Tel-Aviv University –Safety Unit

Standard Operating Procedure for Working with Shigella Spp.

1. Health hazards	Shigella species are pathogenic, gram-negative, rod-shaped bacteria. They are non-motile, non-encapsulated, non-sporulating, and facultative anaerobes. There are currently four recognized species (or subgroups): S. flexneri, S. sonnei, S. boydii, and S. dysenteriae. Each species is further divided into serotypes based on specific antigens. **Infection Process and Symptoms:** Ingested Shigella can survive stomach acidity and cause illness by infecting colonic mucosa. They multiply within colonic epithelial cells and spread laterally to adjacent cells. Clinical symptoms typically appear between 12 hours and 3 days after exposure, and can include fever, tachycardia (rapid heart rate), tachypnea (rapid breathing), and hypotension (low blood pressure). **Severity of Infection:** The severity of a Shigella infection depends on the host's health, the infectious dose, and the specific serotype involved. S. dysenteriae is the most pathogenic species, with a fatality rate of up to 20%. S. sonnei usually causes milder forms of shigellosis. **Laboratory-Acquired Infections:** Shigella species are frequently identified as agents of laboratory-acquired infections due to their high virulence and low infectious dose. **Host Range:** Humans and higher primates are the primary hosts. Shigella has also experimentally infected mice, guinea pigs, domestic pigs, and rabbits, and has been detected in the fecal samples of goats. **Mode of Transmission:** The organisms spread primarily via the fecal-oral route. Transmission commonly occurs through the ingestion of contaminated food (due to poor hygiene) and contaminated drinking water. The spread of infection linked to flies has also been recorded.

	Sources and Specimens: Humans are the most common reservoir, though infections have also been observed in non-human primates. Organisms can be found in stool samples, rectal swabs, and, rarely, in blood samples. **Zoonosis:** Cases of zoonotic transmission of shigellosis between humans and non-human primates have occurred. These instances typically involve occupational contact, where an infected non-human primate transmits the bacteria to a human. **Vectors:** Flies can act as mechanical vectors, helping to disseminate Shigella species.
2. **Housing and Biosafety consideration**	Containment Level 2 facilities. A biological safety cabinet (BSC) or other primary containment devices to be used for activities with open vessels, based on the risks associated with the inherent characteristics of the regulated material, the potential to produce infectious aerosols or aerosolized toxins, the handling of high concentrations of regulated materials, or the handling of large volumes of regulated materials. Use of needles and syringes is to be strictly limited. Additional precautions are required with work involving animals or large-scale activities.
3.Training	Practical experience working in a BSL-2 laboratory, as well as general biosafety, is required.
4. Personal Protective Equipment (PPE)	Gloves, Eyes safety goggles, Lab coat, and closed Shoes. 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 Shigella Spp. if skin is cut or scratched.***

5.General . Precautions	Tools (as, syringe, blades and safety needles where possible) should be adapted for BSL-2. Have a sharps container in close vicinity.
6. Environmental / Ventilation Controls	Work should be conducted in BSL-2 facility, in a class II type A1 or A2 biological cabinet.
7.Decontamination	** Decontaminate work areas with 0.1% bleach for 30 minutes. Follow with water. **Survival outside host :** Shigella spp. can survive up to months on dry surfaces, up to 10 days in citric juices and carbonated soft drinks, several days on contaminated vegetables, over 3 hours on fingers, 2 – 28 days on metal utensils at 15°C or 0 – 13 days at 37°C, in feces for 12 days at 25°C , and water for under 3 days. Growth is possible at 25°C – 37°C and bacteria can survive at 5°C on MacConkey agar. Flies can carry Shigella for up to 20 – 24 days. **Disinfection:** Susceptible to 1% sodium hypochlorite, 2% glutaraldehyde, iodines, phenolics, and formaldehyde. Treatments with ozone (1.6 and 2.2 ppm) for 1-minute decreased S. sonnei population in water by 3.7 and 5.6 log10 cfu/mL, respectively.
8. Spill and Accident Procedures	1. Evacuate area, remove contaminated PPE and allow aerosols to settle for a minimum of 30 minutes. Initiate spill response procedure. 2. Wearing personal protective equipment, gently covering the spill with absorbent material. 3. Starting at the edges carefully pour disinfectant over the absorbed spill, starting at the edges and working towards the center. Saturate the area with disinfectants. 4. Allow sufficient contact time to inactivate the material in the spill. Non-viscous spills require 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. 9. Place bag in a second biohazard bag, secure and disinfect by autoclaving. **Exposure:** 1. In case of skin contact or injection with Shigella 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.
9. Waste Disposal	Organisms can be heat-killed by steam using an autoclave for 1 hour at 100°C under normal atmospheric pressure.
I hereby confirm that I have read the SOP (Standard Operating Procedure) for Working with Shigella Spp. and agree to follow these procedures.	
Name:	Title:
Signature:	Date: