

# Tel-Aviv University –Safety Unit

## Standard Operating Procedure for Working with **Staphylococcus aureus** in Animals

<p>1. Health hazards</p>	<p>Staphylococcus aureus, also known as MRSA (methicillin-resistant Staphylococcus aureus), are Gram-positive, catalase positive cocci belonging to the Staphylococcaceae family. They are approximately 0.5-1.5 µm in diameter, nonmotile, non-spore-forming, facultative anaerobes, that usually form in clusters.</p> <p>Many strains produce staphylococcal enterotoxins, the superantigen toxic shock syndrome toxin (TSST-1), and exfoliative toxins. Staphylococcus aureus are part of human flora, and are primarily found in the nose, skin, groin, axillae, perineal area (males), mucous membranes, the mouth, mammary glands, hair, and the intestinal, genitourinary and upper respiratory tracts.</p> <p>Staphylococcus aureus is an opportunistic pathogen that can cause a variety of self-limiting to life-threatening diseases in humans.</p> <p>The bacteria are a leading cause of food poisoning, resulting from the consumption of food contaminated with enterotoxins, and it is one of the most common causes of skin, soft-tissue, and nosocomial infection.</p> <p><b>Host range:</b> Humans, wild and domestic animals.</p> <p><b>Mode of transmission:</b> Ingestion of food containing enterotoxins, and animal bites. S. aureus is transmitted through aerosol or direct contact with fomites, infected animals, or infected people. Approximately 30% of healthy humans carry S. aureus in their nasopharynx or on their skin. In a laboratory rodent setting, it is more likely for humans to infect animals rather than vice versa.</p> <p><b>Sources/Specimens:</b> Infective stages may be present in CSF (cerebrospinal fluid), joint aspirates, blood, abscesses, aerosols, faeces, and urine.</p> <p><b>** Staphylococcus aureus can grow in a pH of 4.2 to 9.3 and in salt concentrations of up to 15%. Enterotoxins are resistant to temperatures that would destroy the bacteria.</b></p> <p><b>**Sensitive to dry heat treatment of 160-170°C for at least an hour, but not to moist heat treatment.</b></p> <p><b>Drug susceptibility:</b> Antibiotics such as cloxacillin and cephalexin are commonly used to treat staph infections. Susceptibility to extended-spectrum penicillin's.</p> <p><b>Zoonosis:</b> Yes, through direct or indirect contact with an infected animal. Due to its ability to colonize a wide range of species, S. aureus can be readily transmitted from one species to another, including from humans to animals and vice versa.</p> <p><b>Vectors:</b> Non</p>
<p>2. Housing and Biosafety consideration</p>	<p style="text-align: center;">ABSL-2</p>
<p>3. Training</p>	<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> <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><b><i>Personnel should not work with <i>Staphylococcus aureus</i> if skin is cut or scratched.</i></b></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	<p>Work should be conducted in ABSL-2 facility, over absorbent pads in a class II type A1 or A2 biological cabinet.</p>
7. Animal handling practices	<ol style="list-style-type: none"> <li>1. Animals must be housed in filter top cages marked as biohazards (including the name of the pathogen/biohazard). <b>Handling the cages (including bedding) will be done only by the researchers.</b></li> <li>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.</li> <li>3. Infected animals may shed <i>Staphylococcus aureus</i> after treatment; take precautions to avoid the creation of aerosols when changing or washing cages, or cleaning the room.</li> <li>4. Dead animals must be placed in primary plastic bags, which are then placed in biosafety bags for infectious waste incineration.</li> <li>5. All surfaces and racks that may be contaminated will be decontaminated with 0.5% bleach ASAP.</li> <li>6. When changing cages, use a standard microisolator technique: <ul style="list-style-type: none"> <li>• place the cage containing the animals, under the biological safety cabinet and transfer the animals into a clean cage.</li> <li>• spray the dirty cage with virusolve, remove from the safety cabinet and place on a transfer rack .</li> <li>• 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.</li> <li>• 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.</li> </ul> </li> </ol> <p><b>**In cases where the use of autoclave (within the animal facility) is not an option:</b></p> <ul style="list-style-type: none"> <li>• the cages (bedding ) must be emptied inside the BSL-2 cabinet, directly to a double biohazard bags.</li> <li>• Before closing the bags, carefully, add a small amount of water (250ml) to improve the sterilization process.</li> </ul> <p><b><i>Do not close the bag completely/tightly (in order to avoid entering of steam during the sterilization process).</i></b></p> <ul style="list-style-type: none"> <li>• Spray the dirty bag with 0.5% bleach or virusolve.</li> <li>• Remove from the safety cabinet and place on a transfer rack/container.</li> </ul> <p>Put on a pair of new gloves and bring the rack/container, directly to the collection</p>

	point of your department.
8. Decontamination	<p><b>** Decontaminate work areas with 0.5% bleach for 30 minutes. Follow with water.</b></p> <p><b>Survival outside host :</b> Survives on carcasses and organs (up to 42 days), floors (less than 7 days), glass (46 hours), sunlight (17 hours), UV (7 hours), meat products (60 days), coins (up to 7 days), skin (30 minutes to 38 days).</p> <p>Depending on colony size, <i>S. aureus</i> can survive on fabrics from days to months.</p> <p><b>Disinfection:</b> Susceptible to 70% ethanol, clorhexidine, 1% sodium hypochlorite, 2% glutaraldehyde, 0.25% benzalkonium chloride, and formaldehyde.</p>
9. Spill and Accident Procedures	<ol style="list-style-type: none"> <li>1. Evacuate area, remove contaminated PPE and allow agents to settle for a minimum of 30 minutes. Initiate spill response procedure.</li> <li>2. Wearing protective clothing, gently cover the spill with absorbent material. Starting at the edges and work towards the center.</li> <li>3. Carefully pour disinfectant over the absorbed spill, again starting at the edges. Saturate the area with disinfectant.</li> <li>4. Allow sufficient contact period to inactivate the material in the spill. Non-viscous spills require 15-20 minutes: viscous spills require 30 minutes.</li> <li>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</li> <li>6. Discard absorbent material in Chemical waste bags.</li> <li>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.</li> <li>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.</li> <li>9. Place bag in a second biohazard bag, secure and disinfect by autoclaving.</li> </ol> <p><u>Exposure:</u></p> <ol style="list-style-type: none"> <li>1. In case of skin contact or injection with <i>Staphylococcus aureus</i> spp wash the affected area with soap and water for at least 15 minutes. Consult with Employee Health Center.</li> <li>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.</li> </ol>
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 <i>Staphylococcus aureus</i> in Animals, and agree to follow these procedures.	
Name:	Title:
Signature:	Date:

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

