

Tel-Aviv University –Safety Unit

Standard Operating Procedure for Working with **Citrobacter Rodentium** in Animals

<p>1. Health hazards</p>	<p>Citrobacter rodentium, of the Enterobacteriaceae family, is a gram negative bacterium which was previously known as Citrobacter freundii biotype 4280 and Citrobacter species 9. It is one of the 9 species belonging to genus Citrobacter spp. Citrobacter spp. are gram-negative, facultative anaerobic bacteria that appear as rods or coccobacilli at 0.3-1 µm in diameter and 0.6-6 µm long and are motile using their peritrichous flagella. Citrobacter rodentium lacks motility and endospores, which are further significant characteristic of this species. Citrobacter spp. ferment mannitol with production of gaseous H₂S, and they can also use citrate as sole carbon source. The genus can be divided in 43 O-serogroups, based on the O antigen of the lipopolysaccharide (LPS) and in 20 chemogroup, based on the sugar composition of the LPS. Citrobacter rodentium is a rodent equivalent of human Enteropathogenic Escherichia Coli infection. Also, it is a restricted mouse pathogen which lives in mouse colon and can survive with or without oxygen.</p> <p>Citrobacter normally cause urinary tract infections, blood stream infections, intra abdominal sepsis, brain abscesses, and pneumonia and other neonatal infection , such as meningitis, neonatal sepsis, joint infection or general bacteremia. Citrobacter rodentium cause neonatal meningitis that can lead to brain abscesses especially in immunocompromised adult patients.</p> <p>Host range: Hosts include human and animals and aquatic organisms (catfish). Mode of transmission: Citrobacter may be spread by direct contact: person-to-person transmission and through ingestion of environmental sources (fecal-oral route). Parsley contaminated with Citrobacter contained in swine manure has caused an outbreak of Citrobacter in Canada. The outbreak caused 8 urinary tract infection, and 1 death. Reservoir: Human and animal intestines, soil, water, sewage and food. Zoonosis: Non Vectors: 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>
<p>4. Personal Protective Equipment</p>	<p>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</p>

(PPE)	<p>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>Citrobacter Rodentium</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	<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"> 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>Citrobacter Rodentium</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 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. <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: Citrobacter Rodentium can survive in Soil and water.</p> <p>Disinfection: 1% sodium hypochlorite, 70% ethanol, formaldehyde, glutaraldehyde, iodophore and paracetic acid are effective against Citrobacter.</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><u>Exposure:</u></p> <ol style="list-style-type: none"> 1. In case of skin contact or injection with Citrobacter Rodentium 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 Citrobacter Rodentium in Animals, and agree to follow these procedures.	
Name:	Title:
Signature:	Date:

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