Current guidance in the *NIH Guidelines for Research Involving Recombinant DNA Molecules* and *Biosafety in Microbiological and Biomedical Laboratories (4th edition)*

1) The current (April 2002) *NIH Guidelines* and Influenza research

Under Appendix B of the *NIH Guidelines*, Influenza viruses are listed as risk group 2 agents:

> **Orthomyxoviruses**
> **--Influenza viruses types A, B, and C**

2) Recombinant DNA experiments involving DNA from or into a risk group 2 agent are covered under Section III-D of the *NIH Guidelines*

These experiments, under the *NIH Guidelines*, are usually conducted under Biosafety Level-2 containment conditions

Experiments under Section III-D require approval by the Institutional Biosafety Committee before initiation

Section III-D states:

Prior to the initiation of an experiment that falls into this category, the Principal Investigator must submit a registration document to the Institutional Biosafety Committee which contains the following information: (i) the source(s) of DNA; (ii) the nature of the inserted DNA sequences; (iii) the host(s) and vector(s) to be used; (iv) if an attempt will be made to obtain expression of a foreign gene, and if so, indicate the protein that will be produced; and (v) the containment conditions that will be implemented as specified in the *NIH Guidelines*. For experiments in this category, the registration document shall be dated, signed by the Principal Investigator, and filed with the Institutional Biosafety Committee. The Institutional Biosafety Committee shall review and approve all experiments in this category prior to their initiation. Requests to decrease the level of containment specified for experiments in this category will be considered by NIH (see Section IV-C-1-b-(2)-(c), *Minor Actions*).

Section III-D-1. Experiments Using Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems (See Section II-A, Risk Assessment)

Section III-D-1-a. Experiments involving the introduction of recombinant DNA into Risk Group 2 agents will usually be conducted at Biosafety Level (BL) 2 containment. Experiments with such agents will usually be conducted with whole animals at BL2 or BL2-N (Animals) containment.

Section III-D-2. Experiments in Which DNA From Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents is Cloned into Nonpathogenic Prokaryotic or Lower

Eukaryotic Host-Vector Systems

Section III-D-2-a. Experiments in which DNA from Risk Group 2 or Risk Group 3 agents (see Section II-A, *Risk Assessment)* is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment. Experiments in which DNA from Risk Group 4 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment after demonstration that only a totally and irreversibly defective fraction of the agent's genome is present in a given recombinant. In the absence of such a demonstration, BL4 containment shall be used. The Institutional Biosafety Committee may approve the specific lowering of containment for particular experiments to BL1. Many experiments in this category are exempt from the *NIH Guidelines* (see Section III-F, *Exempt Experiments*). Experiments involving the formation of recombinant DNA for certain genes coding for molecules toxic for vertebrates require NIH/OBA approval (see Section III-B-1, *Experiments Involving the Cloning of Toxin Molecules with* LD_{50} *of Less than 100 Nanograms Per Kilogram Body Weight*) or shall be conducted under NIH specified conditions as described in Appendix F, *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*.

Section III-D-3. Experiments Involving the Use of Infectious DNA or RNA Viruses or Defective DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems

Caution: Special care should be used in the evaluation of containment levels for experiments which are likely to either enhance the pathogenicity (e.g., insertion of a host oncogene) or to extend the host range (e.g., introduction of novel control elements) of viral vectors under conditions that permit a productive infection. In such cases, serious consideration should be given to increasing physical containment by at least one level.

Note: Recombinant DNA or RNA molecules derived therefrom, which contain less than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family (see Section V-J, *Footnotes and References of Sections I-IV*) being considered identical (see Section V-K, *Footnotes and References of Sections I-IV*), are considered defective and may be used in the absence of helper under the conditions specified in Section III-E-1, *Experiments Involving the Formation of Recombinant DNA Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus*.

Section III-D-3-a. Experiments involving the use of infectious or defective Risk Group 2 viruses (see Appendix B-II, *Risk Group 2 Agents*) in the presence of helper virus may be conducted at BL2.

Section II-A-3, Comprehensive Risk Assessment:
In deciding the appropriate containment for an experiment, the initial risk assessment from Appendix B should be followed by a thorough consideration of the agent and how it is to be manipulated. Factors to be considered in determining the level of containment include agent factors such as: virulence, pathogenicity, infectious dose, environmental

stability, route of spread, communicability, operations, quantity, availability of vaccine or treatment, and gene product effects such as toxicity, physiological activity, and allergenicity. Any strain that is known to be more hazardous than the parent (wild-type) strain should be considered for handling at a higher containment level. Certain attenuated strains or strains that have been demonstrated to have irreversibly lost known virulence factors may qualify for a reduction of the containment level compared to the Risk Group assigned to the parent strain (see Section V-B, *Footnotes and References of Sections I-IV*).

A final assessment of risk based on these considerations is then used to set the appropriate containment conditions for the experiment (see Section II-B, *Containment*). The containment level required may be equivalent to the Risk Group classification of the agent or it may be raised or lowered as a result of the above considerations. The Institutional Biosafety Committee must approve the risk assessment and the biosafety containment level for recombinant DNA experiments described in … III-D, *Experiments that Require Institutional Biosafety Committee Approval Before Initiation.*

***Biosafety in Microbiological and Biomedical Laboratories* (4th Edition)**

Agent: Influenza

Laboratory-associated infections with influenza are not normally documented in the literature, but by informal accounts and published reports are known to have occurred, particularly when new strains showing antigenic drift or shift are introduced into a laboratory for diagnostic/research purposes. (49)

Laboratory animal-associated infections are not reported; however, there is a high possibility of human infection from infected ferrets and vice-versa.

Laboratory Hazards: The agent may be present in respiratory tissues or secretions of humans or most infected animals, and in the cloaca of many infected avian species. The virus may be disseminated in multiple organs in some infected animal species.

The primary laboratory hazard is inhalation of virus from aerosols generated by infected animals, or by aspirating, dispensing, or mixing virus-infected samples. Genetic manipulation has the potential for altering the host range, pathogenicity, and antigenic composition of influenza viruses. There is unknown potential for introducing into man transmissible viruses with novel antigenic composition.

Recommended Precautions: Biosafety Level 2 practices and facilities are recommended when receiving and inoculating routine laboratory diagnostic specimens. Autopsy material should be handled in a biological safety cabinet using Biosafety Level 2 procedures.

Activities Utilizing Noncontemporary Virus Strains: Biosafety considerations should take into account the available information about infectiousness and virulence of the

strains being used, and the potential for harm to the individual or society in the event that laboratory-acquired infection and subsequent transmission occurs. Research or production activities utilizing contemporary strains may be safely performed using Biosafety Level 2 containment practices. Susceptibility to infection with older noncontemporary human strains, with recombinants, or with animal isolates warrant the use of Biosafety Level 2 containment procedures. However, there is no evidence for laboratory-acquired infection with reference strains A/PR/8/34 and A/WS/33, or its commonly used neurotropic variants.

Transfer of Agent: For a permit to import this agent, contact CDC. An importation or domestic transfer permit for this agent can be obtained from USDA/APHIS/VS.