

Tel-Aviv University –Safety Unit

Standard Operating Procedure for Working with **Fusobacterium spp.** in Animals

1. Health hazards

Fusobacterium are anaerobic gram-negative bacilli, non-sporulating, slender cells with tapered ends or pleomorphism.

Fusobacterium spp., are part of the normal flora of the oropharyngeal, gastrointestinal and genital tracts. Infections may occur after surgical or accidental trauma, edema, anoxia, tissue destruction, and animal bites.

Fusobacterium spp. are pathogenic species in the genus Fusobacterium, include: F. necrophorum, F.nucleatum, F. canifelinum, F. gonidiaformans, F. mortiferum, F. naviforme, F. necrogenes, F. russii, F.ulcerans, F. varium.

F. nucleatum is the most common source of infection, while F. necrophorum is the most virulent species (may cause severe infections in children and young adults (i.e. pharyngotonsillitis)).

Host range: Humans and animals, including horses, cattle, sheep, goats, pigs, fowl.

Mode of transmission: Infections can occur by contact with mucous membranes as well as accidental inoculation and transfer of bodily fluids.

Fusobacterium can be transmitted from human-to-human by bite wounds, and there is also some evidence that Fusobacterium can be transferred in bodily fluids.

Sources/Specimens: Sources of Fusobacterium include feces, necrotic tissues, respiratory tract tissues, urogenital specimens, gut contents, litter, and soil.

Drug susceptibility: Treatment of Fusobacterium infections depend on the site of infections. Metronidazole, piperacillin/tazobactam, ticarcillin/clavulanate, amoxicillin/sulbactam, ampicillin/sulbactam, ertapenem, imipenem, meropenem, clindamycin, and cefoxitin are all used therapeutically to treat infections associated with Fusobacterium. Fusobacterium may be resistant to penicillin and there is widespread resistance to erythromycin and other macrolides.

Survival Outside Host: Fusobacteria have been known to **persist in soil for up to 18 weeks**. They survive well in wet soil with high manure content. studies of aerated fecal slurry showed that the levels of Fusobacterium were below the level of detection after 24 hours.

In non-aerated fecal slurry, no change in Fusobacterium levels were observed in the first 24 hours, and Fusobacteria were no longer present after 6 days.

Survival on BHIA medium exposed to air ranges from six hours to seven days depending on species.

Zoonosis: Yes - Fusobacterium can be passed to humans from animal bites or handling of animals with open sore.

Vectors: Non

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)	<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><i>Personnel should not work with <i>Fusobacterium spp.</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	Work should be conducted in ABSL-2 facility, over absorbent pads 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>Fusobacterium spp.</i> after treatment; take precautions to avoid the creation of aerosols when changing or washing cages, or cleaning the room. <i>Fusobacteria</i> have been known to persist in soil for up to 18 weeks, therefore the cages and the bedding will be considered as biohazards, for the whole time. 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 (or virusolve). 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 (or virusolve), 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. Fusobacteria are susceptible to solutions of 1% sodium hypochlorite, 0.2% chlorhexidine, 70% ethanol, 2% glutaraldehyde, 3% hydrogen peroxide, formaldehyde, phenolics, iodophores, calcium hydroxide, formocresol, triclosan and 1% sodium hypochlorite solution.</p> <p>Physical Inactivation: Fusobacterium can be inactivated by UV light with a wavelength 254nm, and is also susceptible to moist heat of 121 °C for at least 15 minutes and dry heat of 170 °C for at least 1 hour.</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 or use paper towels . 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 Fusobacterium 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 <i>Fusobacterium</i> spp. in Animals, and agree to follow these procedures.	
Name:	Title:
Signature:	Date:

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