Standard Operating Procedure for Working with *Aspergillus spp.* in Animals	
1. Health hazards	*Aspergillus spp.* contain approximately 184 species, 40 of which have been reported to cause human or animal infections. *Aspergillus* spp. reproduce by producing conidia. Aspergillosis is a common term used to describe infections caused by different species of *Aspergillus* . Most cases of aspergillosis are caused by *A. fumigatus*, with *A. flavus* and *A. niger* being the second most common pathogenic *Aspergillus* spp. Worldwide. Diseases caused by *Aspergillus*spp. include clinical allergies (allergic bronchopulmonary aspergillosis, rhinitis, farmers's lung), superficial and local infections (cutaneous infections, otomycosis, tracheobronchitis), infections associated with damaged tissue (aspergilloma, osteomyelitis), and invasive pulmonary and extrapulmonary infections. Conidia of *Aspergillus* are present in decomposing organic matter and soil . ***Aspergillus* may be transmitted by:** • Water and food may act as reservoirs of transmission of *Aspergillus* spp. • Inhalation of airborne conidia, through contaminated water or animals bedding . • Fabrics and plastics may serve as transmission source of *Aspergillus* spp. • Penetration of the skin via puncture or absorption (though scratches, cuts, abrasions, dermatitis or other lesions) • Mucous membrane exposure of the eyes, nose, and mouth(through direct contact or aerosols)
2. Designated Area	ABSL-2 facility.
3.Training	Practical experience with animal care/maintenance, as well as general biosafety, is required.
4. Personal Protective Equipment (PPE)	Gloves, Eyes safety goggles, Lab coat, Disposable shoe covers and Animal handling gown. N-99 respirator mask covering the mouth and nose when not working in a Class II Biosafety Cabinet (BSC). Appropriate PPE recommended for lower arms such as sleeve covers or securing gloves over the sleeves of laboratory coat. ***Personnel should not work with Aspergillus spp. if skin is cut or scratched.***

5.General . Precautions for Animal Use	Tools (as, syringe, blades and safety needles where possible) should be adapted for BSL-2. Have a sharps container in close vicinity. Animals should be restrained or anesthetized during injection.
6. Environmental / Ventilation Controls	Work should be conducted in ABSL-2 facility, over absorbent pads in a class II type A1 or A2 biological cabinet.
7. **Animal handling practices**	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 *Aspergillus spp.* 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 ASAP. 6. When changing cages, use a standard microisolator technique: • 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, remove from the safety cabinet and place on a transfer rack . • 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. **In cases where the use of autoclave (within the animal facility) is not an option: • 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. ***Do not close the bag completely/tightly*** *(in order to aloud entering of steam during the sterilization process).* • 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	** Decontaminate work areas with 0.5% bleach for 30 minutes. Follow with water. In general: *A. niger* and *A. fumigatus* are sensitive to 0.5% alkaline solution of glutaraldehyde *A. niger* is also sensitive to 0.125% butyl paraban ester A 1:50 dilution of a phenolic disinfectant containing 15% 2-phenylphenol and 6.3% 4-ter-amylphenol has shown to be effective against *A. fumigatus* but not *A. nigerAspergillus* spp. are also susceptible to sodium hypochlorite and cupric sulphate
9. Spill and Accident Procedures	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 requite 15-20 minutes: viscous spills requite 30 minutes. 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 biohazard 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 (soaked with disinfectant) in Chemical bag, and

	any contaminated PPE (pay special attention to gloves and shoe covers) in a biohazard bag. Close and secure the bags . 9. Place bag in a second biohazard bag, secure and disinfect by autoclaving. <u>Exposure</u>: 1. In case of skin contact or injection with *Aspergillus* spp., wash the affected area with soap and water for at least 15 minutes. Consult with Employee Health Center. 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 121ºC/250°F, 15psi of steam pressure).
I hereby confirm that I have read the SOP (Standard Operating Procedure) for Working with Aspergillus spp. in Animals, and agree to follow these procedures.	
Name:	Title:
Signature:	Date:

Biological Safety Office, : 640-9966