Tel-Aviv University –Safety Unit

Standard Operating Procedure for Working with **Streptococcus Pneumoniae** in Animals	
1. Health hazards	Streptococcus Pneumoniae , is a Gram-positive, alpha-hemolytic (under aerobic conditions) or beta-hemolytic (under anaerobic conditions), facultative anaerobic member of the genus Streptococcus. Streptococcus Pneumoniae is a group of bacteria (around 90 serotypes), that causes numerous infections and diseases in the body, including meningitis, bacteremia, ear infections, sinus infections and pneumonia. S. pneumoniae colonizes in the mucosal surfaces of the nasopharynx and upper respiratory airway, and symptoms of inflammation appear as the bacteria migrate into the sterile parts of the airway. S pneumoniae infection is an important cause of bacterial co-infection in patients with influenza and can increase the morbidity and mortality in these patients. Incidence of infection is highest in people under 2 years of age or over 60 years of age, or in people afflicted by alcoholism, diabetes mellitus, chronic renal disease, or absence of normal spleen function. **Host range**: Humans, mice, rats, guinea pigs, chimpanzees, rhesus monkeys, and mammals that live in association with humans. **Mode of transmission:** Infectious cells can be disseminated via microaerosol droplets created by coughing or sneezing, or person-person oral contact, and by bites from infected animals. **Sources/Specimens**: Sputum, nasal or throat swabs, blood, bite-wounds from infected animals, cerebrospinal fluids, and respiratory secretions. **Prophylaxis**: Currently, a pneumococcal polysaccharide vaccine (PPV23) is present, which is effective against 23 serotypes. **Zoonosis:** None. **Vectors:** None.
2. **Housing and Biosafety consideration**	ABSL-2
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 Streptococcus Pneumoniae if skin is cut or scratched.*** Personnel should be vaccinated against the Streptococcus Pneumoniae (pneumococcal polysaccharide (PPV23)).
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 *Streptococcus Pneumoniae* 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 virusolve, 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 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 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. Survival outside host : Streptococcus spp. can survive in dental plaque for up to 7 days, in dust for up to 20 days, on glass for 1 – 11 days, and up to 180 days in frozen fish, in mouse carcasses for 180 to 270 days, sputum at room temperature survives 7 days. Disinfection: Exposure to 0.5% glutaraldehyde, 1% sodium hypochlorite, iodines, 70% ethanol, and formaldehyde (effective at higher temperatures than 20˚C) have been shown to disinfect S. pneumonia.
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 Streptococcus Pneumoniae 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 Streptococcus Pneumoniae in Animals, and agree to follow these procedures.	
Name:	Title:
Signature:	Date: