

**Biocontainment for Research with Partial
Genomes of Viruses in Tissue Culture
under the *NIH Guidelines for Research with
Recombinant DNA Molecules*
(*NIH Guidelines*)**

Proposed Revisions

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Outline

- **Review of Section III-E-1: Biocontainment for Research with Partial Genomes of Eukaryotic Viruses in Tissue Culture.**
- **Impetus for Review and Proposed Amendments.**
- **Evolution of Proposed Changes to date.**
- **Revised Proposal.**

Section III-E-1 Current Requirements

- Section III-E-1 of the *NIH Guidelines* allows investigators to initiate research with partial viral genomes in tissue culture at Biosafety Level (BL) 1 containment upon registration of their experiment with the Institutional Biosafety Committee (IBC).
 - The IBC is nonetheless required to review and approve the containment for the research.
- To work under this section the virus must contain less than two-thirds of the genome from any family of viruses and there must be no helper virus present.
- Section III-E of the *NIH Guidelines* is designed to facilitate initiation of low-risk research.

Impetus for Proposed Changes

- **RAC review of research with synthetic nucleic acids and biosafety led to discussions of Section III-E-1**
 - **A question arose as to whether synthetic techniques might be used to generate a functional virus containing less than two-thirds of the genome.**
 - **There was also recognition that rescue of a replication competent virus could occur in the absence of helper virus through mechanisms that involved the recovery of autonomous viral replication functions through recombination of nucleic acid sequences contained in a defective virus with those present in complementing cell lines.**

March 2009 Proposed Revisions

- In March 2009, OBA published a proposal in the Federal Register (74 FR 9411) to amend Section III-E-1 by:
 - Changing the criterion regarding the size of the virus genome deletion:
 - From two-thirds to ONE-HALF
 - In addition to demonstrating the absence of any helper virus, the PI would also be required to provide evidence that the resulting nucleic acids in the tissue culture cells are not capable of producing a replication competent virus.

Public Comments – March 2009 Proposal

- Public comment on this proposal raised two issues:
 - There is research that has been safely conducted for many years under this section with viruses that contain more than one-half of the genome but less than two-thirds: e.g., viral replicon particles of Venezuelan Equine Encephalomyelitis.
 - Rather than a quantitative standard based on deletion size, does the current understanding of virus biology allow for a reduction in containment based on a functional impairment?

Revised Proposal – April 2010

- After further consultation with the RAC, OBA proposed additional amendments:
 - Retain the proposed criterion that one could work under this section if only one-half of the genome was present.
 - Clarified that this only applied to Risk Group (RG) 3 and 4 viruses because research with less than one-half of the genome of a RG2 virus is already exempt from the *NIH Guidelines* (see Appendix C)

Revised Proposal – April 2010 (cont'd)

- **Include functional criteria that would allow reduction of containment to be based on the removal of one or more viral genes that are essential for cell-to-cell transmission even if these genes accounted for less than 1/3 of the genome.**
 - **Removal of such genes should prevent the propagation of virus and its ability to cause disease.**
- **Clarified that containment for research with retroviruses and lentiviruses that have the potential to transduce human cells should not be less than BL2.**

Section III-E-1 – Proposed Language

Published in 75 FR 21008 - April 22, 2010

An investigator can initiate work at BL1 containment in tissue culture upon notification of the IBC if:

- 1) No more than half of the eukaryotic viral genome is present**
- OR**
- 2) There is a complete deletion in one or more essential viral capsid, envelope, or polymerase genes required for cell-to-cell transmission of viral nucleic acids and the investigator provides the IBC with evidence such as sequence or other appropriate data to demonstrate that there is a complete deletion of the nucleic acid sequence such that these functions can not be rescued through homologous recombination.**

Section III-E-1 – Proposal (cont'd)

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AND

In both situations there must be evidence that:

1. The resulting nucleic acids are not capable of producing a replication competent virus in a cell line that would normally support replication of the wild-type virus
2. There is no helper virus present

In addition, a minimum of BL2 containment is required for experiments with retroviruses and lentiviruses that have the potential to transduce human cells and cause insertional mutagenesis.

Additional Biosafety Concerns

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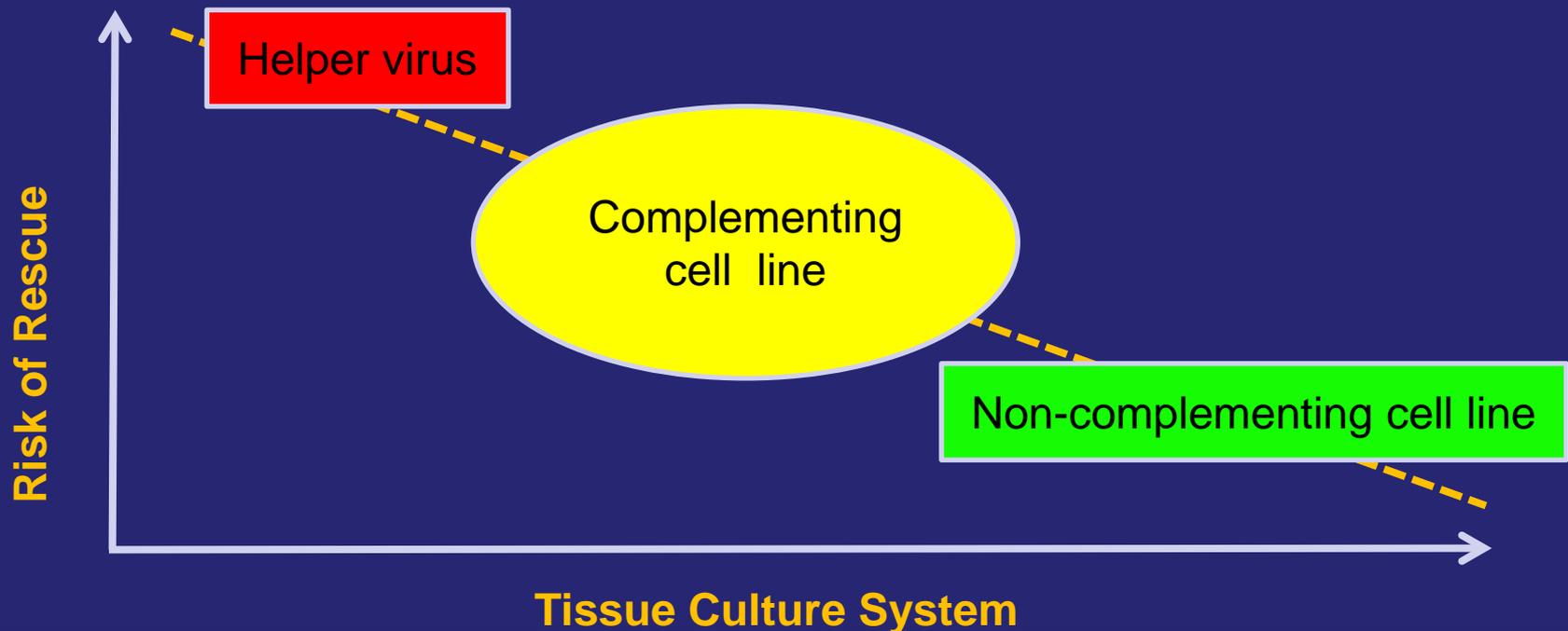
- Although no public comments were offered in response to the April 2010 FR notice, the review of research to create a defective RG4 agent raised concerns about the advisability of working with these viruses under lower containment prior to IBC review.
 - The possibility of a rare event resulting from homologous and/or non-homologous recombination could result in the rescue of a potentially lethal virus at lower containment.
 - Because illegitimate (i.e. non-homologous) recombination events are independent of nucleic acid sequence similarity, the amount of sequence removed from a viral genome has no influence on the potential for rescue of a replication competent virus. Thus a quantitative deletion standard is not a reliable measure of biosafety.

Section III-E-1 – Objectives

- This section is designed to facilitate initiation of low-risk research in tissue culture at Biosafety Level (BL1) containment upon registration of an experiment with the Institutional Biosafety Committee (IBC).
- We propose to retain the notion that only “low-risk” research should fall under Section III-E-1, and to change the criterion of a “defective” virus from a quantitative definition ($<2/3$ of genome) to a functional definition
- Restrict work with defective viruses under this section to non-complementing cells as these present the lowest possible potential for rescue of a replication competent virus.

Biosafety Considerations for Work with Defective Viruses in Tissue Culture

The key biosafety issue is whether a replication competent virus could be rescued. Rescue events are dependent on viral (or cellular) replication and are more likely to occur in the presence of helper virus, to a lesser extent in complementing cells, and to the least extent in non-complementing cells.



Biosafety Analysis – RG4 Viruses

- Risk Group (RG) 4 viruses should not be included under Section III-E-1
 - Under the *NIH Guidelines* most research with RG 4 viruses will need to be conducted at Biosafety Level 4
 - Lowering of containment for the RG4 viruses should be based on data that is reviewed by OBA and the Institutional Biosafety Committee (IBC) before the work begins.

Biosafety Analysis- RG3 Viruses

- **Work with defective RG3 viruses in non-complementing cell lines would also not be included under Section III-E but would qualify for a reduction in containment to BL2, and in certain circumstances to BL1, after IBC review of appropriate biological safety data**
 - **Initiation of research should not proceed until there has been an independent review of safety data to assure that the data documenting the absence of replication competent virus and the low probability of a rescue event are scientifically valid.**

Biosafety Analysis- RG3 Viruses (cont'd)

Consequently for the RG3 viral agents:

- Containment for research in tissue culture with all RG3 defective virus constructs will be reviewed under Section III-D and maintained at BL3 unless the IBC authorizes the lowering of containment to BL2 for experiments performed exclusively in tissue culture cells that cannot complement the deleted viral functions. The tissue culture system used at BL2 containment must not allow for cell to cell transmission of a defective virus.

Biosafety Analysis- RG3 Viruses (cont'd)

Consequently for the RG3 viral agents:

- Following completion of a risk assessment that should include an analysis of the data gathered to examine the presence of replication competent virus, the IBC may lower containment to BL2.
- BL1 containment with BL2 practices may be considered if the specific experimental conditions or procedures cannot be performed within a BL2 facility, e.g. use of specialized equipment located in BL1 environments.

Biosafety Analysis- RG3 Viruses (cont'd)

Consequently for the RG3 viral agents:

- Unlike most experiments reviewed by the IBC under Section III-D, this change will grant the IBC authority to lower containment for experiments using defective RG3 viruses in non-complementing cell lines.

Biosafety Analysis- RG2 Viruses

- Under the proposed Section III-E, Investigators may initiate work with RG2 viruses in non-complementing cell lines at BL1 containment upon registration with the IBC.
- The rationale for allowing containment to be lowered to BL1 without IBC review of the experiment is based on :
 - The differences between BL2 and BL1 containment for tissue culture experiments primarily involve the implementation of biosafety practices; no specialized equipment is mandatory in most cases.

Research Involving Defective Viral Genomes in Tissue Culture Systems

Proposed Section III–E–1. The following experiments may be initiated upon registration with the IBC

Recombinant nucleic acids from a Risk Group 2 eukaryotic virus may be used in cells in tissue culture at BL1 if there is a complete deletion in one or more essential viral capsid, envelope or polymerase genes required for cell-to-cell transmission of viral nucleic acids, and the tissue culture system used is not capable of complementing the deleted viral functions.

A minimum of BL2 containment is required for experiments with retroviruses and lentiviruses that have the potential to transduce human cells and cause insertional mutagenesis.

Research Involving Defective Viral Genomes in Tissue Culture Systems (cont'd)

Proposed new Section under III-D

Experiments with defective recombinant nucleic acids from a Risk Group 3 eukaryotic virus in cells in tissue culture will usually be conducted at BL2 containment if there is a complete deletion in one or more essential viral capsid, envelope or polymerase genes required for cell-to-cell transmission of viral nucleic acids, and the tissue culture system used is not capable of complementing the deleted viral functions. BL1 containment with BL2 practices may be considered if the specific experimental conditions or procedures cannot be performed within a BL2 facility, e.g. use of specialized equipment located in BL1 environments.

Research Involving Defective Viral Genomes in Tissue Culture Systems (cont'd)

Propose new Section under III-D:

A minimum of BL2 containment is required for experiments with retroviruses and lentiviruses that have the potential to transduce human cells and cause insertional mutagenesis.

Research Involving Defective Viral Genomes in Tissue Culture Systems (cont'd)

In both cases (i.e. under **proposed** Section III-E for RG2 or III-D for RG3) to qualify for a reduction of biocontainment, the following data must be provided:

- i. Results of experimental assays demonstrating that the defective virus cannot be transmitted from cell to cell when transfected in a cell line that would normally support cell-to-cell transmission of the intact virus. Such documentation should include evidence that the number of virus-infected cells does not increase on multiple serial passages of the transfected cells. Experimental assays should be designed to detect the presence of replication competent virus and should be appropriately controlled for sensitivity and limits of detection.

Research Involving Defective Viral Genomes in Tissue Culture Systems (cont'd)

- ii. It must also be demonstrated that the cells lack helper viruses for each specific Family of defective virus being used. If helper virus is present, review will proceed under Section III–D–3, *Experiments Involving the Use of Infectious Animal or Plant DNA or RNA Viruses or Defective Animal or Plant DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems.*

Potential Impact on Current Research :

- As the comments from researchers using VEE attest, there are investigators working in complementing cell lines with defective RG3 viruses (e.g. VEE replicons) at BL1 or BL2 containment under the current Section III-E-1 of the *NIH Guidelines*.
- If the changes discussed are implemented, under the revised *NIH Guidelines* this work in complementing cell lines would require BL3 containment.
- Investigators may ask OBA to lower containment for such research by submitting supporting documentation. The IBC can then perform a risk assessment taking into account OBA's recommendation.

Next Steps

Implementation of the proposal will require:

- **Publication of a Federal Register Notice and request for public comment on the proposed changes to Sections III-D-3 and III-E-1 of the *NIH Guidelines*.**