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Policy for Validation and Use of Attenuated Organisms

I. Purpose

To validate the identity of attenuated organisms derived from virulent organisms that originally required BSL3 containment when the attenuation results in a reduction of required biosafety containment for possession or use.

II. Applicability

This policy applies to registrations of attenuated pathogens sought to be used at BSL-2 when the wild type organism required BSL-3 containment. COMS and the institutional biosafety officer will review the biological inactivation procedures. This policy does not apply to pathogens irreversibly inactivated (e.g., chemically) in a BSL3 laboratory. Principal Investigators must ensure they are in compliance with the federal select agent program requirements for excepted organisms.

III. Definitions

A. *Master Stock*

A "master" stock is a culture of a particular organism that has been validated and serves as the reference strain. It is created by selecting 1 colony forming unit (CFU) or plaque forming unit (PFU) and expanding in a small volume. It is used to produce the seed stock.

B. *Seed Stock:*

A "seed" stock is a vial(s) of stock culture that is prepared from the "master" stock. Each master and seed stock vial is only be used once. A new vial of seed stock is used to prepare a "working" stock for a single routine experiment.

IV. Implementation of Procedures

A. Registration of the attenuated RG3 organism

1. As is the case with all proposed studies at institutions under COMS purview, work with any organism cannot be initiated without prior approval by COMS.
2. The investigator must submit a registration describing the proposed work and the validation procedures that will be or have been used.

3. Documentation of the results of validation testing must be submitted to COMS for review and permanent archiving.
4. COMS will review the validation results by appointing two reviewers with the necessary expertise.
5. COMS will present the results at a convened COMS meeting and the committee will vote on whether to accept the results.
6. The investigator will be informed of the COMS decision.
7. Unless COMS grants an exception, until attenuation is validated and COMS approves the results, the attenuated RG3 organisms must be stored in BSL3, and all experiments with attenuated RG3 organisms must take place at BSL3. COMS will consider applications for lesser containment (exceptions) on a case-by-case basis. The source and/or documentation of the strain may be considered in this context.

B. Validation

1. Harvard University has no BSL4 laboratories and so attenuated risk group 4 (RG4) agents cannot be validated at Harvard.
2. If the organism cannot be validated as attenuated, it must be used at the containment level of the wild-type organism (BSL3).
3. Validation of the original organism sample and subsequent master and seed lots prepared must be performed in a laboratory equipped for and experienced with strain validation. This laboratory may be at a Harvard-COMS institution or may be at an off-campus validation laboratory. Refer to Appendix A for validation methods.
4. After the initial validation of the original organism, the attenuated RG3 organism must be grown and aliquoted in quantities sufficient for use until the project is completed. Consequently, vials of a master stock that can be used to prepare batches of seed stock are necessary. Each vial of seed stock is to be used for one set of experiments and then discarded without prolonged propagation.
5. Attenuated RG3 organisms must be stored in a secured, limited access facility.

6. A detailed, legible log of each vial used (master and seed lots) must be kept by the investigator.

C. Maintenance

1. Vials of master stock and seed stock derived from the master stock must be decontaminated and discarded after a single use.
2. Hence, numerous aliquots must be generated by the receiving laboratory.
   a. Seed Stock must be derived only from the master stock.
   b. Revalidation is also acceptable if Seed Stock is not available

D. Transfer to another Laboratory

1. Attenuated RG3 organisms or their derivatives may not be transferred to another laboratory without COMS approval.

V. Policy Authority

The Committee on Microbiological Safety shall enforce this policy.

VI. References

- Centers for Disease Control and Prevention/Division of Select Agents and Toxins Select Agent Program Exclusion List
- http://www.selectagents.gov/SelectAgentsandToxinsExclusions.html

Appendix A: Suggested Laboratory Methods for Attenuated Organism Validation

1. It is best practice to isolate a single clone of the organism and expanding prior to performing the validation, for example by limited dilution cloning. This master stock is then used to reproduce a seed stock. The master stock is only used to prepare a batch of seed stock; hence, the number of vials of master stock prepared must be small.
2. A "seed" stock is prepared from the "master" stock. A master and seed stock vial is to be used as "single" use only; a vial of seed stock is for preparing a "working" stock for a single routine experiment. Ideally, the number of vials of seed stock prepared should be sufficient for the duration of the research funding.
3. Validation must include direct testing for the presence or absence of the virulence factors lost in attenuation by phenotypic or genetic means (or both). This is preferred to methods that demonstrate a genetic match between the attenuated organism and a control strain without testing for the virulence factors themselves (e.g. pulse field gel electrophoresis).
4. Controls for the validation must include properties of the wild-type strain (positive control) and an appropriate negative control.
5. A formal Validation Report must be submitted to COMS for review and approval prior to going forward with a COMS-approved research registration.