**New July 18, 2024**

# Requirements for H5 Highly Pathogenic Avian Influenza Research

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## Purpose

This document outlines requirements set by the IBC for research involving H5 highly pathogenic avian influenza viruses. It does not cover and is not applicable to diagnostic and surveillance activities performed by clinical or public health laboratories.

## Containment Levels at a glance

| Research type | Containment level |
|---|---|
| Live H5 HPAI virus | BSL3/ABSL3 |
| Samples from infected animals | BSL3 |
| Samples from infected patients | BSL3 |
| Samples that may contain live H5 HPAI virus | BSL3 (high risk) or BSL2 (low risk) |
| Inactivated H5 HPAI samples | BSL2 |
| Nucleic acids from H5 HPAI virus | BSL1 (individual genes) or BSL2 (extracted/full genome) |
| Proteins from H5 HPAI virus | BSL1 |

## Background on H5 Highly Pathogenic Avian Influenza

Influenza is a contagious viral disease of the respiratory tract. Common symptoms include fever, headache, malaise, muscle aches, sore throat, and cough. There are four serotypes of Influenza: A, B, C, and D. In nature, influenza disease transmission occurs primarily through inhalation or ingestion, and may occur through direct contact with contaminated surfaces and fomites. Laboratory hazards include mucosal exposure to droplets and inhalation of aerosols resulting from pipetting, mixing, centrifuging, or other aerosol generating activities with viral cultures or infected cells, tissues, or animals.

Influenza A viruses are subtyped by the surface glycoproteins hemagglutinin (HA or H) and neuraminidase (NA or N). The HA protein mediates viral attachment and entry into host cells and must be cleaved into two subunits for infection to occur. Avian Influenza A viruses are classified as Low Pathogenic (LPAI) or Highly Pathogenic (HPAI) viruses based on amino acid differences in the HA cleavage site. In LPAI, only proteases found in the respiratory and gastrointestinal tracts perform this cleavage. HPAI is caused by certain influenza A(H5) and A(H7) viruses that have specific amino acids at the HA cleavage site that allow ubiquitously expressed proteases to cleave the HA protein. HPAI viruses produce systemic disease in birds characterized by high morbidity and mortality, often including severe neurological symptoms.

Influenza A viruses circulate in nature in birds or bats. These can be transmitted by wild birds to domestic animals including poultry and other species. Some HPAI viruses also infect mammals, including domesticated species. Pathogenicity in mammals may differ from that seen in avian species due to the expression of different cellular receptors for influenza viruses (see Figure). In birds, HPAI viruses are found in high concentrations in saliva, nasal secretions, and feces. HPAI viruses can remain viable for long periods in infected tissues, feces from infected animals, and contaminated water, but are readily inactivated by heat and disinfectants.

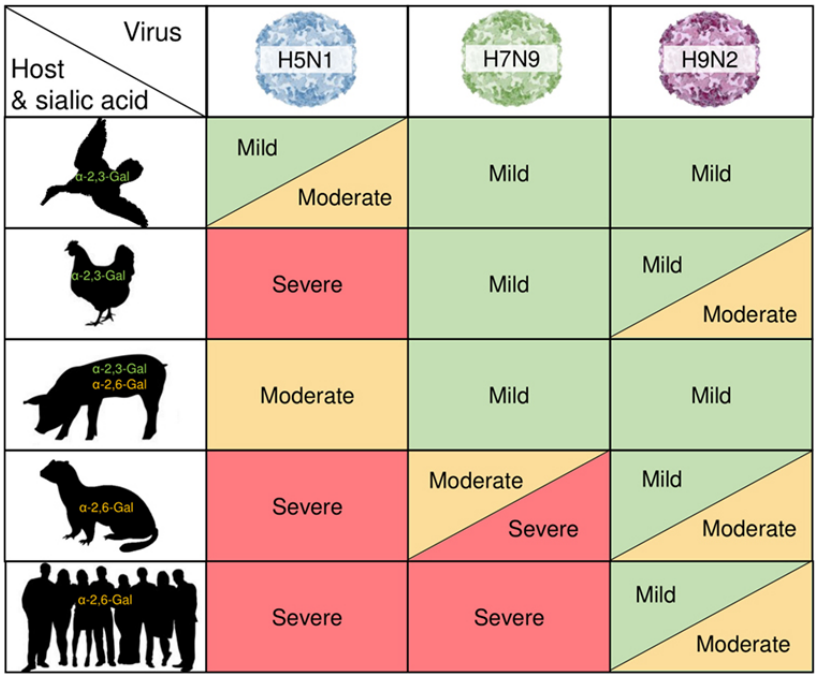


**Figure: Highly Pathogenic Avian Influenza (HPAI) Types and Clinical Severity Across Host Species**
Horman et al., Front. Immunol., 08 August 2018 (https://doi.org/10.3389/fimmu.2018.01812)

Although human infections with HPAI are rare, sporadic human infections with HPAI A(H5N1) virus have been reported in 23 countries since 1997 and have a case fatality rate greater than 50%. In spring 2024, HPAI A(H5N1) was reported in dairy cattle, followed closely by human cases in dairy workers exposed to infected cattle. High viral loads have been detected in raw milk from infected cows and HPAI virus has been found on milking equipment. The U.S. Department of Agriculture (USDA), the U.S. Food and Drug Administration (FDA), and the Centers for Disease Control and Prevention (CDC), along with state partners, continue to investigate outbreaks and provide public and laboratory guidance.

## Regulatory requirements for H5 HPAI research

HPAI viruses are considered select agents by the USDA Veterinary Services and are subject to select agent regulations 9 CFR Part 121.

**Temporary exemption from select agent regulations**

On June 6, 2024, the Administrator of the USDA Animal and Plant Health Inspection Service (APHIS) has utilized his exemption authority under 9 C.F.R. § 121.5(f) in the select agent and toxin regulations to exempt H5 avian influenza viruses from the requirements of the regulations listed in 9 C.F.R. Part 121 for a period of three years. **This exemption is temporary.** At the end of three years, or sooner if revoked, H5 HPAI viruses will once again fall under the requirements of the select agent regulations. H7 HPAI viruses do not fall under this exemption and remain subject to select agent regulations.

Importantly, APHIS has clarified that this exemption does not abrogate permitting requirements, and any laboratories – both diagnostic and research – may store and work with H5 viruses only if the correct permit is in place (see below).

**Permit requirements**

HPAI viruses may not be moved or transferred from one entity to another or imported unless the receiving entity has a valid permit. For the duration of the H5 HPAI virus exemption, the APHIS Veterinary Services Organisms and Vectors (OV) Permitting Unit will issue permits for importation and interstate transportation of all H5 HPAI viruses pursuant to 9 C.F.R. Part 122. Although the HPAI viral genome by itself is not considered a select agent, APHIS also regulates the movement of HPAI viral RNA, mRNA, and cDNA, RNA segments from HPAI viruses, and expressed HPAI viral proteins. The APHIS OV Permitting Unit can be contacted by email at apie@usda.gov.

An import permit from the Centers for Disease Control and Prevention's Import Permit Program is also required for all H5 HPAI variants that are known or suspected to cause human disease. If any imported agent is determined to not cause disease in humans (e.g., attenuated strains that are no longer infectious), then an importer certification statement should be included to avoid potential shipping delays. The Import Permit Program can be contacted by email at importpermit@cdc.gov.

Laboratories (diagnostic and research) may store and work with H5 viruses only if the correct permit is in place. Containment, facility requirements, personnel practices, and restrictions may be applied by APHIS as conditions of the permit for the possession and handling of animal-origin and zoonotic viruses. This may also include laboratory data/results to exclude the possibility of contamination with select agent HPAI viruses in specimens.

**Institutional Biosafety Committee review and approval**

As an institution that receives federal funding, UW-Madison is required to comply with the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acids (NIH Guidelines)*. Experiments that fall under sections III-A through III-D of the *NIH Guidelines* require approval from the UW-Madison Institutional Biosafety Committee (IBC) prior to initiation, even if low risk. Experiments that fall under section III-E of the *NIH Guidelines* may commence simultaneously with IBC notification (i.e., assignment of a biosafety protocol or amendment to an IBC meeting), but still require IBC review and approval.

Experiments that fall under section III-F of the *NIH Guidelines* (i.e., exempt experiments) or that are not subject to the *NIH Guidelines* because they do not involve recombinant or synthetic nucleic acids may still require a biosafety protocol and be subject to IBC review and approval. The IBC is charged by the Chancellor with responsibility for oversight of biological materials that entail a potential risk to humans, animals, plants, or the environment. The IBC is authorized to approve, require modifications to secure approval, or disapprove work with biohazardous materials. The IBC is also authorized to suspend or revoke authorization of work that is not conducted in accordance with their requirements. To determine if your planned research requires a biosafety protocol, please refer to the Bio-ARROW webpage or contact OBS at 608-263-2037 or biosafety@fpm.wisc.edu.

The IBC meets the first Wednesday of every month unless otherwise posted; please see the IBC webpage or contact OBS at 608-263-2037 or biosafety@fpm.wisc.edu for upcoming meeting information. Protocols and amendments are pre-reviewed by OBS staff prior to assignment to an IBC meeting. The cutoff for assignment to an IBC meeting is twelve days prior to the meeting date. **To allow time to complete the pre-review process it is recommended that new protocols and amendments are submitted at least one month prior to the IBC meeting date**.

## General considerations for H5 HPAI research

**H5 HPAI virus is classified as a risk group 3 (RG3) virus.** When planning any H5 HPAI research project, the following should be considered:

- In the event of an exposure to research materials, is there a risk of a lab-acquired H5 HPAI virus infection (i.e., will you be working with live H5 HPAI virus or materials that contain or potentially contain live H5 HPAI virus, such as clinical samples)?
- If exposure to live virus is possible, then:
    - What is the level of exposure risk? (e.g., Will the virus be isolated or cultured? Will samples come from known H5 HPAI veterinary or human patients or are they unknown diagnostic specimens? What type of tissue samples or bodily fluids will be used?)
    - What is the laboratory's experience with Influenza or other respiratory pathogens?
    - Who will train personnel to work with the virus/potentially infectious materials?
    - Has the laboratory developed all the necessary SOPs for working with the virus/potentially infectious materials (e.g., donning and doffing personal protective equipment (PPE), disinfection, spill protocols, emergency response)?
    - Does the laboratory have an H5 HPAI virus occupational health plan?
    - Does the laboratory have a quarantine policy?
    - Have laboratory staff been offered an Influenza vaccine?
- Does the laboratory have the facilities and containment equipment appropriate for the level of risk (as outlined in the sections below)?

- Does the laboratory have access to PPE appropriate for the proposed work (e.g., PAPR, Tyvek)?
- What other hazards are associated with this research (e.g., bloodborne pathogens, viral vectors, sharps), and how will their risk be mitigated?
- If you will be using human subjects or samples collected from human subjects, do you have Institutional Review Board (IRB) approval for this research?
- If you will be using animal models, do you have Institutional Animal Care and Use Committee (IACUC) approval for this research?

The sections below outline the typical requirements for different types of H5 HPAI research. As many factors affect risk (e.g., genetic modifications, equipment, assays, and procedures), OBS and the IBC will work with you to determine the appropriate containment level, location, practices, and PPE needed for your specific research. For questions, please contact OBS at 608-263-2037 or biosafety@fpm.wisc.edu.

**Reassortment**

Influenza A is an enveloped virus with a single stranded, negative-sense RNA genome. The viral genome is segmented into eight regions that each encode 1-2 proteins. These gene segments may be exchanged between different influenza viruses in co-infected cells via a process called reassortment. Reassortment facilitates rapid virus evolution and contributes to the risk of laboratory research with influenza viruses. LPAI viruses may be converted into HPAI viruses through reassortment, and new subtypes can arise from reassortment of mammalian and avian viruses. While HPAI viruses typically are not efficiently spread from one human to another, a HPAI virus could become more transmissible in humans if reassortment occurred between an avian HPAI virus and a seasonal strain of influenza. The possibility of generating a pandemic HPAI virus that poses a danger to humans, agriculture, and natural ecosystems must be taken into account. Enhanced practices must be employed to mitigate risk of reassortment when working with HPAI viruses and HPAI-containing materials. Specific practices are described in the sections below.

**Inventory requirements**

When the temporary exemption of H5 HPAI viruses from the select agent regulations ends, all H5 HPAI containing materials will need to be destroyed or transferred to a select agent-registered facility. Each laboratory must track the use of HPAI from creation until the final disposition by chemical inactivation or heat destruction, including every usage of the inventory. This includes tracking of animals, animal tissues, clinical specimens, environmental samples, food samples, plants, and plant tissues intentionally or accidentally exposed to or infected with HPAI.

The specifics for each laboratory's inventory must be described in the laboratory's biosafety manual and standard operating procedures (SOPs), and must be maintained according to the Select Agent regulations (see Federal Select Agent Program guidance). Inventory SOPs require approval of both the IBC and the Select Agent Responsible Official (RO). Inventory records must:

- Maintain information in a form that can be conveniently reviewed.
- Be made available on request of the RO, Alternate Responsible Officials (AROs), or relevant federal agencies.
- Be accessible to approved lab personnel when inventory changes need to be recorded.
- Be kept in a secure area or secured location when not in use.
- Be made in indelible ink to prevent and detect unauthorized changes or entries.

- Be reconciled monthly and provided electronically to the RO via a secure method (e.g., BOX). Inventory records must not be emailed.

Any suspicious alteration or compromise of inventory records must be reported immediately to the University of Wisconsin Police Department (UWPD) at 608-262-2957 and the RO or ARO.

## Research involving live H5 HPAI virus

Research involving the isolation, enrichment, culture, or concentrated stocks of live H5 HPAI virus requires BSL3 or ABSL3 containment and must be approved by the IBC prior to initiation. In addition to IBC approval, project review by the Dual Use Research of Concern (DURC) Subcommittee is required for work with H5 HPAI virus that has or will be mutated via serial selection in cells or animals or through recombinant modification (e.g., insertions, deletions, point mutations, substitutions) of infectious clones. H5 HPAI viruses generated through recombinant or synthetic means and chimeric viruses containing nucleic acids from H5 HPAI viruses are also subject to the requirements of Section III-D-7 of the *NIH Guidelines*, not all of which are detailed here. Unless otherwise indicated, the following requirements apply to both laboratory and vivarium spaces.

**Facility requirements**

Not all UW-Madison BSL3 laboratories are suitable for work with HPAI viruses. If you are planning research with H5 HPAI virus, please contact OBS at 608-263-2037 or biosafety@fpm.wisc.edu.

For live H5 HPAI virus work, a BSL3 laboratory, ABSL3 laboratory, or ABSL3 vivarium must meet the following requirements:

- Fan failure testing has been performed within the last 12 months.
- Laboratory must have room pressure monitoring.
- Air must be single-pass (100% exhausted) and not re-entrained or recirculated into any part of the building.
- Exhaust air must be HEPA-filtered.
- Exhaust system has sealed ductwork from the containment barrier to the filter.
- Supply and exhaust air handling systems must be interlocked.
- Air handling systems are isolated from other areas.
- It is preferred that there are independent air supply and exhaust systems.
- Laboratory must have an anteroom that is large enough to change out of street clothing, don and doff PPE, and provide adequate storage for PPE.
- Shower must be available, preferably in the containment/non-containment interface.

Please note that other aspects of the laboratory layout and workflow (e.g., location of autoclave, shared use of the space) will be considered as part of the risk assessment by the IBC.

**Required PI approval and training**

The PI must provide their CV or biosketch and a letter explaining their qualifications and experience related to HPAI research for review and approval by the IBC and RO. The laboratory training plan must also be approved by the IBC and RO. All personnel working with H5 HPAI viruses are required to have documented formal BSL3 training. The documented training must be completed prior to starting work with H5 HPAI and include demonstrated competency for working in high containment, training specific to respiratory pathogens in high containment, and H5 HPAI virus specific training.

**Administrative controls and practices**

Good microbiological practices must be followed when working with any microorganism, though this is especially true when working with an RG3 virus. Work with live H5 HPAI requires the following:

- The laboratory or vivarium must have established processes to address occupational health considerations for personnel involved in the research. For more information, please see the Occupational Health Guidance for HPAI (H5N1) research at UW facilities on the Biosafety H5 HPAI Information webpage. The occupational health plan must include:
    - Hazard communication that includes the risk and symptoms of Influenza.
    - Individuals entering any facility where H5 HPAI is used or stored must have received annual vaccination with a currently licensed influenza vaccine. H5 vaccine is required when it becomes available. Any employee declining a vaccine shall be directed to their respective Office of Human Resources, which will consult with Occupational Medicine.
    - Signed informed consent for those working with H5 HPAI.
    - Pathogen-specific emergency response procedures in the event of known potential exposures and development of symptoms with no known exposures.
        - All exposures or development of symptoms must be immediately reported to the RO/AROs.
        - In all cases of exposure, individuals will be quarantined until H5 HPAI infection can be ruled out. The location of quarantine will be determined by the RO/AROs and will depend on the location of the individual, risk of the exposure, and the involved strain.
        - Laboratory staff must report all symptoms consistent with influenza to the RO/AROs and immediately self-isolate if any symptoms of Influenza develop. Self-isolation will continue until released by the RO/AROs.
    - Individuals who have worked with confirmed mammalian-transmissible HPAI viruses cannot travel outside of the state of Wisconsin during the 10-day incubation period.

  OBS has developed an occupational health plan template that may be used for this purpose. It can be found on the Biosafety H5 HPAI Information webpage.
- Access to the laboratory must be controlled and restricted to those involved in the research while active work (i.e., hands-on manipulation) with H5 HPAI is being performed.
    - Signage is posted at the laboratory entry regarding the restricted access.
- Concurrent influenza virus experiments may not be performed that carry the risk of unintended reassortment among HPAI H5N1 and other influenza viruses. In addition, the following measures must be taken to prevent reassortment:
    - Effective decontamination practices in accordance with the approved biosafety protocol and BSL3 manual must be performed between each experiment. A waiting period may be required between experiments involving other influenza viruses.
    - Between experiments, all clothing and PPE must be changed or disinfected prior to handling a different influenza virus in the same work area.
- Concomitant work with H5 HPAI and any other viruses or pathogens (e.g., LPAI, SARS-CoV-2) in the same laboratory space is prohibited unless a plan to avoid cross-contamination is approved by the IBC.
- All aerosol-generating activities (e.g., pipetting, homogenization) are performed inside a certified class II biosafety cabinet (BSC) or other approved containment device.

- Shower-out procedures are used to reduce the risk of fomite transmission.
    - Ideally, personal showers should be located at the containment/non-containment interface.
    - Laboratories are required to have a showering SOP in the BSL3 manual, which must be approved by the IBC.
    - The first step of showering is blowing the nose.
    - Shower must be at least 5 minutes and including washing the hair.
- Liquid effluents from the laboratory are collected and chemically disinfected or heat treated or collected in a central effluent decontamination system prior to release into the sanitary sewer system. The decontamination of shower and toilet effluents is not required.
- Training on SOPs for lab entry/exit including donning and doffing PPE are documented.
- EPA registered disinfectants effective against avian influenza (list M) are used for decontamination of work surfaces and equipment.

**PPE**

Street clothes are not permitted in the BSL3 laboratory when working with H5 HPAI virus; dedicated shoes with disposable shoe covers and dedicated scrubs with full Tyvek coveralls or equivalent is required. No uncovered skin is permitted in the BSL3 laboratory. Other required PPE includes two layers of disposable gloves, disposable hood or head cover, face and respiratory protection. A powered air purifying respirator (PAPR) with full head shroud is preferred for face and respiratory protection over N95/N100 respirator with safety goggles and face shield. Safety glasses are not suitable; if a PAPR is not worn, eye protection must be unvented goggles or a full facepiece respirator. For work with mammalian-transmissible H5 HPAI viruses, protective sleeves shall be worn while working in a biosafety cabinet. Disposable PPE may not be reused. PPE must be sprayed or wiped down with disinfectant prior to leaving containment. The entering and exiting of the BSL3 laboratory should allow for the proper donning, doffing, disinfection, storage, and disposal of PPE.

**Personnel quarantine policy**

Laboratory staff and visitors that enter a laboratory space where H5 HPAI viruses are used are restricted from having contact with susceptible avian species for a minimum of 5 days after last working with the virus. The prohibition includes avian wildlife, backyard poultry, pet birds, fair birds, commercial poultry operations, and zoos. Laboratories are required to have a written quarantine policy that is read and signed by staff and visitors. OBS has developed a quarantine policy template that may be used for this purpose, which may be found on the Biosafety H5 HPAI Information webpage. UW Madison researchers subject to this policy are additionally prohibited from having domicile backyard poultry, pet birds, or fair birds at their residence. The quarantine policy is reviewed and approved by the IBC and RO. The quarantine policy is made available to the IACUC.

**Visitors**

All visitors (i.e., anyone that is not authorized personnel including but not limited to veterinary staff, personnel in training, Environmental Health and Safety staff, and representatives of federal agencies) are required to adhere to the parameters as described for authorized personnel. Visitor training must be documented, and includes hazard communication, information on agent, signs and symptoms, entry and exit requirements, occupational health requirements, quarantine policy, and what to do in the event of symptoms including how and where to seek medical attention. A visitor entry log is required.

**Animal models of H5 HPAI**

Research animals that are infected with H5 HPAI virus require ABSL3 containment. Primary biocontainment housing may be achieved by a containment cage or rack system, flexible film isolator, or glove box. Where possible, animals infected with H5 HPAI virus must be housed in microisolator cages. Caging must be ventilated, and the exhaust air HEPA-filtered in all instances. Static micro-isolator cages may not be used. Static micro-isolators are not effective in achieving the desired effect of preventing air leakage into the laboratory space.

For species that do not have microisolator caging available, animal cages or pens must be housed within an approved HEPA-filtered containment device or within a BSL3-Ag facility. Non-Human Primate (NHP) research is performed in a HEPA-filtered containment device or within a BSL3-Ag facility. Please note that not all UW-Madison BSL3 laboratories are considered suitable for housing animals, and locations for animal use must be approved by the IBC, RO, and IACUC.

Laboratories are required to care for their own animals and approval from the IACUC is required. No animal husbandry for H5 HPAI-infected animals is to be performed by animal caretakers. Veterinary staff are subject to all visitor requirements; they must be escorted, sign the quarantine policy, and trained as visitors of the lab.

All animal tissues, carcasses, bedding, wastes, and cages must be decontaminated by an effective and validated method (e.g., autoclaving) before removal from the laboratory or vivarium.

## Research involving samples from animals naturally or experimentally infected with H5 HPAI

Research with samples from animals that have known or suspected H5 HPAI infections or test positive for H5 HPAI at the time of sample collection follows the provisions described in the "Research involving live H5 HPAI virus" section above. For the purposes of this guidance, samples collected from animals with a natural infection are equivalent to samples from animals experimentally infected with H5 HPAI virus. This applies to all tissues, cells, bodily fluids, secretions, and excretions. Exceptions must be approved by the IBC and require supporting evidence of lower risk (i.e., a published reference or data).

If you will be receiving samples from animals infected with any H5 HPAI virus from a collaborator or commercial source, please provide information about the source in the "Organs, tissues, or biological specimens other information" text box. Samples harvested from animals listed on your biosafety protocol do not need to be listed on the "Cells, Organs, Tissues or Biological Specimens" page; however, any samples harvested from animals housed at ABSL3 cannot be removed from the ABSL3 laboratory unless inactivated using an approved method or explicitly approved as an exception by the IBC and RO.

## Research involving samples from patients infected with H5 HPAI

Research with samples that have not been inactivated from human subjects or patients that have known or suspected H5 HPAI infections or test positive for H5 HPAI at the time of sample collection follows the provisions described in the "Research involving live H5 HPAI virus" section above. This applies to all tissues, cells, bodily fluids, secretions, and excretions. Exceptions must be approved by the IBC and require supporting evidence of lower risk (i.e., a published reference or data).

## Samples that may contain live H5 HPAI virus

Samples of unknown H5 HPAI status from birds, cows, or cats, including all tissues, cells, bodily fluids, secretions, and excretions, must be handled at BSL3 following the provisions described in the "Research involving live H5 HPAI virus" section above if any of the following apply:

- Samples were obtained from individuals with symptoms consistent with HPAI (for that species) who may be suspected to have the disease
- Samples were obtained from a local population (e.g., flock, herd, or barnyard community) with known or suspected H5 HPAI cases and ongoing infections are suspected
- Samples were obtained from individuals that have had a known exposure to H5 HPAI within 10 days of sample collection

Containment and practices for specimens from other susceptible species will be considered on a case-by-case basis. Handling specimens from susceptible species, including human subjects, collected from local populations that are free of known HPAI viruses are generally considered lower risk and may be handled at BSL2 with standard precautions. However, if the testing subsequently detects the presence of HPAI virus, viral proteins, or viral nucleic acids, materials must immediately be inactivated, disposed, or transferred to BSL3 and handled following the provisions described in the "Research involving live H5 HPAI virus" section above.

## Research involving inactivated H5 HPAI samples

Although it is typically not required to list inactivated materials (e.g., fixed tissues) on a biosafety protocol, since H5 HPAI virus is a RG3 pathogen and only temporarily exempt from select agent regulations, investigators are asked to list inactivated H5 HPAI samples. Relevant samples include cells, tissues, bodily fluids, secretions, or excretions from H5 HPAI infected animals or human patients, and cultured cells or tissues infected with H5 HPAI virus. In accordance with APHIS Guidelines for Avian Influenza Viruses work with inactivated H5 HPAI samples require BSL2 containment using standard precautions. However, receipt and inactivation of materials containing live H5 HPAI virus must be performed at BSL3 following the provisions described in the "Research involving live H5 HPAI virus" section above.

Inactivation of H5 HPAI samples must be performed using a method with demonstrated efficacy against H5 HPAI or related Influenza A viruses. The lab should provide a published reference or data demonstrating viral inactivation, including inactivation time and concentration of reagents. If the laboratory is receiving inactivated H5 HPAI samples from a collaborator or commercial source, they should request and retain documentation verifying inactivation of the samples using a validated method.

If you will be working with only inactivated H5 HPAI samples, please do not list H5 HPAI on the Microbes page in Bio-ARROW. Rather, please list the inactivated materials in the "Organs, tissues or biological specimens" section and provide a description of the samples, their source, and the inactivation method used in the "Organs, tissues, or biological specimens other information" text box.

## Research involving inactivated nucleic acids from H5 HPAI virus

Research involving the subcloning, expression, or analysis of individual genes from H5 HPAI *in vitro*, in microbes, or in cultured cells may be performed at BSL1 if no other agents used in the experiments require a higher level of containment. Recombinant or synthetic nucleic acid constructs and

recombinantly modified microbes and cells must be listed on your biosafety protocol and may require concomitant notification of the IBC or IBC approval prior to initiation.

The RNA genome of H5 HPAI is not considered infectious by itself. However, the method used to extract H5 HPAI nucleic acids must ensure that no cross-contamination with viable virus occurs. Research involving nucleic acids that have been extracted from H5 HPAI-containing samples using a validated method to ensure inactivation may be performed at BSL2 using standard precautions. However, receipt and inactivation of materials containing live H5 HPAI virus must be performed at BSL3 following the provisions described in the "Research involving live H5 HPAI virus" section above. Relevant samples include cells, tissues, or fluids from H5 HPAI infected animals or human patients, and cultured cells or tissues infected with H5 HPAI. For research involving nucleic acids extracted from H5 HPAI samples without inactivation, the containment level and precautions will be set by the IBC and RO.

If you will be working with only extracted inactivated H5 HPAI RNA or cDNA (e.g., deep sequencing experiments), please do not list H5 HPAI on the Microbes page in Bio-ARROW. Rather, please list the nucleic acids in the "Organs, tissues or biological specimens" section and provide a description of the material and its source in the "Organs, tissues, or biological specimens other information" text box.

## Research involving proteins H5 HPAI virus

Research involving only isolated H5 HPAI viral proteins does not require a biosafety protocol or OBS/IBC review if all the following are true:

- The proteins are being produced in cells or RG1 microbes through expression of recombinant protein and no H5 HPAI virus is involved in any step of their production
- Your laboratory is not doing any of the recombinant work but is just receiving the purified protein from a collaborator or commercial source*
- The research does not involve any other recombinant or biohazardous materials

By themselves, H5 HPAI proteins do not cause disease. Therefore, there are no enhanced biosafety requirements for experiments involving only H5 HPAI proteins in the absence of its RNA genome.

*If you will be expressing recombinant H5 HPAI proteins in cells or microbes in your laboratory, please refer to the "Research involving inactivated nucleic acids from H5 HPAI virus" section for requirements.