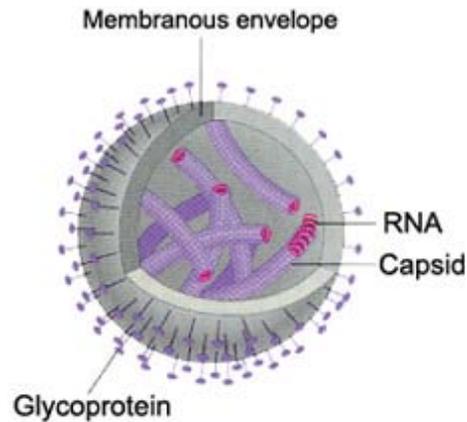




Section III-E-1 Experiments in Tissue Culture with Partial Viral Genomes



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Section III-E-1: Tissue Culture Experiments

- ❑ **Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family being considered identical) may be propagated and maintained in cells in tissue culture using BL1 containment if**
 - **It is demonstrated that the cells lack helper virus for the specific Families of defective viruses being used.**
- ❑ **The DNA may contain fragments of the genome of viruses from more than one Family but each fragment shall be less than two-thirds of a genome.**



Section III-E-1: RAC Review

- ❑ **Concern that synthetic viral agents derived from multiple sources might be able to function with less than 2/3 genome present, therefore**
 - **The 2/3 genome proposed to be changed to 1/2**
- ❑ **Generation of replication-competent virus could arise from mechanisms other than presence of helper virus**
 - **Proposed to amend this section to require a demonstration that the preparation(s) are free of replication-competent virus which may be generated by homologous recombination with endogenous proviruses or in the presence of helper virus.**



Section III-E-1: March 2009 - Proposed

- **BL-1 containment permitted for experiments involving risk Group 3 and 4* viruses with less than one-half of any eukaryotic viral genome provided evidence is also submitted attesting that:**
 - **The resulting NA molecules in these cells are not capable of producing a replication-competent virus and**
 - **The cells lack helper virus for the specific Families of defective viruses being used**
- * Viruses in the RG2 category and below, with less than one-half of the genome are exempt from the *NIH Guidelines per Appendix C-I***



Section III-E-1: March 2009 FR Public Comments

- One respondent proposed that the criterion for lowering containment should be based on the nature of a functional impairment (e.g. an irreversible biological defect).**
- Another respondent noted that a requirement for a 50% deletion would force VEE-based vaccine work (*i.e.* replicons) to be conducted at BL3.**



Section III-E-1 - Revised

- ❑ **Decision made to include criteria based on impairments to structural or functional genes in addition to a quantitative standard.**



Section III-E-1 Revised – *cont. . .*

- **Well characterized viruses can be safely disabled by the removal of certain critical genes – *i.e.* capsid, envelope and polymerase genes – that are essential components for replication and for cell-to-cell transmission of infectious virions.**
 - **Deletion of a gene must be complete; partial gene deletions may be rescued by homologous recombination. Point mutations and frame-shift mutations can be reversed.**



Section III-E-1 Revised – *cont. . .*

- For emerging and less characterized viruses, impairment of replication and transmission can be assured by a minimum 50% deletion of the viral genome.**



Section III-E-1: April 2010 Proposal *

- Recombinant nucleic acids from a eukaryotic virus (excluding *Variola major* and *V. minor*) and/or synthetic nucleic acids molecules based on a sequence from a eukaryotic virus (excluding *Variola major* and *V. minor*) may be propagated and maintained in cells in tissue culture using BL1 containment if:**



III-E-1 Proposed

- **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, or**
- **For Risk Group 3 or 4 viruses no more than half of the genome is present, (all viruses from a single Family being considered identical). The nucleic acids may contain fragments of the genome of viruses from more than one Family but each fragment shall be less than one-half of a genome.**



III-E-1 Proposed

- ❑ **In addition, there must be evidence that 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.**
- ❑ **If a gene deletion is the basis for a reduction in containment, sequence or other appropriate data should be submitted to the IBC to demonstrate that the deleted function(s) cannot be rescued by homologous recombination.**



III-E-1 Proposed – Revision

- ❑ **In addition, there must be evidence that 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.**
- ❑ **If a gene deletion is the basis for a reduction in containment, evidence should be presented to the IBC that the cell culture cannot complement the missing gene(s) or reconstitute the infectivity of the virus by homologous recombination between genes resident in the cells and the viral nucleic acid sequences introduced into the cell culture. Such evidence could consist of a demonstration that the cell culture does not contain a contiguous copy of the nucleic acid sequences deleted from the virus.**



III-E-1 Proposed

- It must be demonstrated that the cells lack helper virus for specific Families of defective viruses being used. If helper virus is present, Section III-D-3 applies and IBC review is required prior to initiation.**
- A minimum of BL2 containment is required for experiments with retroviruses that have the potential to transduce human cells and cause insertional mutagenesis.**



Public Comments: April 2010 FR Notice

- **American Biological Safety Association:**
Unclear why the proposed revisions to Section III-E only pertain to research with RG 3 and RG 4 agents.
 - Currently, experiments in tissue culture with viruses that are not RG 3 or 4 agents from in which the genome is deleted for at least 1/2 do not require IBC review and in fact are exempt (Appendix C-I). Text will be added to Section III-E-1 in order to emphasize that the current Appendix C-I is applicable for agents that are not RG 3 or 4 .



III-E-1 Proposed Revised

- **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, or**
- **For Risk Group 3 or 4 viruses no more than half of the genome is present, (all viruses from a single Family being considered identical). The nucleic acids may contain fragments of the genome of viruses from more than one Family but each fragment shall be less than one-half of a genome.**
Experiments in tissue culture with less than 50% of a genome from viruses that are not RG 3 or 4 agents are exempt from the NIH Guidelines (see Appendix C-I)



Public Comments: April 2010 FR Notice

- **American Biological Safety Association: Requested Guidance Document be developed for this revision.**
 - **In addition to the final Federal Register notice that will explain the changes, OBA will develop Guidance for IBCs and Investigators**



Comments *cont. . .*

- **Division of Select Agents and Toxins – Centers for Disease Control and Prevention**

Proposed changes could allow for the generation of a wild type virus from two (or more) constructs each of which contains less than 1/2 of a viral genome



Comments *cont. . .*

- Currently, for research to fall under Section III-E, the sum total of nucleic acids from a **single viral Family** may not exceed $2/3$ of the genome (at least $1/3$ deleted); all viruses from a single viral Family are considered to be identical. The proposed change from a $1/3$ to $1/2$ deletion reduces the amount of nucleic acid that may be present from a **single viral Family**.
- While viruses within families could potentially recombine, extremely unlikely, if at all possible, for viruses from different families to not recombine and form an infectious virus.



Comments *cont. . .*

- **Division of Select Agents and Toxins – CDC**

If an investigator divided the viral genome into fragments, e.g. 3 and had each fragment separately but worked with all 3 at the same time or in the same space there could be a chance of accidental reconstitution of replication competent virus? Should there be temporal or spatial separation?



Comments *cont. . .*

- ❑ **Given each fragment should be incapable of replication or cell to cell transmission the likelihood of such an event is extremely remote.**
- ❑ **Requiring spatial and temporal separation has the potential of creating a burden on the investigator and was not required previously under this section (III-E-1).**
- ❑ **The final FR notice will emphasize the need for good biosafety practices, including labeling of specimens and separation of reagents .**
- ❑ **Temporal and spatial separation is only required for work with RG 3 Influenza viruses due to the propensity for these viruses to reassort and their ability to spread person to person.**



RAC DISCUSSION