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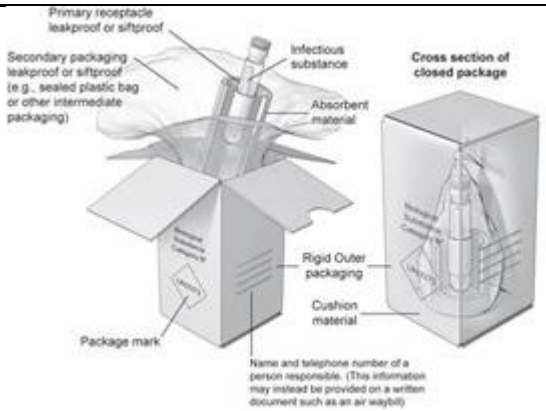
Tel-Aviv University –Safety Unit

Standard Operating Procedure for Working with **Salmonella enterica spp.** in Animals

1. Health hazards	Salmonella is a rod-shaped (0.7-1.5 by 2.0-5.0 µm in size), motile, aerobic and facultative anaerobe, non-spore forming and gram-negative organism. Salmonella is a general name used for a group of more than 2,000 closely related bacteria that cause illness by reproducing in the digestive tract. Each salmonella serotype shares common antigens. There are two species of Salmonella: Salmonella bongori and Salmonella enterica, the latter of which is divided into six subtypes: enterica, salamae, arizonae, diarizonae, houtenae and indica. Salmonella is closely related to the Escherichia genus and are found worldwide in cold- and warm-blooded animals (including humans), and in the environment. The usual habitat for subspecies enterica is warm-blooded animals. All species of Salmonella can infect humans. Salmonella enterica subspecies enterica has 2610 different serotypes; the most well known being serotypes Typhi, Paratyphi, Enteriditis, Typhimurium and Choleraesuis. The serotypes are characterized by three surface antigens: the flagellar "H" antigen, the oligosaccharide "O" antigen and the polysaccharide "Vi" antigen (found in Typhi and Paratyphi serotypes). Salmonella enterica can cause four different clinical manifestations: gastroenteritis, bacteremia, enteric fever, and an asymptomatic carrier state. Salmonella can grow from 5°C up to 47°C, with an optimum temperature of 37°C. **Host range**: Humans, animals: domestic and wild animals such as cattle, swine, poultry, wild birds, and pets (particularly reptiles) as well as flies. **Mode of transmission:** Human infection usually occurs when consuming contaminated foods and water, contact with infected feces, as well as contact with infective animals, animal feed, or humans. Flies can infect foods which can also be a risk for transmission to humans. Humans can spread the disease for as long as they shed the bacterium in their feces. Certain carriers shed the bacteria for years and 5 % of patients recovering from non-typhoidal salmonellosis can shed the bacteria for 20 weeks. ** Animals can have a latent or carrier state where they excrete the organism briefly, intermittently or persistently. **Incubation Period:** For non-typhoidal salmonellosis, the incubation period is variable, depends on the inoculum size, and usually ranges between 5 and 72 hours. For typhoid fever, the incubation period can be between 3 and 60 days, although most infections occur 7-14 days after contamination. **Drug susceptibility**: Susceptible to chloramphenicol, ciproflaxin, amoxicillin, co-trimoxazole, trimethprim-sulfonamid, cephalosporins and norfloxacin. Some resistance to chloramphenicol has been reported and, in 1989, 32% of strains were multi-drug resistant.

	Zoonosis: Yes. Transmission between animals and humans occur when humans are in contact with infective animals and their feces. **Vectors:** Flies are a possible indirect vector as they may transmit the bacterium to foods. **Sources/specimens**: All Salmonella enterica subspecies (with the exception of serotype Typhi) are found in blood, urine, feces, food and feed and environmental materials. Serotype Typhi is found in blood, urine, feces and bile
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 Salmonella enterica 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. when not working in a Class II Biosafety Cabinet (BSC), use an absorbent pads and respirator mask .
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 *Salmonella enterica 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 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: Serotype Choleraesuis can survive in wet swine feces for at least 3 months and in dry swine feces for at least 13 months. Serotype Dublin can survive in feces spread on concrete, rubber, and polyester for almost six years. Serotype Typhimurium can survive in cattle slurry for 19-60 days, cattle manure for 48 days, soil for 231 days, and water for up to 152 days. Flies have been shown to excrete certain serotypes for 8 days and bed bugs can excrete bacilli for up to 21 days. Certain serotypes have been shown to survive on fingertips for up to 80 minutes, depending on the inoculum size. Disinfection: Gram negative bacteria are susceptible to 2-5% phenol, 1% sodium hypochlorite, 4% formaldehyde, 2% glutaraldehyde, 70% ethanol, 70% propanol, 2% peracetic acid, 3-6% hydrogen peroxide, quaternary ammonium compounds and iodophors; however, Salmonella spp. is resistant to nitrites.
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 Salmonella 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).
11. Transporting Biological Materials	 **Regulations on the transportation of biological agents**: the requirements for the packaging and transport of biologicals in a manner that will minimize the threat of release via container breakage during transport. • All materials must be transported and stored in a secondary container to prevent breakage. A secondary container is capable of containing the materials if the primary container breaks or leaks. Absorbent materials must be included in the secondary container to absorb any liquids. Cushion the materials to prevent container breakage. • Small amounts of biological materials in sealed containers can be transported in a cooler with a latching lid. The cooler will act as the approved secondary container. Inside this cooler must be enough absorbent or cushioning to prevent shifting during transport. The cooler must also be secured to prevent it from sliding or toppling during transport. • Include on the cooler or container a sheet listing the name of the suspected infectious agent(s) or materials being transported (the biohazard level of the agent needs to be respected) and the below-listed emergency phone numbers. • When moving material **on campus** the package will be put in secondary leak proof containment and clearly marked with the appropriate biohazard signs. All efforts will be made to prevent a spill or aerosol. The outer container needs to be properly surface decontaminated and the agent will never be left unattended while in transit between laboratories. The biohazard level of the agent needs to be respected; you may not take a biohazard agent in a

	laboratory that is of lower containment specification even if the secondary container is not opened in that laboratory.

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

Name:	Title:
Signature:	Date:

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