NCI-FREDERICK
INSTITUTIONAL BIOSAFETY COMMITTEE

Minutes
November 13, 2007
NCI-Frederick

The NCI-Frederick Institutional Biosafety Committee was convened at 12:05 p.m. in the Building 549 Cafeteria with the following members in attendance:

Dr. Michael Baseler	Ms. Diana Boissey
Dr. Stephen Creekmore	Dr. Bruce Crise
Dr. David Garfinkel	Dr. Henry Hearn
Dr. Jeannie Herring	Dr. Dan McVicar
Dr. Randall Morin	Ms. Alberta Peugeot

Members not in attendance: Dr. Melinda Hollingshead, Ms. Theresa Bell, Dr. Stephen Hughes, Mr. Lucien Winegar

Others in attendance: Dr. Scott Keimig, Ms. Gail Housaman, Ms. Robin Pickens, Mr. William Brady, Ms. Pat Marshall, Dr. John Madsen, Dr. Jason Gall, Dr. Criss Tarr

INTRODUCTION
Dr. Morin called the meeting to order.

PROTOCOL REVIEWS

NEW BUSINESS

07-60 (Tarr – Manufacturing of Adenoviral Vector Vaccines Containing HIV Inserts)
- BSL-2 large scale requires surveillance of employees. This should include HIV testing before the start and after the completion of the campaign.

- The Class II BioPROtect II is a recirculating BSC. A catastrophic spill (e.g. 10^{10} ifu) may result in contamination of the laboratory. For instance, the capacity of the HEPA filters could be compromised.

- An important issue includes the potential for exposure in the QC lab, which is a large

and open area. Good, clear protocols are needed for how they will work safely with infectious material in the QC lab so that workers are not exposed. The work in the QC lab should be done in a BSC. Any work done outside the BSC should be done in a way that rigorously prevents the escape of infectious material into the QC lab environment.

- Particular attention should be paid to work being done with aerosol-transmissible agents, especially with infectious materials under pressure (for example chromatography and centrifugation).

- During the chromatography process there is no secondary containment. Two people will monitor the process at all times and wear N-100s.

- Protocol should address how to contain large-scale batches of infectious material in the production labs. If the safety/containment setup is designed to work at the level of the room/lab, then anyone working in the lab is at risk. It is always better to have some form of containment for the fermenter, column, etc., within a production lab to help protect the workers if there is a spill or accident. It also makes it much easier to clean up a spill if the whole room isn't grossly contaminated.

- Address PPE, including eye protection in case of splashes.

- How will decontamination of equipment be validated?

- Indicate that BSC's, other containment devices are ducted/vented out.

- Methanol fixative – protocols states that this is effective against adenovirus. Documentation should be provided to show this is accurate.

- Before use, the viral seed banks are tested and shown to be negative for detectable replication competent adenovirus using the assay described in the submission.

- Are tests performed for replication-deficient recombinants capable of cytopathic effects? Check at each scale. The VPP explained that materials are tested for the presence of replication deficient recombinants.

- To ensure containment, VPP will perform riboflavin testing of all proposed virus-containing operations prior to the use of virus.

- An audit with the VPP Safety Officer should be conducted.

- A2: States "The cell line has been used by the VRC in the past to generate multiple lots of adenoviral vectors, which are currently in clinical trials and have been successfully tested in hundreds of volunteers." Is this statement correct? The understanding is that this is the first vector production in the facility. Other work performed was vialing.

- A6b: As this is a new application reiteration of the transportation of the finished product should be noted here. Yes is selected but an explanation must be provided.

- B5e3: This should be yes. Adenovirus is amphotrophic.

- B5g: This should be yes. The purpose of the product is to infect cells of the recipient and force expression of the HIV proteins.

- B5gl: The material is "infectious" not "replication competent".

- B5j: Why are two groups listed?

- B6: Must be answered. Subsections a, b, and c need to be accurately filled out.

- B6c: The resulting virus is capable of infecting human cells; therefore, this should be yes.

- B8a: This response may be accurate, but misleading. The HIV proteins are expressed in the 293 cells.

- B8b: Note that sero-converting to HIV has been considered.

- B8b: Administration of any high dose of antigenic protein can have a hazard associated with it due to the resulting immune response. In this case, workers could potentially seroconvert causing significant concern for them. These issues should be acknowledged.

Appendix 4

- The layout demonstrates that everyone in the room is subject to possible exposure from the adenovectors; therefore, the safety and surveillance measures employed should apply to all the staff working with the adenovectors.

Appendix 5

- Semi-Micro Spec PU: Is this cuvette quartz? Clarify that this cuvette will be transported in secondary containers. Are contingencies in place to decontaminate the Spec and the cuvettes in case of accidental contamination? VPP explained that analysis does not contain live virus. The UV spec is under secondary containment.

- A suggestion was made to use disposable cuvettes with caps, rather than parafilm. The Plexiglass box is validated every six months.

- Focus Forming Unit Assay: Secondary containment is required here.

-What's the fixative? Are the plates transported in a secondary container between the BSC and incubator.

- LAL: How is the plate sealed? Are contingencies in place for decontamination of the plate reader?

- Transgene Expression Western Blot: This is a significant manipulation. Is TTX 100 lysis used here? How are lysates centrifuged prior to sample preparation and loading?

- Is centrifugation done within a cabinet?

- HPLC PU Assay: Are there any contingency plans for cleaning up the HPLC?

- Reverse Phase HPLC: How is the HPLC decontaminated--both internally and the injection portheedle? VPP explained that the Autosampler is sealed. Decontamination is done with bleach or NaOH.

- Host Cell Protein Western: Procedure should include transporting the swinging bucket rotor cup back to the BSC before opening the lid.

- El Revertant Assay: Proteinease K is not a standard method for virus inactivation. Has inactivation by this method been validated. Why not use chaotropic salts?

- Transgene Expression: The percentage of SDS is not listed.

- SDS-PAGE analysis: Procedure includes transporting the swinging bucket rotor cup back to the BSC before opening the lid.

- Benzonase ELISA: (1) The 0.5M sulfuric acid volume must exceed the volume of the sample and any of the wash steps to ensures contaminated surfaces are treated. (2) When incubating the plate outside the BSC, it should be in a secondary container.

- Note at the end of the appendix: "Aerosol resistant pipets are used to prevent aeorsolization." There will be no manipulation of infectious materials on the benchtop with or without aerosol resistant pipet tips. This is for all processes, just not QC processes.

Appendix 8

- Concentration and Enzyme Treatment: How are the large harvest bags transported?

- Transporting the infected materials is to take place in leak proof secondary containers.

- All processes that are in volumes of 10L or less should take place in devices that are capable of holding the entire liquid volume used.

- At the preparative stages (40L) all activities that involve placing liquid with infectious materials under pressure are to be carried out in an engineering control capable of containing aerosols.

Appendix 9
- Purification Process Step: Is pouring appropriate here?

- The drawing indicates that a large column is sitting in the open, not within a containment device. The device alarm is set at -22psi. The catch basin at the bottom of the column does not appear sufficient to hold the volume of the material being pumped through the column.

- How are the columns cleaned and decontaminated after use?

Appendix 10
- Spill control: Contact times are not specified. Should respirators be used during spill cleanup? This procedure seems relatively superficial for the potential spill of liters of active virus. More detail is needed regarding spill contingency plans and worker training. Discussion at meeting regarding sloped floor, drain, and decon validation.

- Is there secondary containment for the large bags of virus?

Production Runs:
- At the largest production run levels (400L), is the production room floor designed so it is capable of containing the entire production run volume? Are the floor drains open or closed during the run?

- How is the fermenter decontaminated after use?

- How is the final production run volumes removed from the fermenter? Is the process under pressure? How are the receptacles contained?

Dr. Creekmore made a motion to conditionally approve this registration pending the modifications noted above, Dr. Crise seconded and all were in favor.

<u>07-61 (Van Dyke – Investigation of the molecular mechanisms of astrocytoma development using inducible genetically engineered mice)</u>
- Will change stations be used in building 429 or are BSCs used in the quarantine area?

- Tamoxifen should be used in a BSC. Animals excrete tamoxifen in feces/urine and can be aerosolized; therefore, animal caretakers must be on respiratory program.

- Are any genetic elements capable of being mobilized?

- How will the adenoviral vector be introduced into the mice?

- After the vector is put into the mice, how will the mice be caged, handled, etc.? This is particularly important for a week or so after infection when the mice might be shedding virus.

- The Ad-Cre virus is a commercial stock. It is supposed to be helper free; however, Ad vector stocks usually have some replication competent viruses in them, and this should be acknowledged.

- The protocol states that there is no aerosol hazard from Ad virus. This is an overly optimistic view.

- The protocol states that Cre is not a hazard. The toxicity is not well defined, but there are hints that long term expression is toxic, presumably because Cre causes genome rearrangements. It should not be considered entirely safe.

- More information about the handling and introduction of the modified tamoxifen should be provided.

- E6e1: This is correct in the context of this protocol, but misleading in the larger sense. The language should be fixed. (Mice are full of endogenous viruses which can be mobilized, and some endogenous mouse viruses can infect humans. The protocol under consideration does not raise the risk, which appears to be quite low in immunocompetent humans)

- E6g: The risk of generating a replication competent Ad virus arises when the Ad vector stock is generated by the vendor, a time when the sequences missing in the vector are supplied in trans. The experiments proposed here do not raise this risk; however, the risk is built into the use of this vector system.

- Although it is probably a negligible risk, humans can carry Ad viruses and infected humans can bring complementing replication competent Ad viruses to the experiment

Dr. Crise made a motion to defer approval, Dr. Baseler seconded, and all were in favor.

<u>07-62 (Yoshimura- Generation of formyl peptide receptor (fpr) 1 and 2 double knockout mice)</u>

- No virus work is conducted in this registration

- All standard methods are performed with well characterized segments of DNA.

Dr. Garfinkel made a motion to approve, Dr. Crise seconded, and all were in favor.

<u>06-34 (Parchment - Specimens for pharmacologic and toxicologic assessments)</u>

- This registration was submitted as a renewal (new #07-63).

- Standard pathogen screening is conducted.

- Signatures of the PI and staff have been received and training has been completed.

Dr. Creekmore made a motion to approve, Dr. Garfinkel seconded, and all were in favor.

P150205SSA01 (Sei - Screening of NCI tumor cell line panel to oncolytic vaccinia virus-induced cytopathic effect)

- There were no outstanding issues with this amendment and was approved prior to the meeting by the designated reviewer.

Other Business

- Ms. Peugeot reported on 3 potential biological exposures
  1) Needlestick with human prostate cancer cell lines while injecting a mouse; Cell line was screened for multiple viruses, all negative. No recognized potential for exposure.
  2) Puncture via dropped scalpel while dissecting mouse embryos; no recognized potential for exposure.
  3) Needlestick with contaminated needle while giving antibiotic compound injection to mouse. Potential for exposure, patient treated with medication.

- IBC/ACUC subcommittee – on hold until all players are available

- BBP training update: 1002/1089 = 92% compliant

The meeting was adjourned at 1:42 p.m.

Theresa D. Bell, MPH, CBSP	Ms. Cara Leitch
IBC Secretary	IBC Coordinator
Biological Safety Officer, EHS	Sr. Safety Specialist, EHS

APPROVED:

Randall S. Morin, Dr. P.H.	Date
Chairman, NCI-Frederick IBC	
Director, EHS	

xc: Dr. Reynolds

Mr. Wheatley
Dr. Arthur
Mr. Bufter