

Tel-Aviv University –Safety Unit

Standard Operating Procedure for Working with **Pseudomonas spp.** in Animals

1. Health hazards	<p>Pseudomonas spp. are motile gram-negative aerobic bacteria, 2 – 4 µm long plump-shaped rods, with polar flagella, belonging to the family Pseudomonadaceae (containing 191 validly described species).</p> <p>They are non-spore forming and can produce pigments (such as pyocyanine (green-blue) and pyorubrin (yellow-green) fluorescence).</p> <p>Pseudomonas aeruginosa can produce a large variety of extracellular toxins, including exotoxin A and enterotoxins. Other substances such as hydrocyanic acid, proteolytic enzymes, toxic surface slime, and hemolytic substances may also contribute to the pathogenicity of this species.</p> <p>Pseudomonades are natural residents of soil and water.</p> <p>Pseudomonas spp. common site of infection is the lower respiratory tract, and severity ranges from colonization without immunological response to severe necrotizing bronchopneumonia. Infections also include endocarditis, osteomyelitis, urinary tract infections, gastrointestinal infections, meningitis, and, commonly, septicaemia. A primary skin infections in healthy individuals, usually may cause skin rash or folliculitis.</p> <p>The bacteria colonize on lenses and produce proteases to kill or invade corneal cells, an infection that can lead to scarring and vision loss. <i>P. aeruginosa</i> is also associated with swimmer's ear (otitis externa).</p> <p>In immunocompromised patients, systemic infections can occur, which may be severe and associated with a high mortality.</p> <p>Host range: Humans, animals (wild, domestic, livestock), and plants (flora and fungi). Mode of transmission: <i>P. aeruginosa</i> have been found to survive within droplet nuclei and can remain in aerosols for long periods of time, thus there is evidence of potential airborne transmission and lung exposure from inhaling aerosols. The bacterial can often enter the body through injuries and wounds.</p> <p>Sources/Specimens: Blood cultures, urine, skin, sputum, soft tissue samples, lower respiratory tract secretions, wound exudates, contaminated water samples, and mechanical ventilator equipment</p> <p>Drug susceptibility: <i>Pseudomonas spp.</i> are resistant to many antibiotics. Susceptibility to extended-spectrum penicillin's</p> <p>Zoonosis: Non</p> <p>Vectors: Non</p>
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)	<p>Gloves, Eyes safety goggles, Lab coat, Disposable shoe covers and Animal handling gown.</p> <p>N-99 respirator mask covering the mouth and nose when not working in a Class II Biosafety Cabinet (BSC).</p> <p>Appropriate PPE recommended for lower arms such as sleeve covers or securing gloves over the sleeves of laboratory coat.</p> <p><i>Personnel should not work with <i>Pseudomonas spp.</i> if skin is cut or scratched.</i></p>
5.General . Precautions for Animal Use	<p>Tools (as, syringe, blades and safety needles where possible) should be adapted for BSL-2. Have a sharps container in close vicinity.</p> <p>Animals should be restrained or anesthetized during injection.</p>
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	<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>Pseudomonas spp.</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 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, 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. <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 allow 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. <p>Put on a pair of new gloves and bring the rack/container, directly to the collection point of your department.</p>
8. Decontamination	<p>** Decontaminate work areas with 0.5% bleach for 30 minutes. Follow with water.</p> <p>Survival outside host: Pseudomonas can survive for months on dry surfaces and inanimate objects, humidity can improve persistence.</p> <p>Growth observed in distilled water , pseudomonas can survive up to months with minimal nutrients</p> <p>Disinfection: 1% sodium hypochlorite-bleach (recommended)</p> <p>70% ethanol, 2% glutaraldehyde, and formaldehyde, isopropyl alcohol 4% v/v or ethyl alcohol 6% v/v are effective disinfectants.</p> <p>It has been found to be resistant to chlorine, chloramines, ozone, and iodine.</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. 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 require 15-20 minutes; viscous spills require 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. <p>Exposure:</p> <ol style="list-style-type: none"> 1. In case of skin contact or injection with Pseudomonas 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 121°C/250°F, 15psi of steam pressure).
I hereby confirm that I have read the SOP (Standard Operating Procedure) for Working with Pseudomonas spp. in Animals, and agree to follow these procedures.	
Name:	Title:
Signature:	Date:

Dr. Esther Michael - Biological Safety Office, : 640-9966

