
STANDARD OPERATING PROCEDURES (SOP)

Reassortant influenza A viruses

1. SCOPE

All subtypes of influenza A virus occur naturally in wild birds. While wild birds usually do not become ill when infected with influenza A virus, infected domesticated poultry such as chickens do become ill and may die. Influenza A viruses are categorized as low pathogenicity (“LPAI”; temporary illness in domesticated poultry such as decreased egg production) or high pathogenicity (“HPAI”; 90% or higher mortality in domesticated poultry within 48 hours of initial infection.) Humans can become ill from exposure to avian influenza viruses, usually by close contact with infected poultry or their secretions. Some HPAI such as H5N1 have resulted in documented human fatalities in the past 10 years.

Work with HPAI is currently regulated under the Select Agent Program (Title 9, Code of Federal Regulations, Part 121.) Recently, reassortant viruses built on a backbone of LPAI such as A/PR/8/34, and containing hemagglutinin (HA) and neuraminidase (NA) genes from avian influenza subtype H5N1, were made available to the research community by the Centers for Disease Control and Prevention (CDC.) These viruses are exempt from the Select Agent Program. This SOP was designed to establish a system of information and safeguards that should be followed at the University of Pittsburgh when using these reassortant viruses.

2. PROCEDURE

- 2.1 **Agent-** Reassortant avian influenza viruses containing one or more gene sequences from highly pathogenic avian influenza.
- 2.2 **Employees at risk-** Cultures of reassortant viruses, experimentally infected laboratory animals, or experimentally infected materials of animal origin, such as poultry eggs, are a potential source of infection to exposed laboratory personnel.
- 2.3 **Laboratory hazards**
 - 2.3.1 Ingestion, parenteral inoculation, and droplet or aerosol exposure of mucous membranes or broken skin with infectious fluids or tissues, are the primary hazards to laboratory personnel.
 - 2.3.2 Human infections resulting from exposure to strains of the subtypes of LPAI used in these reassortant viruses have not been documented.
- 2.4 **Required Procedures**
 - 2.4.1 All Principal Investigators (PIs) using reassortant influenza A viruses must complete an application for work with recombinant DNA through the Institutional Biosafety/rDNA Committee (rDNA@pitt.edu and www.rcco.pitt.edu/rDNA/.)
 - 2.4.2 All PIs using reassortant influenza A viruses must also complete an EH&S registration workbook for the project. Registration workbook may be obtained from the web site www.ehs.pitt.edu or by calling EH&S at 624-9505.
 - 2.4.3 All PIs who wish to work with reassortant influenza A viruses must obtain a permit from the United States Department of Agriculture (USDA.) Forms are available at

www.aphis.usda.gov/vs/ncie. USDA will contact the investigator to schedule an inspection of the lab.

- 2.4.4 If available, commissioned Biosafety Level 3 facilities and practices should be utilized for manipulations of reassortant influenza A viruses.
- 2.4.5 If BSL-3 facilities are not available, then Biosafety Level 2 facilities with BSL-3 practices, also known at the University as Biosafety Level 2+, is required for all activities involving the use or manipulation of reassortant influenza A viruses. The specific additional practice requirements for BSL-2+, involving reassortant influenza A viruses, include the following:
 - 2.4.5.1 An operations manual must be prepared for the BSL-2+ agent. See University Lentivirus SOP for details.
 - 2.4.5.2 Access is restricted when work is in progress.
 - 2.4.5.3 Principal Investigator is responsible for ensuring that all personnel demonstrate proficiency in the practices and operations of the facility prior to beginning work with the organisms.
 - 2.4.5.4 Any vacuum line is protected with liquid disinfectant traps and/or HEPA filters.
 - 2.4.5.5 Class II or III Biological Safety Cabinets (BSCs) are used for all manipulations involving infectious materials.
 - 2.4.5.6 Centrifuge Safety Cups must be used for centrifugation outside of a biosafety cabinet. Safety Cups must only be opened in a Biosafety Cabinet.
 - 2.4.5.7 Generation of aerosols must be minimized.
 - 2.4.5.8 The use of sharps is restricted or eliminated.
 - 2.4.5.9 Personnel should wear additional protective gear while handling reassortant viruses, infected animals, and infected materials of animal origin such as poultry eggs. Protective gear includes an N-95 respirator or equivalent, hair cover, liquid barrier gloves, a gown (preferably liquid barrier and disposable), eye protection, and shoe covers. Workers must be fit-tested to the N-95 respirator and enrolled in the University's respiratory protection program.
 - 2.4.5.10 No other experiments should be conducted in the designated lab for handling reassortant viruses.
 - 2.4.5.11 All surfaces, equipment, and wastes, must be disinfected (chemically or by steam sterilization) at the end of each work session. Potentially infectious materials associated with reassortant viruses may not be left in the lab.
 - 2.4.5.12 After the work session with reassortant viruses is completed, at least 30 minutes must pass before other workers may enter the lab and handle other biological agents.
 - 2.4.5.13 Each lab must be evaluated by EH&S on a case-by-case basis for suitability for work with reassortant viruses. The lab must be maintained under negative pressure with respect to adjacent labs or corridors at all times.
- 2.4.6 Laboratories are inspected by EH&S to verify appropriate BSL-2+ or BSL-3 containment and practices.
- 2.4.7 If a BSL-2+ facility is used, then it is preferred that a facility which includes an anteroom is used, in which garb can be donned. If an anteroom is not available, a garb station outside of the laboratory and out of the public thoroughfare should be used to don garb. N-95 respirators may be kept within the laboratory, only if they can be donned prior to work with reassortant viruses. Anyone intending to enter the laboratory when work with reassortant viruses is in progress must don a respirator prior to entering the lab. All personal protective equipment except for the N-95 respirator should be removed in the lab before exiting. Protective equipment should be disposed as biohazardous.
- 2.4.8 Personnel shall be offered and strongly encouraged to receive the seasonal influenza vaccine.

- 2.4.9 Personnel should monitor their body temperature daily and report any symptoms to their supervisor which may indicate an influenza infection, such as temperature over 100.4°F, sore throat, cough, labored breathing. The supervisor must contact Employee Health Services (647-3695) for confidential medical evaluation of the employee. See Absenteeism SOP for more information.
- 2.4.10 Staff must be advised that stocks of Tamiflu (oseltamivir) and other antiviral treatments are kept on hand by Employee Health Services, and that occupational exposures must be immediately reported to the supervisor or PI.
- 2.4.11 All transport of infectious materials within the BSL-2+ or BSL-3 laboratory must be performed in leak-proof sealed containers.
- 2.4.12 Non-lab personnel, such as janitors or trades workers, may not enter the laboratory when reassortant viruses are in use. All work areas must be disinfected prior to their entry.
- 2.4.13 If it is intended to inactivate the virus and then handle it further in another laboratory outside of the designated laboratory, then EH&S and the Institutional Biosafety/rDNA Committee must review and approve the inactivation protocol. Inactivated virus may be handled in a BSL-2 laboratory without respiratory protection.

3. APPROVAL

The University of Pittsburgh's Biohazards Committee and EH&S have reviewed and approved this SOP as attested by the signatures of the Committee Chairperson and the Biosafety Officer.

Committee Chairperson

Date

Biosafety Officer

Date