

Tel-Aviv University –Safety Unit

Standard Operating Procedure for Working with *PR8 influenza A virus strain* in Animals.

1. Health hazards

PR8 influenza A virus strain is a members of the Orthomyxoviridae family of segmented, negative sense, single-stranded RNA viruses.

The main antigenic determinants of influenza A viruses are the haemagglutinin (HA) and the neuraminidase (NA) genes. Based on the antigenicity of these membrane-associated glycoproteins, sixteen HA (H1–H16) and nine NA (N1–N9) subtypes have been described until now.

Antigenic alterations occur frequently in influenza HA and NA antigenic sites and are the mechanism for virus adaptation to the host and survival.

HOST RANGE: Humans, swine, horses, domestic and wild avian species (predominantly ducks), rodents, and shorebirds.

Infected humans can shed detectable amounts of influenza virus the day before symptoms begin. Adults usually shed the virus for 3 to 5 days, and up to 7 days in young children

MODE OF TRANSMISSION: Transmission of influenza can occur via respiratory infection by aerosols and droplets (from coughing and sneezing) or from contact transmission from contaminated surfaces. Transmission of influenza virus from donors who are shedding large amounts of virus can be infective for 2 to 8 hours via stainless steel surfaces and for a few minutes via paper tissues.

Inhalation of virus from aerosols generated when aspirating, dispensing, or mixing virus-infected samples (tissues, faeces, secretions) from infected animals. Laboratory infection can also occur from direct inoculation of mucous membranes via virus contaminated gloves following the handling of tissues, faeces and/or secretions from infected animals.

The infection progression in mice, starts in the respiratory tract in areas close to large conducting airways and later spreads to deeper sections of the lungs.

The PR8 influenza virus strain is an attenuated virus, and has no ability to replicate in humans, as a result of over 100 passages in each of mice, ferrets and embryonated chicken eggs.

2. Designated Area	<p>ABSL-2 facility.</p> <p>The safety level was determined in order to prevent infecting the animals found at animal facility.</p>
3. Training	<p>Practical experience with animal care/maintenance, as well as general biosafety, is required.</p>
4. Personal Protective Equipment (PPE)	<p>Gloves, Eyes safety goggles, Lab coat, Disposable shoe covers and Animal handling gown.</p>
5. General . Precautions for Animal Use	<p>The main goal is to prevent infecting the animals found at animal facility.</p>
6. Environmental / Ventilation Controls	<p>Work should be conducted in ABSL-2 facility, over absorbent pads in a class II type A1 or A2 biological cabinet.</p> <p>SURVIVAL OUTSIDE HOST: Influenza A virus can survive for 24 to 48 hours on hard, nonporous surfaces such as stainless steel and plastic and for approximately 8 to 12 hours on cloth, paper and tissues</p>
7. Animal handling practices	<ol style="list-style-type: none"> 1. Animals must be housed in filter top cages marked as biohazards (including the name of the pathogen/biohazard). Handling the cages (including bedding) will be done only by the researchers. 2. Use a class II Biological Safety Cabinet at all times (especially during injection or any surgical procedure), when performing work on these animals and/or when moving animals from dirty to clean cages. 3. Infected animals may shed <i>influenza</i> after treatment; take precautions to avoid the creation of aerosols when changing or washing cages, or cleaning the room. 4. Dead animals must be placed in primary plastic bags, which are then placed in biosafety bags for infectious waste incineration. 5. All surfaces and racks that may be contaminated will be decontaminated with 0.5% bleach (or virusolve), ASAP. 6. When changing cages, use a standard microisolator technique: <ul style="list-style-type: none"> • place the cage containing the animals, under the biological safety cabinet and transfer the animals into a clean cage. • spray the dirty cage with 0.5% bleach (or virusolve), remove from the safety

	<p>cabinet and place on a transfer rack .</p> <ul style="list-style-type: none"> • when all cages have been changed, spray the dirty cages and rack again with 0.5% bleach, and cover the rack. Put on a pair of new gloves and bring the rack directly to the autoclave in the dirty cage wash area. • immediately autoclave the dirty cages (1 hour at 121°C/250° F, 15psi of steam pressure). Once the autoclave cycle is completed, the cages can be emptied and the bedding disposed of in a normal fashion. <p>**In cases where the use of autoclave (within the animal facility) is not an option:</p> <ul style="list-style-type: none"> • the cages (bedding) must be emptied inside the BSL-2 cabinet, directly to a double biohazard bags. • Before closing the bags, carefully, add a small amount of water (250ml) to improve the sterilization process. <p><i>Do not close the bag completely/tightly (in order to avoid entering of steam during the sterilization process).</i></p> <ul style="list-style-type: none"> • Spray the dirty bag with 0.5% bleach or virusolve. • Remove from the safety cabinet and place on a transfer rack/container. • Put on a pair of new gloves and bring the rack/container, directly to the collection point of your department.
8.Decontamination	<p>Influenza A is susceptible to disinfectants, including sodium hypochlorite (freshly made 1:10 dilution of bleach), 60 to 95% ethanol, 2% alkaline glutaraldehyde, 5 to 8% formalin, and 5% phenol.</p> <p>Physical inactivation: Susceptible to moist heat at 121°C for 20 minutes or dry heat at 170°C for 1 hour, 160°C for 2 hours, or 121°C for at least 16 hours.</p>
9. Spill and Accident Procedures	<ol style="list-style-type: none"> 1. Evacuate area, remove contaminated PPE and allow agents to settle for a minimum of 30 minutes. Initiate spill response procedure. 2. Cover the spill with absorbent material. Starting at the edges and work towards the center. 3. Carefully pour disinfectant over the absorbed spill, again starting at the edges. Saturate the area with disinfectant. 4. Allow sufficient contact period to inactivate the material in the spill. Non-viscous spills require 15-20 minutes: viscous spills require 30 minutes.

	<ol style="list-style-type: none"> 5. Use paper towels to wipe up the spill, working from the edge to center. Use tongs or forceps to pick up broken plastics, glass or other sharps that could puncture gloves 6. Discard absorbent material in Chemical waste bags 7. Clean the spill area with fresh paper towels soaked in disinfectant. Thoroughly wet the spill area, and wipe with towels. 8. Discard all cleanup materials in Chemical bag, along with any contaminated PPE (pay special attention to gloves and shoe covers). Close and secure the bag. 9. Place bag in a second Chemical bag, secure and dispose as chemical waste. 10. Discard contaminated PPE (with biohazard materials) in biohazard bag. Place bag in a second biohazard bag, secure and disinfect by autoclaving. <p><u>Exposure:</u></p> <ol style="list-style-type: none"> 1. In case of skin contact or injection with <i>influenza</i>, wash the affected area with soap and water for at least 15 minutes. 2. For eye exposure, flush with water for at least 15 minutes. Consult with Employee Health Center,. Report incident to supervisor. Supervisor reports the accident/injury to the Biosafety Unit.
10. Waste Disposal	Autoclave all waste (1 hour at 121°C/250 °F, 15psi of steam pressure).
I hereby confirm that I have read the SOP (Standard Operating Procedure) for Working with PR8 influenza A virus strain in Animals, and agree to follow these procedures.	
Name:	Title:
Signature:	Date:

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