.3	1.0 *	1.2	QNS	1.4 *	0.5	1.0	1.9 *	QNS	0.8	0.6
	1	5	1.2	3.1	1.0	1.2	2.1	1.9	1.1	1.4	2.4	1.2	QNS	1.3	2.2
	1	7	0.9	0.9	0.9 *	0.7	1.0	0.9	0.8	0.8	QNS	1.3	0.9	0.5	0.4 *
	1	9	3.1	1.3	1.8	0.9	1.1	1.4	1.1	1.6	2.0	2.8	QNS	2.4	1.2
		Average	1.8	1.6	1.3	1.0	1.7	1.3	1.1	1.1	1.8	1.9	0.9	1.6	0.9
		Std Dev	1.0	0.9	0.4	0.2	0.9	0.4	0.2	0.5	0.7	0.7	0.0	1.0	0.8
	2	13	1.8	1.8	1.2	1.8	0.9	1.0	1.4	QNS	QNS	1.2	QNS	2.7	0.3 *
	2	15	1.2	2.4	1.7	1.3	2.6	2.1	2.2	2.2	1.7	1.3	QNS	3.3	1.0
	2	19	2.4	2.1	4.1	2.9	1.2	3.3	5.5	3.5	1.4	1.8 *	2.1	5.2	1.2 *
	2	25	0.8	2.1	1.1	0.7	1.3	1.0	0.8	1.0	1.5	1.3	QNS	0.9	0.8
	2	28	1.5	1.5	1.2	1.5	1.7	1.8	1.2	1.9	0.9	1.0	0.9	0.9	0.6
	2	30	1.2	1.0	0.9	0.9	1.5	1.0	1.7	1.0	1.2	1.3	QNS	1.1	0.7
	2	34	1.5	1.9	0.9	1.3	0.9	3.1	1.1	0.9	2.1	1.4	0.8	0.7	0.6
		Average	1.5	1.8	1.6	1.5	1.4	1.9	2.0	1.8	1.5	1.3	1.3	2.1	0.7
		Std Dev	0.5	0.5	1.1	0.7	0.6	1.0	1.6	1.0	0.4	0.2	0.7	1.7	0.3
	3	11	1.8	1.9	1.0	3.0	1.5	1.4	1.2	1.8	1.8	5.0	4.2	4.1	1.5 *
	3	12	1.0	1.2	0.9 *	1.2	QNS	0.9	1.0	0.9	1.0	QNS	2.4	QNS	2.9
	3	14	2.4	2.3	1.7	2.6	1.3	1.6	1.3	1.6	1.7	3.4	2.1	QNS	**6.9** *
	3	18	1.1	1.7	1.0	1.9	0.9	QNS	2.3	QNS	3.4	0.8	QNS	1.9	0.8 *
	3	2	1.1	1.3	0.8	QNS	0.9	0.9	--	--	--	--	--	--	--
	3	32	1.9	1.2	2.2	2.5	1.1	QNS	2.7	QNS	1.5	QNS	1.3	0.9	0.8 *
	3	8	1.4	1.9	5.7	2.7	1.7	2.1	2.0	QNS	4.3	QNS	2.1	2.8	0.9
		Average	1.5	1.6	1.9	2.3	1.2	1.4	1.8	1.4	2.3	3.1	2.4	2.4	2.3
		Std Dev	0.5	0.4	1.8	0.7	0.3	0.5	0.7	0.5	1.3	2.1	1.1	1.4	2.4
	4	21	3.4	3.1	1.6	1.3	1.3	1.8	1.4	QNS	QNS	3.2	4.1	2.2	2.4
	4	27	1.3	1.6	QNS	1.3	0.9	1.0	1.7	--	--	--	--	--	--
	4	31	2.4	2.1	QNS	2.7	5.3	--	--	--	--	--	--	--	--
	4	33	2.6	1.0	QNS	1.8	1.1 *	--	--	--	--	--	--	--	--
	4	38	1.7	3.8	1.5	1.7	2.7	4.2	4.8	**8.2**	**8.3**	4.9	4.5	1.7	2.0 *
	4	39	1.8	1.2	0.9	1.3	1.9	2.5	1.1	1.5	1.7	1.3	0.8	3.1	1.0
	4	6	1.7	3.6	5.0	3.3	--	--	--	--	--	--	--	--	--
		Average	2.1	2.3	2.3	1.9	2.2	2.4	2.3	4.9	5.0	3.1	3.1	2.3	1.8
		Std Dev	0.7	1.2	1.9	0.8	1.7	1.4	1.7	4.7	4.7	1.8	2.0	0.7	0.7

ATC 10.4.10

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QNS - Sample Volume not Sufficient for analysis

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ATC 10.4.10

Parameter	Group	Animal ID	Day -3	Day 2	Day 4	Day 9	Day 11	Day 16	Day 18	Day 23	Day 25	Day 30	Day 32	Day 37	Day 39
Eosinophils Percentage	1	37	1.2	2.6	QNS	2.9 *	1.7	3.5 *	4.0	QNS	QNS	2.9	QNS	2.8	**8.3** *
(0.1 - 7 %)	1	40	2.6	3.1	3.8	3.5 *	3.1	QNS	2.6 *	2.9	3.6	3.5 *	QNS	3.3	3.0
	1	5	2.2	0.9	1.2	1.4	2.5	2.2	2.3	2.1	1.5	2.3	QNS	2.0	2.7
	1	7	3.8	3.3	4.6 *	2.4	2.3	2.1	2.4	2.4	QNS	2.3	3.2	2.9	**7.8** *
	1	9	2.8	2.7	2.2	1.8	2.6	5.8	4.9	4.5	3.7	3.1	QNS	3.3	3.5
		Average	2.5	2.5	3.0	2.4	2.4	3.4	3.2	3.0	2.9	2.8	3.2	2.9	5.1
		Std Dev	0.9	0.9	1.5	0.8	0.5	1.7	1.2	1.1	1.2	0.5	0.0	0.5	2.8
	2	13	3.1	2.7	2.2	1.6	2.1	1.9	2.1	QNS	QNS	2.0	QNS	2.2	4.4 *
	2	15	2.7	2.1	2.1	2.1	1.8	2.1	1.5	1.5	1.9	1.7	QNS	1.6	1.7
	2	19	2.5	4.3	3.3	3.1	3.1	3.2	4.1	3.7	4.0	3.3 *	2.9	3.4	4.3 *
	2	25	1.7	1.4	1.3	1.6	2.1	1.6	1.2	1.6	2.2	1.4	QNS	1.4	1.3
	2	28	3.2	3.6	4.2	3.1	2.8	2.6	2.2	3.9	3.1	3.3	2.4	2.9	4.6
	2	30	2.0	1.9	2.2	1.4	2.0	2.1	2.4	2.6	2.8	1.7	QNS	3.0	2.8
	2	34	1.2	2.6	1.9	1.8	1.5	1.9	2.0	1.9	3.1	2.9	2.3	2.6	2.6
		Average	2.3	2.7	2.5	2.1	2.2	2.2	2.2	2.5	2.9	2.3	2.5	2.4	3.1
		Std Dev	0.7	1.0	1.0	0.7	0.6	0.5	0.9	1.1	0.7	0.8	0.3	0.7	1.3
	3	11	3.8	3.8	3.1	2.2	2.8	2.9	2.7	2.0	2.4	2.9	2.8	2.3	5.4 *
	3	12	3.2	2.1	3.5 *	2.5	QNS	2.5	3.1	2.4	2.8	QNS	1.8	QNS	4.1
	3	14	2.4	1.7	1.9	2.3	2.2	1.8	1.9	1.8	1.5	3.9	4.2	QNS	5.0 *
	3	18	2.1	3.5	3.6	3.6	2.5	QNS	1.1	QNS	3.3	3.5	QNS	2.0	2.4 *
	3	2	4.3	2.9	3.0	QNS	3.2	2.7	--	--	--	--	--	--	--
	3	32	3.0	4.1	5.9	4.3	3.4	QNS	3.4	QNS	2.9	QNS	2.6	3.0	4.5 *
	3	8	1.9	2.2	2.2	2.2	2.5	2.6	2.1	QNS	3.3	QNS	2.4	2.5	1.9
		Average	3.0	2.9	3.3	2.9	2.8	2.5	2.4	2.1	2.7	3.4	2.8	2.5	3.9
		Std Dev	0.9	0.9	1.3	0.9	0.5	0.4	0.8	0.3	0.7	0.5	0.9	0.4	1.4
	4	21	2.0	3.6	3.8	3.1	4.1	3.1	2.9	QNS	QNS	2.4	1.8	2.8	2.5
	4	27	2.6	1.8	QNS	1.8	1.2	1.3	1.5	--	--	--	--	--	--
	4	31	1.6	2.2	QNS	2.7	2.5	--	--	--	--	--	--	--	--
	4	33	3.1	1.4	QNS	1.8	3.5 *	--	--	--	--	--	--	--	--
	4	38	1.9	2.3	2.3	2.0	2.5	2.1	1.4	0.7	0.9	1.4	1.8	3.2	4.4 *
	4	39	1.3	1.6	2.4	1.7	1.8	1.4	1.0	1.7	1.0	1.7	2.0	1.9	1.4
	4	6	2.0	1.1	1.4	1.8	--	--	--	--	--	--	--	--	--
		Average	2.1	2.0	2.5	2.1	2.6	2.0	1.7	1.2	1.0	1.8	1.9	2.6	2.8
		Std Dev	0.6	0.8	1.0	0.5	1.1	0.8	0.8	0.7	0.1	0.5	0.1	0.7	1.5

ATC 10·4·10

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QNS - Sample Volume not Sufficient for analysis

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ATC 10·4·10

Parameter	Group	Animal ID	Day -3	Day 2	Day 4	Day 9	Day 11	Day 16	Day 18	Day 23	Day 25	Day 30	Day 32	Day 37	Day 39
Basophils Percentage	1	37	5.3	7.7	QNS	6.0 *	5.8	6.9 *	8.5	QNS	QNS	**10.9**	QNS	6.3	3.0 *
(2.2 - 9.7 %)	1	40	4.1	6.1	5.8	7.4 *	6.1	QNS	7.8 *	4.2	6.5	6.6 *	QNS	6.7	5.6
	1	5	1.0	1.0	1.0	0.9	1.3	1.4	1.6	1.6	1.3	1.5	QNS	1.5	1.1
	1	7	6.2	5.2	4.4 *	4.8	3.9	4.7	4.7	5.7	QNS	4.9	5.5	3.8	2.9 *
	1	9	1.6	2.2	1.6	1.5	1.8	2.0	2.7	3.8	4.0	5.4	QNS	6.4	4.3
		Average	3.6	4.4	3.2	4.1	3.8	3.8	5.1	3.8	3.9	5.9	5.5	4.9	3.4
		Std Dev	2.3	2.8	2.3	2.8	2.2	2.5	3.0	1.7	2.6	3.4	0.0	2.2	1.7
	2	13	6.0	4.6	4.7	5.0	5.3	5.1	4.7	QNS	QNS	4.3	QNS	4.4	2.9 *
	2	15	5.6	6.0	6.5	5.9	5.9	5.1	7.3	7.2	7.1	6.4	QNS	6.6	6.6
	2	19	3.0	3.8	4.3	3.7	3.8	3.1	4.6	4.9	3.0	3.2 *	5.1	3.8	3.2 *
	2	25	0.9	1.0	1.1	1.5	2.6	1.9	2.5	2.6	2.2	2.2	QNS	2.2	2.5
	2	28	2.8	2.9	3.8	3.0	3.3	3.7	3.0	3.9	3.6	5.6	5.7	4.5	4.0
	2	30	1.0	1.1	0.8	1.5	0.8	1.6	1.4	1.6	1.7	1.7	QNS	2.3	1.7
	2	34	5.2	4.9	6.9	6.4	5.8	6.1	6.4	**10.0**	**10.4**	9.5	**10.3**	8.3	4.2
		Average	3.5	3.5	4.0	3.9	3.9	3.8	4.3	5.0	4.7	4.7	7.0	4.6	3.6
		Std Dev	2.1	1.9	2.4	2.0	1.9	1.7	2.1	3.1	3.4	2.7	2.8	2.2	1.6
	3	11	4.5	5.2	5.0	4.3	5.3	5.4	4.3	5.0	4.5	5.6	6.6	5.8	4.4 *
	3	12	3.6	2.9	2.3 *	3.4	QNS	3.8	2.1	2.3	2.4	QNS	1.9	QNS	1.1
	3	14	1.6	1.8	2.0	2.5	2.3	2.4	2.8	1.9	2.4	3.2	4.5	QNS	4.6 *
	3	18	3.9	5.9	7.3	7.8	3.9	QNS	1.7	QNS	4.6	3.3	QNS	3.0	3.1 *
	3	2	5.0	1.9	1.4	QNS	1.6	0.8	--	--	--	--	--	--	--
	3	32	5.2	6.9	5.8	6.1	3.7	QNS	3.7	QNS	5.0	QNS	5.4	4.8	3.1 *
	3	8	1.6	2.0	2.2	3.9	4.4	3.5	4.1	QNS	6.3	QNS	6.9	5.5	5.4
		Average	3.6	3.8	3.7	4.7	3.5	3.2	3.1	3.1	4.2	4.0	5.1	4.8	3.6
		Std Dev	1.5	2.1	2.3	1.9	1.4	1.7	1.1	1.7	1.5	1.4	2.0	1.3	1.5
	4	21	1.5	2.6	3.1	3.8	3.8	4.8	4.3	QNS	QNS	6.8	4.8	5.8	5.5
	4	27	1.8	1.7	QNS	1.9	2.1	2.1	1.6	--	--	--	--	--	--
	4	31	2.8	3.5	QNS	3.5	2.6	--	--	--	--	--	--	--	--
	4	33	1.2	0.6	QNS	0.7	0.9 *	--	--	--	--	--	--	--	--
	4	38	3.7	5.4	5.4	4.7	4.8	4.7	3.5	2.6	3.5	4.1	5.0	**10.7**	5.7 *
	4	39	2.7	3.2	2.3	3.0	2.9	2.9	2.1	3.1	2.1	2.3	3.3	3.0	1.4
	4	6	0.9	0.7	0.9	0.9	--	--	--	--	--	--	--	--	--
		Average	2.1	2.5	2.9	2.6	2.9	3.6	2.9	2.9	2.8	4.4	4.4	6.5	4.2
		Std Dev	1.0	1.7	1.9	1.5	1.4	1.3	1.2	0.4	1.0	2.3	0.9	3.9	2.4

ATC 10.4.10

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ATC 10.4.10

S-17

Parameter	Group	Animal ID	Day -3	Day 2	Day 4	Day 9	Day 11	Day 16	Day 18	Day 23	Day 25	Day 30	Day 32	Day 37	Day 39
Neutrophils	1	37	**4.54**	2.70	QNS	1.55 *	2.62	1.24 *	1.27	QNS	QNS	1.79	QNS	1.64	0.17 *
(0.8 - 2.9 ×10³/uL)	1	40	1.65	1.27	1.50	0.87 *	1.26	QNS	0.86 *	0.24	0.88	0.60 *	QNS	1.37	0.68
	1	5	1.90	1.38	1.76	1.45	1.26	1.51	1.19	1.45	1.56	1.46	QNS	1.50	0.86
	1	7	1.17	1.58	0.31 *	1.37	1.59	1.59	1.59	1.64	QNS	1.41	1.39	1.55	0.24 *
	1	9	0.83	1.20	2.03	1.15	0.71	1.28	1.02	1.72	1.71	1.15	QNS	1.59	1.62
		Average	2.02	1.63	1.40	1.28	1.49	1.41	1.19	1.26	1.38	1.28	1.39	1.53	0.71
		Std Dev	1.47	0.62	0.76	0.27	0.71	0.17	0.28	0.69	0.44	0.44	0.00	0.10	0.58
	2	13	1.65	1.23	1.29	1.21	1.31	1.33	1.35	QNS	QNS	1.37	QNS	1.81	0.43 *
	2	15	1.56	1.55	1.68	1.32	1.48	1.63	1.27	1.90	1.26	1.87	QNS	1.96	1.12
	2	19	2.87	2.02	1.81	1.75	1.65	1.52	1.20	1.74	1.68	1.68 *	1.37	1.35	0.47 *
	2	25	1.32	1.57	1.80	1.74	2.49	1.70	1.60	1.89	1.77	1.36	QNS	1.61	1.21
	2	28	0.93	0.92	1.21	1.12	1.34	1.02	1.41	1.58	1.17	1.20	1.32	1.21	1.04
	2	30	1.51	0.92	1.20	1.24	1.27	1.26	1.23	1.08	1.14	0.98	QNS	0.96	0.83
	2	34	2.06	2.22	2.11	1.67	1.30	1.43	1.05	1.43	1.44	1.49	1.07	1.14	0.55
		Average	1.70	1.49	1.59	1.44	1.55	1.41	1.30	1.60	1.41	1.42	1.25	1.43	0.81
		Std Dev	0.62	0.51	0.36	0.27	0.44	0.23	0.17	0.31	0.27	0.30	0.16	0.37	0.33
	3	11	1.97	1.40	1.52	1.36	1.38	1.40	1.43	**4.21**	1.72	1.64	2.34	1.46	0.23 *
	3	12	1.13	1.34	0.96 *	1.28	QNS	1.23	1.42	2.20	0.71	QNS	2.33	QNS	2.56
	3	14	1.92	1.47	1.59	1.78	1.77	1.79	1.80	1.81	1.79	2.10	1.47	QNS	1.35 *
	3	18	2.45	0.93	2.07	1.98	2.10	QNS	2.48	QNS	2.41	1.54	QNS	1.51	1.41 *
	3	2	1.06	1.09	1.51	QNS	1.25	1.10	--	--	--	--	--	--	--
	3	32	1.70	1.10	1.03	1.27	1.27	QNS	0.95	QNS	1.73	QNS	0.99	1.15	0.58 *
	3	8	1.50	1.19	0.81	1.42	1.66	1.23	1.28	QNS	1.76	QNS	1.70	1.51	0.71
		Average	1.68	1.22	1.36	1.52	1.57	1.35	1.56	2.74	1.69	1.76	1.77	1.41	1.14
		Std Dev	0.49	0.19	0.44	0.29	0.33	0.27	0.53	1.29	0.55	0.30	0.58	0.17	0.83
	4	21	**3.72**	1.37	1.18	1.40	1.43	0.77	1.49	QNS	QNS	1.21	**3.50**	1.26	1.22
	4	27	2.24	1.90	QNS	2.53	2.73	1.89	**3.27**	--	--	--	--	--	--
	4	31	2.42	**3.24**	QNS	2.16	2.74	--	--	--	--	--	--	--	--
	4	33	1.87	2.53	QNS	2.73	0.88 *	--	--	--	--	--	--	--	--
	4	38	2.61	2.11	2.79	1.96	2.32	2.08	2.65	**8.71**	**8.23**	**7.32**	**5.40**	1.35	2.51 *
	4	39	1.97	1.73	1.95	1.29	1.59	1.31	1.28	1.87	1.09	1.40	1.46	1.33	0.87
	4	6	2.07	2.00	**5.26**	2.32	--	--	--	--	--	--	--	--	--
		Average	2.41	2.13	2.80	2.06	1.95	1.51	2.17	5.29	4.66	3.31	3.45	1.31	1.53
		Std Dev	0.63	0.61	1.77	0.55	0.76	0.59	0.95	4.84	5.05	3.47	1.97	0.05	0.86

ATC 10·4·10

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QNS - Sample Volume not Sufficient for analysis

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ATC 10·4·10

Parameter	Group	Animal ID	Day -3	Day 2	Day 4	Day 9	Day 11	Day 16	Day 18	Day 23	Day 25	Day 30	Day 32	Day 37	Day 39
Lymphocytes	1	37	3.02	3.29	QNS	2.54 *	2.25	3.34 *	2.81	QNS	QNS	4.00	QNS	3.90	0.98 *
(2.2 - 5.3 ×10³/uL)	1	40	3.29	3.22	3.20	3.29 *	3.87	QNS	3.80 *	2.64	3.17	2.78 *	QNS	3.70	3.66
	1	5	**5.35**	**5.73**	**5.62**	**7.84**	**5.74**	**5.85**	**6.96**	**6.82**	**5.86**	**6.59**	QNS	**7.04**	4.91
	1	7	2.81	4.49	1.13 *	4.68	4.58	4.73	4.74	4.36	QNS	4.74	4.49	4.81	1.12 *
	1	9	3.02	3.39	3.52	3.40	3.58	3.61	3.94	3.45	3.77	3.62	QNS	3.40	3.66
		Average	3.50	4.02	3.37	4.35	4.00	4.38	4.45	4.32	4.27	4.35	4.49	4.57	2.87
		Std Dev	1.05	1.09	1.84	2.10	1.29	1.15	1.56	1.81	1.41	1.44	0.00	1.48	1.74
	2	13	5.14	**6.34**	**7.61**	**7.38**	**5.60**	**6.64**	**5.71**	QNS	QNS	4.81	QNS	5.22	2.27 *
	2	15	4.55	**5.69**	**5.73**	**5.56**	**5.60**	4.72	**5.62**	4.75	4.27	**5.71**	QNS	5.10	5.16
	2	19	4.41	4.83	4.39	4.92	4.50	4.37	3.06	3.68	4.43	3.96 *	3.27	3.35	1.92 *
	2	25	4.62	**6.05**	**5.63**	**5.96**	**5.73**	**6.79**	**7.80**	**5.84**	**6.09**	**6.03**	QNS	**6.65**	**5.89**
	2	28	3.82	3.63	3.80	3.84	3.64	4.16	4.85	4.03	3.79	4.31	3.75	3.63	3.20
	2	30	4.46	4.08	4.38	4.98	5.19	4.79	4.65	4.46	3.73	4.55	QNS	3.66	3.48
	2	34	**6.46**	**5.57**	4.78	**5.50**	**5.91**	3.97	4.96	3.58	3.06	3.78	3.69	3.90	2.81
		Average	4.78	5.17	5.19	5.45	5.17	5.06	5.24	4.39	4.23	4.74	3.57	4.50	3.53
		Std Dev	0.84	1.02	1.27	1.09	0.82	1.17	1.43	0.84	1.03	0.85	0.26	1.20	1.47
	3	11	4.40	4.31	4.64	4.36	3.83	4.52	4.88	3.72	4.39	4.45	3.53	4.01	0.86 *
	3	12	3.12	3.03	2.46 *	3.05	QNS	3.35	2.53	3.11	3.00	QNS	2.91	QNS	2.00
	3	14	3.96	4.73	5.25	4.98	**5.43**	4.81	**5.64**	**5.76**	**5.70**	**5.96**	4.91	QNS	1.50 *
	3	18	**5.35**	3.01	2.40	2.36	4.08	QNS	3.28	QNS	3.61	3.30	QNS	4.01	4.46 *
	3	2	3.60	3.35	3.41	QNS	3.29	3.33	--	--	--	--	--	--	--
	3	32	3.75	3.11	3.27	3.67	3.91	QNS	2.83	QNS	3.25	QNS	3.33	3.22	2.48 *
	3	8	4.49	4.56	4.10	4.57	**5.70**	4.74	5.14	QNS	3.40	QNS	3.20	3.50	3.84
		Average	4.10	3.73	3.65	3.83	4.37	4.15	4.05	4.20	3.89	4.57	3.58	3.69	2.52
		Std Dev	0.73	0.77	1.07	0.99	0.96	0.75	1.33	1.39	1.00	1.33	0.78	0.39	1.38
	4	21	4.07	3.82	4.29	4.54	4.64	4.27	**6.39**	QNS	QNS	3.28	3.44	4.02	3.97
	4	27	4.87	**5.35**	QNS	**5.77**	**6.84**	**6.33**	**6.47**	--	--	--	--	--	--
	4	31	3.57	3.70	QNS	2.93	3.02	--	--	--	--	--	--	--	--
	4	33	2.00	1.63	QNS	2.30	1.90 *	--	--	--	--	--	--	--	--
	4	38	4.62	3.99	4.32	4.43	4.74	5.29	**6.24**	**9.10**	**7.41**	**6.18**	4.35	3.68	4.00 *
	4	39	**6.62**	**8.25**	**6.66**	**5.37**	**6.06**	5.23	**7.12**	**6.49**	**8.14**	**7.86**	**6.11**	**6.86**	5.28
	4	6	**5.53**	**5.47**	4.69	5.19	--	--	--	--	--	--	--	--	--
		Average	4.47	4.60	4.99	4.36	4.53	5.28	6.56	7.80	7.78	5.77	4.63	4.85	4.42
		Std Dev	1.47	2.05	1.13	1.29	1.84	0.84	0.39	1.85	0.52	2.32	1.36	1.75	0.75

ATC 10-4-10

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ATC 10-4-10

Parameter	Group	Animal ID	Day -3	Day 2	Day 4	Day 9	Day 11	Day 16	Day 18	Day 23	Day 25	Day 30	Day 32	Day 37	Day 39
Monocytes	1	37	0.23	0.09	QNS	0.04 *	0.17	0.06 *	0.06	QNS	QNS	0.16	QNS	0.17	0.00 *
(0 - 0.4 ×10³/uL)	1	40	0.07	0.06	0.07	0.05 *	0.07	QNS	0.08 *	0.02	0.04	0.07 *	QNS	0.04	0.03
	1	5	0.09	0.23	0.07	0.12	0.15	0.15	0.09	0.12	0.19	0.11	QNS	0.11	0.13
	1	7	0.04	0.06	0.01 *	0.04	0.07	0.06	0.06	0.05	QNS	0.08	0.06	0.03	0.01 *
	1	9	0.13	0.07	0.11	0.04	0.05	0.08	0.06	0.09	0.12	0.15	QNS	0.13	0.07
		Average	0.11	0.10	0.07	0.06	0.10	0.09	0.07	0.07	0.12	0.11	0.06	0.10	0.05
		Std Dev	0.07	0.07	0.04	0.03	0.05	0.04	0.01	0.04	0.08	0.04	0.00	0.06	0.05
	2	13	0.14	0.15	0.11	0.17	0.07	0.09	0.11	QNS	QNS	0.08	QNS	0.21	0.01 *
	2	15	0.08	0.19	0.14	0.10	0.21	0.15	0.17	0.16	0.11	0.11	QNS	0.26	0.07
	2	19	0.19	0.16	0.29	0.22	0.08	0.21	0.28	0.21	0.09	0.11 *	0.11	0.28	0.03 *
	2	25	0.05	0.17	0.09	0.06	0.11	0.09	0.08	0.08	0.12	0.10	QNS	0.08	0.06
	2	28	0.08	0.07	0.07	0.08	0.09	0.10	0.08	0.12	0.05	0.06	0.05	0.05	0.03
	2	30	0.07	0.05	0.05	0.06	0.10	0.06	0.11	0.06	0.06	0.08	QNS	0.05	0.03
	2	34	0.14	0.17	0.07	0.11	0.07	0.19	0.07	0.05	0.11	0.09	0.05	0.04	0.02
		Average	0.11	0.14	0.12	0.11	0.10	0.13	0.13	0.11	0.09	0.09	0.07	0.14	0.04
		Std Dev	0.05	0.05	0.08	0.06	0.05	0.06	0.07	0.06	0.03	0.02	0.03	0.11	0.02
	3	11	0.13	0.12	0.07	0.19	0.09	0.09	0.08	0.15	0.12	0.35	0.28	0.26	0.02 *
	3	12	0.05	0.06	0.03 *	0.06	QNS	0.04	0.04	0.05	0.04	QNS	0.13	QNS	0.15
	3	14	0.15	0.15	0.12	0.19	0.10	0.11	0.11	0.13	0.14	0.30	0.15	QNS	0.24 *
	3	18	0.09	0.08	0.05	0.10	0.06	QNS	0.14	QNS	0.23	0.04	QNS	0.11	0.05 *
	3	2	0.06	0.06	0.04	QNS	0.04	0.04	--	--	--	--	--	--	--
	3	32	0.11	0.06	0.11	0.14	0.06	QNS	0.11	QNS	0.08	QNS	0.06	0.04	0.03 *
	3	8	0.09	0.11	0.31	0.18	0.14	0.14	0.14	QNS	0.26	QNS	0.12	0.16	0.04
		Average	0.10	0.09	0.10	0.14	0.08	0.08	0.10	0.11	0.15	0.23	0.15	0.14	0.09
		Std Dev	0.04	0.04	0.10	0.05	0.04	0.04	0.04	0.05	0.09	0.17	0.08	0.09	0.09
	4	21	0.28	0.18	0.10	0.08	0.09	0.10	0.12	QNS	QNS	0.16	0.32	0.13	0.14
	4	27	0.10	0.12	QNS	0.12	0.09	0.08	0.17	--	--	--	--	--	--
	4	31	0.15	0.16	QNS	0.15	0.34	--	--	--	--	--	--	--	--
	4	33	0.11	0.04	QNS	0.10	0.03 *	--	--	--	--	--	--	--	--
	4	38	0.13	0.26	0.12	0.12	0.21	0.35	**0.47**	**1.66**	**1.50**	**0.73**	**0.49**	0.10	0.15 *
	4	39	0.17	0.12	0.08	0.09	0.15	0.17	0.10	0.14	0.17	0.13	0.07	0.28	0.06
	4	6	0.13	0.28	**0.54**	0.26	--	--	--	--	--	--	--	--	--
		Average	0.15	0.17	0.21	0.13	0.15	0.18	0.22	0.90	0.84	0.34	0.29	0.17	0.12
		Std Dev	0.06	0.08	0.22	0.06	0.11	0.12	0.17	1.07	0.94	0.34	0.21	0.10	0.05

ATC 10.4.10

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QNS - Sample Volume not Sufficient for analysis

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ATC 10.4.10

S-20

Parameter	Group	Animal ID	Day -3	Day 2	Day 4	Day 9	Day 11	Day 16	Day 18	Day 23	Day 25	Day 30	Day 32	Day 37	Day 39
Eosinophils	1	37	0.10	0.18	QNS	0.13 *	0.09	0.18 *	0.19	QNS	QNS	0.20	QNS	0.18	0.11 *
(0 - 0.4 ×10³/uL)	1	40	0.14	0.16	0.20	0.16 *	0.18	QNS	0.14 *	0.09	0.16	0.13 *	QNS	0.19	0.14
	1	5	0.17	0.07	0.09	0.14	0.19	0.17	0.20	0.18	0.12	0.19	QNS	0.18	0.16
	1	7	0.17	0.22	0.07 *	0.16	0.15	0.14	0.17	0.16	QNS	0.15	0.21	0.20	0.12 *
	1	9	0.12	0.13	0.13	0.08	0.12	0.31	0.26	0.26	0.22	0.16	QNS	0.19	0.20
		Average	0.14	0.15	0.12	0.13	0.15	0.20	0.19	0.17	0.17	0.17	0.21	0.19	0.15
		Std Dev	0.03	0.06	0.06	0.03	0.04	0.08	0.04	0.07	0.05	0.03	0.00	0.01	0.04
	2	13	0.24	0.22	0.21	0.15	0.16	0.16	0.16	QNS	QNS	0.13	QNS	0.17	0.13 *
	2	15	0.19	0.17	0.18	0.16	0.15	0.14	0.11	0.11	0.12	0.14	QNS	0.13	0.11
	2	19	0.20	0.33	0.23	0.23	0.21	0.21	0.21	0.23	0.27	0.20 *	0.15	0.18	0.11 *
	2	25	0.11	0.11	0.10	0.12	0.18	0.15	0.12	0.13	0.18	0.11	QNS	0.13	0.09
	2	28	0.17	0.18	0.23	0.17	0.15	0.14	0.15	0.24	0.17	0.20	0.14	0.15	0.21
	2	30	0.12	0.10	0.13	0.09	0.14	0.14	0.15	0.15	0.14	0.10	QNS	0.15	0.13
	2	34	0.11	0.22	0.15	0.15	0.12	0.12	0.13	0.11	0.16	0.18	0.13	0.15	0.09
		Average	0.16	0.19	0.18	0.15	0.16	0.15	0.15	0.16	0.17	0.15	0.14	0.15	0.12
		Std Dev	0.05	0.08	0.05	0.04	0.03	0.03	0.03	0.06	0.05	0.04	0.01	0.02	0.04
	3	11	0.27	0.25	0.21	0.14	0.16	0.19	0.18	0.17	0.16	0.21	0.19	0.14	0.07 *
	3	12	0.15	0.10	0.13 *	0.12	QNS	0.13	0.13	0.13	0.11	QNS	0.10	QNS	0.21
	3	14	0.15	0.11	0.14	0.17	0.17	0.13	0.15	0.14	0.12	0.35	0.31	QNS	0.18 *
	3	18	0.18	0.15	0.18	0.18	0.17	QNS	0.07	QNS	0.23	0.18	QNS	0.12	0.15 *
	3	2	0.22	0.14	0.15	QNS	0.16	0.13	--	--	--	--	--	--	--
	3	32	0.18	0.20	0.29	0.24	0.19	QNS	0.14	QNS	0.16	QNS	0.12	0.15	0.15 *
	3	8	0.12	0.13	0.12	0.14	0.20	0.17	0.15	QNS	0.20	QNS	0.13	0.14	0.10
		Average	0.18	0.15	0.17	0.17	0.18	0.15	0.14	0.15	0.16	0.25	0.17	0.14	0.14
		Std Dev	0.05	0.05	0.06	0.04	0.02	0.03	0.04	0.02	0.05	0.09	0.09	0.01	0.05
	4	21	0.16	0.21	0.22	0.20	0.28	0.18	0.25	QNS	QNS	0.12	0.14	0.16	0.14
	4	27	0.19	0.14	QNS	0.16	0.12	0.11	0.16	--	--	--	--	--	--
	4	31	0.10	0.17	QNS	0.15	0.16	--	--	--	--	--	--	--	--
	4	33	0.13	0.06	QNS	0.09	0.10 *	--	--	--	--	--	--	--	--
	4	38	0.15	0.16	0.18	0.14	0.20	0.18	0.14	0.15	0.17	0.21	0.20	0.19	0.33 *
	4	39	0.12	0.17	0.22	0.12	0.15	0.10	0.09	0.15	0.10	0.17	0.16	0.17	0.09
	4	6	0.16	0.09	0.15	0.14	--	--	--	--	--	--	--	--	--
		Average	0.14	0.14	0.19	0.14	0.17	0.14	0.16	0.15	0.14	0.17	0.17	0.17	0.19
		Std Dev	0.03	0.05	0.03	0.03	0.06	0.04	0.07	0.00	0.05	0.05	0.03	0.02	0.13

AC 10.4.10

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AC 10.4.10

Parameter	Group	Animal ID	Day -3	Day 2	Day 4	Day 9	Day 11	Day 16	Day 18	Day 23	Day 25	Day 30	Day 32	Day 37	Day 39
Basophils	1	37	0.44	0.52	QNS	0.27 *	0.32	0.36 *	0.40	QNS	QNS	**0.76**	QNS	0.39	0.04 *
(0.1 - 0.6 ×10³/uL)	1	40	0.22	0.31	0.31	0.35 *	0.35	QNS	0.41 *	0.13	0.30	0.25 *	QNS	0.38	0.27
	1	5	0.08	0.08	0.08	0.09	0.10	0.11	0.14	0.14	0.10	0.13	QNS	0.13	0.07
	1	7	0.28	0.35	0.07 *	0.32	0.26	0.32	0.33	0.38	QNS	0.33	0.36	0.26	0.04 *
	1	9	0.07	0.11	0.09	0.07	0.08	0.11	0.15	0.22	0.25	0.29	QNS	0.36	0.25
		Average	0.22	0.27	0.14	0.22	0.22	0.23	0.29	0.22	0.22	0.35	0.36	0.30	0.13
		Std Dev	0.15	0.18	0.12	0.13	0.12	0.13	0.13	0.12	0.10	0.24	0.00	0.11	0.12
	2	13	0.46	0.38	0.46	0.47	0.40	0.44	0.36	QNS	QNS	0.29	QNS	0.35	0.08 *
	2	15	0.38	0.49	0.54	0.45	0.46	0.35	0.57	0.54	0.44	0.54	QNS	0.53	0.46
	2	19	0.24	0.29	0.31	0.27	0.26	0.21	0.23	0.30	0.20	0.20 *	0.26	0.20	0.08 *
	2	25	0.06	0.08	0.08	0.12	0.23	0.17	0.24	0.21	0.19	0.17	QNS	0.19	0.19
	2	28	0.15	0.15	0.21	0.16	0.18	0.21	0.20	0.24	0.19	0.34	0.32	0.24	0.19
	2	30	0.06	0.06	0.05	0.09	0.05	0.10	0.09	0.09	0.09	0.10	QNS	0.11	0.08
	2	34	0.48	0.43	0.53	0.51	0.45	0.37	0.43	0.57	0.56	0.58	0.57	0.47	0.15
		Average	0.26	0.27	0.31	0.30	0.29	0.26	0.30	0.33	0.28	0.32	0.38	0.30	0.18
		Std Dev	0.18	0.17	0.21	0.18	0.15	0.12	0.16	0.19	0.18	0.18	0.16	0.16	0.14
	3	11	0.32	0.34	0.34	0.27	0.31	0.35	0.30	0.43	0.30	0.39	0.45	0.36	0.05 *
	3	12	0.17	0.14	0.08 *	0.16	QNS	0.19	0.09	0.13	0.09	QNS	0.11	QNS	0.05
	3	14	0.10	0.12	0.15	0.18	0.18	0.17	0.23	0.15	0.19	0.29	0.33	QNS	0.16 *
	3	18	0.33	0.26	0.37	0.39	0.26	QNS	0.10	QNS	0.31	0.17	QNS	0.18	0.19 *
	3	2	0.26	0.09	0.08	QNS	0.08	0.04	--	--	--	--	--	--	--
	3	32	0.31	0.33	0.29	0.35	0.21	QNS	0.15	QNS	0.28	QNS	0.25	0.23	0.10 *
	3	8	0.10	0.12	0.12	0.26	0.35	0.23	0.29	QNS	0.38	QNS	0.38	0.31	0.27
		Average	0.23	0.20	0.20	0.27	0.23	0.20	0.19	0.24	0.26	0.28	0.30	0.27	0.14
		Std Dev	0.10	0.11	0.13	0.09	0.10	0.11	0.09	0.17	0.10	0.11	0.13	0.08	0.09
	4	21	0.13	0.15	0.18	0.25	0.26	0.27	0.37	QNS	QNS	0.35	0.37	0.34	0.32
	4	27	0.13	0.13	QNS	0.17	0.21	0.18	0.16	--	--	--	--	--	--
	4	31	0.18	0.26	QNS	0.20	0.17	--	--	--	--	--	--	--	--
	4	33	0.05	0.03	QNS	0.04	0.03 *	--	--	--	--	--	--	--	--
	4	38	0.29	0.37	0.42	0.33	0.38	0.39	0.35	0.53	**0.63**	**0.62**	0.55	**0.64**	0.43 *
	4	39	0.25	0.34	0.21	0.21	0.24	0.20	0.18	0.27	0.20	0.23	0.27	0.27	0.09
	4	6	0.08	0.05	0.10	0.07	--	--	--	--	--	--	--	--	--
		Average	0.16	0.19	0.23	0.18	0.22	0.26	0.27	0.40	0.42	0.40	0.40	0.42	0.28
		Std Dev	0.09	0.14	0.14	0.10	0.12	0.09	0.11	0.18	0.30	0.20	0.14	0.20	0.17

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QNS - Sample Volume not Sufficient for analysis

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AC 10.4.10

Parameter	Group	Animal ID	Day -3	Day 2	Day 4	Day 9	Day 11	Day 16	Day 18	Day 23	Day 25	Day 30	Day 32	Day 37	Day 39
Neutrophils/Lymphocytes Ratio	1	37	1.503	0.821	QNS	0.610 *	1.164	0.371 *	0.452	QNS	QNS	0.448	QNS	0.421	0.173 *
	1	40	0.502	0.394	0.469	0.264 *	0.326	QNS	0.226 *	0.091	0.278	0.216 *	QNS	0.370	0.186
	1	5	0.355	0.241	0.313	0.185	0.220	0.258	0.171	0.213	0.266	0.222	QNS	0.213	0.175
	1	7	0.416	0.352	0.274 *	0.293	0.347	0.336	0.335	0.376	QNS	0.297	0.310	0.322	0.214 *
	1	9	0.275	0.354	0.577	0.338	0.198	0.355	0.259	0.499	0.454	0.318	QNS	0.468	0.443
		Average	0.610	0.432	0.408	0.338	0.451	0.330	0.289	0.295	0.332	0.300	0.310	0.359	0.238
		Std Dev	0.506	0.224	0.140	0.162	0.404	0.050	0.109	0.179	0.105	0.094	0.000	0.098	0.115
	2	13	0.321	0.194	0.170	0.164	0.234	0.200	0.236	QNS	QNS	0.285	QNS	0.347	0.189 *
	2	15	0.343	0.272	0.293	0.237	0.264	0.345	0.226	0.400	0.295	0.327	QNS	0.384	0.217
	2	19	0.651	0.418	0.412	0.356	0.367	0.348	0.392	0.473	0.379	0.424 *	0.419	0.403	0.245 *
	2	25	0.286	0.260	0.320	0.292	0.435	0.250	0.205	0.324	0.291	0.226	QNS	0.242	0.205
	2	28	0.243	0.253	0.318	0.292	0.368	0.245	0.291	0.392	0.309	0.278	0.352	0.333	0.325
	2	30	0.339	0.225	0.274	0.249	0.245	0.263	0.265	0.242	0.306	0.215	QNS	0.262	0.239
	2	34	0.319	0.399	0.441	0.304	0.220	0.360	0.212	0.399	0.471	0.394	0.290	0.292	0.196
		Average	0.357	0.289	0.318	0.270	0.305	0.287	0.261	0.372	0.342	0.307	0.354	0.323	0.231
		Std Dev	0.134	0.086	0.090	0.061	0.084	0.063	0.065	0.079	0.071	0.080	0.065	0.061	0.046
	3	11	0.448	0.325	0.328	0.312	0.360	0.310	0.293	1.132	0.392	0.369	0.663	0.364	0.267 *
	3	12	0.362	0.442	0.390 *	0.420	QNS	0.367	0.561	0.707	0.237	QNS	0.801	QNS	1.280
	3	14	0.485	0.311	0.303	0.357	0.326	0.372	0.319	0.314	0.314	0.352	0.299	QNS	0.900 *
	3	18	0.458	0.309	0.862	0.839	0.515	QNS	0.756	QNS	0.668	0.467	QNS	0.377	0.316 *
	3	2	0.294	0.325	0.443	QNS	0.380	0.330	--	--	--	--	--	--	--
	3	32	0.453	0.354	0.315	0.346	0.325	QNS	0.336	QNS	0.532	QNS	0.297	0.357	0.234 *
	3	8	0.334	0.261	0.198	0.311	0.291	0.259	0.249	QNS	0.518	QNS	0.531	0.431	0.185
		Average	0.405	0.332	0.406	0.431	0.366	0.328	0.419	0.718	0.443	0.396	0.518	0.382	0.530
		Std Dev	0.074	0.056	0.215	0.204	0.079	0.046	0.198	0.409	0.159	0.062	0.222	0.034	0.452
	4	21	0.914	0.359	0.275	0.308	0.308	0.180	0.233	QNS	QNS	0.369	1.017	0.313	0.307
	4	27	0.460	0.355	QNS	0.438	0.399	0.299	0.505	--	--	--	--	--	--
	4	31	0.678	0.876	QNS	0.737	0.907	--	--	--	--	--	--	--	--
	4	33	0.935	1.552	QNS	1.187	0.463 *	--	--	--	--	--	--	--	--
	4	38	0.565	0.529	0.646	0.442	0.489	0.393	0.425	0.957	1.111	1.184	1.241	0.367	0.628 *
	4	39	0.298	0.210	0.293	0.240	0.262	0.250	0.180	0.288	0.134	0.178	0.239	0.194	0.165
	4	6	0.374	0.366	1.122	0.447	--	--	--	--	--	--	--	--	--
		Average	0.603	0.607	0.584	0.543	0.472	0.281	0.336	0.623	0.622	0.577	0.833	0.291	0.367
		Std Dev	0.252	0.468	0.397	0.324	0.231	0.089	0.154	0.473	0.691	0.535	0.526	0.089	0.237

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Bold: > Normal Range
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Parameter	Group	Animal ID	Day -3	Day 2	Day 4	Day 9	Day 11	Day 16	Day 18	Day 23	Day 25	Day 30	Day 32	Day 37	Day 39
Neutrophils/Lymphocytes Percentage Ratio	1	37	1.503	0.821	QNS	0.612 *	1.162	0.370 *	0.454	QNS	QNS	0.448	QNS	0.419	0.170 *
	1	40	0.502	0.393	0.470	0.266 *	0.327	QNS	0.227 *	0.090	0.279	0.217 *	QNS	0.370	0.186
	1	5	0.356	0.241	0.313	0.185	0.218	0.259	0.170	0.213	0.265	0.222	QNS	0.213	0.176
	1	7	0.417	0.352	0.275 *	0.292	0.347	0.336	0.336	0.376	QNS	0.298	0.309	0.324	0.213 *
	1	9	0.274	0.355	0.578	0.340	0.198	0.356	0.257	0.498	0.455	0.316	QNS	0.468	0.443
		Average	0.610	0.432	0.409	0.339	0.450	0.330	0.289	0.294	0.333	0.300	0.309	0.359	0.237
		Std Dev	0.506	0.225	0.141	0.163	0.403	0.050	0.110	0.179	0.106	0.094	0.000	0.098	0.116
	2	13	0.320	0.193	0.169	0.164	0.234	0.199	0.236	QNS	QNS	0.285	QNS	0.347	0.190 *
	2	15	0.343	0.274	0.293	0.236	0.264	0.346	0.226	0.399	0.295	0.327	QNS	0.386	0.216
	2	19	0.652	0.419	0.413	0.355	0.368	0.349	0.392	0.472	0.380	0.424 *	0.419	0.404	0.248 *
	2	25	0.287	0.260	0.319	0.293	0.434	0.250	0.206	0.323	0.292	0.226	QNS	0.243	0.206
	2	28	0.242	0.253	0.319	0.291	0.368	0.245	0.292	0.392	0.308	0.278	0.351	0.333	0.326
	2	30	0.337	0.225	0.273	0.249	0.245	0.263	0.264	0.242	0.306	0.216	QNS	0.262	0.238
	2	34	0.319	0.398	0.443	0.303	0.220	0.360	0.213	0.400	0.472	0.394	0.291	0.293	0.195
		Average	0.357	0.289	0.318	0.270	0.304	0.288	0.261	0.372	0.342	0.307	0.354	0.324	0.231
		Std Dev	0.134	0.086	0.091	0.061	0.084	0.063	0.065	0.079	0.071	0.079	0.064	0.061	0.047
	3	11	0.448	0.325	0.327	0.312	0.359	0.311	0.293	1.131	0.390	0.369	0.662	0.366	0.271 *
	3	12	0.362	0.442	0.390 *	0.419	QNS	0.368	0.562	0.708	0.236	QNS	0.800	QNS	1.284
	3	14	0.486	0.312	0.302	0.357	0.326	0.373	0.319	0.313	0.313	0.351	0.301	QNS	0.901 *
	3	18	0.458	0.310	0.863	0.843	0.516	QNS	0.756	QNS	0.667	0.469	QNS	0.377	0.315 *
	3	2	0.295	0.326	0.441	QNS	0.380	0.330	--	--	--	--	--	--	--
	3	32	0.451	0.354	0.315	0.346	0.325	QNS	0.335	QNS	0.534	QNS	0.298	0.359	0.233 *
	3	8	0.334	0.262	0.197	0.312	0.291	0.259	0.249	QNS	0.517	QNS	0.532	0.432	0.184
		Average	0.405	0.333	0.405	0.431	0.366	0.328	0.419	0.718	0.443	0.396	0.518	0.384	0.531
		Std Dev	0.073	0.055	0.216	0.205	0.079	0.047	0.198	0.409	0.159	0.064	0.221	0.033	0.453
	4	21	0.916	0.358	0.275	0.308	0.309	0.182	0.232	QNS	QNS	0.370	1.016	0.314	0.308
	4	27	0.462	0.354	QNS	0.439	0.399	0.299	0.505	--	--	--	--	--	--
	4	31	0.679	0.876	QNS	0.740	0.908	--	--	--	--	--	--	--	--
	4	33	0.935	1.557	QNS	1.186	0.462 *	--	--	--	--	--	--	--	--
	4	38	0.564	0.529	0.646	0.442	0.488	0.392	0.424	0.957	1.110	1.183	1.241	0.368	0.629 *
	4	39	0.297	0.210	0.293	0.240	0.263	0.251	0.180	0.288	0.134	0.178	0.239	0.195	0.166
	4	6	0.374	0.366	1.121	0.447	--	--	--	--	--	--	--	--	--
		Average	0.604	0.607	0.584	0.543	0.472	0.281	0.335	0.623	0.622	0.577	0.832	0.292	0.368
		Std Dev	0.252	0.469	0.397	0.324	0.231	0.088	0.154	0.473	0.690	0.533	0.525	0.088	0.237

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QNS - Sample Volume not Sufficient for analysis

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APPENDIX T
INDIVIDUAL C-REACTIVE PROTEIN RESULTS

C-Reactive Protein (CRP)

Parameter	Group	Animal ID	Day -3	Day 2	Day 4	Day 9	Day 11	Day 16	Day 18	Day 23	Day 25	Day 30	Day 32	Day 37	Day 39
C-Reactive Protein	1	37	1.48	1.14	-- QNS	0.55	1.27	<0.5	<0.5	-- QNS	<0.5	<0.5	<0.5	<0.5	<0.5
(mg/dL)	1	40	0.98	0.89	0.72	<0.5	<0.5	-- QNS	<0.5	<0.5	-- QNS	-- QNS	<0.5	<0.5	<0.5
	1	5	<0.5	<0.5	-- QNS	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	1	7	<0.5	0.56	-- QNS	-- QNS	<0.5	-- QNS	1.57	<0.5	<0.5	<0.5	<0.5	<0.5	-- /////
	1	9	<0.5	0.71	1.49	0.65	<0.5	0.57	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
		Average	0.64	0.71	1.11	0.43	0.45	0.36	0.51	0.25	0.25	0.25	0.25	0.25	0.25
		Std Dev	0.57	0.34	0.54	0.21	0.46	0.18	0.59	0.00	0.00	0.00	0.00	0.00	0.00
	2	13	<0.5	<0.5	0.54	<0.5	-- QNS	<0.5	<0.5	-- QNS	-- QNS	<0.5	-- QNS	<0.5	2.20
	2	15	<0.5	0.52	0.56	<0.5	<0.5	-- QNS	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	2	19	0.67	0.69	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	-- /////	0.67	<0.5	<0.5
	2	25	<0.5	<0.5	<0.5	<0.5	0.64	<0.5	<0.5	-- QNS	<0.5	<0.5	<0.5	<0.5	<0.5
	2	28	<0.5	-- /////	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
	2	30	<0.5	<0.5	<0.5	<0.5	<0.5	-- QNS	<0.5	<0.5	<0.5	<0.5	-- QNS	-- /////	<0.5
	2	34	1.36	1.23	0.92	<0.5	<0.5	1.21	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
		Average	0.47	0.53	0.43	0.25	0.32	0.44	0.25	0.25	0.25	0.25	0.33	0.25	0.53
		Std Dev	0.42	0.39	0.26	0.00	0.16	0.43	0.00	0.00	0.00	0.00	0.19	0.00	0.74
	3	11	<0.5	<0.5	<0.5	<0.5	1.78	1.49	0.66	-- QNS	0.50	<0.5	<0.5	<0.5	<0.5
	3	12	<0.5	0.61	-- QNS	<0.5	-- QNS	<0.5	<0.5	<0.5	<0.5	-- QNS	1.16	<0.5	0.61
	3	14	<0.5	<0.5	<0.5	<0.5	<0.5	0.56	<0.5	<0.5	<0.5	<0.5	<0.5	-- /////	<0.5
	3	18	<0.5	<0.5	0.71	-- QNS	<0.5	-- QNS	<0.5	-- QNS	<0.5	-- QNS	<0.5	-- /////	<0.5
	3	2	<0.5	<0.5	<0.5	-- QNS	<0.5	<0.5	--	--	--	--	--	--	--
	3	32	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	-- QNS	<0.5	-- QNS	<0.5	<0.5	<0.5
	3	8	<0.5	<0.5	<0.5	<0.5	0.50	<0.5	<0.5	-- QNS	<0.5	-- QNS	2.39	<0.5	<0.5
		Average	0.25	0.30	0.33	0.25	0.55	0.51	0.32	0.25	0.29	0.25	0.76	0.25	0.31
		Std Dev	0.00	0.14	0.19	0.00	0.61	0.50	0.17	0.00	0.10	0.00	0.88	0.00	0.15
	4	21	1.20	0.61	<0.5	<0.5	<0.5	-- QNS	0.75	<0.5	0.50	-- /////	<0.5	0.62	0.64
	4	27	<0.5	<0.5	-- QNS	<0.5	<0.5	<0.5	1.27	--	--	--	--	--	--
	4	31	<0.5	1.44	-- QNS	1.19	-- QNS	--	--	--	--	--	--	--	--
	4	33	1.12	1.35	-- QNS	-- QNS	0.82	--	--	--	--	--	--	--	--
	4	38	0.50	3.00	1.01	<0.5	<0.5	1.23	4.78	7.42	5.45	1.91	1.24	2.32	0.54
	4	39	<0.5	0.92	0.69	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	-- /////	<0.5
	4	6	<0.5	<0.5	<0.5	1.24	--	--	--	--	--	--	--	--	--
		Average	0.55	1.12	0.55	0.57	0.36	0.58	1.76	2.64	2.07	1.08	0.58	1.47	0.48
		Std Dev	0.43	0.96	0.37	0.50	0.25	0.57	2.05	4.14	2.93	1.17	0.57	1.20	0.20

PHC 11-2-10

-- No Value
- Negative; + Slight; ++ Moderate
+++ Many; ++++ Severe
QNS Sample vol. not sufficient for analysis
///// Instrument unable to calculate results
<0.5 - Value < LOD, use LOD/2 in calculation of Avg. and Std Dev.

QA AUDIT COMPLETED
BY/DATE: [signature] 11/4/10

QC/Tech Rev. By/Date: PHC 11-2-10

C-Reactive Protein (CRP)

Parameter	Group	Animal ID	Day -3		Day 2		Day 4		Day 9		Day 11		Day 16		Day 18		Day 23		Day 25		Day 30		Day 32		Day 37		Day 39	
Hemolysis Index	1	37	--	-	--	-	--	QNS	--	-	--	-	--	+	--	-	--	QNS	--	-	--	-	--	+	--	-	--	+
	1	40	--	+	--	-	--	-	--	+	--	+	--	QNS	--	+	--	+	--	QNS	--	QNS	--	+	--	-	--	-
	1	5	--	-	--	-	--	QNS	--	-	--	+	--	-	--	-	--	+	--	-	--	+	--	+	--	+	--	-
	1	7	--	+	--	-	--	QNS	--	QNS	--	+	--	QNS	--	-	--	-	--	+	--	-	--	-	--	-	--	+
	1	9	--	-	--	-	--	+	--	-	--	+	--	+	--	-	--	-	--	-	--	-	--	+	--	-	--	-
	2	13	--	++	--	-	--	+	--	-	--	QNS	--	-	--	-	--	QNS	--	QNS	--	+	--	QNS	--	-	--	+
	2	15	--	+	--	-	--	-	--	-	--	-	--	QNS	--	+	--	-	--	+	--	-	--	+	--	+	--	+
	2	19	--	+	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	+	--	-	--	-	--	-	--	+
	2	25	--	-	--	+	--	-	--	-	--	+	--	+	--	-	--	QNS	--	-	--	+	--	+	--	+	--	-
	2	28	--	+	--	++	--	-	--	-	--	+	--	+	--	++	--	-	--	-	--	-	--	++	--	-	--	-
	2	30	--	+	--	+	--	-	--	-	--	+	--	QNS	--	+	--	+	--	+	--	-	--	QNS	--	+	--	-
	2	34	--	+	--	+	--	-	--	-	--	-	--	-	--	+	--	+	--	-	--	-	--	-	--	-	--	-
	3	11	--	-	--	-	--	-	--	-	--	-	--	-	--	+	--	QNS	--	-	--	-	--	+	--	-	--	+
	3	12	--	-	--	-	--	QNS	--	-	--	QNS	--	+	--	+	--	-	--	+	--	QNS	--	+	--	+	--	-
	3	14	--	-	--	-	--	-	--	-	--	-	--	+	--	-	--	-	--	-	--	+	--	+	--	+	--	+
	3	18	--	+	--	+	--	-	--	QNS	--	-	--	QNS	--	-	--	QNS	--	-	--	QNS	--	+	--	-	--	-
	3	2	--	+	--	+	--	-	--	QNS	--	-	--	+	--		--		--		--		--		--		--	
	3	32	--	++	--	-	--	-	--	-	--	+	--	++	--	-	--	QNS	--	+	--	QNS	--	-	--	-	--	+
	3	8	--	-	--	-	--	-	--	-	--	-	--	+	--	+	--	QNS	--	-	--	QNS	--	-	--	+	--	-
	4	21	--	-	--	-	--	-	--	-	--	-	--	QNS	--	-	--	+	--	-	--	-	--	-	--	+	--	-
	4	27	--	+	--	-	--	QNS	--	+	--	+	--	-	--	+	--		--		--		--		--		--	
	4	31	--	-	--	-	--	QNS	--	-	--	QNS	--		--		--		--		--		--		--		--	
	4	33	--	-	--	-	--	QNS	--	QNS	--	-	--		--		--		--		--		--		--		--	
	4	38	--	-	--	-	--	+	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	+	--	-
	4	39	--	-	--	-	--	-	--	-	--	-	--	+	--	-	--	+	--	-	--	-	--	-	--	+	--	-
	4	6	--	+	--	+	--	-	--	-	--		--		--		--		--		--		--		--		--	

ATC 11-2-10

QA AUDIT COMPLETED
BY/DATE: [signature] 11/4/10

-- No Value
- Negative; + Slight; ++ Moderate
+++ Many; ++++ Severe
QNS Sample vol. not sufficient for analysis
///// Instrument unable to calculate results
<0.5 - Value < LOD, use LOD/2 in calculation of Avg. and Std Dev.

QC/Tech Rev. By/Date: ATC 11-2-10

C-Reactive Protein (CRP)

Parameter	Group	Animal ID	Day -3		Day 2		Day 4		Day 9		Day 11		Day 16		Day 18		Day 23		Day 25		Day 30		Day 32		Day 37		Day 39	
Lipemia Index	1	37	--	-	--	-	--	QNS	--	-	--	-	--	-	--	-	--	QNS	--	-	--	-	--	-	--	-	--	-
	1	40	--	-	--	-	--	-	--	-	--	-	--	QNS	--	-	--	-	--	QNS	--	QNS	--	-	--	-	--	-
	1	5	--	-	--	-	--	QNS	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-
	1	7	--	-	--	-	--	QNS	--	QNS	--	-	--	QNS	--	-	--	-	--	-	--	-	--	-	--	-	--	-
	1	9	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-
	2	13	--	-	--	-	--	-	--	-	--	QNS	--	-	--	-	--	QNS	--	QNS	--	-	--	QNS	--	-	--	-
	2	15	--	-	--	-	--	-	--	-	--	-	--	QNS	--	-	--	-	--	-	--	-	--	-	--	-	--	-
	2	19	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-
	2	25	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	QNS	--	-	--	-	--	-	--	-	--	-
	2	28	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-
	2	30	--	-	--	-	--	-	--	-	--	-	--	QNS	--	-	--	-	--	-	--	-	--	QNS	--	-	--	-
	2	34	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-
	3	11	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	QNS	--	-	--	-	--	-	--	-	--	-
	3	12	--	-	--	-	--	QNS	--	-	--	QNS	--	-	--	-	--	-	--	-	--	QNS	--	-	--	-	--	-
	3	14	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-
	3	18	--	-	--	-	--	-	--	QNS	--	-	--	QNS	--	-	--	QNS	--	-	--	QNS	--	-	--	-	--	-
	3	2	--	-	--	-	--	-	--	QNS	--	-	--	-	--		--		--		--		--		--		--	
	3	32	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	QNS	--	-	--	QNS	--	-	--	-	--	-
	3	8	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	QNS	--	-	--	QNS	--	-	--	-	--	-
	4	21	--	-	--	-	--	-	--	-	--	-	--	QNS	--	-	--	-	--	-	--	-	--	-	--	-	--	-
	4	27	--	-	--	-	--	QNS	--	-	--	-	--	-	--	-	--		--		--		--		--		--	
	4	31	--	-	--	-	--	QNS	--	-	--	QNS	--		--		--		--		--		--		--		--	
	4	33	--	-	--	-	--	QNS	--	QNS	--	-	--		--		--		--		--		--		--		--	
	4	38	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-
	4	39	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-	--	-
	4	6	--	-	--	-	--	-	--	-	--		--		--		--		--		--		--		--		--	

ATC 11·2·10

-- No Value
- Negative; + Slight; ++ Moderate
+++ Many; ++++ Severe
QNS Sample vol. not sufficient for analysis
///// Instrument unable to calculate results
<0.5 - Value < LOD, use LOD/2 in calculation of Avg. and Std Dev.

QA AUDIT COMPLETED
BY/DATE: Jkn 11/4/10

QC/Tech Rev. By/Date: ATC 11·2·10

APPENDIX U
PATHOLOGY REPORT

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List of Acronyms

CFU .... colony forming units
mm .... millimeter
SOP .... standard operating procedure

1.0 Introduction

The objective of this Study was to determine physiological markers of disease following multiple exposures to *Bacillus anthracis* Ames strain spores. This narrative addresses gross and microscopic findings in selected tissues.

Prior to challenge, New Zealand white male rabbits were randomized into three groups of seven and one control group of five. Each rabbit was aerosol challenged with targeted doses of *B. anthracis* Ames strain spores as outlined in Table 1.

Table 1. Study Design and Challenge Doses

Group	Spore Dose (CFU)	Number of Spore Challenges[b]	Number of Rabbits
1 (Negative) Control[a]	10,000[a]	15	5
2	100	15	7
3	1000	15	7
4	10,000	15	7

[a] Negative controls were challenged with irradiated spores.
[b] Rabbits were challenged once a day for five straight working days (Monday through Friday) each week for three straight weeks.

Complete necropsies were performed on all rabbits following spontaneous death or euthanasia, including rabbits surviving to study termination on Study Day 39, according to Standard Operating Procedure (SOP) PATH X1-001. Protocol-specified tissues (lungs and gross lesions) were sampled and preserved in 10% neutral buffered formalin. Standard sections of these tissues from all rabbits were processed to slides, stained with hematoxylin and eosin, and interpreted by a board-certified veterinary 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. The incidence summary of microscopic observations with weighted average severity is presented in Table 3 (survivors on Day 39) and

Table 4 (unscheduled-death rabbits). In all 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).

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

One rabbit (14%) in Group 3 and four rabbits (57%) in Group 4 died or became moribund and were euthanized between 10 and 21 days after the initial challenge (see Table 2).

Table 2. Mortality in Rabbits Aerosol Challenged with *B. anthracis* Spores

Group Legend: 1= Control; 2=100 cfu; 3=1,000 cfu; 4=10,000 cfu				
Group:	**1[a]**	**2**	**3**	**4**
Mortality (%)	0	0	14	57
Number Dead	0	0	1	4
Number of Animals	5	7	7	7

[a] Control animals (Group 1) were challenged with 10,000 cfu irradiated spores.

Gross lesions consistent with anthrax in rabbits (Zaucha, *et al.,* 1998) included discoloration of the lungs, foci in the appendix, "accumulation" in the cecum, and/or enlargement of a mediastinal lymph node; and were found in rabbits [305 (12), 401 (6), 402 (33), and 403 (27)]. These lesions correlated microscopically with hemorrhage, necrosis, edema/fibrin, and suppurative (largely heterophilic admixed with bacteria and/or necrotic debris) inflammation. Gross lesions in the lungs correlated with multiple foreign body granulomas/pyogranulomas [rabbit 407 (38)] microscopically and were attributed to anthrax (indirectly). Hindlimb/abdominal skin "lacerations" were diagnosed grossly in two rabbits [305 (12) and 407 (38)]. These lesions correlated microscopically with necrosuppurative inflammation but were not associated with bacteria as seen in the single dose rabbits in a related study (Study 1020-CG920503). While anthrax may have been a contributing factor, these lesions were more likely due to trauma. Gross and microscopic lesions are summarized in Table 5 and are listed in the Individual Gross and Microscopic Observations Table 6.

3.0 Histopathology

Sections of left apical and right diaphragmatic lung lobes and gross lesions were examined microscopically for evidence of anthrax. There were no missing tissues.
Microscopic findings consistent with anthrax (*Zaucha, et al, 1998*) were present in tissues from all rabbits. Lesions typical of anthrax in this Study included suppurative inflammation, necrosis, lymphocyte necrosis/depletion, hemorrhage, edema, and/or large rod shaped bacteria (bacilli) in the lungs, cecum, appendix, and mediastinal lymph nodes. Lung lesions attributed to *B. anthracis* were primarily interstitial and consisted of minimal to mild suppurative interstitial inflammation and interstitial and/or intravascular bacteria.

Multinucleated giant cells as well as foreign body granulomas/pyogranulmas were present in the lungs of challenged rabbits but were not seen in control rabbits in this Study. In a related single-dose study (1020-CG9290503), multinucleated giant cells were noted in both exposed and control animals. However, the lesions were more severe in challenged rabbits. These multinucleated cells and granulomas/pyogranulomas surrounded foreign material (foreign bodies) consistent with organic debris [e.g. food particles or hair and debris from vascular access ports (*Taketoh, et al, 2009*)]. As with Study 1020-CG920503, these lesions were likely the result of altered foreign particle clearance by alveolar macrophages (macrophage dysfunction). Macrophage dysfunction has been reported to occur in late sepsis (*Pahuja, et al, 2008*). Prolonged bacteremia/sepsis attributed to anthrax could alter foreign particle clearance by alveolar macrophages. One lesion, perivascular eosinophils in the lungs, was likely attributed to vascular access port placement and has been observed in rodent studies (*Taketoh, et al, 2009*). Necrosis in the skin was likely due to self-inflicted trauma.

Table 3. Incidence Summary of Microscopic Nonneoplastic Graded Observations with Average Severity - Males, Day 39

Tissue/Observation	Group:	Number Observed Per Group			
		1	2	3	4
Appendix	Number Examined:	0	0	0	0
Hemorrhage		-	-	-	-
	Average Severity:	-	-	-	-
Infiltration Cellular, Macrophages		-	-	-	-
	Average Severity:	-	-	-	-
Necrosis/Depletion, Lymphoid		-	-	-	-
	Average Severity:	-	-	-	-
Cecum	Number Examined:	0	0	0	0
Edema		-	-	-	-
	Average Severity:	-	-	-	-
Hemorrhage		-	-	-	-
	Average Severity:	-	-	-	-
Necrosis		-	-	-	-
	Average Severity:	-	-	-	-
Lung	Number Examined:	5	7	6	3
Bacteria		0	0	0	0
	Average Severity:	0.0	0.0	0.0	0.0
Foreign Body		0	1	1	1
	Average Severity:	0.0	0.3	0.2	1.0
Granuloma/Pyogranulomatous		0	0	0	1
	Average Severity:	0.0	0.0	0.0	1.0
Hemorrhage		0	0	0	0
	Average Severity:	0.0	0.0	0.0	0.0

Group Legend: 1=CONTROL; 2=100 CFU; 3=1,000 CFU; 4=10,000 CFU

Table 3. Incidence Summary of Microscopic Nonneoplastic Graded Observations with Average Severity - Males, Day 39 (Continued)

Tissue/Observation	Group:	Number Observed Per Group			
		1	2	3	4
Inflammation, Suppurative, Interstitial		0	0	0	0
	Average Severity:	0.0	0.0	0.0	0.0
Multinucleated Giant Cells		0	1	1	1
	Average Severity:	0.0	0.3	0.3	0.3
Perivascular Eosinophils		2	3	3	1
	Average Severity:	0.4	0.6	0.5	0.3
Lymph Node, Mediastinal	Number Examined:	0	0	0	0
Bacteria		-	-	-	-
	Average Severity:	-	-	-	-
Edema/Fibrin		-	-	-	-
	Average Severity:	-	-	-	-
Hemorrhage		-	-	-	-
	Average Severity:	-	-	-	-
Necrosis/Depletion, Lymphoid		-	-	-	-
	Average Severity:	-	-	-	-
Skin	Number Examined:	0	0	1	1
Inflammation, Necrosuppurative		-	-	1	1
	Average Severity:	-	-	4.0	3.0
Artery Thrombosis		-	-	-	1
	Average Severity	-	-	-	2.0

Group Legend: 1=CONTROL; 2=100 CFU; 3=1,000 CFU; 4=10,000 CFU

Table 4. Incidence Summary of Microscopic Nonneoplastic Graded Observations with Average Severity - Males, Unscheduled

Tissue/Observation	Group:	Number Observed Per Group			
		1	2	3	4
Appendix	Number Examined:	0	0	0	1
Hemorrhage		-	-	-	1
	Average Severity:	-	-	-	2.0
Infiltration Cellular, Macrophages		-	-	-	1
	Average Severity:	-	-	-	3.0
Necrosis/Depletion, Lymphoid		-	-	-	1
	Average Severity:	-	-	-	3.0
Cecum	Number Examined:	0	0	0	1
Edema		-	-	-	1
	Average Severity:	-	-	-	2.0
Hemorrhage		-	-	-	1
	Average Severity:	-	-	-	3.0
Necrosis		-	-	-	1
	Average Severity:	-	-	-	3.0
Lung	Number Examined:	0	0	1	4
Bacteria		-	-	1	3
	Average Severity:	-	-	1.0	1.3
Foreign Body		-	-	0	0
	Average Severity:	-	-	0.0	0.0
Granuloma/Pyogranulomatous		-	-	0	0
	Average Severity:	-	-	0.0	0.0
Hemorrhage		-	-	1	1
	Average Severity:	-	-	1.0	0.3

Group Legend: 1=CONTROL; 2=100 CFU; 3=1,000 CFU; 4=10,000 CFU

Table 4. Incidence Summary of Microscopic Nonneoplastic Graded Observations with Average Severity – Males, Unscheduled (Continued)

Tissue/Observation	Group:	Number Observed Per Group 1	2	3	4
Inflammation, Suppurative		-	-	1	3
	Average Severity:	-	-	1.0	1.0
Multinucleated Giant Cells		-	-	0	0
	Average Severity:	-	-	0.0	0.0
Perivascular Eosinophils		-	-	0	3
	Average Severity:	-	-	0.0	0.8
Lymph Node, Mediastinal	Number Examined:	0	0	0	1
Bacteria		-	-	-	1
	Average Severity:	-	-	-	4.0
Edema/Fibrin		-	-	-	1
	Average Severity:	-	-	-	2.0
Hemorrhage		-	-	-	1
	Average Severity:	-	-	-	1.0
Necrosis/Depletion/Lymphoid		-	-	-	1
	Average Severity:	-	-	-	4.0
Skin	Number Examined:	0	0	0	0
Inflammation, Necrosuppurative		-	-	-	-
	Average Severity:	-	-	-	-
Thrombosis, Artery		-	-	-	-
	Average Severity:	-	-	-	-

Group Legend: 1=CONTROL; 2=100 CFU; 3=1,000 CFU; 4=10,000 CFU

Table 5. Summary of Individual Gross and Microscopic Observations, Males

Group Number	Animal Number/ Death Status[a]	Gross Findings	Microscopic Findings
Control	101 (40)/FS		Lung: Unremarkable.
	102 (7)/FS		Lung: Unremarkable.
	103 (5)/FS		Lung: Perivascular eosinophils, minimal.
	104 (9)/FS		Lung: Perivascular eosinophils, minimal.
	105 (37)/FS		Lung: Unremarkable.
100 CFU	201 (13)/FS		Lung: Perivascular eosinophils, minimal.
	202 (34)/FS		Lung: Foreign body, mild. Lung: Multinucleated giant cells, mild.
	203 (25)/FS		Lung: Unremarkable.
	204 (15)/FS		Lung: Perivascular eosinophils, minimal.
	205 (30)/FS		Lung: Unremarkable.
	206 (28)/FS		Lung: Perivascular eosinophils, mild.
	207 (19)/FS		Lung: Unremarkable.
1000 CFU	301 (14)/FS		Lung: Perivascular eosinophils, minimal.
	302 (11)/FS		Lung: Perivascular eosinophils, minimal.
	303 (2)/FD		Lung: Hemorrhage, minimal. Lung: Inflammation, suppurative, minimal. Lung: Bacteria, minimal.
	304 (8)/FS		Unremarkable.
	305 (12)/FS	Skin: Laceration(s), hindlimb, red, left hindlimb, 40 x 20 mm	Lung: Foreign body, minimal. Lung: Multinucleated giant cells, mild. Skin: Inflammation, necrosuppurative, marked.

[a]FD = Found Dead, FS = Final Phase Sacrifice

Table 5. Summary of Individual Gross and Microscopic Observations, Males (Continued)

Group Number	Animal Number/ Death Status[a]	Gross Findings	Microscopic Findings
1000 CFU	306 (18)/FS		Lung: Unremarkable.
	307 (32)/FS		Lung: Perivascular eosinophils, minimal.
10,000 CFU	401 (6)/FD	Cecum: Accumulation (gas). Samples of cecum, colon, jejunum, and appendix were collected to confirm lesion.	Cecum: Edema, mild. Cecum: Edema, hemorrhage and necrosis. Cecum: Hemorrhage, moderate. Cecum: Necrosis, moderate. Lung: Perivascular eosinophils, minimal.
	402 (33)/FD	Lymph Node, Mediastinal: Enlarged, dark, 3x.	Lung: Bacteria, mild. Lung: Hemorrhage, minimal. Lung: Inflammation, suppurative, mild. Lung: Perivascular eosinophils, minimal. Lymph Node, Mediastinal: Bacteria, Marked. Lymph Node, Mediastinal: Edema, fibrin, mild. Lymph Node, Mediastinal: Hemorrhage, minimal. Lymph Node, Mediastinal: Necrosis/depletion, lymphoid, marked.
	403 (27)/FD	Appendix: Foci, multiple, red, up to 2 x 2 mm.	Appendix: Hemorrhage, mild. Appendix: Necrosis/depletion, lymphoid, moderate.

[a]FD = Found Dead, FS = Final Phase Sacrifice

Table 5. Summary of Individual Gross and Microscopic Observations, Males (Continued)

Group Number	Animal Number/ Death Status[a]	Gross Findings	Microscopic Findings
10,000 CFU	403 (27)/FD (Continued)		Appendix: Infiltration cellular, macrophages, moderate. Appendix: Note: hemorrhage and necrosis. Lung: Bacteria, minimal. Lung: Inflammation, suppurative, minimal. Lung: Perivascular eosinophils, minimal.
	404 (31)/FD		Lung: Bacteria, mild. Lung: Inflammation, suppurative, minimal.
	405 (39)		Lung: Foreign body, minimal. Lung: Multinucleated giant cells, minimal.
	406 (21)		Lung: Unremarkable.
	407 (38)	Lung: Discoloration(s), apical lobe, pale, firm. Skin: Laceration(s), abdominal, red, 20 x 15 mm.	Lung: Foreign body, moderate. Lung: Granuloma/pyrogranuloma, moderate. Lung: Perivascular eosinophils, minimal. Skin: Inflammation, necrosuppurative, moderate. Skin: Thrombosis, artery, mild.

[a]FD = Found Dead, FS = Final Phase Sacrifice
Blank Space = No gross lesions observed on tissue

Table 6. Individual Gross and Microscopic Observations, Males

Animal ID: 101 (40) Group: CONTROL
Day of Death: 39 (Final Phase Sacrifice)

Tissue	Gross Observation(s)	Microscopic Observation(s)
Lung	No gross lesions observed on tissue.	Unremarkable.

Animal ID: 102 (7) Group: CONTROL
Day of Death: 39 (Final Phase Sacrifice)

Tissue	Gross Observation(s)	Microscopic Observation(s)
Lung	No gross lesions observed on tissues.	Unremarkable.

Animal ID: 103 (5) Group: CONTROL
Day of Death: 39 (Final Phase Sacrifice)

Tissue	Gross Observation(s)	Microscopic Observation(s)
Lung	No gross lesions observed on tissue.	Perivascular eosinophils, minimal.

Animal ID: 104 (9) Group: CONTROL
Day of Death: 39 (Final Phase Sacrifice)

Tissue	Gross Observation(s)	Microscopic Observation(s)
Lung	No gross lesions observed on tissue.	Perivascular eosinophils, minimal.

Table 6. Individual Gross and Microscopic Observations, Males (Continued)

Animal ID: 105 (37) Group: CONTROL
Day of Death: 39 (Final Phase Sacrifice)

Tissue	Gross Observation(s)	Microscopic Observation(s)
Lung	No gross observed on tissue.	Unremarkable.

Animal ID: 201 (13) Group: 100 CFU
Day of Death: 39 (Final Phase Sacrifice)

Tissue	Gross Observation(s)	Microscopic Observation(s)
Lung	No gross lesions observed on tissue.	Perivascular eosinophils, minimal.

Animal ID: 202 (34) Group: 100 CFU
Day of Death: 39 (Final Phase Sacrifice)

Tissue	Gross Observation(s)	Microscopic Observation(s)
Lung	No gross lesions observed on tissue.	Foreign body, mild. Multinucleated giant cells, mild.

Animal ID: 203 (25) Group: 100 CFU
Day of Death: 39 (Final Phase Sacrifice)

Tissue	Gross Observation(s)	Microscopic Observation(s)
Lung	No gross lesions observed on tissue.	Unremarkable.

Table 6. Individual Gross and Microscopic Observations, Males (Continued)

Animal ID: 204 (15) Group: 100 CFU
Day of Death: 39 (Final Phase Sacrifice)

Tissue	Gross Observation(s)	Microscopic Observation(s)
Lung	No gross lesions observed on tissue.	Perivascular eosinophils, minimal.

Animal ID: 205 (30) Group: 100 CFU
Day of Death: 39 (Final Phase Sacrifice)

Tissue	Gross Observation(s)	Microscopic Observation(s)
Lung	No gross lesions observed on tissue.	Unremarkable.

Animal ID: 206 (28) Group: 100 CFU
Day of Death: 39 (Final Phase Sacrifice)

Tissue	Gross Observation(s)	Microscopic Observation(s)
Lung	No gross lesions observed on tissue.	Perivascular eosinophils, mild.

Animal ID: 207 (19) Group: 100 CFU
Day of Death: 39 (Final Phase Sacrifice)

Tissue	Gross Observation(s)	Microscopic Observation(s)
Lung	No gross lesions observed on tissue.	Unremarkable.

Table 6. Individual Gross and Microscopic Observations, Males (Continued)

Animal ID: 301 (14) Group: 1,000 CFU
Day of Death: 39 (Final Phase Sacrifice)

Tissue	Gross Observation(s)	Microscopic Observation(s)
Lung	No gross lesions observed on tissue.	Perivascular eosinophils, minimal.

Animal ID: 302 (11) Group: 1,000 CFU
Day of Death: 39 (Final Phase Sacrifice)

Tissue	Gross Observation(s)	Microscopic Observation(s)
Lung	No gross lesions observed on tissue.	Perivascular eosinophils, minimal.

Animal ID: 303 (2) Group: 1,000 CFU
Day of Death: 18 (Found Dead)

Tissue	Gross Observation(s)	Microscopic Observation(s)
Lung	No gross lesions observed on tissue.	Hemorrhage, minimal. Inflammation, suppurative, minimal. Bacteria, minimal.

Animal ID: 304 (8) Group: 1,000 CFU
Day of Death: 39 (Final Phase Sacrifice)

Tissue	Gross Observation(s)	Microscopic Observation(s)
Lung	No gross lesions observed on tissue.	Unremarkable.

Table 6. Individual Gross and Microscopic Observations, Males (Continued)

Animal ID: 305 (12) Group: 1,000 CFU
Day of Death: 39 (Final Phase Sacrifice)

Tissue	Gross Observation(s)	Microscopic Observation(s)
Lung	No gross lesions observed on tissue.	Foreign body, minimal. Multinucleated giant cells, mild.
Skin	Laceration(s), hindlimb, red, left hindlimb, 40x20mm.	Inflammation, necrosuppurative, marked.

Animal ID: 306 (18) Group: 1,000 CFU
Day of Death: 39 (Final Phase Sacrifice)

Tissue	Gross Observation(s)	Microscopic Observation(s)
Lung	No gross lesions observed on tissue.	Unremarkable.

Animal ID: 307 (32) Group: 1,000 CFU
Day of Death: 39 (Final Phase Sacrifice)

Tissue	Gross Observation(s)	Microscopic Observation(s)
Lung	No gross lesions observed on tissue.	Perivascular eosinophils, minimal.

Animal ID: 401 (6) Group: 10,000 CFU
Day of Death: 11 (Found Dead)

Tissue	Gross Observation(s)	Microscopic Observation(s)
Cecum	Accumulation (gas). Samples of cecum, colon, jejunum, and appendix were collected to confirm lesion.	Edema, mild. Hemorrhage, moderate. Necrosis, moderate.
Lung	No gross lesions observed on tissue.	Perivascular eosinophils, minimal.

Table 6. Individual Gross and Microscopic Observations, Males (Continued)

Animal ID: 402 (33) Group: 10,000 CFU
Day of Death: 13 (Found Dead)

Tissue	Gross Observation(s)	Microscopic Observation(s)
Lung	No gross lesions observed on tissue.	Perivascular eosinophils, minimal. Hemorrhage, minimal. Inflammation, suppurative, mild. Bacteria, mild.
Lymph Node, Mediastinal	Enlarged, dark, 3x.	Necrosis/depletion, lymphoid, marked. Bacteria, marked. Hemorrhage, minimal. Edema/fibrin, mild.

Animal ID: 403 (27) Group: 10,000 CFU
Day of Death: 21 (Found Dead)

Tissue	Gross Observation(s)	Microscopic Observation(s)
Appendix	Foci, multiple, red, up to 2x2mm.	Hemorrhage, mild. Necrosis/depletion, lymphoid, moderate. Infiltration cellular, macrophages, moderate.
Lung	No gross lesions observed on tissue.	Perivascular eosinophils, minimal. Inflammation, suppurative, minimal. Bacteria, minimal.

Animal ID: 404 (31) Group: 10,000 CFU
Day of Death: 15 (Found Dead)

Tissue	Gross Observation(s)	Microscopic Observation(s)
Lung	No gross lesions observed on tissue.	Inflammation, suppurative, minimal. Bacteria, mild.

Table 6. Individual Gross and Microscopic Observations, Males (Continued)

Animal ID: 405 (39) Group: 10,000 CFU
Day of Death: 39 (Final Phase Sacrifice)

Tissue	Gross Observation(s)	Microscopic Observation(s)
Lung	No gross lesions observed on tissue.	Foreign body, minimal. Multinucleated giant cells, minimum.

Animal ID: 406 (21) Group: 10,000 CFU
Day of Death: 39 (Final Phase Sacrifice)

Tissue	Gross Observation(s)	Microscopic Observation(s)
Lung	No gross lesions observed on tissue.	Unremarkable.

Animal ID: 407 (38) Group: 10,000 CFU
Day of Death: 39 (Final Phase Sacrifice)

Tissue	Gross Observation(s)	Microscopic Observation(s)
Lung	Discoloration(s), apical lobe, pale, firm.	Perivascular eosinophils, minimal. Granuloma/pyogranuloma, moderate. Foreign body, moderate.
Skin	Laceration(s), abdominal, red, 20x15mm.	Inflammation, necrosuppurative, moderate. Thrombosis, artery, mild.

4.0 Conclusions

In this multiple-dose anthrax Study, lesions typical of anthrax were generally acute and consisted of suppurative inflammation, hemorrhage, edema, lymphocyte destruction, and/or intravascular and intralesional bacilli. Multinucleated giant cells (as seen with Study 1020-CG920503) were also frequently present in the lungs of challenged animals and were attributed to anthrax septicemia. Additionally, one multiple-dosed rabbit also had well-formed granulomas/pyogranulomas distributed randomly throughout one lung lobe. Multinucleated giant cells and/or foreign body granulomas of the severity observed in these single and multiple dosed rabbits are not typical of anthrax.

5.0 References

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.

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Pahuja, M., Tran, C., Wang, H., and Yin, K. (2008) Alveolar macrophage suppression in sepsis is associated with high Mobility Group Box 1 Transmigration. *Shock,* (29): 754-760.

Battelle Study No. 1078-CG920794

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APPENDIX I

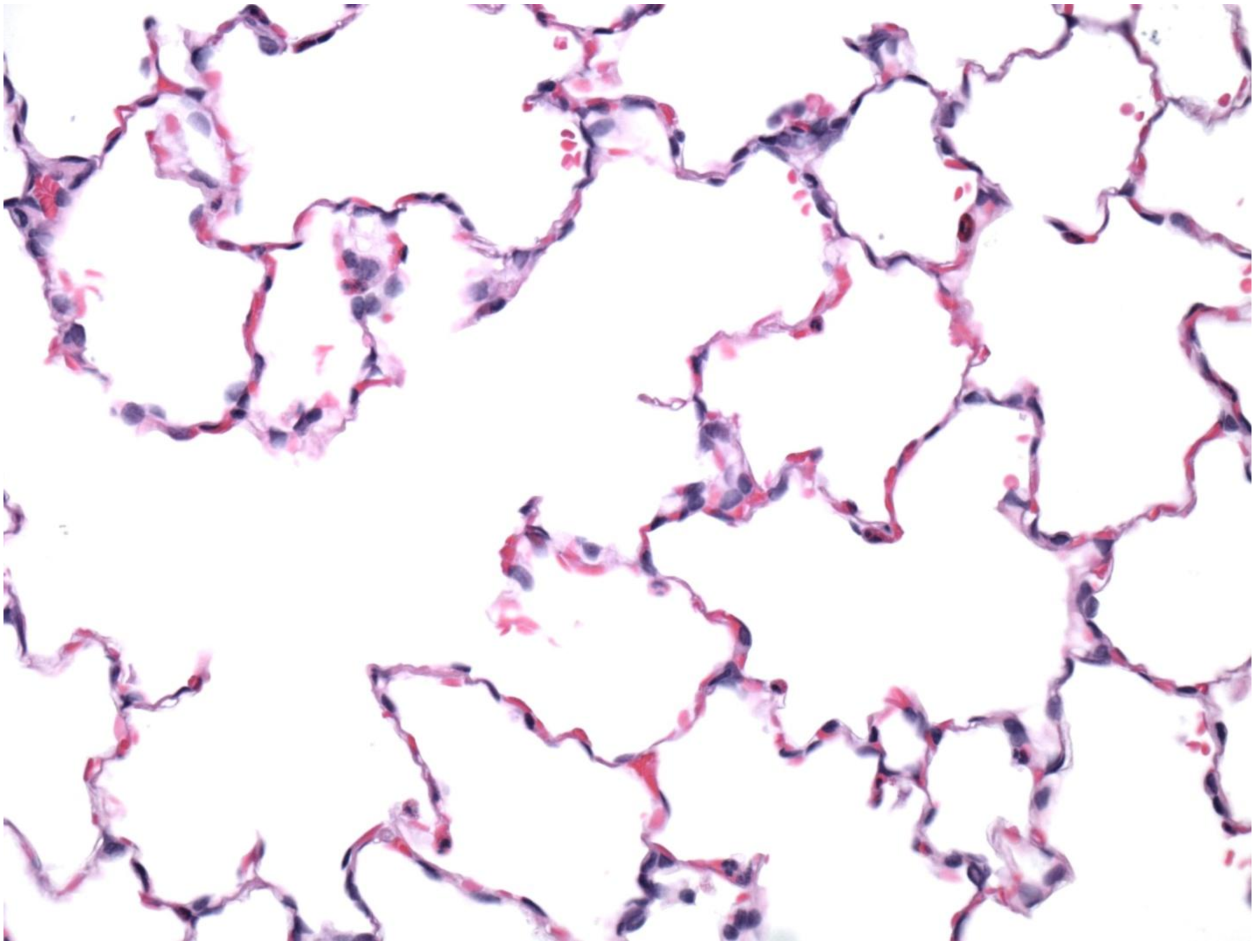

Figure I-1. Animal# 103 (37): Lung; normal alveoli (Control). H & E Stain. 40X

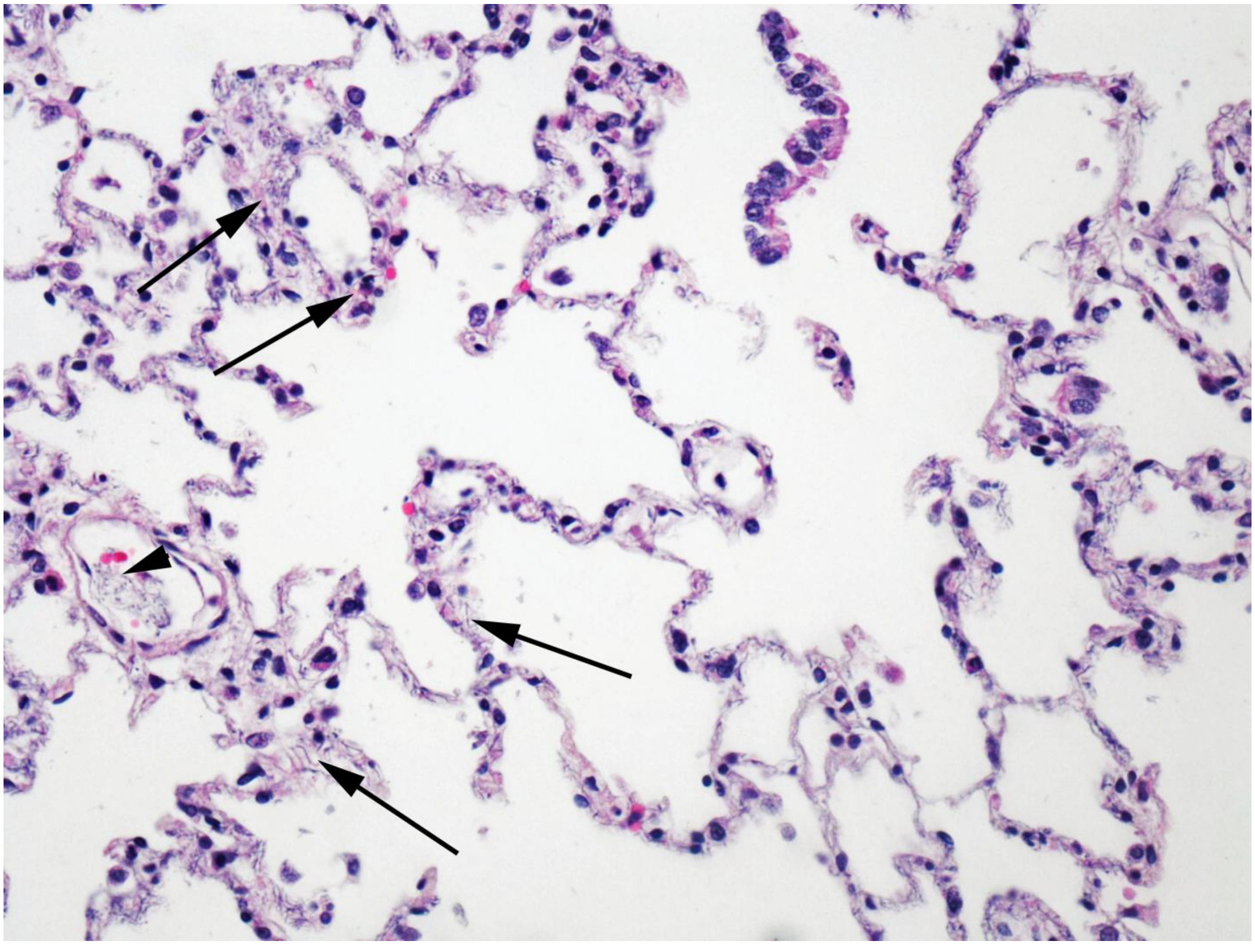

Figure I-2. Animal# 404 (31): Lung; alveoli contain interstitial suppurative inflammation and anthrax bacilli (arrows). Alveolar vessels contain anthrax bacilli (arrows). H&E Stain. 40X

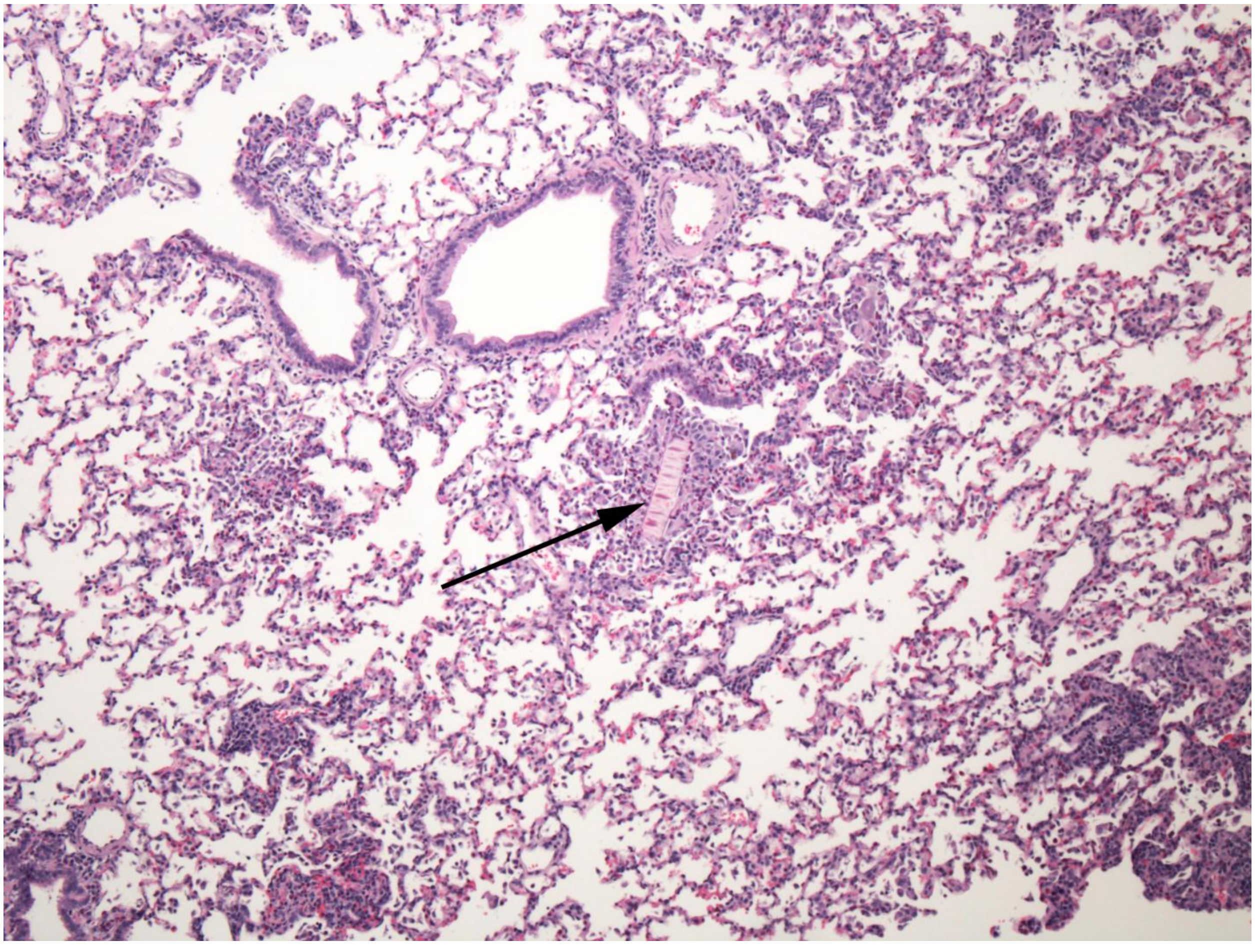

Figure I-3. Animal# 407 (38): Lung, alveoli; aggregates of inflammatory cells surrounding a foreign body (arrow). H&E Stain. 10X

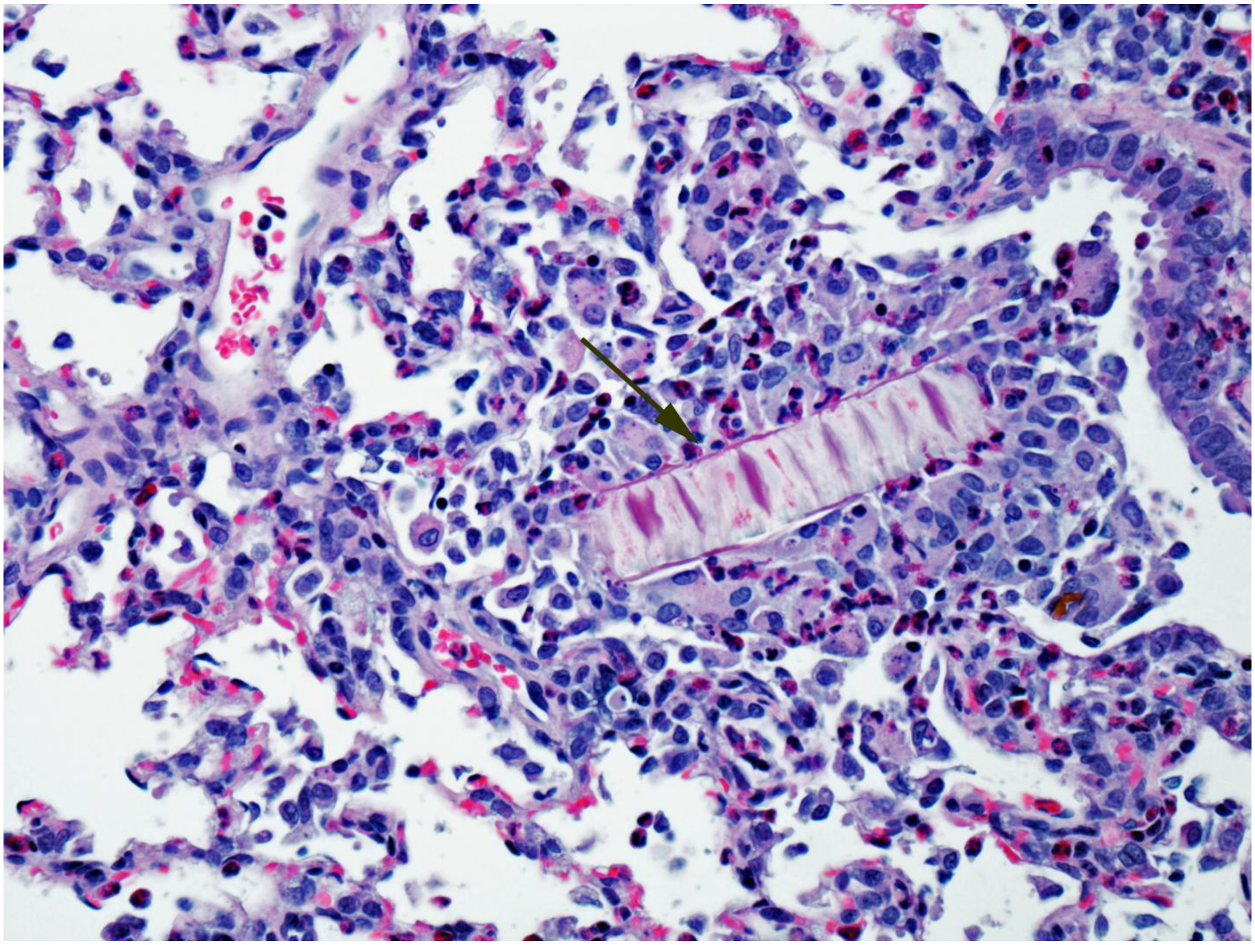

Figure I-4. Animal# 407 (38): Lung, alveoli; pyogranulomatous (epithelioid macrophages, lymphocytes, and neutrophils) inflammatory reaction to a foreign body (arrow). H&E Stain. 40X

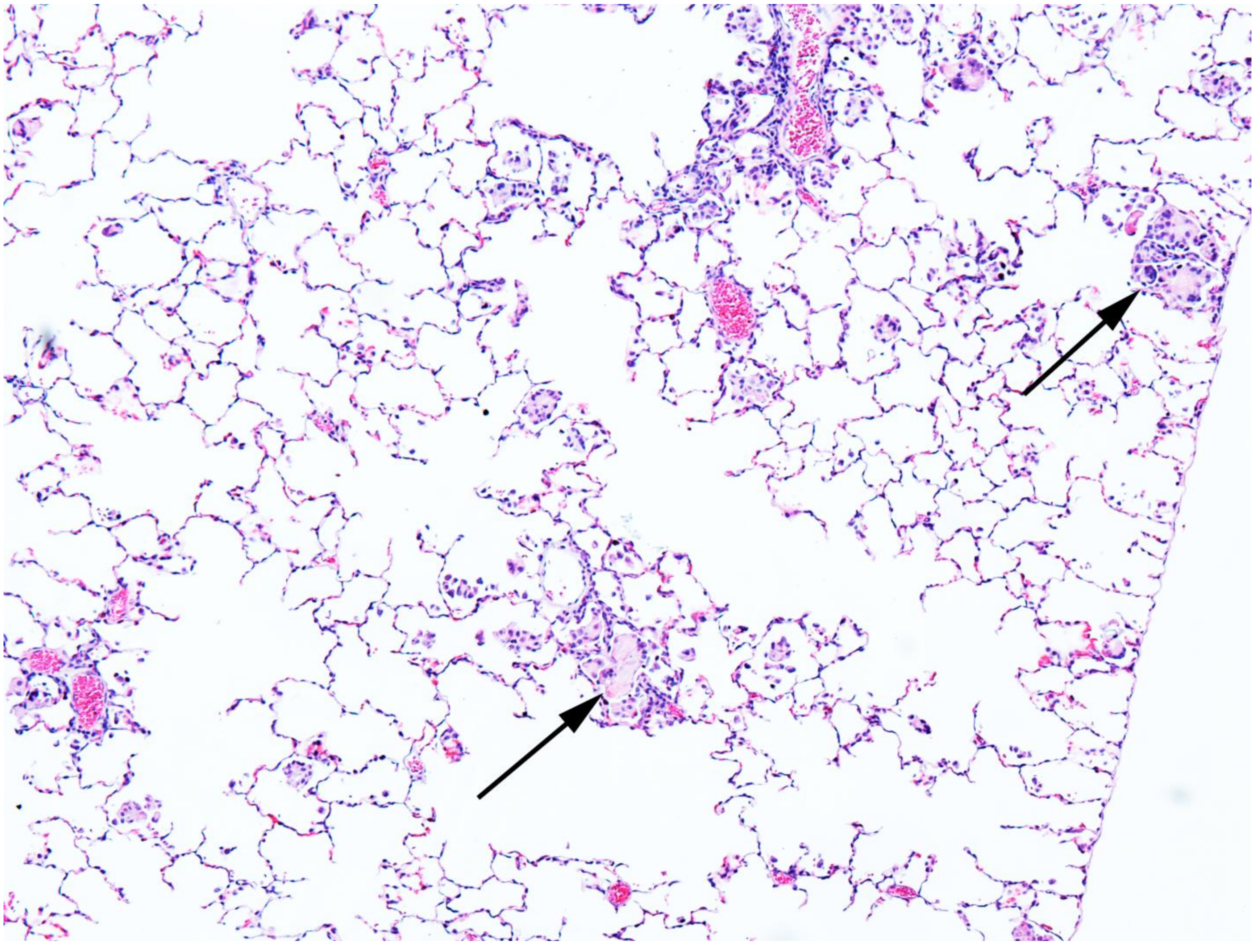

Figure I-5. Animal# 202 (34): Lung, alveoli; multinucleated giant cells are found within alveolar spaces (arrows). H&E Stain. 10X

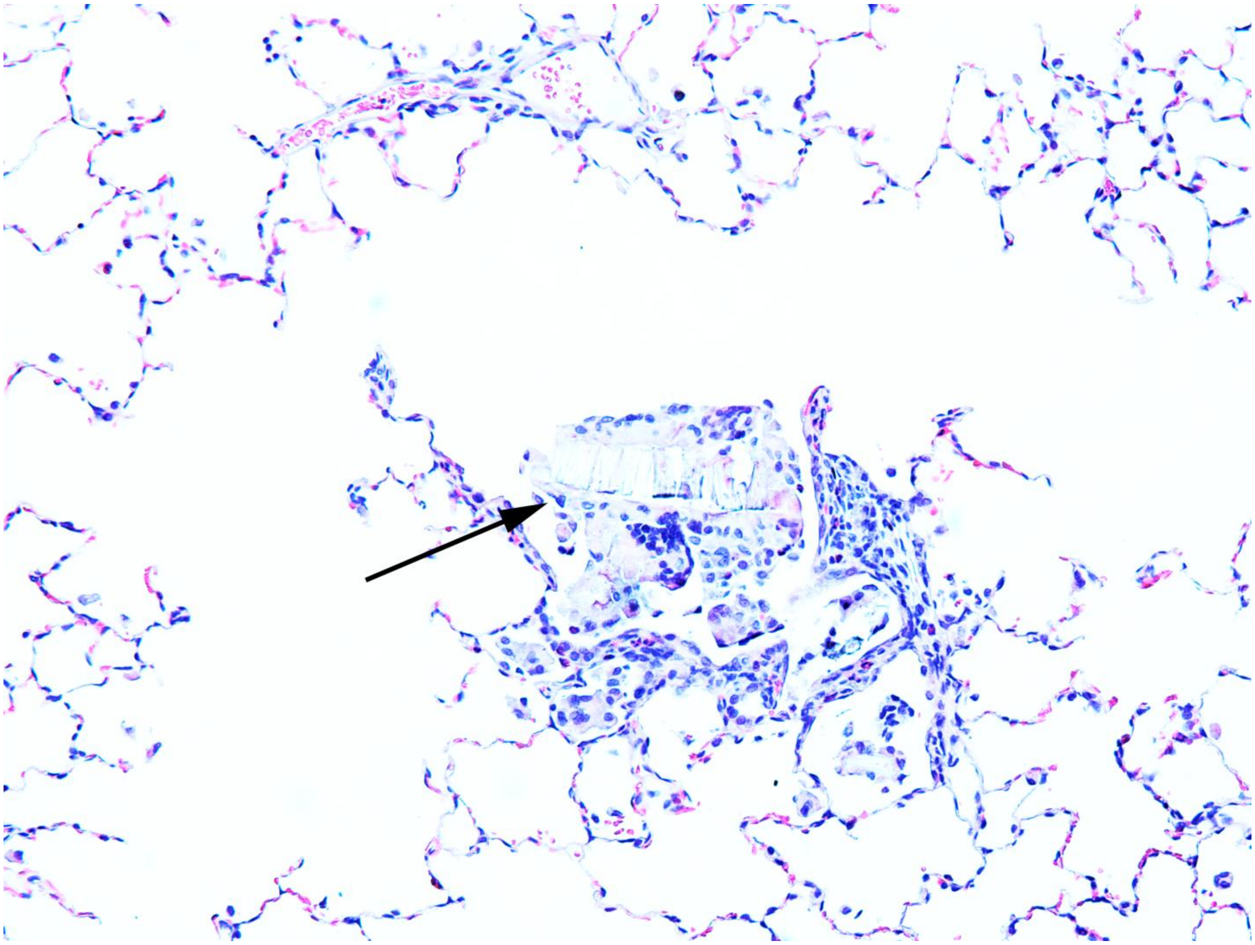

Figure I-6. Animal# 202 (34): Lung, alveoli; multinucleated giant cells surrounding a foreign body (arrow). H&E Stain. 40X

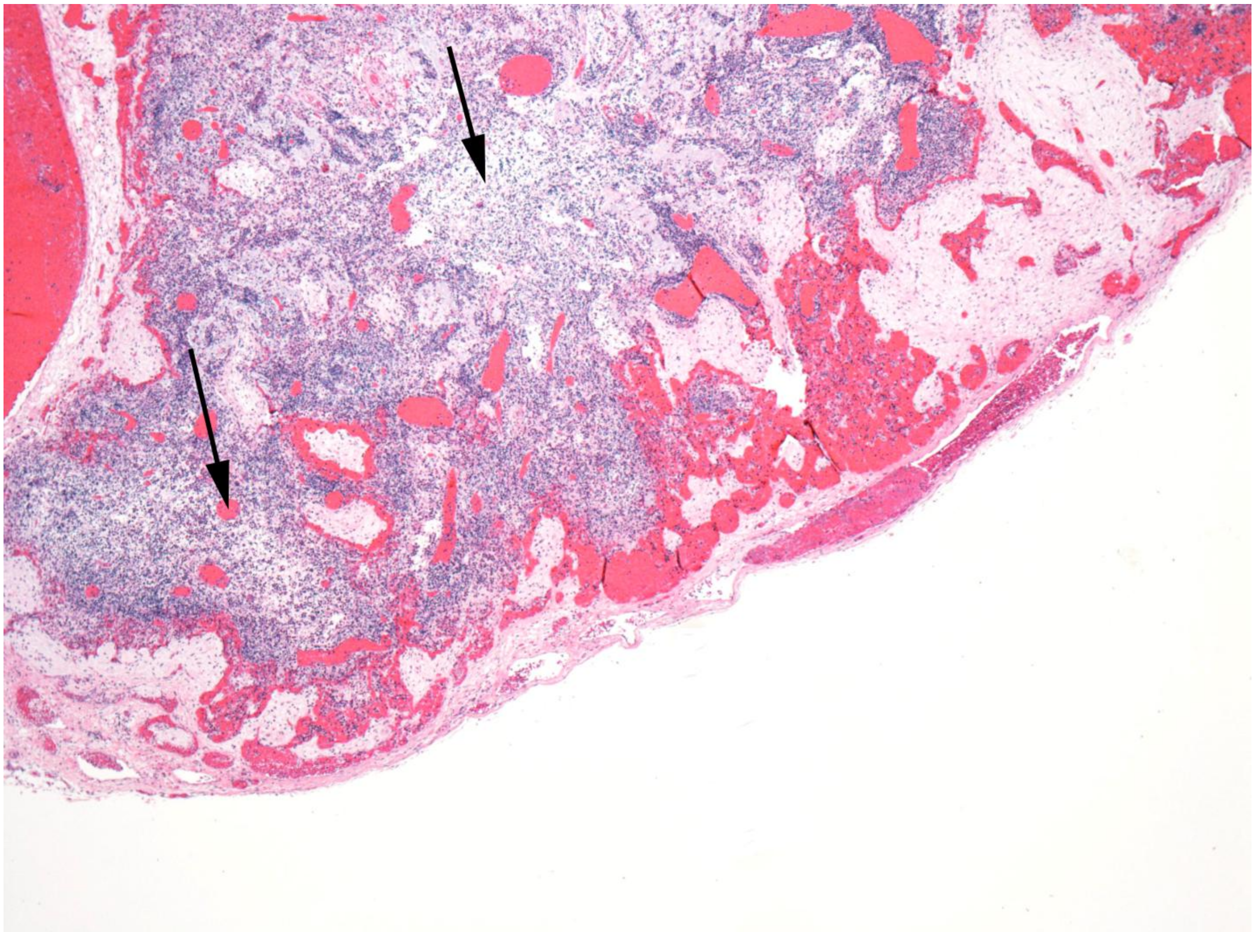

Figure I-7. Animal# 407 (38): Lymph node, mediastinal; lymph node congestion and lymphoid follicles necrosis/depletion. H&E Stain. 4X

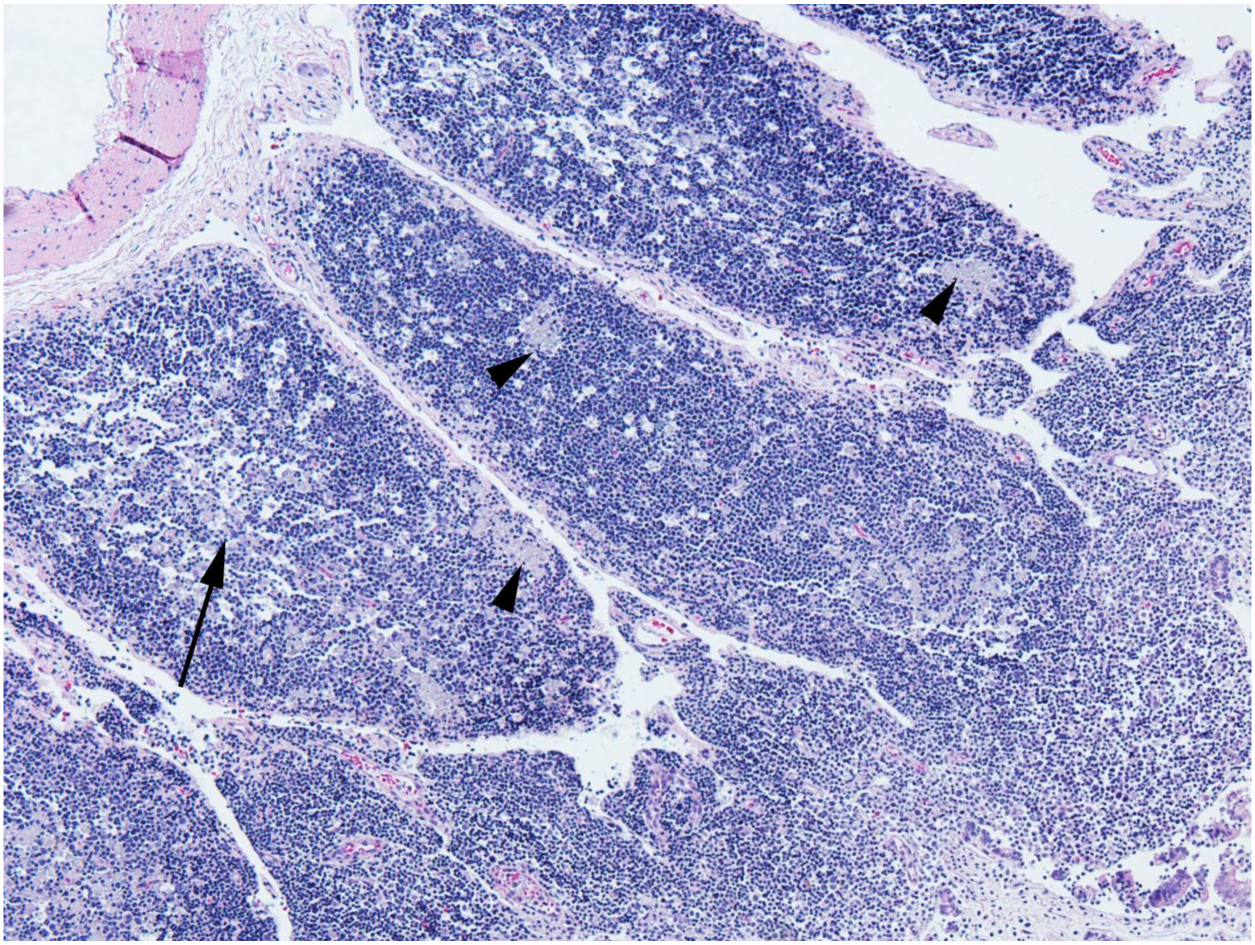

Figure I-8. Animal# 407 (38): Appendix; lymphocytes undergoing excessive apoptosis (arrow) with macrophage infiltration (arrowheads). H&E Stain. 10X

APPENDIX V
BENCHMARK DOSE STUDY REPORT

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Acronyms and Abbreviations

ADD	Average Daily Dose per Animal
AIC	Akaike Information Criterion
BMD_x	Benchmark Dose at an x level of BMR
$BMDL_x$	Benchmark Dose Limit at an x level of BMR
BMDS	Benchmark Dose Software
BMRx	Benchmark Response at an x level
CFU	Colony Forming Units
EPA	US Environmental Protection Agency
GSD	geometric standard deviation
HEC	Human Equivalent Concentration
HED	Human Equivalent Dose
LD_{50}	median lethal dose
MMAD	median aerodynamic diameter
RDDR	Regionally Deposited Dose Ratio
TAD	Total Aggregate Dose per Animal

1. Introduction

A benchmark dose analysis was conducted using Study 1078 data developed from rabbit inhalation exposures to *Bacillus anthracis* spores over a 15-dose series. The outputs of the benchmark dose analysis were then used as the inputs for an interspecies extrapolation to derive human equivalent dose (HED) and human equivalent concentration (HEC) values. One potential use of the calculated HED and HEC values is the development of cleanup goals to evaluate the hazard posed by *B. anthracis* releases.

2. Methods

2.1 Calculation of Doses

Individual rabbit inhaled doses (Colony Forming Units [CFU]/animal) were obtained from the 1078-CG920794 Inhalation Exposure Report (Tables 16 through 30). The nominal doses (i.e., 100 CFU/animal/day, 1,000 CFU/animal/day, and 10,000 CFU/animal/day) were not used as inputs in the dose-response analysis. For noninteger[1] reported inhaled doses, the integer of the inhaled dose was used in all dose calculations.

Two dose metrics of inhaled dose were evaluated in the benchmark dose analysis - the average daily dose per animal (ADD) and the total aggregate dose per animal (TAD). The ADD (CFU/Animal/Day) was calculated as shown in Equation 1. For the ADD, daily inhaled doses were averaged across all exposure days until the death of the animal or the exposure duration for those animals that survived the length of the study. The exposure duration of the study was 19 days, which captures the total number of study days including nondosing days to allow for calculation of an average daily dose consistent with EPA guidance for discontinuous exposure assessment (US EPA, 2002).[2] The dates when each animal died during the study were obtained from Appendix N. The TAD (Total CFU/Animal) was calculated as shown Equation 2. For the TAD, daily inhaled doses were summed across all exposure days until the death of the animal or the exposure duration for those animals that survived the length of the study. The calculated ADD and TAD dose values and the presence or absence of the study endpoint (i.e., death or no death) by individual animal are shown in Table 1.

Calculation of Average Daily Dose (1)

$$Average\ Daily\ Dose\ per\ Animal\ (ADD) = \frac{\sum Daily\ Inhaled\ Dose\ (\frac{CFU}{Animal})}{Total\ Number\ of\ Days\ of\ Exposure\ \ (Days)}$$

[1] The value for the air concentration was quantified using the arithmetic average of triplicate plate counts from *B. anthracis* spores captured by the measurement impingers. As such, the air concentrations and calculated daily inhaled doses as reported included noninteger values.

[2] Rabbit Number 27 died three days (i.e., August 16th) after the study exposures were completed on August 13th. For the calculation of that individual ADD, the total number of days of exposure was calculated the same as for all survivors (i.e., 19 days of exposure, the full period of the study) and the individual rabbit was identified as exhibiting the lethality endpoint.

Calculation of Total Aggregate Dose (2)

$$Total\ Aggregate\ Dose\ per\ Animal\ (TAD) = \sum Daily\ Inhaled\ Dose\ (\frac{CFU}{Animal})$$

Table 1. Raw Data Used in Benchmark Dose Analysis by Individual Animal

Nominal Dose Group (CFU/Animal/Day)	Rabbit Number	Average Daily Dose (Inhaled CFU/Animal)	Total Aggregate Dose (Inhaled CFU/Animal)	Death During Study
100	13	304	5,780	
	34	250	4,760	
	25	220	4,190	
	15	250	4,760	
	30	214	4,070	
	28	184	3,510	
	19	182	3,480	
1,000	14	582	11,100	
	11	883	16,800	
	2	1,040	18,600	Death
	8	1,110	21,200	
	12	1,030	19,600	
	18	958	18,200	
	32	1,140	21,600	
10,000	6	5,240	57,700	Death
	33	7,500	97,500	Death
	27	8,360	159,000	Death
	31	9,140	137,000	Death
	39	11,300	216,000	
	21	10,400	198,000	
	38	10,000	191,000	

2.2 Dose-Response Analysis Using Benchmark Dose Software

2.2.1 Benchmark Dose Models for Dose-Response Relationships

For the benchmark dose evaluation, the current version of the US Environmental Protection Agency's (EPA) Benchmark Dose Software (BMDS) (BMDS 2.1.2 Version 2.1.2.60, Build 06/11/10) (US EPA 2010a) was used to fit models to the dose-response data. Models from the BMDS dichotomous and dichotomous-alternative model suites were used for analysis: Weibull model, Weibull model run as exponential (with the power coefficient fixed as one), probit, log_e probit, logistic, log_e logistic, Gamma model, dichotomous Hill, probit-background response, log_e probit-background response, logistic-background response, and log_e logistic-background response.

The overall goal of benchmark dose analysis is to fit a mathematical function that best describes the dose-response relationship in the observable low dose region of the data to enable extrapolation to doses lower than those tested and/or interpolation among the test doses to identify a given response level. Benchmark dose analysis estimates the dose, termed a benchmark dose (BMD), for a specified level of benchmark dose response (BMR) observed. The BMR is defined as the level of change in the response rate. For example, a BMR of 10% would be equivalent to a 10% response rate of the endpoint of interest. The BMDS allows for the change in response rate to be calculated as one of added or extra risk; extra risk was selected for all analyses.

EPA (2008a) recommends a BMR value of 0.10 for use with dichotomous data sets for chemical hazards when deriving a point of departure value, although users may make data-specific determinations to select other values. To date, EPA has not developed guidance for the selection of BMRs when conducting microbial benchmark dose analysis. For this assessment, BMRs of 0.50, 0.10, and 0.01 were reported to allow for comparison of different model estimates at various points in the dose-response relationship. When used as inputs to the calculation of BMDs, these BMR values correspond to estimates of 50% lethality (i.e., LD_{50}), 10% lethality, and 1% lethality; the resulting BMDs would be written BMD_{50}, BMD_{10}, and BMD_{01}, respectively. The Benchmark Dose Limit (BMDL) is the 95% lower statistical confidence limit of the calculated BMD when the 95% lower confidence limit is applied to the estimated slope parameter value.

The BMDS software places a number of default restrictions on the slope and power values for specified models. These restrictions operate in the slope parameter for the log_e probit and log_e logistic models, where the value of the slope parameter is restricted to be equal or greater than one, and in the power term for the gamma, Weibull, log_e logistic, and log_e probit models, where the value of the power term is restricted to be greater than or equal to one. All default slope and power term restrictions were maintained in this analysis which prevents the modeling of supra-linear response in the low dose region.[3]

The background parameter was directly specified as zero for those models allowing this selection (i.e., $\log_e$ logistic, $\log_e$ probit, Weibull, and Weibull run as exponential) and the g parameter was specified as zero for the dichotomous Hill model to ensure model fits did not incorporate a background incidence of lethality.

Statistically valid model fits and BMD values for a given data set were identified using EPA guidance (US EPA, 2008a). For each model, two BMDS outputs describing the fit of an individual model to the data were evaluated: the global goodness of fit as measured by the model-calculated Chi-square p-value and the scaled residuals calculated for each dose group. The p-value reflects the overall goodness of fit, and a p-value of greater than 0.1 was used to identify a statistically valid fit. The scaled residual is the difference between the model estimate of response for an individual or dose group relative to its measured value. Scaled residuals closest to the BMD are of most concern for benchmark dose analysis as they indicate the fit of the model to the data in the dose region of greatest interest.

When comparing the fit of different models with valid statistical fits and equivalent restrictions, the lowest BMDL was selected when the calculated BMDLs were not within a three-fold range (US EPA, 2008a). However, if the BMDLs were within a three-fold range, the model with the lowest calculated value of the Akaike Information Criterion (AIC) was selected (US EPA, 2008a). The AIC value was calculated using the log-likelihood at the maximum likelihood estimates for the model parameters and the number of model degrees of freedom. The AIC value is more appropriately used to compare fits across models than the Chi-square p-values because these p-values cannot be used to compare the fits among different families of models or models with differing numbers of parameters.

2.2.2 ten Berge Models

The ten Berge model (ten Berge, 1986), also known as Concentration x Time model, is also available for evaluation using the BMDS. This model is appropriate for “data that identifies

[3] Historically, microbial dose-response models (i.e., exponential, beta-Poisson) have exhibited linearity in the low dose region and are mathematically precluded from displaying supra-linear behavior. Since the use of the power term default value of one maintains this limit on supra-linear behavior in the low dose region, the power term default that was originally recommended for use with chemical hazards was maintained.

concentration (or dose values) and durations of exposure (the time component, typically shorter-term durations), as well as responses (dichotomous response rates) to estimate a concentration-time-response relationship" (U.S. EPA, 2008b). The BMDS calculates a Chi-square p-value for each tested model and a Student t value is produced to determine the statistical significance of model coefficients (U.S. EPA, 2008b).

2.3 Calculation of Human Equivalent Dose and Human Equivalent Concentration

An interspecies extrapolation was conducted using the assumptions identified in Table 2 and the $BMDL_{10}$ value calculated using the best fitting mathematical model identified during the benchmark dose analysis. With the exception of generating a particle size distribution-specific pulmonary deposition rate using the Regionally Deposited Dose Ratio (RDDR) Model (U.S. EPA, 1994), the approach to calculate the HED and HEC followed that presented in U.S. EPA (2010b).

Table 2. Assumptions Used to Generate Human Equivalent Dose and Human Equivalent Concentration

Parameter	Value	Units	Source
Rabbit Pulmonary Deposition Rate	0.056	Unitless	Calculated with RDDR Model v. 2.3 (U.S. EPA, 1994) with Inputs of MMAD = 0.82 and GSD=1.53 (Data Source: Figure 3, Aerosol Exposure Report), Body Weight of 2850 g (Arithmetic Average of Body Weight on Days, 2, 9, and 16), and Minute Volume of 1.3 L (Average of Calculated Minute Volume from Days 2, 9 and 15)
Human Inhalation Rate	16	m^3/day	31 to <51 yrs, Mean Value, Table 6-1, Exposure Factors Handbook, EPA 2009
Human Deposition Rate	0.2	Unitless	Higher End of the Range of Human Depositional Values for 1 to 2 µM particles, Figure 6-6 , U.S. EPA 2004

GSD - geometric standard deviation
MMAD – median aerodynamic diameter

3. Results

3.1 Average Daily Dose

The following models exhibited acceptable fits as measured by p-values and scaled residuals at BMDLs of interest: Dichotomous-Hill, Log_e Logistic, and Weibull (run as Exponential) (Table 3). The calculated BMDL values were within a three-fold range when compared at $BMDL_{10}$ values (Table 4). Therefore, the model with the lowest AIC value was selected per existing EPA benchmark dose guidance (US EPA, 2008a). Accordingly, the log_e logistic model was identified as the best fitting model to the data.

This model calculated a $BMDL_{50}$ of 2,600 inhaled CFU and a $BMDL_{10}$ of 290 inhaled CFU (Table 4). Calculated BMDs and BMDLs for identified BMRs are provided in Table 4.

Table 3. Model Parameters, Standard Errors, 95% Confidence Limits, and AIC Values for the Statistically Significant Mathematical Model Fits for the Average Daily Dose Data

Model	Slope (Standard Error) 95% Confidence Limit	Intercept (Standard Error) 95% Confidence Limit	Power (Standard Error) 95% Confidence Limit	v and g Parameters (Standard Error) 95% Confidence Limit	AIC Values	Value of Scaled Residual Closest to BMD_{10}
Dichotomous-Hill (p=0.80)	5.88 (12.4) -18.4 to 30.2	-41.7 (85.9) -210 to 127	Parameter Not in Model	v: 0.568 (0.189) 0.199 to 0.938 g: Parameter Specified as 0	20.9394	-0.299
Log_e Logistic (p=0.71)	1 (*) *	-8.83 (*) *	Parameter Not in Model	Parameters Not in Model	18.9504	-0.36
Weibull (Run as Exponential) (p=0.48)	9.47E-5 (4.31E-05) 1.02E-05 to 0.000179	Parameter Not in Model	Power Set to 1	Parameters Not in Model	19.6503	-0.333

***Standard Error not calculated by BMDS due to recognized error in its calculation**

Table 4. The BMD and BMDL at Identified BMRs for the Average Daily Dose Data

	BMR = 0.50	**BMR = 0.10**	**BMR = 0.01**
Dichotomous-Hill	BMD_{50} = 1,700 $BMDL_{50}$ = 980	BMD_{10} = 920 $BMDL_{10}$ = 230	BMD_{01} = 600 $BMDL_{01}$ = 19
Log_e Logistic ***(Best Fitting Model)***	BMD_{50} = 6,800 $BMDL_{50}$ = 2,600	BMD_{10} = 760 $BMDL_{10}$ = 290	BMD_{01} = 68 $BMDL_{01}$ = 25
Weibull (Run as Exponential)	BMD_{50} = 7,300 $BMDL_{50}$ = 3,700	BMD_{10} = 1,100 $BMDL_{10}$ = 570	BMD_{01} = 110 $BMDL_{01}$ = 54

3.2 Total Aggregate Dose

The following models exhibited acceptable fits as measured by p-values and scaled residuals at BMDLs of interest: Dichotomous-Hill, Log_e Logistic, and Weibull (Run as Exponential) (Table 5). The calculated BMDL values were within a three-fold range when compared at $BMDL_{10}$ values (Table 6). Therefore, the model with the lowest AIC value was selected per existing EPA benchmark dose guidance (US EPA, 2008a). Accordingly, the log_e logistic model was identified as the best fitting model to the data.

This model calculated a $BMDL_{50}$ of 44,000 total inhaled CFU and a $BMDL_{10}$ of 4,900 total inhaled CFU (Table 6). Calculated BMDs and BMDLs for identified BMRs are provided in Table 6.

3.3 ten Berge

The dose-response data sets using the ADD and TAD dose metrics were unable to be successfully fit to the ten Berge model using the BMDS.

Table 5. Model Parameters, Standard Errors, 95% Confidence Limits, and AIC Values for the Statistically Significant Mathematical Model Fits for the Aggregate Dose Data

Model	Slope (Standard Error) 95% Confidence Limit	Intercept (Standard Error) 95% Confidence Limit	Power (Standard Error) 95% Confidence Limit	v and g Parameters (Standard Error) 95% Confidence Limit	AIC Values	Value of Scaled Residual Closest to BMD_{10}
Dichotomous-Hill (p=0.75)	4.27 (4.56) -4.66 to 13.2	- 42.9 (45.0) -131 to 45.2	Parameter Not in Model	v: 0.563 (0.190) 0.192 to 0.935 g: Parameter Specified as 0	21.3862	-0.352
Log_e Logistic (p=0.62) (Best Fitting Model)	1 (*) *	-11.7 (*) *	Parameter Not in Model	Parameters Not in Model	20.3447	-0.309
Weibull (Run as Exponential) (p=0.33)	5.24E-06 (2.37E-06) 5.87E-7 to 9.89E-06	Parameter Not in Model	Power Set to 1	Parameters Not in Model	21.549	-0.329

***Standard Error not calculated by BMDS due to recognized error in its calculation**

Table 6. The BMD and BMDL at Identified BMRs for the Total Aggregate Dose Data

	BMR = 0.50	BMR = 0.10	BMR = 0.01
Dichotomous-Hill	BMD_{50} = 38,000 $BMDL_{50}$ = 19,000	BMD_{10} = 16,000 $BMDL_{10}$ = 4,500	BMD_{01} = 9,100 $BMDL_{01}$ = 380
Log_e Logistic *(Best Fitting Model)*	BMD_{50} = 120,000 $BMDL_{50}$ = 44,000	BMD_{10} = 13,000 $BMDL_{10}$ = 4,900	BMD_{01} = 1,200 $BMDL_{01}$ = 450
Weibull (Run as Exponential)	BMD_{50} = 130,000 $BMDL_{50}$ = 68,000	BMD_{10} = 20,000 $BMDL_{10}$ = 10,000	BMD_{01} = 1,900 $BMDL_{01}$ = 980

3.4 Human Equivalent Dose and Human Equivalent Concentration

Using the ADD $BMDL_{10}$ value from the log_e logistic model, the calculated values for the HED and HEC were 1,400 inhaled CFU and 87 CFU/m^3, respectively. The values for the intermediate calculations of the interspecies extrapolation are provided in Figure 1.

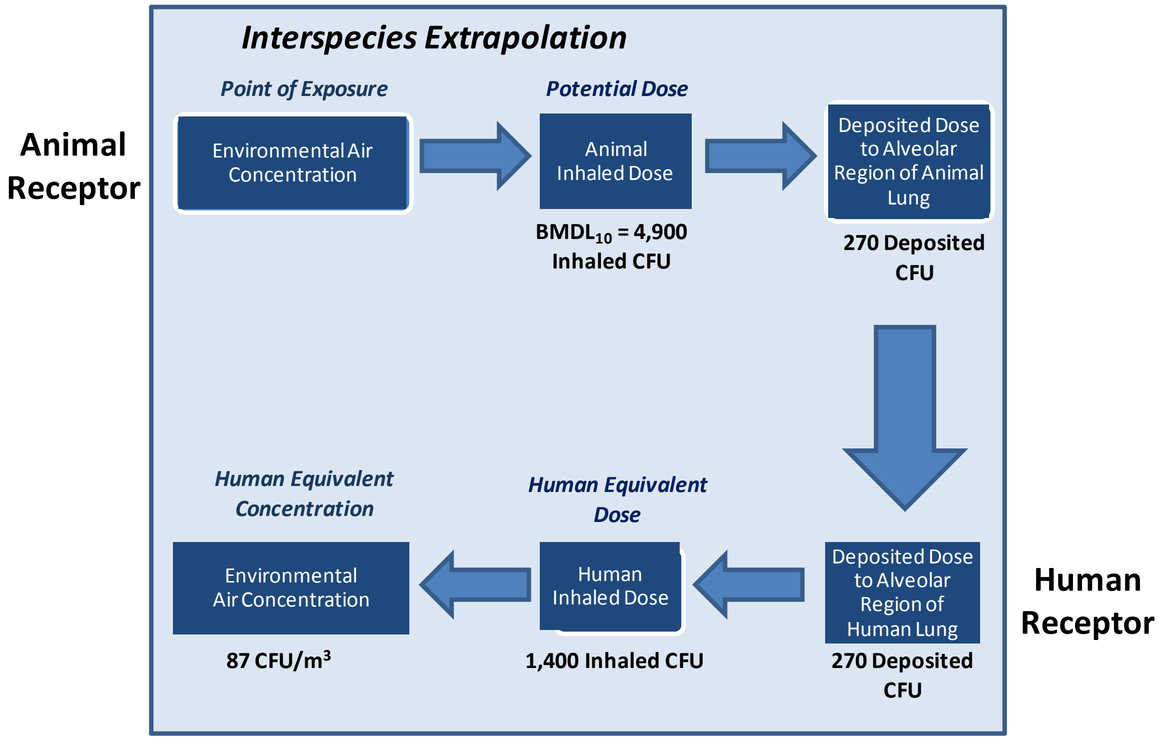

Figure 1. Interspecies extrapolation using BMDS results.

4. Discussion

Study 1078 represents the first reported study designed to derive the dose-response relationship of a multiple dose inhalation exposure to *B. anthracis* spores in the rabbit animal model. While the rabbit is recognized as a suitable animal model for *B. anthracis* disease modeling (Leffel and Pitt, 2006), few dose-response data sets suitable for analysis are found in the literature. Study 1078 is a continuation of the single dose acute study reported by Comer (2010) that built upon the single dose study design to include multiple doses while maintaining consistency with the protocol of the previous study.

The dose-response data for Study 1078 may also provide preliminary evidence that a threshold in the ADD may be present below which lethality is unlikely to occur in a healthy, adult male rabbit population. This is evidenced by of the survival rates of the two lowest dose groups. As shown in Table 1, no lethality was exhibited in the 100 CFU/animal/day nominal dose group and only 14% lethality was exhibited in the 1,000 CFU/animal/day nominal dose group. Further testing of levels between these two doses may allow modeling of this threshold value.

When comparing the benchmark dose analyses for data from these two studies as reported in Hines et al. (2011) for the first study and detailed in this report for Study 1078, there are preliminary indications that a discernable relationship may be present in the measured endpoint of lethality with the administered dose, number and timing of the administered doses (Table 7). The basis for this hypothesis is that the total aggregate dose $BMDL_{10}$ of 4,900 CFU is approximately 3.5 times the $BMDL_{10}$ of 1,400 CFU from the single dose acute study and the total aggregate dose $BMDL_{50}$ of 44,000 CFU is approximately 3.4 times the $BMDL_{50}$ of 13,000 CFU from the same single dose acute study. This implies that Haber's Law, or the microbial equivalent of the independent action hypothesis[4], may not hold for *B. anthracis* exposures in rabbits for the tested dose ranges and timing of doses evaluated in Study 1078 and Comer (2010). Haber's Law, originally derived during the early 1900's for acute inhalation exposures to volatile chemicals, describes a dose-response relationship whereby the product of concentration (or dose, in this case) and time (or, number of doses as was tested here) is the sole determinant of

[4] One aspect of the independent hypothesis for microbial effects predicts that the length of time of dose administration (e.g., number of doses over which total dose administered) should not affect the probability of response (Rubin, 1987).

toxicity. The law also assumes that each element, concentration and time, contributes equally to the toxic effect. However, this law does not hold for acute inhalation exposures to even a small set of tested volatile chemicals (ten Berge, 1986). The model developed by ten Berge (ten Berge, 1986) allows for an evaluation of differing exponents (i.e., other than one) on the concentration x time equation terms. The fit of the study data to the ADD was evaluated using the BMDS and the ten Berge model was unable to converge with the data and provide reportable results. However, further analysis of this potential relationship using other software or models is still desirable to definitively assess the potential for this relationship. For example, preliminary work is being conducted using dynamic dose-response response modeling as a mechanism to capture the time dependence of dosing on response (Mayer et al., 2010). Given the limited availability of dose-response data sets for *B. anthracis* exposures, techniques to model the relationship between dose, concentration, number of doses, and exposure duration may provide useful information to further define the hazard posed by acute and short-term exposure scenarios to low levels of *B. anthracis* contamination.

Table 7. Comparison of Single Dose and Multiple Dose Study Results for Rabbit Inhalation Exposures to *Bacillus anthracis* (Ames) Spores

	BMR = 0.50	**BMR = 0.10**	**Other Reported Measures or Study Notes**
Single Dose Study Results			
Hines et al., 2011 (Dichotomous-Hill Model)	**BMD_{50} = 52,000** **$BMDL_{50}$ = 13,000**	**BMD_{10} = 5,700** **$BMDL_{10}$ = 1,400**	**NA**
Gutting et al., 2008	**NA**	**NA**	**When Dosed with up to 3,360 Inhaled *B. anthracis* CFU, 4 out of 4 Rabbits Survived the Exposure**
Zaucha et al., 1998 (Probit Model)	**BMD_{50} = 105,000**	**NA**	**NA**
Multiple Dose Study Results			
Study 1078 (ADD, Daily Dose over 19 Days) (Log_e Logistic Model)	**BMD_{50} = 6,800** **$BMDL_{50}$ = 2,600**	**BMD_{10} = 760** **$BMDL_{10}$ = 290**	**NA**
Study 1078 (TAD, Aggregate Dose over 19 Days) (Log_e Logistic Model)	**BMD_{50} = 120,000** **$BMDL_{50}$ = 44,000**	**BMD_{10} = 13,000** **$BMDL_{10}$ = 4,900**	**NA**

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