

# **Cloning cDNA from Risk Group 4 Viruses Ebola and Marburg: Biosafety & Biosecurity**

**Proposal from the University of Wisconsin**

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# Agenda

- Recommended Biosafety Level under the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*
- Summary of previous Biosafety and Biosecurity recommendations for similar work with Ebola, Marburg, Nipah and Hendra Risk Group 4 (RG4) Mononegavirales from Rocky Mountain Laboratories (RML), NIAID, NIH
- Differences between the RML proposal and the University of Wisconsin proposal

# Containment under the *NIH Guidelines*

- The *NIH Guidelines* permits the cloning of a RG4 genome in a prokaryotic cell under Biosafety Level 2 (BL2) conditions only if it is an irreversibly defective fraction of the genome (Section III-D-2-a)
  - The full cDNA of a RG 4 mononegavirales is not a fraction of the viral genome nor is it irreversibly defective in mammalian cells
- The *NIH Guidelines* requires that research with the full cDNA be done at BL4 unless a determination is made by OBA, in consultation with the RAC as needed, that lowering containment is appropriate

# Containment Considerations for Research with Full-Length cDNA of RG4 Mononegavirales in *E. coli*

- Biosafety: the full-length cDNA of any of these viruses can produce infectious virus in mammalian cells only if the full-length RNA genome is produced and then only with the addition of 3 or 4 essential viral proteins.
- Biosecurity: the full-length cDNA plasmid transformed into a prokaryotic cell is not a Select Agent, but a trained laboratory worker could intentionally rescue the virus in mammalian cells. The infectious virus is a Select Agent.

# Previous RAC Recommendations Regarding Research with Full-Length cDNA of RG4 Mononegavirales in *E. coli*

Biosafety Level 2 practices are appropriate provided additional biosecurity measures are in place:

- Dedicated secure space for work with the cDNA
- Procedures by which access is limited to those personnel who are properly trained and who have undergone an appropriate background investigation
- An inventory system that can track access and movement of all materials
- Separation of cDNA from the complementing plasmids required to rescue live virus in a mammalian cell line

# Previous RAC Recommendations

## continued

- A written plan outlining the flow of materials into and out of the secure space, including procedures used to transfer reagents from one location, laboratory or institution to another
- A written plan for proper decontamination and disposal of biological reagents / waste

# Previous RAC Recommendations: Institutional Obligations

- Appointment of an institutional official to oversee the biosafety and biosecurity plans
- An written incident reporting and response plan
  - Including an occupational health plan
- A formal training program covering both the biosafety and biosecurity requirements for this research
- Annual reporting to the IBC and OBA

# Key Differences between Current Proposal and Prior Proposals

- The cDNA will be transferred from the University of Wisconsin to the Rocky Mountain Laboratories for the generation of infectious virus under BL4 containment
- Liquid waste will be decontaminated using a phenolic disinfectant and discarded in the public waste stream

# Questions for Discussion Identified by Biosafety Working Group

- Is there a formal agreement between University of Wisconsin and RML that specifically addresses:
  - The flow of materials between the Institutions
  - That separation of the full cDNAs and the plasmids necessary to rescue full virus will be maintained
- Is the current liquid waste decontaminated plan adequate

# University of Wisconsin

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# Evaluation of University of Wisconsin Proposal

- Dedicated secure space for work with the cDNA
- Procedures by which access is limited to those personnel who are properly trained and who have undergone an appropriate background investigation
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# Evaluation of University of Wisconsin Proposal

- A written plan outlining the flow of materials into and out of the secure space, including procedures used to transfer reagents from one location, laboratory or institution to another
- A written plan for proper decontamination and disposal of biological reagents / waste
  - Has disposal of liquid waste been addressed?

# Evaluation of University of Wisconsin Proposal

- Appointment of an institutional official to oversee the biosafety and biosecurity plans
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  - Including an occupational health plan
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- Annual reporting to the IBC and OBA

# Evaluation of University of Wisconsin Proposal

- A formal agreement between University of Wisconsin and RML that specifically addresses:
  - The flow of materials between the Institutions
  - That separation of the full cDNAs and the plasmids necessary to rescue full virus will be maintained throughout the process