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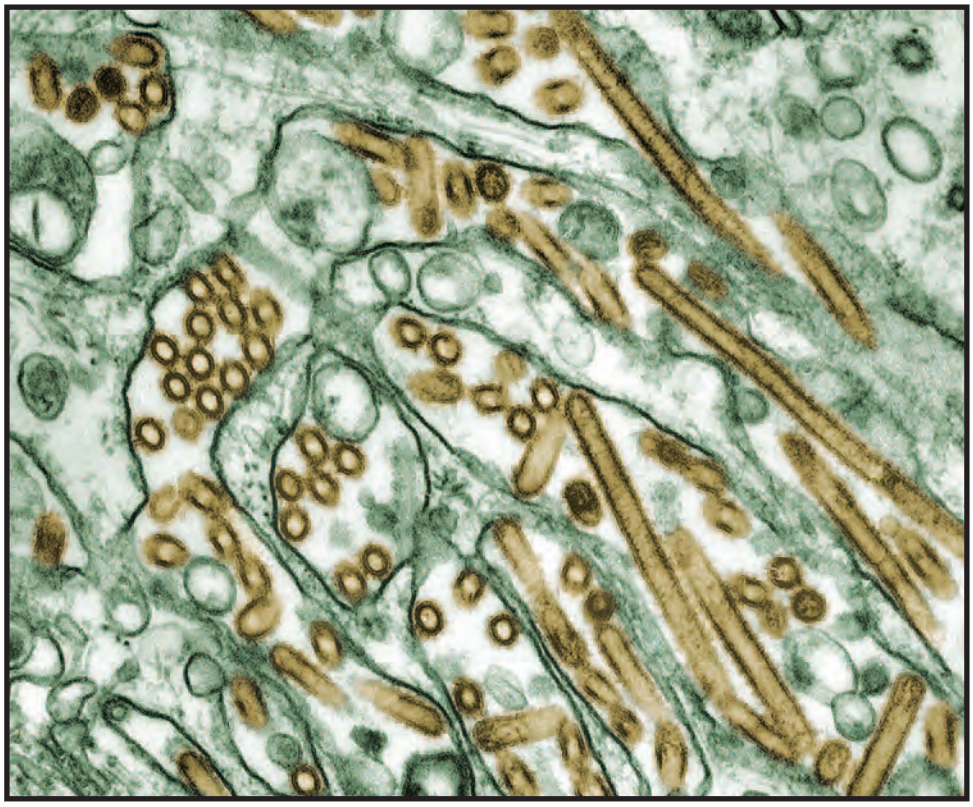
# Biosafety Recommendations for Work with Influenza Viruses Containing a Hemagglutinin from the A/goose/Guangdong/1/96 Lineage



**U.S. Department of Health and Human Services**
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**Front cover photo:** Transmission electron micrograph of avian influenza A H5N1 viruses (*gold*) grown in Madin-Darby canine kidney (MDCK) cells (*green*).

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# Biosafety Recommendations for Work with Influenza Viruses Containing a Hemagglutinin from the A/goose/Guangdong/1/96 Lineage

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## Summary

*The CDC and National Institutes of Health (NIH)* Biosafety in Microbiological and Biomedical Laboratories *(BMBL) manual describes biosafety recommendations for work involving highly pathogenic avian influenza (HPAI) (US Department of Health and Human Services [HHS], CDC. Biosafety in microbiological and biomedical laboratories, 5th ed. Atlanta, GA: CDC; 2009. HHS publication no. [CDC] 21-1112. Available at http://www.cdc.gov/biosafety/publications/bmbl5). The U.S. Department of Agriculture* Guidelines for Avian Influenza Viruses *builds on the BMBL manual and provides additional biosafety and biocontainment guidelines for laboratories working with HPAI (US Department of Agriculture, Animal and Plant Health Inspection Service, Agricultural Select Agent Program. Guidelines for avian influenza viruses. Washington, DC: US Department of Agriculture; 2011. Available at http://www.selectagents.gov/Guidelines_for_Avian_Influenza_Viruses.html). The recommendations in this report, which are intended for laboratories in the United States, outline the essential baseline biosafety measures for working with the subset of influenza viruses that contain a hemagglutinin (HA) from the HPAI influenza A/goose/Guangdong/1/96 lineage, including reassortant influenza viruses created in a laboratory setting. All H5N1 influenza virus clades known to infect humans to date have been derived from this lineage (WHO/OIE/FAO H5N1 Evolution Working Group. Continued evolution of highly pathogenic avian influenza A [H5N1]: updated nomenclature. Influenza Other Respir Viruses 2012;6:1–5). In 2009, the NIH* Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules *were amended to include specific biosafety and biocontainment recommendations for laboratories working with Recombinant Risk Group 3 influenza viruses, including HPAI H5N1 influenza viruses within the Goose/Guangdong/1/96-like H5 lineage. In February 2013, the NIH guidelines were further revised to provide additional biosafety containment enhancements and practices for research with HPAI H5N1 viruses that are transmissible among mammals by respiratory droplets (i.e., mammalian-transmissible HPAI H5N1) (National Institutes of Health, Office of Biotechnology Activities. NIH guidelines for research involving recombinant or synthetic nucleic acid molecules. Appendix G-II-C-5: biosafety level 3 enhanced for research involving risk group 3 influenza viruses. Bethesda, MD: National Institutes of Health; 2013. Available at http://oba.od.nih.gov/rdna/nih_guidelines_oba.html). The recent revisions to the NIH guidelines focus on a smaller subset of viruses but are applicable and consistent with the recommendations in this report.*

*The biosafety recommendations in this report were developed by CDC with advice from the Intragovernmental Select Agents and Toxins Technical Advisory Committee, which is a panel composed of federal government subject-matter experts, and from public input received in response to the request for information that was published in the* Federal Register *on October 17, 2012 (US Department of Health and Human Services, CDC. Influenza viruses containing the hemagglutinin from the Goose/ Guangdong/1/96 lineage; proposed rule; request for information and comment. 42 CFR, Part 73. Federal Register 2012;77:63783–5). Work with HPAI H5N1 virus should be conducted, at a minimum, at biosafety level 3 (BSL-3), with specific enhancements to protect workers, the public, animal health, and animal products. Original clinical specimens suspected of containing viruses of this lineage can only be handled at BSL-2 if the procedures do not involve the propagation of the virus. An appropriate biosafety level should be determined in accordance with a biosafety risk assessment. Additional information on performing biosafety risk assessments and establishing effective biosafety containment is available in the BMBL manual.*

The material in this report originated in the Division of Select Agents and Toxins, Office of Public Health Preparedness and Response, Robbin S. Weyant, PhD, Director.
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# Introduction

During 2003–2011, the World Health Organization (WHO) received reports of approximately 600 confirmed cases of human infections with highly pathogenic avian influenza (HPAI) H5N1 viruses, with a mortality rate of approximately 59% (*1*). To date, almost all human cases have been linked to close contact with infected poultry. Several reports of family clusters indicate that human-to-human transmission might occur, although infrequently, after prolonged and close contact with an infected person (*2*). All human cases reported during 2003–2011 have been caused by viruses containing the hemagglutinin from the A/goose/Guangdong/1/96 lineage. Epidemiologic evidence indicates that once transmitted into a human host, HPAI H5N1 viruses might result in more severe disease than other subtypes of influenza (*3*). Recent studies designed to identify genetic elements related to the transmissibility of these viruses have produced mammalian and avian influenza viruses containing the hemagglutinin from the A/goose/Guangdong/1/96 lineage that are transmissible via respiratory droplets in the ferret model (*4*,*5*), which is considered to be the animal model most representative of human influenza *(6)*. In response to these developments, CDC has developed the following biosafety guidelines for working with this lineage and specimens suspected of containing these viruses.

# Background

## Influenza A Nomenclature and the Importance of the Hemagglutinin Subtype

Influenza viruses are RNA viruses in the family Orthomyxoviridae. The influenza viruses are categorized into three main types on the basis of their antigenic properties: A, B, and C. Influenza A viruses, which are the most virulent to humans, are divided into subtypes and identified on the basis of two surface glycoproteins, hemagglutinin (HA or H) and neuraminidase (NA or N). Sixteen HA subtypes (H1 to H16) and nine NA subtypes (N1 to N9) are known that circulate in wild birds; more divergent H and N subtypes recently have been isolated from bats (*7*,*8*). For example, an influenza A virus that has an H5 HA and an N1 NA would be designated as an H5N1 virus. The nomenclature of avian influenza viruses is based on a standardized format representing the influenza type, the host origin, the place of collection, the virus identification number, the year of isolation, and the influenza A subtype designation in parentheses. For example, A/chicken/Texas/309402/04 (H5N2) is an H5N2 influenza A virus that was collected from a chicken in Texas in 2004 and assigned the strain number 309402 (*9*,*10*).

Avian influenza virus strains are further classified as low or highly pathogenic on the basis of specific molecular determinants of the HA protein (e.g., nucleotide sequence) and the biologic behavior of the virus during in vitro and in vivo tests. Most avian influenza viruses are associated with mild disease in poultry and are referred to as low pathogenic avian influenza (LPAI) viruses. In contrast, avian influenza viruses that meet HPAI criteria typically are associated with severe illness and high mortality in poultry (*11*). All poultry outbreaks of HPAI have been caused by influenza A viruses of the subtypes H5 or H7 (*12*).

Cleavage of the HA molecule by host proteases is required for influenza viruses to infect cells and replicate. The cleavage site of the HA protein of many influenza viruses is recognized by trypsinlike proteases expressed on surface cells lining the respiratory and gastrointestinal tracts, which limits disease to those tissues. The polybasic cleavage site of HPAI viruses is recognized by a wide range of proteases that are distributed ubiquitously, resulting in virus replication in many tissues and systemic disease. Extrapulmonary dissemination of H5N1 virus has been documented in some fatal cases of H5N1 virus infections in humans (*13*). The HA molecule also mediates binding of the influenza virus to host cells in the respiratory tract. Human influenza viruses preferentially bind to different receptors than avian influenza viruses (*14*). Although human influenza virus receptors are more prevalent in the upper respiratory tract, avian influenza virus receptors are more prevalent in the lower respiratory tract of humans. The ability of H5N1 viruses to bind and infect cells in the lungs might contribute to the severity of H5N1-induced viral pneumonia (*15*–*17*). Furthermore, a change from avian- to human-type receptor-binding specificity, as occurred with the pandemic strains of 1918 (H1N1), 1957 (H2N2), and 1968 (H3N2), is considered a critical step in the adaptation of avian influenza viruses to humans and the ability to transmit efficiently among humans (*18*–*20*). Findings from recent influenza studies in the ferret animal model indicate that laboratory-modified influenza viruses with certain mutations can be transmitted via respiratory droplets between ferrets (*4*,*5*). Ferrets are regarded in the scientific community as the best surrogate animal model for human infection with influenza viruses (*6*).

Because all H5N1 influenza virus clades known to infect humans have been derived from the A/goose/Guangdong/1/96 lineage, and because the HA confers the ability to infect a wide range of host cells and therefore plays a key role in the pathogenicity of this virus in poultry and likely also in humans, biosafety guidelines are imperative for work with influenza viruses that contain an HA from the A/goose/Guangdong/1/96 lineage (*21*).

## Applicable Regulations

All HPAI viruses are regulated by the U.S. Department of Agriculture (USDA) as select agents pursuant to section 121.3(b) of Title 9 of the Code of Federal Regulations (9 CFR §121.3[b]). The select agent regulatory requirements include but are not limited to biosafety, security, incident response, training, and notification of theft, loss, and release (available at http://www.selectagents.gov). Avian influenza viruses classified as low pathogenic viruses, including H5 and H7 subtypes, are regulated by USDA pursuant to part 122 (Organisms and Vectors) of Title 9 of the CFR (9 CFR part 122). Additional information on the regulation of reassortant avian influenza viruses is available from USDA's *Guidelines for Avian Influenza Viruses* (*11*).

On October 17, 2012, CDC published a request for information in the *Federal Register* to obtain input from interested parties of the public on the potential regulation of the agent and biosafety recommendations when working with influenza viruses containing an HA from the A/goose/Guangdong/1/96 lineage (*22*). Responses to this notice, in conjunction with input from subject-matter experts, will be used by CDC to determine whether to regulate these viruses as U.S. Department of Health and Human Services select agents. The input received in response to this notice has been included in this report. The recommendations in this report, which are intended for laboratories in the United States, outline the essential baseline biosafety measures for working with the subset of influenza viruses that contain an HA from the HPAI influenza A/goose/Guangdong/1/96 lineage, including reassortant influenza viruses created in a laboratory setting.

## Guidelines for Working with Influenza Viruses Containing an HA from the A/goose/Guangdong/1/96 Lineage

In response to publications examining the genetic changes that might enable currently circulating strains of influenza viruses within the Goose/Guangdong/95-like lineage to transmit among mammals by respiratory droplets (*4*,*5*), CDC developed biosafety recommendations for work with these viruses. The biosafety recommendations in this report were developed by CDC with advice from the Intragovernmental Select Agents and Toxins Technical Advisory Committee, which is a panel composed of federal government subject-matter experts, and from public input received in response to the request for information that was published in the *Federal Register* on October 17, 2012 (*22*). Specific guidelines regarding work with influenza viruses containing an HA from the A/goose/Guangdong/1/96 lineage are presented in the following sections. The biosafety and biocontainment guidelines that follow apply to both laboratory-generated and natural isolates of HPAI viruses.

## Occupational Health Guidelines for Work with Influenza Viruses Containing an HA from the A/goose/Guangdong/1/96 Lineage

In addition to the biocontainment guidance in the following section, the following guidelines apply to work at all biosafety levels with influenza viruses containing an HA from the A/goose/Guangdong/1/96 lineage. Before working with these agents, an occupational health plan should be developed in consultation with persons with the appropriate clinical expertise. This plan should include the following:

- A properly constituted respiratory protection program should exist that complies with Occupational Safety and Health Administration regulations 29 CFR §1910.134. A primary prerequisite before entering a laboratory for work with influenza viruses containing the HA from the A/goose/Guangdong/1/96 lineage is respiratory protection. Either a powered air-purifying respirator (PAPR) with a high-efficiency particulate air (HEPA) filter or a properly fitted full-face respirator with HEPA or N95 particulate protection is required for work with these viruses and is applicable to all BSLs, with the exception of BSL-4 suit laboratories. The selection of an appropriate respirator depends on the type of activity anticipated in the laboratory. Training in the effective use of respirators is mandatory and requires yearly certification by the occupational health and safety program of each laboratorian's organization or laboratory.
- Baseline serum samples should be stored in accordance with institutional policy for as long as the laboratory workers have the potential for exposure to influenza viruses containing an HA from the A/goose/Guangdong/1/96 lineage.
- Laboratory workers must be required to be vaccinated against seasonal influenza unless an absolute medical contraindication exists.
- If a licensed HPAI H5N1 vaccine is available and no medical contraindications exist, the vaccine should be administered to all laboratory workers. A postvaccination serum sample should be collected, assessed for immune response, and stored in accordance with institutional policy, at least for the time in which the person continues to have the potential for exposure to influenza viruses

containing an HA from the A/goose/Guangdong/1/96 lineage. An assessment of the immune response should be made in consultation with appropriate medical professionals. If an Investigational New Drug (IND) is available, it should be considered in consultation with appropriate medical professionals.
- All staff working with influenza viruses containing an HA from the A/goose/Guangdong/1/96 lineage should be enrolled in a medical surveillance program, based on institutional policy. A medical surveillance program should include a response plan to a confirmed or suspected exposure and the use of vaccination and antiviral treatment. Counseling and training should be provided regarding disease symptoms and protocols for reporting possible exposures and symptoms.
- Isolation and antiviral treatment protocols should be in place for exposed personnel on the basis of a site-specific risk assessment and the health-care practitioner's clinical judgment.

## Biocontainment Guidelines for Work with Influenza Viruses Containing an HA from the A/goose/Guangdong/1/96 Lineage

A list of appropriate work practices, safety equipment, and engineering controls for the containment levels listed in the following sections are available in the BMBL manual (*23*). The biosafety level selected depends on a site-specific risk assessment. Work with influenza viruses containing the HA from the A/goose/Guangdong/1/96 lineage may only be conducted in laboratories meeting one of the following four biosafety level options. (Guidelines derived from the BMBL manual and USDA *Guidelines for Avian Influenza Viruses* [*11*].)

### 1. BSL-4 and Animal BSL-4

Work with influenza viruses containing the HA from the A/goose/Guangdong/1/96 lineage may be conducted in BSL-4 and animal BSL-4 (ABSL-4) containment levels. A comprehensive list of BSL-4 and ABSL-4 practices, procedures, containment, equipment, and facility requirements are available in the BMBL manual (*23*). No additional provisions for work with influenza viruses containing the HA from the A/goose/Guangdong/1/96 lineage are provided in this document for BSL-4 and ABSL-4 containment levels.

### 2. BSL-3 Agriculture, with the Following Provisions

BSL-3 agriculture (BSL-3-Ag) conditions are appropriate for work with open-caged or loosely housed animals infected with influenza viruses containing an HA from the A/goose/Guangdong/1/96 lineage. BSL-3-Ag facilities have containment features of ABSL-3 facilities, as well as enhancements of features common in BSL-4 facilities. A list of BLS-3-Ag biocontainment features, work practices, equipment, and engineering controls are outlined in the BMBL manual and USDA *Guidelines for Avian Influenza Viruses* (*11*,*23*). In addition, the following provisions also are recommended.
- **Personnel quarantine policy:** Laboratory staff members and visitors are restricted from having contact with any susceptible avian species for at least 5 days after last possible contact with the virus. The prohibition includes but is not limited to contact with any avian wildlife, pet birds, backyard poultry, birds at county and state fairs, commercial poultry operations, and zoos. Local institutional policy must require that employees and visitors read the quarantine policy and acknowledge by signature their agreement to comply with that policy. Laboratories that work with HPAI viruses should not be close to avian colonies. No commercial poultry flocks should be located within 0.5 mile (0.8 km) of the facility or any other avian colonies (e.g., backyard flock, aviary, or zoo) within 328 feet (100 m) of the facility.
- **Personal protective equipment (PPE):** PPE used should include the following: 1) disposable hood or head cover; 2) protective eyewear (e.g., safety glasses); 3) respiratory protection (preferably PAPRs); 4) disposable double gloves; 5) disposable protective suit (e.g., wrap-back disposable gown and olefin protective suit or coveralls); 6) and disposable shoe or foot covers. Before leaving the containment area, PPE must be decontaminated by an effective and validated method (e.g., use of an autoclave or sprayed disinfectant) with activity against influenza virus.

### 3. BSL-3 Enhanced, with the Following Provisions

These conditions are appropriate for in vitro work with influenza viruses containing the HA from the A/goose/Guangdong/1/96 lineage.
- **Personnel quarantine policy (see previous section)**
- **PPE (see previous section)**
- **Air handling:** HEPA filtration of laboratory exhaust air is mandatory. The exhaust system must have a sealed ductwork system from the containment barrier to the filter. The heating, ventilation, and air conditioning system must

be designed and operated in a way that prevents airflow reversals under failure conditions.

- **Showers:** A gown-in, shower-out protocol should be used to enforce changing out of street clothes.
- **Decontamination of laboratory liquid effluents:** Liquid effluents originating from laboratories should be collected locally and chemically disinfected or heat treated or collected and processed in a central effluent decontamination system before being released into the local sanitary system. The decontamination of shower and toilet effluents is not a requirement if appropriate practices and procedures are in place for primary containment.

### 4. Animal BSL-3 Enhanced, with the Following Provisions

ABSL-3 conditions are appropriate for work with influenza viruses containing the HA from the A/goose/Guangdong/1/96 lineage that involves animals housed in caging systems.

- **Personnel quarantine policy (see previous section)**
- **Air handling (see previous section)**
- **Showers (see previous section)**
- **Decontamination of laboratory liquid effluents (see previous section)**
- **PPE (see previous section)**
- **Special caging:** Animals infected with influenza viruses containing the HA from the A/goose/Guangdong/1/96 lineage must be housed in primary biocontainment units. Containment at the cage level can be achieved in several ways depending on preference and animal size. For example, primary biocontainment housing can include a containment cage or rack system, flexible film isolator, or glove box. Caging should be ventilated, and all the exhaust air should be HEPA filtered. The primary barrier system housing the animal cages must be installed and operated in a way that prevents airflow reversals and minimizes the effect on adjacent spaces. Static microisolators do not prevent air from leaking into the laboratory space.
- **Decontamination of solid animal wastes:** All 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 an autoclave is not conveniently located in areas where infectious materials or animals (or both) are housed or are manipulated, distinct practices should be developed for transport of infectious materials to designated alternative locations within the facility. An appropriate final method of disposal of carcasses of large infected animals (i.e., incineration or other approved means) should be used.

## Diagnostic Specimens

Laboratory workers who process samples or perform non–culture-based diagnostic testing on original specimens suspected to contain influenza viruses containing the HA from the A/goose/ Guangdong/1/96 lineage virus should perform the work in a BSL-2 or higher laboratory with the following enhancements:

- All personnel must wear a disposable, wrap-around gown; shoe covers; an N95 respirator with eye protection or a face shield; or PAPR and must put on two pairs of gloves inside the room before beginning work. Persons must be appropriately trained and those wearing N95 respirators fit tested in accordance with 29 CFR 1910.134 (available at http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_id=12716&p_table=standards).
- When exiting the laboratory room, personnel must discard both pairs of gloves, shoe covers, gowns, and respirator in a biohazard bag inside the room. Personnel should not wear PPE outside of the laboratory and must remove PPE before exiting the laboratory.
- Personnel who wear eyeglasses without a face shield while working in the BSL-2 laboratory must thoroughly wash the eyeglasses with a germicidal soap before taking them out of the laboratory.
- All activities involving infectious materials must be conducted in biological safety cabinets or other physical containment devices within the laboratory. Work conducted in open vessels should not be performed on an open bench.
- Work surfaces of biological safety cabinets and other containment equipment must be decontaminated when work with infectious materials is finished.
- Small hand tools and similar items to be removed from the laboratory must first be decontaminated with appropriate disinfectants using suggested contact times per institutional biosafety guidelines. If feasible, the laboratory should have special engineering and design features to ensure directional airflow from clean to potentially contaminated areas.

A list of appropriate work practices, safety equipment, and engineering controls for BSL-2 containment are available in the BMBL manual (*23*). This work might include the use of the polymerase chain reaction or other in vitro diagnostic methods but not virus propagation. The sample may be placed in lysis buffer and only then handled outside of a biosafety cabinet in BSL-2 for molecular testing. The method of destruction (e.g., lysis buffer) must be safety tested to verify destruction of the live virus before removal of the agent to a lower biosafety level.

After a sample is identified as positive for influenza virus containing the HA from the A/goose/Guangdong/1/96 lineage, storage of the material may be continued under these BSL-2 enhanced conditions. However, any other manipulations or additional work with the material or any subsequent related virus isolates should be conducted only in BSL-3 enhanced or higher containment, as described in a previous section for work with the virus (*24*).

### NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

The *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* remain applicable to this and other research involving recombinant DNA, including review and approval by an Institutional Biosafety Committee (see section III-D-7 and Appendix G-II-C-5 of the NIH guidelines) (*25*). The NIH guidelines are available online (http://oba.od.nih.gov/oba/rac/Guidelines/NIH_Guidelines.htm).

The recent amendment to the NIH guidelines regarding mammalian-transmissible HPAI H5N1 virus focuses on the subset of research with the HPAI H5N1 viruses that are transmissible among mammals by respiratory droplets. The biosafety recommendations in this document apply to all research with influenza viruses containing an HA from the A/goose/Guangdong/1/96 lineage, and they are consistent with the NIH guidelines for research with mammalian-transmissible HPAI H5N1 virus.

## Conclusion

The recommendations in this report are intended to provide persons and laboratories with baseline guidance for working safely with influenza viruses containing an HA from the A/goose/Guangdong/1/96 lineage. Application of these recommendations should be carried out in conjunction with a site-specific and agent-specific risk assessment; biosafety containment and practices may be further enhanced or customized for a specific research project.

These recommendations are an important addition to the collective set of biosafety recommendations for research with these specific influenza viruses and will be reviewed periodically and refined as additional experience is gained. The ultimate effectiveness of these recommendations in preventing laboratory-acquired infections and other releases of these viruses out of biocontainment depends on the work being conducted by properly trained workers who understand the hazards posed by these materials.

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