Tel-Aviv University –Safety Unit

Standard Operating Procedure for Working with **Mycobacterium bovis/BCG** in Rodents

1. Health hazards	Mycobacterium tuberculosis complex (including M. bovis, M. africanum, M. pinnipedii, M. microti, M. caprae and M. canettii) are acid-fast, aerobic, non-spore forming, non-motile bacteria and the causative agent of tuberculosis. Mycobacterium tuberculosis has a thick, lipid-rich cell wall that renders bacilli resistant to harsh treatments including alkali and detergents. M. bovis is primarily found in animals but can also infect humans. It is spread to humans, primarily children, by consumption of non-pasteurized milk and dairy products, by handling of infected carcasses, or by inhalation. Human-to-human transmission of M. bovis via aerosols is possible. M. tuberculosis and M. bovis infections are a proven hazard to laboratory personnel and others who may be exposed to infectious aerosols in the laboratory, autopsy rooms, and other healthcare facilities. Mycobacterium bovis bacillus Calmette-Guérin (BCG) is a vaccine for tuberculosis (TB) disease. It is partly named after its inventors Albert Calmette and Camille Guérin. **HOST RANGE:** Monkeys, humans, parrots, cattle, sheep, goats, dogs, cats. **MODE OF TRANSMISSION:** Transmission can be airborne (inhalation of droplet nuclei carrying M. tuberculosis). Other modes of transmission include exposure to autopsy material, venereal transmission, and even percutaneous transmission (direct injury to the skin and mucous membranes through breaks in skin and needlesticks). Tubercle bacilli may be present in sputum, gastric lavage fluids, CSF, urine, and in a variety of tissues. Infected animals can spread the infection to laboratory workers through aerosols, fomites, bites. **Experimentally-infected guinea pigs and mice do not pose the same hazard because droplet nuclei are not produced by coughing in these species; however, litter from infected animal cages may become contaminated and serve as a source of infectious aerosols. **SURVIVAL OUTSIDE HOST:** M. tuberculosis can survive for months on dry inanimate surfaces. M. bovis can survive on dry surfaces at 4°C. M. tuberculosis can survive in cockroach feces for 8 weeks, sputum on carpet (19 days) and wood (over 88 days), moist and dry soil (4 weeks), and in the environment for more than 74 days if protected from light (possibly longer if in feces). **RESERVOIR:** Humans, and diseased animals. **ZOONOSIS:** Yes, through monkeys, parrots, cattle, sheep, goat, dogs, cats. **VECTOR:** None.

2. **Housing and Biosafety consideration**	**ABSL-2+ or ABS-3** ABSL-3 practices are recommended for animal studies using experimentally or naturally infected NHPs or immunocompromised mice, as high titers may be found in organs from immunocompromised animals. Animal studies using rodents (e.g., guinea pigs, rats, rabbits, mice) can be conducted at ABSL-2 with ABSL-3 practices. All airborne infections of rodents using any of the subspecies of the M. tuberculosis complex, must be performed in an appropriate ABSL-3 laboratory. Manipulation of small quantities of the attenuated vaccine strain M. bovis Bacillus Calmette-Guérin (BCG) can be performed at BSL-2 in laboratories that do not culture M. tuberculosis. **However**, considerable care is suggested to verify the identity of the strain and to ensure that cultures are not contaminated with virulent M. tuberculosis or other M. bovis strains. BSL-3 practices, containment equipment, and facilities are recommended for laboratory activities in the propagation and manipulation of cultures of any of the subspecies of the M. tuberculosis complex.
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. Appropriate PPE recommended for lower arms such as sleeve covers or securing gloves over the sleeves of laboratory coat. **Surveillance Annual or semi-annual skin testing with purified protein derivative (PPD) or FDA-approved Interferon-Gamma Release Assay (IGRA) of previously skin-test-negative personnel can be used as a surveillance procedure. ***Personnel should not work with Mycobacterium bovis/BCG, 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 at ABSL-2 with ABSL-3 practices, over absorbent pads in a class II type A1 or A2 biological cabinet (BSC).

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 *Mycobacterium bovis/BCG* after treatment; take precautions to avoid the creation of aerosols when changing or washing cages, or cleaning the room. 4. Dead animals placed in primary plastic bags, and 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 virusolve, remove from the safety cabinet and place on a transfer rack. • When all cages have changed, spray the dirty cages and rack again with virusolve, 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 121ºC/250°F, 15psi of steam pressure). Once the autoclave cycle is completed, the cages can empty 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 emptied inside the BSL-2 cabinet, directly to a double biohazard bag. • 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. • 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	**SUSCEPTIBILITY TO DISINFECTANTS:** Mycobacterium species show greater resistance to disinfectants than vegetative bacteria. M. tuberculosis is more resistant than M. bovis. 0.05 % to 0.5% sodium hypochlorite can be used for surface disinfection (30 minutes incubation, follow with water). Higher concentrations of chlorine are required for efficacy against M. tuberculosis (1%). A 2% solution of aqueous glutaraldehyde is required to kill M. tuberculosis within 10-20 minutes at room temperature. *Some disinfectants such as quaternary ammonium compounds, chlorhexidine gluconate, and iodophor have been reported to be ineffective against M. tuberculosis.*

	PHYSICAL INACTIVATION: UV light can be used for surface disinfection. Most bacteria are sensitive to moist heat (121°C for at least 15 min). Mycobacteria are easily killed by heat (> 65 °C for at least 30 min).
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. Wearing protective clothing, gently 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 Chemical waste bags. 7. Clean the spill area with fresh paper towels soaked in disinfectant. Thoroughly wet the spill area, allow to disinfect for 15-20 minutes longer, 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 Mycobacterium bovis/BCG 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 121°C/250°F, 15psi of steam pressure).

I hereby confirm that I have read the SOP (Standard Operating Procedure) for Working with Mycobacterium bovis/BCG in Animals, and agree to follow these procedures.

Name:	Title:
Signature:	Date:

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