A Case Study for Animal Care Post Approval Monitoring

The 55th Annual Biosafety Conference of the American Biological Safety Association-Orlando, Florida
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October 20, 2012

Introduction

This presentation is designed to inform the audience of the considerations for the determination of containment for experiments involving a recombinant virus and the factors involved with the assessment of risk. In addition, you will learn why it is essential to involve your animal care staff and veterinarians in the review of the experimental scope of work.

Key Considerations in the Review Process

- Key Points-Biosafety
  - Protect public health, primarily with regard to animal care workers to biological hazards
  - Document appropriate control measure by use of a biosafety plan-required in California
  - Protect environment
- Key Points-Animal Care
  - Quarantine/Need to separate vulnerable species
  - Use of Appropriate Biological Containment
  - Humane animal care policies

The Agent-Rabies Virus

- The agent in use is Rabies Virus. In particular, the virus has been modified with a GFP (green florescent protein) insert for use in imaging.
- The tropism of the virus is nervous tissue and its especially promising for brain and neural tissue studies.

The Agent-Rabies Virus

- Rabies virus is classified by National Institutes of Health, Guidelines for the Use of Recombinant DNA Molecules at Risk Group 2
- The recommended Centers For Disease Control and Prevention Biosafety Level is BSL 2 for cultures, BSL 3 for high concentration, aerosol droplet generation

The Agent-Rabies Virus

- The virus used in this experiment was a glycoprotein–deleted variant of the SAD-B19 strain of rabies virus encoding GFP, known as SAD△G-GFP.
- This vector can be used to trace neural activity and can be viable for weeks.

The Agent-Rabies Virus

- SAD△G-GFP and other variants allow the investigator to trace neural circuitry.
- This vector can be used with transgenic mice for direct gene expression.
- Several genes have been amplified, mostly marker and Cre-recombinase.

The Agent-Rabies Virus

- The problem with rabies virus is the pathogenicity with mammalian species-The virus is very effective in infecting its host but the route of exposure of the virus can be either through percutaneous injury such as an animal bite or the inhalation of droplets
- This experiment's aerosol generating procedures are regulated by the *California Airborne Transmissible Disease Standard*, 8 CCR 5199.

The Agent-Rabies Virus
Replication Competency

- The PI said that the replication competency of the virus was possible by an inactivation procedure
  - however, the scientist who devised the procedure and created the viral vector did not have a validation test to ensure replication deficiency, nor did he state that his organization performed this experiment under a minimum containment level of Biosafety Level 2.

Animal Care Considerations

- The use of rabies virus was an obvious concern because of the nature of the virus and several mammalian species that could be a reservoir.
- The rabies virus could be used in vitro in a tissue culture hood (preparation of syringes, titers of virus) at BSL2.

Animal Care Considerations

- The rabies virus could be experimentally performed in vivo in a ABSL 2 procedure room within the animal care facility.
- However, an issue of concern arose with regard to the housing and examination of the animals.

Animal Care Considerations

- The investigator had an imager and camera in her lab that was not part of the animal facility-it was in an adjacent laboratory building.
- The animals could be safely brought out to the imager and be photographed with the camera using a cloaked cages and a cart.

Animal Care Considerations

- Infected animals could not be brought back to the vivaria; animal care policies stated that animals that were brought out of the “barrier” must be sacrificed and not brought back.
- The animals were part of a dose tolerance study where the intensity of the imaged animals had a direct bearing on the research related results and sacrificing the animals would mean the investigator would only get a snapshot of the imaging process.

Biosafety Committee Considerations

- This experiment would be under the California Airborne Transmissible Disease Standard if it involved the use of wild type rabies virus.
- In the assessment of risk, the source of the virus had not devised a validation test to prove that the containment of the virus could be less than BSL2.
- The Institutional Biosafety Committee had no choice but to require ABSL2 containment and consequently, the experiment as devised could not occur.

Biosafety Committee Considerations

- The resulting backlash of the non approval of the experiment was centered on the IBC's refusal to perform this work at anything less than BSL2.
- When calm and rational debate ensued, the focus was not on the committee's decision, it was an animal care and housing issue that the camera and the imager could not be accommodated in the animal care facility and therefore, the animals could not be housed in a quarantine area and remain in the facility.

Biosafety Committee Considerations

- The PI mistakenly thought if she could get approval at ABSL1, then animals would be unrestricted in moving to and from the animal facility to her lab.
- She could not understand that just because the virus was replication deficient and recombinant that without a validation procedure, wild type virus could result from recombination or other untoward events.

Resolution of Research Concerns

Engineered Solution

- The IBC approved the application at BSL2/ABSL2 but the PI could not perform the work outside the animal facility.
- NIH/OBA reviewed our case and notified the University that internal downgrades to a lower level of containment were not permitted under the Guidelines, even with a validation test. An exception would need to be granted.

Engineered Solutions

- The NIH explained that validation tests did not always ensure the prevention or detection of wild type virus.
- In addition, the request for reduction must have scientific merit, and not be a specious argument, such as the lack of facilities or lack of space.

Engineered Solutions

- The IBC examined ways that containment could be maintained – one possible solution was used at another University: the Biobubble.
- A Biobubble is a flexible film HEPA-filtered Ultra-Clean and Containment Enclosures for SPF, immunodeficient, and transgenic animal models and infectious disease containment.
- The Biobubble, essentially is a containment device that allows BSL2 containment to be maintained.

Engineered Solutions

- The PI is currently is bringing her animals out of the facility to her lab and then sacrificing them after imaging and photographing them.
- Several Biobubbles have been ordered for other scientists who wish to use that imager and camera. All of the PIs are using Risk Group 2 agents at a containment level of BSL2.
- All of the PIs currently sacrifice the animals and are not allowed to bring them back into the animal facility.

Issues Summarized

- The PI could not understand that:
  - just because the virus was replication deficient, it did not warrant BSL1 containment;
  - That the IBC approved her experiment at BSL2/ABSL2 and that the issue was animal housing, not a biosafety issue.
  - Bringing infected animals in an out of the animal facility jeopardized other animals.

Summary

This experiment reinforced the need to work closely with the Campus Veterinarian and IBC, and Institutional Animal Care and use Committee. Training for the PI and also the department helped to educate why animals cannot be arbitrarily brought in and out of the animal facility. Lastly, it was important that our institution learned that NIH/OBA approval is required to work with agents at a lower level of containment after committee approval.