

# Tel-Aviv University –Safety Unit

## Standard Operating Procedure for Working with *Porphyromonas gingivalis* in Animals

1. Health hazards	<p><i>Porphyromonas gingivalis</i> belongs to the phylum Bacteroidetes and is a nonmotile, Gram-negative, non-spore forming, rod-shaped, anaerobic, pathogenic bacterium. The bacteria found mostly in subgingival sites and occasionally in the tongue and tonsil.</p> <p><i>P. gingivalis</i> plays an important role in the progression of periodontal disease and the destruction of tissue and bone. Though it is found in low abundance in the oral cavity, it causes a microbial shift of the oral cavity, allowing for uncontrolled growth of the commensal microbial community and increasing the virulence of other commensal bacterium.</p> <p>Collagen degradation observed in chronic periodontal disease results in part from the collagenase enzymes of this species. <i>P. gingivalis</i> has been linked to rheumatoid arthritis.</p> <p><i>P.gingivalis</i> is implicated in certain forms of periodontal disease, as well as the upper gastrointestinal tract, respiratory tract, and in the colon.</p> <p><i>P. gingivalis</i> involved in periodontal disease can become loose and enter the bloodstream during regular dental procedures, chewing, brushing, flossing.</p> <p><b>Host range:</b> Humans and animals, including : primates, murine, dogs, rodents and sheep.</p> <p><b>Mode of transmission:</b> Infections can occur by contact with mucous membranes as well as accidental inoculation and transfer of bodily fluids.</p> <p><i>P. gingivalis</i> can be transmitted to humans from animal bites or between humans via direct contact with human saliva.</p> <p><b>Sources/Specimens:</b> Sources of <i>P. gingivalis</i> include feces, necrotic tissues, respiratory tract tissues, urogenital specimens, gut contents, litter, and soil.</p> <p><b>Survival in the Host :</b> <i>P. gingivalis</i> can invade human gingival fibroblasts and can survive in them in the presence of considerable concentrations of antibiotics.</p> <p><i>P. gingivalis</i> also invades gingival epithelial cells in high numbers, in which cases both bacteria and epithelial cells survive for extended periods of time.</p> <p><b>Zoonosis:</b> Yes - <i>P. gingivalis</i> can be passed to humans from animal bites or handling of animals with open sore.</p> <p><b>Vectors:</b> Non</p>
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><b>Personnel should not work with <i>P. gingivalis</i> if skin is cut or scratched.</b></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"> <li>1. Animals must be housed in filter top cages marked as biohazards (including the name of the pathogen/biohazard). <b>Handling the cages (including bedding) will be done only by the researchers.</b></li> <li>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.</li> <li>3. Infected animals may shed <i>P. gingivalis</i> after treatment; take precautions to avoid the creation of aerosols when changing or washing cages, or cleaning the room. <i>P. gingivalis</i> have been known to persist for a long period of time, therefore the cages and the bedding will be considered as biohazards, for the whole time.</li> <li>4. Dead animals must be placed in primary plastic bags, which are then placed in biosafety bags for infectious waste incineration.</li> <li>5. All surfaces and racks that may be contaminated will be decontaminated with 0.5% bleach ASAP (or virusolve).</li> <li>6. When changing cages, use a standard microisolator technique: <ul style="list-style-type: none"> <li>• place the cage containing the animals, under the biological safety cabinet and transfer the animals into a clean cage.</li> <li>• spray the dirty cage with 0.5% bleach (or virusolve), remove from the safety cabinet and place on a transfer rack .</li> <li>• 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.</li> <li>• 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.</li> </ul> </li> </ol> <p><b>**In cases where the use of autoclave (within the animal facility) is not an option:</b></p> <ul style="list-style-type: none"> <li>• the cages (bedding ) must be emptied inside the BSL-2 cabinet, directly to a double biohazard bags.</li> <li>• Before closing the bags, carefully, add a small amount of water (250ml) to</li> </ul>

	<p>improve the sterilization process.</p> <p><b>Do not close the bag completely/tightly</b> (in order to avoid entering of steam during the sterilization process).</p> <ul style="list-style-type: none"> <li>• Spray the dirty bag with 0.5% bleach or virusolve.</li> <li>• Remove from the safety cabinet and place on a transfer rack/container.</li> </ul> <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. susceptible to glutaraldehyde, sodium hypochlorite, hydrogen peroxide and sulfathiazole .</p> <p>Physical Inactivation: <i>P. gingivalis</i> is susceptible to moist heat of 121 °C for at least 30 minutes and dry heat of 170 °C for at least 1 hour.</p>
9. Spill and Accident Procedures	<ol style="list-style-type: none"> <li>1. Evacuate area, remove contaminated PPE and allow agents to settle for a minimum of 30 minutes. Initiate spill response procedure.</li> <li>2. Wearing protective clothing, gently cover the spill with absorbent material, starting at the edges and work towards the center or use paper towels .</li> <li>3. Carefully pour disinfectant over the absorbed spill, again starting at the edges. Saturate the area with disinfectant.</li> <li>4. Allow sufficient contact period to inactivate the material in the spill. Non-viscous spills require 15-20 minutes: viscous spills require 30 minutes.</li> <li>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</li> <li>6. Discard absorbent material in Chemical waste bags.</li> <li>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.</li> <li>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.</li> <li>9. Place bag in a second biohazard bag, secure and disinfect by autoclaving.</li> </ol> <p><u>Exposure:</u></p> <ol style="list-style-type: none"> <li>1. In case of skin contact or injection with <i>P. gingivalis</i> wash the affected area with soap and water for at least 15 minutes. Consult with Employee Health Center.</li> <li>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.</li> </ol>
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 *P. gingivalis* in Animals, and agree to follow these procedures.

Name:

Title:

Signature:

Date:

**Dr. Esther Michael - Biological Safety Office, : 640-9966**