

**NOTICE PERTINENT TO THE OCTOBER 2011 VERSION OF THE  
NIH GUIDELINES FOR RESEARCH INVOLVING  
RECOMBINANT DNA MOLECULES  
(NIH GUIDELINES)**

**EFFECTIVE IMMEDIATELY (February 21, 2013)**

The following portions of the *NIH Guidelines* include amendments that address biocontainment for research with strains of HPAI H5N1 virus that may transmit among mammals by respiratory droplets, as demonstrated in an animal model or clinically (mammalian-transmissible HPAI H5N1). The section and appendices below address research with Risk Group 3 influenza viruses, including mammalian-transmissible HPAI H5N1. Other sections of the *NIH Guidelines* may apply.

**These changes are effective immediately and will be incorporated into the March 2013 edition of the  
*NIH Guidelines*.**

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Changes in language from the October 2011 version of the *NIH Guidelines* are italicized.

**Section III-D-7.** Experiments with influenza viruses generated by recombinant methods (e.g., generation by reverse genetics of chimeric viruses with reassorted segments, introduction of specific mutations) shall be conducted at the biosafety level containment corresponding to the risk group of the virus that was the source of the majority of segments in the recombinant virus (e.g., experiments with viruses containing a majority of segments from a RG3 virus shall be conducted at BL3). Experiments with influenza viruses containing genes or segments from 1918-1919 H1N1 virus (1918 H1N1), human H2N2 virus (1957-1968) and highly pathogenic avian influenza H5N1 virus strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1), *including, but not limited to, strains of HPAI H5N1 virus that are transmissible among mammals by respiratory droplets, as demonstrated in an appropriate animal model or clinically in humans (hereinafter referred to as mammalian-transmissible HPAI H5N1 virus)*, shall be conducted at BL3 enhanced containment (see Appendix G-II-C-5, Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses) unless indicated below.

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**Appendix G-II-C. Biosafety Level 3 (BL3)** (See Appendix G-III-P, *Footnotes and References of Appendix G*)

**Appendix G-II-C-1. Standard Microbiological Practices (BL3)**

**Appendix G-II-C-1-a.** Work surfaces are decontaminated at least once a day and after any spill of viable material.

**Appendix G-II-C-1-b.** All contaminated liquid or solid wastes are decontaminated before disposal.

**Appendix G-II-C-1-c.** Mechanical pipetting devices are used; mouth pipetting is prohibited.

**Appendix G-II-C-1-d.** Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the work area.

**Appendix G-II-C-1-e.** Persons wash their hands: (i) after handling materials involving organisms containing recombinant DNA molecules, and handling animals, and (ii) when exiting the laboratory.

**Appendix G-II-C-1-f.** All procedures are performed carefully to minimize the creation of aerosols.

**Appendix G-II-C-1-g.** Persons under 16 years of age shall not enter the laboratory.

**Appendix G-II-C-1-h.** If experiments involving other organisms which require lower levels of containment are to be conducted in the same laboratory concurrently with experiments requiring BL3 level physical containment, they shall be conducted in accordance with all BL3 level laboratory practices.

#### **Appendix G-II-C-2. Special Practices (BL3)**

**Appendix G-II-C-2-a.** Laboratory doors are kept closed when experiments are in progress.

**Appendix G-II-C-2-b.** Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.

**Appendix G-II-C-2-c.** The Principal Investigator controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

**Appendix G-II-C-2-d.** The Principal Investigator establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures entering the laboratory or animal rooms.

**Appendix G-II-C-2-e.** When organisms containing recombinant DNA molecules or experimental animals are present in the laboratory or containment module, a hazard warning sign incorporating the universal biosafety symbol is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the Principal Investigator or other responsible person(s), and indicates any special requirements for entering the laboratory such as the need for immunizations, respirators, or other personal protective measures.

**Appendix G-II-C-2-f.** All activities involving organisms containing recombinant DNA molecules are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench.

**Appendix G-II-C-2-g.** The work surfaces of biological safety cabinets and other containment equipment are decontaminated when work with organisms containing recombinant DNA molecules is finished. Plastic-backed paper toweling used on non-perforated work surfaces within biological safety cabinets facilitates clean-up.

**Appendix G-II-C-2-h.** An insect and rodent program is in effect.

**Appendix G-II-C-2-i.** Laboratory clothing that protects street clothing (e.g., solid front or wrap-around gowns, scrub suits, coveralls) is worn in the laboratory. Laboratory clothing is not worn outside the laboratory, and it is decontaminated prior to laundering or disposal.

**Appendix G-II-C-2-j.** Special care is taken to avoid skin contamination with contaminated materials; gloves should be worn when handling infected animals and when skin contact with infectious materials is unavoidable.

**Appendix G-II-C-2-k.** Molded surgical masks or respirators are worn in rooms containing experimental animals.

**Appendix G-II-C-2-l.** Animals and plants not related to the work being conducted are not permitted in the laboratory.

**Appendix G-II-C-2-m.** Laboratory animals held in a BL3 area shall be housed in partial-containment caging systems, such as Horsfall units (see Appendix G-III-K, *Footnotes and References of Appendix G*), open cages placed in ventilated enclosures, solid-wall and -bottom cages covered by filter bonnets or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet in radiation lamps and reflectors.

**Note:** Conventional caging systems may be used provided that all personnel wear appropriate personal protective devices. These protective devices shall include at a minimum wrap-around gowns, head covers, gloves, shoe covers, and respirators. All personnel shall shower on exit from areas where these devices are required.

**Appendix G-II-C-2-n.** All wastes from laboratories and animal rooms are appropriately decontaminated before disposal. *For research involving mammalian-transmissible HPAI H5N1 virus, liquid effluents should be chemically disinfected or heat-treated, or collected and processed in a central effluent decontamination system. Decontamination of shower and toilet effluents is not required, provided appropriate practices and procedures are in place for primary containment of mammalian-transmissible HPAI H5N1 virus. Animal tissues, carcasses, and bedding originating from the animal room must be decontaminated by an effective and validated method (e.g., use of an autoclave) preferably before leaving the containment barrier. If waste must be transported, special practices should be developed for transport of infectious materials to designated alternate location(s) within the facility.*

**Appendix G-II-C-2-o.** Vacuum lines are protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.

**Appendix G-II-C-2-p.** Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

**Appendix G-II-C-2-q.** Spills and accidents which result in overt or potential exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, and NIH/OBA. Reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.

**Appendix G-II-C-2-r.** Baseline serum samples for all laboratory and other at-risk personnel should be collected and stored *in accordance with institutional policy and at least for the time period in which the personnel continues to work with the agent at biosafety level 3 containment. Such samples must be collected and stored for laboratory and other at risk personnel who will work with mammalian-transmissible HPAI H5N1 virus.* Additional serum specimens may be collected periodically depending on the agents handled or the function of the laboratory.

**Appendix G-II-C-2-s.** A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow the instructions on practices and procedures.

#### **Appendix G-II-C-2-t. Alternative Selection of Containment Equipment (BL3)**

Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified may be conducted in the BL3 laboratory using containment equipment specified for the BL2 level of physical containment. Experimental procedures involving a host-vector system that provides a one-step lower level of biological containment than that specified may be conducted in the BL3 laboratory using containment equipment specified for the BL4 level of physical containment. Alternative combination of containment safeguards are shown in Appendix G-Table 1, *Possible Alternate Combinations of Physical and Biological Containment Safeguards.*

#### **Appendix G-II-C-3. Containment Equipment (BL3)**

**Appendix G-II-C-3-a.** Biological safety cabinets (Class I, II, or III) (see Appendix G-III-L, *Footnotes and References of Appendix G*) or other appropriate combinations of personal protective or physical containment devices (e.g., special protective clothing, masks, gloves, respirators, centrifuge safety cups, sealed centrifuge rotors, and containment caging for animals) are used for all activities with organisms containing recombinant DNA molecules which pose a threat of aerosol exposure. These include: manipulation of cultures and of those clinical or environmental materials which may be a source of aerosols; the aerosol challenge of experimental animals; the harvesting of infected tissues or fluids from experimental animals and embryonate eggs; and the necropsy of experimental animals.

#### **Appendix G-II-C-4. Laboratory Facilities (BL3)**

**Appendix G-II-C-4-a.** The laboratory is separated from areas which are open to unrestricted traffic flow within the building. Passage through two sets of doors is the basic requirement for entry into the laboratory from access corridors or other contiguous areas. Physical separation of the high containment laboratory from access corridors or other laboratories or activities may be provided by a double-doored clothes change room (showers may be included), airlock, or other access facility which requires passage through two sets of doors before entering the laboratory.

**Appendix G-II-C-4-b.** The interior surfaces of walls, floors, and ceilings are water resistant so that they can be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate decontaminating the area.

**Appendix G-II-C-4-c.** Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

**Appendix G-II-C-4-d.** Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.

**Appendix G-II-C-4-e.** Each laboratory contains a sink for hand washing. The sink is foot, elbow, or automatically operated and is located near the laboratory exit door.

**Appendix G-II-C-4-f.** Windows in the laboratory are closed and sealed.

**Appendix G-II-C-4-g.** Access doors to the laboratory or containment module are self-closing.

**Appendix G-II-C-4-h.** An autoclave for decontaminating laboratory wastes is available preferably within the laboratory.

**Appendix G-II-C-4-i.** A ducted exhaust air ventilation system is provided. This system creates directional airflow that draws air into the laboratory *from uncontaminated spaces surrounding the laboratory*. The exhaust air is not recirculated to any other area of the building, is discharged to the outside, and is dispersed away from occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the laboratory) is proper. The exhaust air from the laboratory room may be discharged to the outside without being filtered or otherwise treated *unless research is being conducted with mammalian-transmissible HPAI H5N1 virus. For research with mammalian-transmissible HPAI H5N1 virus, exhaust air must be HEPA filtered and there must be sealed ductwork from the containment barrier to the filter. In addition, the air handling system shall be designed such that under failure conditions, the airflow will not be reversed and periodic verification, with annual verification of the HEPA filters, shall be performed. Finally, backup power shall be available for critical controls and instrumentation necessary to maintain containment.*

**Appendix G-II-C-4-j.** The high efficiency particulate air/HEPA filtered exhaust air from Class I or Class II biological safety cabinets is discharged directly to the outside or through the building exhaust system. Exhaust air from Class I or II biological safety cabinets may be recirculated within the laboratory if the cabinet is tested and certified at least every twelve months. If the HEPA-filtered exhaust air from Class I or II biological safety cabinets is to be discharged to the outside through the building exhaust air system, it is connected to this system in a manner (e.g., thimble unit connection (see Appendix G-III-L, *Footnotes and References of Appendix G*)) that avoids any interference with the air balance of the cabinets or building exhaust system.

**Appendix G-II-C-5. Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses.**

*(See Appendices G-II-C-2-n, G-II-C-2-r, and G-II-C-4-i for additional guidance for facilities, waste handling, and serum collection for research involving mammalian-transmissible HPAI H5N1 virus.)*

**Appendix G-II-C-5-a. Containment, Practices, and Training for Research with Risk Group 3 Influenza Viruses (BL3 Enhanced)**

**Appendix G-II-C-5-a-(1).** In addition to standard BL3 practices, the following additional personal protective equipment and practices shall be used: 1) Powered Air-purifying Respirators (PAPR) are worn. 2) Street clothes are changed to protective suit (e.g., wrap-back disposable gown, olefin protective suit). 3) Double gloves (*disposable*) are worn. *For research with mammalian-transmissible HPAI H5N1 viruses, protective sleeves shall be worn over the gown while working in a biosafety cabinet.* 4) Appropriate shoe coverings are worn (e.g., double disposable shoe coverings, single disposable shoe coverings if worn with footwear dedicated to BL3 enhanced laboratory use, or impervious boots or shoes of rubber or other suitable material that can be decontaminated). 5) Showers prior to exiting the laboratory should be considered depending on risk assessment of research activities, *with the exception that showers prior to exiting the laboratory are required for all research with mammalian-transmissible HPAI H5N1 virus, including care of animals infected with mammalian-transmissible HPAI H5N1 virus.* (6) *For research with mammalian-transmissible*

*HPAI H5N1 virus, prior to leaving containment, personal protective equipment shall be sprayed or wiped down with a disinfectant that has activity against influenza viruses. (7) In order to promote adherence to proper practices, including proper removal of personal protective equipment, and reporting of any loss of containment or exposures, at least two individuals should be in the laboratory at all times when research with mammalian-transmissible HPAI H5N1 virus involves experimental procedures with animals or sharps, or when procedures are being conducted whereby the generation of aerosols is reasonably anticipated. Removal of personal protective equipment should be observed.*

**Appendix G-II-C-5-a-(2).** As proper training of laboratory workers is an essential component of biosafety, retraining and periodic reassessments (at least annually) in BL3 enhanced practices, especially the proper use of respiratory equipment, such as PAPRs, and clothing changes, are required. *For research with mammalian-transmissible HPAI H5N1 virus, laboratory workers shall be required to sign a document acknowledging their understanding of and intent to adhere to biosafety, biosecurity, and occupational health requirements. This document shall include a statement that the laboratory worker agrees to report any exposures or accidents, including those by other individuals in the lab.*

**Appendix G-II-C-5-a-(3).** Reporting of all spills and accidents, even if relatively minor, is required as described in Appendix G-II-C-2-q.

**Appendix G-II-C-5-a-(4).** To avoid inadvertent cross contamination of 1918 H1N1, HPAI H5N1 or human H2N2 (1957-1968): (1) Containment facilities and practices appropriate for highest risk group virus shall be used at all times with lower risk group viruses, when studied in the same laboratory room. (2) Tissue cultures with these viruses shall be conducted at separate times (temporal spacing) in the same room. (3) Separate reagents shall be used to minimize risk of cross contamination. (4) A laboratory worker shall not perform concurrent influenza virus experiments that carry the risk of unintended reassortment among 1918 H1N1, human H2N2 (1957-1968), HPAI H5N1 and other human influenza viruses. (5) Two or more laboratory workers shall not perform within the same work area simultaneous influenza virus experiments that carry the risk of unintended segment reassortment between 1918 H1N1, or HPAI H5N1, or human H2N2 (1957-1968) and other human influenza viruses. (6) Between experiments good biosafety decontamination practices (e.g., surface and biosafety cabinet surface decontamination according to standard BL3 procedures) shall be used and there shall be a thirty minute wait period after decontamination before equipment is used for experiments with any other influenza A viruses. (7) Between experiments, in addition to decontamination of the work area, clothing changes and PAPR disinfection shall be performed prior to handling a different influenza virus in the same work area. (Shower-out capability may be required by USDA/APHIS for certain experiments with HPAI H5N1.)

**Appendix G-II-C-5-a-(5).** Continued susceptibility of the reassortant influenza viruses containing genes and/or segments from 1918 H1N1, HPAI H5N1, and human H2N2 (1957-1968) to antiviral agents shall be established by sequence analysis or suitable biological assays. After manipulation of genes that influence sensitivity to antiviral agents, susceptibility to these agents shall be reconfirmed. *If susceptibility to neuraminidase inhibitors or other effective antiviral agents is lost as a result of genetic modification or serial passage of a mammalian-transmissible HPAI H5N1 virus, then any research with this antiviral agent-resistant virus shall be stopped and research shall only proceed after review by the NIH (as outlined in Section III-A-1-a) or the appropriate federal regulatory agency.*

**Appendix G-II-C-5-b. Containment for Animal Research.**

Guidance provided in Appendix G-II-C and Appendix Q-II-C is applicable with the following emphasis on standard BL3 or BL3-N containment or additional enhancements.

**Appendix G-II-C-5-b-(1).** Research with small animals shall be conducted in a class II biosafety cabinet. Small animals such as rodents (e.g. mice, hamsters, rats, guinea pigs) can be housed within a negative

pressure BL3 animal suite using high-density individually vented caging (IVC) systems that independently supply high efficiency particulate air/HEPA-filtered and directional air circulation. Other animals (e.g. rabbits, ferrets) that are of a size or have growth or caging requirements that preclude the use of high-density IVC systems are to be housed in negative pressure bioisolators.

**Appendix G-II-C-5-b-(2).** Large animals such as non-human primates shall be housed in primary barrier environments according to BL3-N containment requirements (see Appendix Q-II-C).

**Appendix G-II-C-5-b-(3).** Specialized training and proven competency in all assigned practices and procedures shall be required for laboratory staff, including staff involved in animal care.

**Appendix G-II-C-5-b-(4).** For HPAI H5N1 research, the *NIH Guidelines* defer to USDA/APHIS recommendations for biocontainment practices for loose housed animals.

**Appendix G-II-C-5-c. Occupational Health.** A detailed occupational health plan shall be developed in advance of working with these agents in consultation, as needed, with individuals with the appropriate clinical expertise. In addition, the appropriate public health authority shall be consulted (e.g., local public health officials) on the plan and a mock drill of this plan shall be undertaken periodically. The plan *shall include a description of the incident reporting system in place for incidents, which includes any loss of containment, spills, accidents, or potential exposures. The plan must specify that all incidents must be reported immediately to the appropriate institutional authorities, and no later than 24 hours to the appropriate public health authorities (e.g., the U.S. Department of Agriculture, the Centers for Disease Control and Prevention, NIH, local and state health authorities).*

**Appendix G-II-C-5-c-(1).** Laboratory workers shall be provided with medical cards which include, at a minimum, the following information: characterization of the influenza virus to which they have been potentially exposed, and 24-hour contact numbers for the principal investigator and institution's occupational health care provider(s).

**Appendix G-II-C-5-c-(2).** A detailed occupational health plan shall include: 1) Unless there is a medical contraindication to vaccination (e.g., a severe egg allergy), annual seasonal influenza vaccination as a prerequisite for research to reduce the risk of influenza-like illness *that would require* isolation and *testing* to rule out infection with experimental *viruses* and *raise the risk for* possible co-infection with circulating influenza strains. 2) Virus-specific vaccination, if available, should be offered *and if a licensed HPAI H5N1 vaccine is available, and there are no medical contraindications, laboratory workers performing research with mammalian-transmissible HPAI H5N1 virus should be vaccinated. A post-vaccination serum sample shall be collected, assessed for immune response, and stored in accordance with institutional policy, at least for the time in which the laboratory worker continues to conduct HPAI H5N1 virus research.* 3) Reporting of all respiratory symptoms and/or fever (i.e., influenza-like illnesses). *For research involving mammalian-transmissible HPAI H5N1 virus, laboratory workers shall be actively monitored for influenza-like illness (i.e., fever and respiratory symptoms).* 4) 24-hour access to a medical facility that is prepared to implement appropriate respiratory isolation to prevent transmission and is able to provide appropriate antiviral agents. Real-time reverse transcription polymerase chain reaction (RT-PCR) assays should be used *for virus detection and* to discriminate these viruses from currently circulating human influenza viruses. For exposures to viruses containing genes from 1918 H1N1 or the HA gene from human H2N2 (1957-1968), specimens shall be sent to the CDC for testing (RT-PCR and confirmatory sequencing).

**Appendix G-II-C-5-c-(3).** In preparing to perform research with 1918 H1N1, human H2N2 (1957-1968), or HPAI H5N1, principal investigators should develop a clear plan specifying who will be contacted in the event of a potential exposure (during and after work hours) to conduct a risk

assessment and make decisions as to the required response, including the need for and extent of isolation of the exposed worker. After any kind of potential exposure, a rapid risk assessment shall be performed by the Principal Investigator, health and biosafety officials, and subsequent actions should depend on the appraised level of risk of respiratory infection for the individual and potential for transmission to others. A laboratory worker performing research with either an influenza virus containing the HA gene from human H2N2 or an influenza virus containing genes and/or segments from 1918 H1N1 or mammalian-transmissible HPAI H5N1 viruses, shall be informed in advance that, in the case of a *known* laboratory exposure with a high risk for infection, e.g., involving the upper or lower respiratory tract or mucous membranes, the laboratory worker will need to be isolated in a predetermined facility, rather than home isolation, until infection can be ruled out by testing (e.g., negative RT-PCR for 1918 H1N1, human H2N2 (1957-1968), or HPAI H5N1) of appropriately timed specimens. Laboratory workers *with a known laboratory exposure with high risk for infection during research with HPAI H5N1 virus strains that are not transmissible among mammals* should be prepared to self-isolate (for example at home) until infection can be ruled out by testing (e.g., RT PCR for HPAI H5N1) of appropriately timed specimens. The action taken for other types of exposures should be based on the risk assessment. In addition, based on the risk assessment: (1) treatment with appropriate antiviral agents shall be initiated, and (2) the appropriate public health authorities shall be notified.

**Appendix G-II-C-5-c-(4). Influenza-like illness.** *If an individual has entered (within ten days) a laboratory conducting research with influenza viruses containing the human H2N2 HA gene or any gene from the 1918 H1N1 or HPAI H5N1 viruses, or housing animals exposed to such viruses, and the individual demonstrates symptoms and/or signs of influenza infection (e.g., fever/chills, cough, myalgia, headache), then he/she shall report by phone to the supervisor/Principal Investigator and other individuals identified in the occupational health plan. If needed, the person with influenza-like illness shall be transported, under the appropriate isolation conditions, to a healthcare facility that can provide adequate respiratory isolation, appropriate medical therapy, and testing to determine whether the infection is due to a recombinant influenza virus. The appropriate public health authorities shall be informed whenever a suspected case is isolated.*

**Appendix G-II-C-5-c-(5).** For 1918 H1N1 research, the use of antiviral agents (e.g., oseltamivir) for pre-exposure prophylaxis shall be discussed with laboratory workers in advance including a discussion of the data on the safety of long term exposure to these agents and their ability to reduce the risk of clinical disease and the limits of the data regarding protection of close contacts and the community.

**Appendix G-II-C-5-c-(6).** Antiviral agents for *an exposure* shall be provided only after medical evaluation. Home supplies shall not be provided in advance for research with 1918 H1N1, mammalian-transmissible HPAI H5N1 or influenza viruses containing the HA gene from human H2N2.