**University of Pittsburgh Institutional Biosafety Committee**
**Guidance on Biosafety Level Assignment for Influenza Viruses**
*Approved March 11, 2024*

## Background

Influenza is an acute viral disease of the respiratory tract, which can occur locally or in epidemics or pandemics, particularly in winter months. The most common clinical manifestations in people are fever, headache, malaise, sore throat, cough, and muscle aches. Gastrointestinal (GI) tract manifestations (e.g., nausea, vomiting, diarrhea) and extrapulmonary complications (e.g., myocarditis, encephalitis) can also occur. Influenza can affect people of all ages, but it has a higher incidence in children. People who are young, elderly, or have chronic disease often have the most severe disease.

Influenza viruses are enveloped viruses with a segmented, negative-sense RNA genome that belong to the *Orthomyxoviridae* family. They can be divided into four serotypes: A, B, C, and D. Influenza A viruses can be further classified into subtypes based on antigenicity of their hemagglutinin (HA) and neuraminidase (NA) proteins. Influenza viruses can replicate in humans as well as several animal species. Influenza evolution can be driven by both antigenic drift (e.g. mutations that result in minor or gradual antigenic changes) and antigenic shift (e.g., major antigenic changes in which the virus can be transmitted between species). Interspecies transmission, genome segment reassortment, and genome recombination of influenza A viruses have been reported in humans, pigs, and birds.

Avian Influenza viruses can be classified based on their pathogenicity in chickens: Highly Pathogenic Avian Influenza (HPAI) or Low Pathogenicity Avian Influenza (LPAI) viruses. HPAI and other influenza viruses (e.g., 1918 H1N1 pandemic strain) are considered Select Agents, requiring registration with the U.S. Centers for Disease Control (CDC) or U.S. Department of Agriculture (USDA) for possession, use, storage, or transfer. Certain governmental requirements and/or restrictions may apply for these viruses.

## NIH and CDC Opinions

The *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (NIH Guidelines, April 2019) state that experiments with influenza viruses containing genes or segments from 1918-1919 H1N1 (1918 H1N1), human H2N2 (1957-1968) and highly pathogenic avian influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1) shall be conducted at biosafety level 3 (BSL-3) containment.

The NIH Guidelines also state that an IBC risk assessment may determine that containment may be lowered to BSL-2 or BSL-2 with enhanced practices (BSL-2+). "The IBC should consider whether, in at least two animal models (e.g., ferret, mouse, Syrian golden hamster, cotton rat, non-human primates), there is evidence that the resulting influenza virus shows reduced replication and virulence compared to the parental RG3 virus at relevant doses. If an influenza virus containing genes from one of these viruses is resistant to both classes of current antiviral agents, adamantanes and neuraminidase inhibitors, higher containment may be required based on the risk assessment

considering transmissibility to humans, virulence, pandemic potential, alternative antiviral agents if available, etc."

Furthermore, the 6th edition of the Biosafety in Microbiological and Biomedical Laboratories (BMBL, June 2020) makes the following recommendations:

- BSL-3/ABSL-3 containment should be used for laboratory work with LPAI that have caused zoonotic infection, particularly with fatal outcomes, or Asian lineage A and non-U.S. LPAI A influenza viruses.
- BSL-2 with enhanced facilities, practices, and procedures (BSL-2+) should be used for work with domestic LPAI A viruses and equine, canine, and swine influenza A viruses.
- BSL-2/ABSL-2 practices are recommended for diagnostic research and production activities utilizing contemporary influenza A, B, and C viruses circulating among humans (e.g., H1/N3/B).
- Increased caution should be given to non-contemporary, wild-type human influenza A H2N2 viruses or reassortants containing non-contemporary H2 or N2 RNA segments.
- Cold-adapted, live attenuated A H2N2 vaccine viruses may be used at BSL-2, but it is recommended that a risk assessment be performed before working with such viruses and special attention should be given to prevent generation of reassortants that have H2 and/or N2 RNA segments that lack attenuating features of the parental attenuated viruses.

## IBC Recommendation

The University of Pittsburgh IBC has adopted NIH recommendations and defined the following biosafety levels for use of influenza viruses:

<u>BSL-2/ABSL-2</u>

- Mechanical pipetting aids shall be used when pipetting all material. Mouth pipetting is prohibited regardless of the material or manipulation.
- Eating, drinking, storing food, expressing breast milk, handling contact lenses and applying cosmetics are not permitted in laboratory areas. Food should not be stored in refrigerators or freezers used to store biohazardous material.
- Hands must be washed immediately after procedures involving biological material manipulation or handling, after glove removal and routinely before leaving the laboratory. All labs using biological materials must be equipped with a sink having hot and cold running water dispensed by a mixing faucet, and have soap and disposable hand towels immediately accessible.
- Workers should decontaminate their work area following work with biological material and immediately after any spill.
- Liquid-barrier gloves should be worn to protect faculty, staff and students from infection through contact with biological materials. Gloves must be removed prior to exiting the laboratory.

- Procedures for the safe handling of sharps must be instituted, and efforts should be made to minimize exposure to potentially infectious material through the evaluation and use of safety-engineered sharps.
- Laboratory coats or gowns should be worn while handling biological material. All protective equipment and laboratory garments must not be worn outside the laboratory. Laboratory clothing must be disinfected or clearly labeled as potentially infectious or dirty before removal from the laboratory.
- All procedures should be performed in a manner that reduces the generation of aerosolized material. Operations such as centrifugation, sonication, and blending are known aerosol-generating procedures. Procedures or activities expected to produce potentially infectious aerosols must be performed in a certified biological safety cabinet or other equipment with integral engineering controls to contain aerosolized material.
- Access to laboratory should be restricted.
- Biohazard warning signs must be posted at each entrance to limit access to authorized individuals, provide contact and agent information, and indicate BSL-2 hazards.
- An autoclave must be accessible for decontamination of infectious waste generated in BSL-2 facilities.
- All BSL-2 facilities must be maintained under negative pressure relative to corridors and adjacent public areas and must have exhaust air that is not re-circulated.
- All infectious materials must be decontaminated prior to disposal and implementation of an accident/incident plan that details exposure follow-up procedures and methods to clean up spills is required.
- Transfer of agent and/or samples containing agent to collaborators within the University (i.e. intra-entity transfer) may require recipient to be listed as approved end user on parent CDC/USDA permit and/or MTA
    - For projects with multiple Pitt investigators working in collaboration EH&S recommends considering listing a single PI as main recipient (MTA) and/or permittee with collaborators added as end users.
- <u>Prior to</u> transfer of agent and/or samples containing agent to collaborators outside of the University (i.e. inter-entity transfer) recipient may need to have an approved CDC/USDA permit and/or contact original provider entity to obtain an approved MTA

<u>BSL-2 with enhancements for influenza virus work (in vitro)</u>

All of the requirements for work at BSL-2, and following influenza-specific recommendations:

- Seasonal influenza vaccine recommended for personnel handling agent or animals infected with agent
- Project- and facility-specific biosafety will be required
- Aerosol-producing procedures must be performed in BSC or other applicable primary containment device.

- Additional risk assessment and mitigation may be needed if known aerosol-producing procedures (e.g. exposure of animals via aerosol) or use of specialized procedures/equipment (e.g. environmental control chambers, nebulizers, flow cytometry with potentially infectious materials, etc.) is planned

- Use of agents may be restricted to specific buildings and rooms with appropriate engineering controls for planned work
- Recommend that the Investigator documents training for personnel in standard microbiological practices and aseptic technique to avoid inadvertent cross-contamination or reassortment if work with multiple viruses will be performed in the laboratory.
    - Training documentation strongly recommended for research programs involving work with multiple viruses that include treatment-resistant and/or regulated strains
        - Investigators planning intentional generation of reassortants that have HX and/or NX segments that lack attenuating features of parental attenuated viruses must contact EH&S and the IBC prior to beginning studies as additional project-specific risk assessment and mitigation measures may be needed.
- Influenza viruses regulated by the CDC Import Program and/or USDA APHIS

  Additional permit-specific enhancements may be required for influenza viruses regulated by the CDC Import Program and/or USDA APHIS and may include:
    - An on-site inspection of laboratory and animal facilities where influenza viruses will be used and stored may be required prior to permit approval.
        - Investigators should contact EH&S immediately for assistance with preparation if a permit inspection is requested. EH&S must be present during the on-site inspection to provide support.
    - Quarantine procedures may need to be developed and documented for all personnel, visitors, and contractors to spaces where agents are in use
        - Restrictions for visiting/having potential contact with commercial or residential animals, agricultural fairs, zoos, aviaries, petting zoos, etc. for specific time periods

<u>ABSL-2 with enhancements for influenza virus work (in vivo)</u>

- All applicable recommendations for *in vitro* work as above, and:
- Prior to beginning *in vivo* studies, coordination between the investigator, DLAR and EH&S is required to ensure that appropriate facilities, caging, occupational health surveillance, and/or other husbandry and veterinary care services are available. Examples of potential considerations include:
    - Specific animal spaces that can accommodate work with influenza viruses in animal species to be used as model

        - Adherence to specific intra- and/or inter-animal facility traffic patterns may be needed
    - Ventilated microisolator or primary containment caging should be available and is recommended for work
    - Enhanced PPE may be required, including substitution of liquid-barrier coveralls for disposable gowns (if used), facility-dedicated shoes or boots or shoe covers, and/or respiratory protection
        - The Investigator and EH&S will need to determine whether personnel protection, uninfected animal protection, or both is needed as this will determine specific types of respirators recommended for the work
    - Documentation that carcasses and wastes are appropriately controlled and disposed may be required and specific document retention periods may apply (e.g. may require modification to standard DLAR veterinary record maintenance period)
- Seasonal influenza vaccine recommended for personnel handling agent or animals infected with agent
    - Investigators should note that recommendation includes DLAR personnel providing animal care/husbandry/vet services and advance notice may be needed to ensure that personnel who may handle animals, caging, bedding, etc have an opportunity be vaccinated

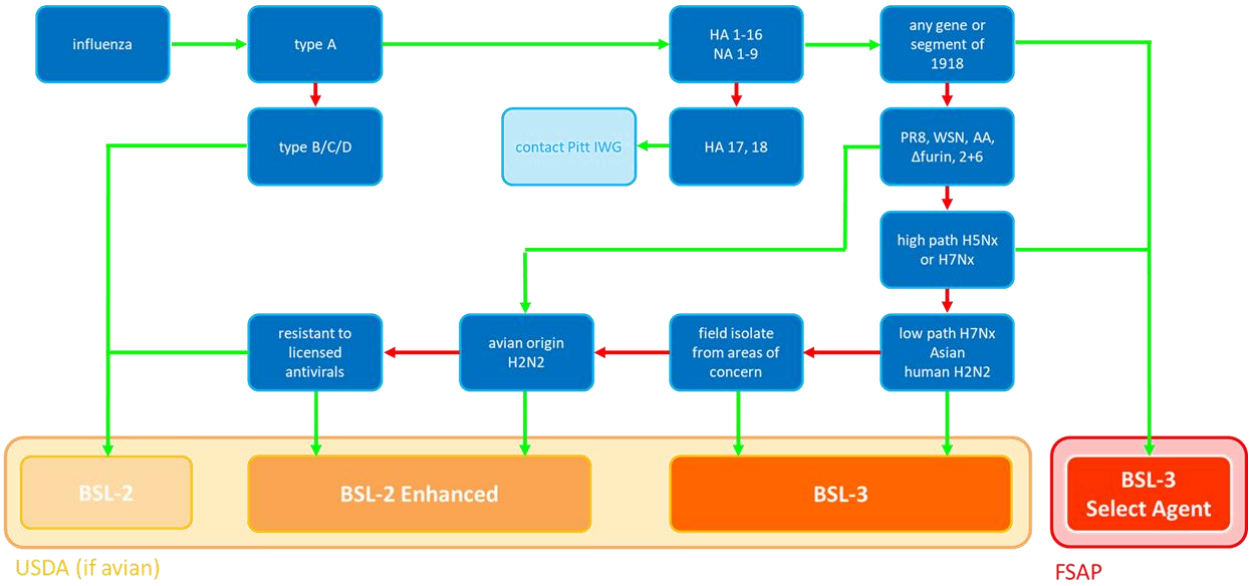
BSL-3/ABSL-3/RBL for Risk Group 3 and/or Federal Select Agent Program (FSAP)-regulated Influenza Viruses

**Investigators must contact an EH&S Biosafety Officer (biosafe@ehs.pitt.edu) before acquiring any Risk Group 3 influenza viruses or influenza viruses regulated by the Federal Select Agent Program (Select Agent and Toxin List).**

Requirements for work at BSL-3, ABSL-3, and in the RBL are defined in facility- and RBL-specific operations manuals, standard operating procedures, PI-specific BSL-3 or RBL biosafety manuals, University Safety Manual Guidelines (Occupational Health Program For BSL-3 Workers; HPAI Guidelines), and other EH&S and Select Agent Program documents.

Investigators should contact an an EH&S Biosafety Officer (biosafe@ehs.pitt.edu) for specific information regarding the University's high containment research program.

In the table below are the specific criteria that were determined by the Influenza Working Group, which has been adopted by the IBC, for the containment of influenza viruses.




FSAP is the [Federal Select Agent Program]