IACUC and Veterinary Considerations for Review of ABSL3 and ABSL4 Research Protocols

Curtis Klages

Animal Care Operations, Division of Research, University of Houston, Houston, Texas, USA.

*Corresponding Author: Curtis Klages, University of Houston, Division of Research, University of Houston, Houston, TX, USA. E-mail: aggiedvm2k@gmail.com.

Abstract

With the recent upswing of infectious disease outbreaks (coronavirus, influenza, Ebola, etc), there is an ever-increasing need for biocontainment animal use protocols to better address the research of emerging diseases and to increase the health of both animals and humans. It is imperative that we as a research community ensure these protocols are conducted with the utmost scrutiny and regulatory compliance for the welfare of the animals as well as the health and safety concerns of the individual conducting these studies. Both the welfare of the animals and the health and safety of the research staff must be balanced with the integrity of the science being studied. Even prior to reviewing biocontainment protocols, the research stakeholders should have professional and collegial interactions across all levels of the proposed project. These stakeholders should include the attending veterinarian, the principal investigator, the sponsor, and any organic institutional health and safety assets (environmental health and safety, occupational health, biosafety personnel, medical personnel, facilities operations and maintenance, etc). At most institutions, these stakeholders are members of the Institutional Animal Care and Use Committee and may not possess the necessary tools to properly assess an Animal Biosafety Level 3 and 4 animal use protocol. It is the goal of this article to review some basic concepts of biocontainment, discuss critical communications and preapprovals, clinical observations, medical interventions and supportive care, scientific and study endpoints, euthanasia criteria, animal manipulations, documentation, training, emergency response and contingency plans, security, and decontamination and provide a scenario-based and informative thought-provoking process Institutional Animal Care and Use Committee members and veterinary staff may consider during Animal Biosafety Level 3 and 4 protocol review. These topics will enhance the ability of all stakeholders to balance the protection of the people with the integrity of the science and ultimately the welfare of the animal.

Key words: ABSL 3; ABSL 4; animal research; biocontainment; biohazard; containment; livestock

Introduction

Three B’s (Biocontainment, Bioexclusion, Bioprotection)

In reviewing almost any animal use protocol, especially those involving infectious diseases, 3 basic concepts need to be addressed: biocontainment, bioexclusion, and bioprotection, or the 3B’s.1

In the simplest terms, one must understand the concept of keeping "stuff" in (biocontainment), keeping "stuff" out (bioexclusion), and keeping "stuff from you" (bioprotection). It may be a fine line between “in and out,” but it is imperative to understand this concept when reviewing almost any infectious disease protocol, especially those in Animal Biosafety Level 3 and 4 (ABSL3 and ABSL4). Biocontainment is keeping “stuff” in, or in other words preventing the release of a pathogen or agent. The Biosafety in Microbiological and Biomedical Laboratories (BMBL) delineates 4 levels of biocontainment, ABSL1 through ABSL4, and outlines the requirements for each level and the necessary equipment and facilities needed to keep stuff in. Bioexclusion essentially is keeping stuff out or prevention of pathogen entry. This concept is identified in almost any quarantine facility in which the inherent goal of quarantine is to keep...
what is inside the quarantine away from the main vivarium. Finally, bioprotection is the concept of keeping the “stuff from you” or developing a risk assessment and methodology for prevention and control of the pathogen to prevent infections. This is accomplished at many facilities in a combination of ways. These include requirements for personal protective equipment (PPE), facility design, institutional environmental health and safety protocols, engineering controls, and appropriate medical and occupational health oversight.

During ABSL3 and ABSL4 protocol review, some questions may need to be answered regarding the 3B’s. Be advised this list is not all inclusive, and many are institute and facility dependent. Some of these are outlined below.

Regarding biocontainment, what is the caging construct? Is the caging primary or secondary containment? Is the caging construct open or closed? What facility constraints are in place? What is the agent? Transmissibility of agent? Risk level of agent? Review of the BMBL facility PPE requirements and infectious agent definitions and protections are critical for individuals to truly assess a protocol within any biocontainment level. Most biocontainment facilities are built on a “box-in-a-box” concept, where the innermost areas contain the ABSL3 or ABSL4 agent, and another “box” keeps the agent in the inner box (Fig. 1).

Regarding bioexclusion, what precautions are in place to keep the agent out? Agent transmissibility? Facility design to exclude agent(s)? Biosafety level?

And lastly, in terms of bioprotection, what about agent? PPE? Facility design/ABSL? Pre-conditioning of staff? Training of personnel? Occupational safety requirements?

Basic Biocontainment Protocol Designs
Most biocontainment protocols can be summarized into 3 very basic protocol designs. These are basic science or pathogenesis, therapeutics, and prophylactics. Usually, the initial biocontainment study starts with an unknown agent of some sort. These studies focus on what the agent is, what the agent does to its host, how infectious the agent is, how the agent enters the body, and the overall lethality of the agent. Some of these research studies also evolve into options for battling the agent and hindering or stopping its progression. The next level of protocols involves options for therapy against the infectious agent. These studies typically are testing some sort of countermeasure against the agent. These therapeutics may include antibody therapy, anti-viral therapy, or any other item noted to stop the progression of the agent during the basic science or pathogenesis studies. The last protocols are research studies to assist in preventing the disease process, or prophylactics. These include vaccines or other therapeutics that prevent the disease process of the agent. Many of these protocols are conducted under good laboratory practice and Food and Drug Administration (FDA) Animal Rule conditions. The Animal Rule allows for drugs and therapeutics to be approved when human efficacy studies are not ethical or feasible. Many therapeutics and biologics have been approved through the Animal Rule pathway.

Critical Communications/Preapproval Review
As noted earlier, it is critical that research stakeholders have professional and collegial relationships across all levels of the proposed project. These relationships will allow for a robust pre-review of the overall project. Going back to Figure 2, stakeholders represent each side of the triangle, with each of them having a critical area of the protocol. Some stakeholders are experts in protecting the people while others focus on the integrity of the science, with hopefully all stakeholders balancing the welfare of the animal(s) on the study. Each study should be considered holistically and from multiple angles to ensure the project meets the regulatory and safety requirements for an ABSL3/ABSL4 animal use protocol. During the prereview, some questions may need to be addressed. These include: Is this the right animal model for the study? Is this an FDA Animal Rule study? Does the agent need to be modified for any reason? What are the veterinary aspects of pain and distress for the animals? What is the agent and its associated biosafety level? Who is performing/conducting the animal manipulations during the approved protocol? Are these individuals trained and qualified to work at the level of biocontainment? What is the facility biosafety requirements for entry into the suite? What PPE is available? Is the facility able to contain the agent? As noted, previously, this list of questions is not exhaustive but may induce a thought-provoking process in reviewing an ABSL3 and ABSL4 protocol.

Clinical Observations/Protocol Procedures
ABSL 3 and ABSL4 animal use protocols tend to be more complicated than a non-containment animal use protocol. Many of these differences deal with the agent being tested, the species of animal, the biocontainment facility, the complexity of the research, the expertise and training of the research staff and veterinary personnel, and the PPE required to accomplish the approved protocol tasks.

One question that should be addressed early in the protocol review process is how approved protocol clinical observations are going to be accomplished and verified. Many biocontainment facilities have the capabilities of both physical and digital methods (via a camera or window) of accomplishing these observations or “rounds.” With some of the more lethal agents and the course and severity of disease, these clinical observations may be more frequent and may include 24-hour observation of the animal to prevent undue pain and distress. However, some institutions may not permit 24-hour access to the containment suite itself due to the human safety factors or during required dwell time post decontamination procedures. These individuals who
need to be in the containment suite and are studying a highly lethal disease put themselves at an elevated/increased risk when entering the containment suite during the dark phase of the light cycle. Some institutions mandate a 2-person rule when entering an ABSL3 or ABSL4 containment suite, thus potentially putting more individuals at risk.

A stringent review of the animal use protocols must also account for the animal’s welfare during the conduct of the study. Institutional Animal Care and Use Committee (IACUC) and veterinary personnel should be cognizant of the timing of these clinical observations so as not to affect the nature of the research and the overall welfare of the animal. In other words, if an animal is near the endpoint or the approved euthanasia clinical score of the study, will checking on the animal during the dark phase of the light cycle cause undue stress and inadvertently push the animal into the criteria for euthanasia? Some approved protocols may also implant and use telemetry to assess an animal from the non-containment area of the facility to prevent disturbing the animal during its sleep cycle.

The use of telemetry implants for an ABSL3 or ABSL4 study will add a layer of complexity to the animal use protocol. During a protocol pre-review, IACUC and veterinary personnel should address some of the following questions regarding telemetry: What bioparameters is the principal investigator (PI) attempting to collect? What is the overall goal of the project? Have the end-point criteria been addressed? Who is performing the implant surgery(ies)? How will surgical instruments be sterilized? How long will the post-surgical recovery be, and when can the animals go into the suite? Will the animals be tethered? Who monitors the telemetry output? How will the telemetry data be delivered? These questions are best answered well before the protocol reaches the IACUC to better address the welfare of the animal(s).

Imaging devices may add even another layer of complexity to an ABSL3 or ABSL4 study. Digital imaging such as radiographs, bioluminescence imaging, and Positron Emission Tomography–Computed Tomography are just some of the possibilities. Are the images real-time? Does the animal need to be sedated or restrained for imaging? Who reads the images and how do they get to the reviewer from the containment suite? Is the equipment able to be in the containment suite? Are the devices able to be decontaminated, and how does this affect the manufacturer’s warranty? Is the facility designed for this imaging capability regarding shielding or other bioexclusion issues to prevent the imaging modality from contamination?

Interventions/Supportive Care
IACUC and veterinary staff need to be aware of each and any possible intervention when reviewing any of the 3 basic ABSL3 and ABSL4 protocols: pathogenesis, therapeutics, and prophylaxis. The interventions within these 3 protocols may include common veterinary medical treatments, any supportive care to the animal, and/or any other medical countermeasure to aid the research project. All members of the research staff and veterinary team need to understand the research goal and animal model to review any possible adverse effects the intervention may have on the overall goal of the project. In other words, regarding a pathogenesis or basic science study, an investigator may not want any intervention(s) to fully characterize the agent’s pathogenesis.

Animal care interventions per the FDA Animal Rule are divided into 3 categories based on the rationale for their use: (1) intervention as part of adequate veterinary care, (2) intervention as supportive care to mimic the human clinical scenario, and (3) intervention to permit the manifestation of the disease or condition of interest for the purpose of model development.

Triggers for intervention should be established for use in animal efficacy studies when needed (eg, post-exposure prophylaxis and treatment indications). The trigger for intervention should be identified based on the natural history studies. For a post-exposure prophylaxis indication, a trigger for intervention should be defined to ensure drug administration within a reasonable time frame after exposure to the challenge agent and prior to the onset of the disease or condition of interest. The time frame should be justified with respect to administration of the drug to humans. Animals cannot simulate the health-seeking behavior manifested by humans; therefore, a clearly defined trigger for intervention for a treatment indication will ensure treatment is not initiated until the disease or injury process is established. If signs observed in the animal model closely resemble those in humans and are predictive for the disease, they may serve as the trigger for intervention.

Some basic definitions of supportive care should also be understood and agreed on before an ABSL3 or ABSL4 protocol is approved. These are the definitions of standard veterinary care, supportive care, and critical care. These are best defined from the critical to the less severe. Critical care usually entails saving life, limb, or eyesight. Supportive care gets a bit trickier because the reviewers need to understand what effects, if any, medical supportive intervention may have on the research being conducted. These supportive care measures may be identified during a literature search or during an initial basic science study. Some therapeutic protocols also need to address any possible parallel human interventions that may already be known. Standard veterinary care is best defined as most normal day-to-day clinical veterinary care, feed, bed, and enrichment. The timing of each of these levels of intervention within a given ABSL3 or ABSL4 study needs to be considered so as not to affect the overall goal of the project as well. Each of these supportive care levels/interventions may have a different definition(s) within the 3 basic containment protocol designs. In other words, one intervention or supportive care modality may not necessarily be warranted for one study or the other.

IACUC reviewers need to also keep in mind regulatory documents when reviewing ABSL3 and ABSL4 protocols. The Animal Welfare Act (AWA), the Guide for the Care and Use of Laboratory Animals, and the Public Health and Safety Policy mandate medical treatments necessary to prevent unacceptable pain and suffering, including euthanasia, is the sole responsibility of the attending veterinarian (AV). Discretionary medical treatment may be carried out based on consensus agreement between the PI and the AV. Interventions and possible supportive care for all animals on the study are a pivotal conversation during the pre-review process. Once agreed on, these interventions and supportive measures should be included in the approved animal use protocol.

Study/Scientific Endpoints
ABSL3 and ABSL4 protocols, as well as any other animal use protocols, requires animal experimentation protocols to include clearly defined experimental/study and humane endpoints. Well defined endpoints ensure animals do not experience unnecessary pain or distress in the completion of one’s scientific objectives and are consistent with the US Government Principles for the Utilization of Animals
Experimental endpoints are typically defined as the time at which an experiment will end due to completion of the scientific objectives. Experimental endpoints are dependent on the goals of the experiment. They should be clearly described in the animal use protocol, justified for experimental reasons, and then strictly adhered to during the study.

Humane endpoints are typically defined in most protocols where animals may be euthanized prior to the defined experimental endpoints due to the health of the animal. For example, if an agent being tested causes unexpected toxic effects to the animals, they should be euthanized early; if animals developed ulcerative tumors or severe abdominal distension before lung metastasis, then the study should be terminated early. Remember the stress and potential for distress increases as the animal reaches these criteria and may be defined as a pre-moribund state. IACUC and veterinary personnel should strive for euthanasia at the earliest applicable endpoint.

Clinical Scoring/Euthanasia Criteria

IACUCs and veterinary staff need to fully understand the animal model (as much as currently known) as well as the infection and disease progression of the agent being studied. Understanding the agent and animal model should be balanced with the overall goal of the study. Most pathogenesis studies tend to be extremely lengthy, and the information and data collected become a foundation for future studies. It is these pathogenesis studies, or basic science studies, which allow all protocol members, especially the veterinary staff, to learn about the agent and build a portfolio of opportunities for future possible intervention(s) to better guard the welfare of the animal.

These initial studies provide an opportunity to develop a clinical scoring system to assist with clinical observations of the animals. These studies are usually completed without intervention or supportive care and in many facilities are called “death as an endpoint,” or USDA category E studies. Building a clinical scoring sheet from these initial studies may identify critical clinical parameters that may be used to select euthanasia criteria in future studies or an area where an intervention or supportive care may be tested. Most clinical scoring sheets are species and agent specific and usually include general appearance, body condition, natural behavior, and responsiveness. Each area on a clinical scoring sheet usually has a specific numerical value that increases as the disease worsens and the animal presents with more severe clinical signs. For example, a normal clinical score may be a 1, whereas an animal in severe distress may be a 5 and warrant a specific pre-determined action to reduce pain and/or distress to the animal. Each clinical scoring sheet should have a legend somewhere on the sheet that signifies an action to be taken at a specific clinical score. The action may include addition of more frequent monitoring, contact of the PI or veterinary staff, a possible intervention or supportive care method, and possible euthanasia of the observed animal. Each clinical scoring sheet should be species specific and have a different scoring range. Some clinical scoring sheets have a set point within each category that may initiate immediate euthanasia of the observed animal. As studies progress, scoring sheets should be re-assessed so modifications can be made to potentially lessen the stress on the animal by possibly inserting an earlier humane endpoint(s). Not all ABSL3 and ABSL4 studies warrant a scoring sheet; some projects use a single clinical sign to identify the endpoint for the animal. For example, if it was found in a pathogenesis study that a 15% weight loss in an animal signifies the animal will not survive, then this becomes the initial criteria for euthanasia and each animal having a >15% weight loss will be immediately euthanized. The BMML is another good source of clinical information to start building a clinical observation scoring sheet if the agent is a known entity or has characteristics of a known entity.

Animal Manupulations in ABSL3/4, Small vs Large Animals

Biosampling or samples collected from the research subjects on study are another item on an ABSL3 or ABSL4 protocol that need addressing. Within each of the 3 basic biocontainment animal use protocols, a reviewer needs to understand the research goals and animal model to better assess the number of “samples” each animal will need to donate. In other words, how much biosample is to be taken from each animal? How is this accomplished? Under anesthesia? How will repetitive anesthesia affect the integrity of the study? What is the volume of the sample needed? How often? Would a serial sacrifice study be better warranted to accumulate the number of sample(s)? What is the species of animal? As projects progress through the 3 basic biocontainment studies, the volume and number of samples tend to decrease, so a researcher should get as much data and sample as possible to help “learn” more about the agent being studied during the earlier stages of the study/studies.

Safety of the research personnel also becomes a factor when collecting biosamples, especially when dealing with larger animal species. How often will the samples be taken? When will the samples be taken? Will there be a biosampling schedule such as every 6 hours? How will an institution’s safety personnel mandate a biosample collection time during the dark/light cycle? How, when, and by whom will the terminal biosamples be taken, that is, euthanasia and necropsies?

Documentation/Animal Health Records

Documentation of all aspects of a research project is another area of ABSL3 and ABSL4 protocol review. This documentation ranges from entry and exit paperwork to the IACUC protocol itself, to the research and veterinary animal health records.
This documentation can be a critical aspect of any study and ultimately a possible FDA approval of a drug or therapeutic. A reviewer needs to be cognizant of how each area of the study is documented, where these documents are located in the suite for daily use, how the document and data integrity are insured, and who has access to manipulate and input the data. Many government regulatory agencies mandate good record keeping, ensuring the proper oversight of any animal use protocol.\(^5,7\)

**Training and Certification of Personnel: Protocol Procedures**

**Figure 2** denotes the “protection of the people,” where an institution’s health and safety personnel become very important. A reviewer of any IACUC protocol, especially those in an ABSL3 and ABSL4 environment, should review the training records of the personnel who are going to complete the animal manipulation(s) on the proposed project. Extensive experience in biocontainment teaches that there are 3 things a person in a biocontainment environment should trust: their training, their equipment, and the people next to them. Most institutions with biocontainment suites have an internal program that can train veterinary and research personnel to enter and exit biocontainment suites.

It is repetitive training that builds trust among the biocontainment personnel. Most of this training deals with safety protocols, entry and exit procedures, and practical use of equipment with the appropriate level of PPE attire for the biocontainment suite. Taking a biosample from an animal is not much different across the biocontainment levels; however, the added layers of PPE (eg, ABSL4 gloves) may make obtaining the biosample much more difficult. Training in a mock laboratory or other training environment becomes critical for the overall success of the personnel and, ultimately, the study.

One question for an institution to consider is who signs off on the training and when can a person ultimately go “hot.” Some facilities have the safety and training departments sign off, whereas others allow the biocontainment-qualified veterinary staff to approve entry into biocontainment and the approved animal manipulations. Other facilities only allow fully trained ABSL3 and ABSL4 veterinary staff to enter the suite and complete the required and approved animal use protocol manipulation(s).

Facilities may also limit the number of staff in the biocontainment suite at any one time. This limited number of personnel may be due to personnel safety procedures in an emergency, a limited or fixed number of air lines, the amount of PPE for the staff, availability of ABSL4 suits, or the overall biocontainment experience of the staff. Institutional health and safety departments may also limit the number of total hours a person may spend in the suite at any one time, especially in an ABSL4 environment.

**Emergency Response/Contingency Plans**

Regulatory agencies mandate an emergency response or contingency plan for any facility with an animal use program.\(^5,7\) Review of any animal use protocol, including those in any biocontainment environment, should address what the procedures are for the animals on the project in the case of an unexpected physical (weather, facility, power outage) or personnel (a fall or injury) emergency. Due to the complexity of a biocontainment suite, this becomes critical for any ABSL3 or ABSL4 environment.

Part of a protocol review should identify any possible emergency that may occur during the conduct of any animal use protocol. Situations change when an emergency occurs either within or outside the biocontainment suite due to some of the complicated entry and exit procedures. For example, most exit procedures from an ABSL4 suite include a 6-to 8-minute chemical decontamination shower and then a personal shower before truly exiting the suite. Most health and safety personnel will outline and train on these exit procedures during the initial entry training. Some institutions also conduct annual emergency exit training for all approved personnel who can enter the suite. This annual training should be coordinated with the local emergency response teams, such as a fire department or emergency medical teams.

**Security**

As with any animal facility, security is a prime concern. It is prudent for institutions to have an adequately informed and trained security staff. This security staff needs to be fully knowledgeable of the inner workings of the building and who does and who does not have access to the animals. Each institution needs to address who controls the access to not only the biocontainment suite but also the animals. Just because a person has access to the suite does not give them access to the animals. A detailed security plan should be part of any research facility, especially those with an ABSL3 and ABSL4 laboratory. The security force should be one of the stakeholders at the table when having a pre-protocol review and reinforces the protection of the people aspect for protocol review.

**Decontamination/Cross-Contamination Prevention/Room Turnover**

Due to the limited space in many ABSL3 and ABSL4 laboratories, some PIs either share space or wait for a room to open for their project. Some of these decisions rest with the facility design whereas others rest with the veterinary staff. Facility design may allow for only 1 study, 1 species, or 1 agent at a time in a room to help prevent agent cross-contamination. However, some caging constructs allow for multiple agents or multiple species to be studied, for example, individually ventilated caging for rodents. These decisions can be discussed and hopefully solved at a pre-protocol meeting to help the PI’s research move forward.

**Possible Questions for a Pre-Protocol Review (“Thinkables”)**

As noted earlier, a pre-protocol review session between all stakeholders is critical to the success of the project. The following paragraphs may assist reviewers during a pre-review of an ABSL3 or ABSL4 protocol. These questions are broken into the three basic biocontainment protocols in a scenario-based format. Each scenario also has clinical protocol observation and biosampling consideration sections. Some questions overlap across each basic biocontainment protocol but allow for the questions and answers to build on themselves as the studies progress. Also, this list of questions may not apply to every situation but is intended to spark dialogue between the stakeholders. Each scenario is given a functional and CDC-accredited ABSL4 laboratory and
a fully qualified ABSL4 veterinary care staff to support each scenario. These scenarios and questions are an attempt to create a dialog between stakeholders, to keep the “red dot” in Figure 2 in the center, and to come to a consensus on the proposed ABSL3/ABSL4 project.

**Scenario #1: Natural History/Pathogenesis**

Dr B requests to put a novel ABSL4 agent (filoX) in an animal model. An outbreak has occurred with confirmation of filoX, causing significant clinical disease and death in humans. She intends to use guinea pigs and then non-human primates. She would like to use equal numbers of males and females and to infect via the intramuscular (IM) route.

**Clinical Observations “Thinkables” for Scenario #1**

Have pre-infection clinical behavioral assessments of the animals been completed?

Have the Veterinary staff worked out the animal quarantine vs acclimation? How often will this be? How long will this be? Will this be in or out of containment?

What if the lethality in IP (guinea pig) vs IM (non-human primates) injections is different?

Are there possible species differences with filoX?

What are the humane and scientific endpoints? What are these based on?

What are the criteria for euthanasia? What is this based on?

Is there a possible starting point for virus and species specific “scoring” sheets?

How long do you use these “scoring sheets”?

Are there possibilities of animal observations without entering the suite?

What interventions/supportive care are possible by veterinarian staff? When will these be provided?

Who makes the “call” on euthanasia?

How will the female menses affect scoring?

Will social housing be allowed?

What happens if FiloX is not uniformly lethal in guinea pigs or non-human primates?

**Biosampling for Scenario #1**

Were pre-exposure biochemical evaluations (baseline) completed by species?

How often and how many biosamples are needed by species?

What about rotational sampling across each species?

By what route are the samples being collected?

Is anesthesia needed to collect the biosample(s)?

Are there anesthesia concerns?

What about terminal samples?

Are there necropsy concerns? Appropriate pathology staff onsite?

How many samples need to be taken? Storage of these samples?

Can a serial sacrifice scheme be acceptable?

What about carcass disposal?

How are the samples or data getting out of containment?

**Scenario #2: Therapeutic**

Dr S (Dr B’s research colleague) has taken data from Dr B’s initial study and now has a possible therapeutic (a monoclonal antibody) that showing promise in cell culture in preventing infection of filoX. She has a moderate budget and requests to use guinea pigs and non-human primates with equal numbers of males and females.

**Clinical Observations for Scenario #2**

Were pre-infection behavioral and biochemical assessments completed?

Do the animals need quarantine or acclimation to the suite?

How often and when is therapeutic given?

How long is therapeutic given?

What route is the therapeutic given (how given to animal)?

Will a clinical scoring be used for observations?

Will the clinical scoring sheets be used beyond the control animals?

Who completes the clinical scoring sheets? Are they trained?

What interventions are allowed beyond the tested therapeutic?

Have the endpoint criteria been established from the initial study?

Are there criteria for euthanasia?

What happens after the controls succumb to infection?

What is the USDA pain level?

**Biosampling for Scenario #2**

Were pre-exposure biochemical evaluations (baseline) completed for each species?

How often and how many biosamples are needed by species?

What about rotational sampling across each species?

By what route are the samples being collected?

Is anesthesia needed to collect the biosample(s)?

Are there anesthesia concerns?

What about terminal samples?

Are there necropsy concerns? Appropriate pathology staff?

How many samples need to be taken? Storage of these samples?

What about carcass disposal?

How are the samples or data getting out of containment?

**Scenario #3: Vaccine or prophylactic**

Dr K (Dr B and Dr S’s post-doc) has developed a possible vaccine candidate for filoX that shows protection from infection. She
would also like to use guinea pigs and non-human primates with 2 different vaccine schedules.

Clinical Observations for Scenario #3
Were pre-infection behavioral and biochemical assessments completed?
Do the animals need quarantine or acclimation to the suite?
What is the vaccine schedule? How many doses?
Will these vaccines be given in or out of the ABSL4 suite?
What route is the vaccine given (how given to animal)?
Will a clinical scoring be used for observations?
Will the clinical scoring sheets be used beyond the control animals?
Who completes the clinical scoring sheets? Are they trained?
Is the observer blinded to the vaccine groups?
What interventions are allowed? Are interventions allowed beyond the control animals?
Have the endpoint criteria been established/reviewed from the initial and therapeutic studies?
Are there criteria for euthanasia?
What happens after the controls succumb to infection?
How long do the animals survive?
What is the USDA pain level?

Biosampling for Scenario #3
Were pre-exposure biochemical evaluations (baseline) completed by species?
How often and how many biosamples are needed by species?
By what route are the samples being collected?
Is anesthesia needed to collect the biosample(s)?
Are there anesthesia concerns?
Do these concerns conflict with the clinical scoring sheet?
What about terminal samples?
What is the disposition of the survivors?
Are there necropsy concerns? Appropriate pathology staff?
How many samples need to be taken? Storage of these samples?
What about carcass disposal?
How are the samples or data getting out of containment?

Conclusion
This article reviewed numerous topics in an attempted to provide IACUC and veterinary personnel some basic tools to help in the review and conduct of an ABSL3 and ABSL4 animal protocol. These topics included the concepts of biocontainment, bioexclusion, and bioprotection. Most ABSL3 and ABSL4 animal use protocols are categorized into 3 basic designs—pathogenesis or basic science, therapeutics, and prophylactics. Throughout the process of review of an ABSL3 or ABSL4 animal use protocol, all institutional research stakeholders should have very professional and collegial relationships throughout the review, approval, and conduct of a proposed project. Veterinary and research staff should also identify any supportive care, interventions, and/or steps to ensure the welfare of the animal in the studies and delineate these steps in the animal use proposal. Research staff, especially the PI, and the AV need to also address study, scientific, and/or humane endpoints, preferably in the pre-review process to better assist the IACUC in the approval of the protocol. Along the same lines as endpoints, the AV and PI should be in close contact throughout the conduct of the study to formulate clinical scoring criteria and readdress these criteria as the study progresses to look out for the welfare of the animal. This article also provided 3 step-wise scenarios within the basic ABSL3 and ABSL4 protocols designs with lists of questions to help stimulate conversation among all stakeholders. In reviewing an ABSL3 or ABSL4, animal use protocol should always balance the integrity of the science with the protection of the people and ultimately the welfare of the animal.

Acknowledgments
I would like to acknowledge the numerous people who allowed me the distinct opportunity to work and make a veterinary career in and out of biocontainment. These individuals are too numerous to mention but span from my time in the military as well as my current duties in academia. These folks are truly mentors and giants in the industry, and I thank them for mentoring me. I only hope to continue mentoring the future personnel, as was done for me, who wish to work in the biocontainment environment.

Potential conflicts of interest. No reported conflicts.

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