

Tel-Aviv University –Safety Unit

Standard Operating Procedure for Working with *LISTERIA MONOCYTOGENES* in Animals.

1. Health hazards

Listeria monocytogenes is a facultatively anaerobic, gram-positive, rod-shaped coccobacillus.

Most commonly, listeria causes a mild febrile illness however, several types of listeriosis disease manifestations are recognized; for instance, listeriosis in pregnancy, listeriosis of the central nervous system (CNS), febrile gastroenteritis, glandular listeriosis, local listeriosis, typhoid listeriosis, and atypical listeriosis

Certain factors predispose individuals to infection with *L. monocytogenes*, such as neonates, pregnancy, leukemia, Hodgkin's disease, diabetes mellitus, alcoholism or cirrhosis and immunosuppressive or cytostatic therapy.

Host range: *L. monocytogenes* has been isolated from many organisms, including humans and other mammals, fish, crustaceans, and insects.

Zoonosis: Yes, through consumption of foodstuffs containing infected animal products, manure contaminated vegetables, and by direct contact with animal tissues during birthing and butchering.

Survival outside host: *L. monocytogenes* is commonly found in nature, particularly in association with soil, is relatively heat resistant, can tolerate cold temperature environments well, and can survive at low pH.

***Listeria monocytogenes* may be transmitted by:**

- Blood, cerebrospinal fluid, faeces, placenta, skin lesions, pus, amniotic fluid, menstrual blood, lochia, respiratory secretions, meconium, gastric aspirate, animal tissues/specimens, and infected organs such as brain and liver
Inhalation of airborne conidia, through contaminated water or animals bedding .
- Accidental autoinoculation, exposure to the tissues of experimentally infected animals, and *L. monocytogenes* cultures.
- Penetration of the skin via puncture or absorption (through scratches, cuts, abrasions, dermatitis or other lesions)
- Mucous membrane exposure of the eyes, nose, and mouth (through direct contact or aerosols)
- Direct contact with diseased animals.

2. Designated Area	ABSL-2 facility.
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>Those at greater risk of infection (pregnant women or immunocompromised individuals) should take extra care when working in a laboratory where <i>L. monocytogenes</i> is propagated or handled.</p> <p><i>Personnel should not work with Listeria monocytogenes 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, 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>Listeria monocytogenes</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.

	<p>5. All surfaces and racks that may be contaminated will be decontaminated with 0.5% bleach ASAP.</p> <p>6. When changing cages, use a standard microisolator technique:</p> <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 avoid 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. • Put on a pair of new gloves and bring the rack/container, directly to the collection point of your department.
8. Decontamination	<p>** Decontaminate work areas with 0.5% bleach for 30 minutes. Follow with water.</p> <p>In general: <i>Listeria monocytogenes</i> is sensitive to 0.5% sodium hypochlorite, iodophor compounds, and quaternary ammonium compounds.</p> <p>Five to 10-fold higher concentrations of the above compounds are required at 4 °C.</p> <p>Physical inactivation: <i>L. monocytogenes</i> can be inactivated by ozone, high pressure (500MPa), and high temperatures (at least 70 °C for 2 minutes)</p>
9. Spill and Accident	<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. Cover the spill with absorbent material. Starting at the edges and work towards

Procedures	<p>the center.</p> <ol style="list-style-type: none"> 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, and wipe with towels. 8. Discard all cleanup materials in Chemical bag, along with any contaminated PPE (pay special attention to gloves and shoe covers). Close and secure the bag. 9. Place bag in a second Chemical bag, secure and dispose as chemical waste. 10. Discard contaminated PPE (with biohazard materials) in biohazard bag. Place bag in a second biohazard bag, secure and disinfect by autoclaving. <p><u>Exposure:</u></p> <ol style="list-style-type: none"> 1. In case of skin contact or injection with <i>Listeria monocytogenes</i>, 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 LISTERIA MONOCYTOGENES in Animals, and agree to follow these procedures.	
Name:	Title:
Signature:	Date:

