1. PURPOSE

This Standard Operating Procedure (SOP) describes the methods for routine monitoring for potential microbial pathogens in laboratory animal facilities.

The goals of the program are the following:

- To ensure that sanitization procedures result in a significant decrease in bacterial surface contamination.
- Eliminate pathogens from items that are mechanically washed.
- Demonstrate a significant decrease in bacterial loads on items that are washed by hand, with particular concern for pathogens.
- Ensure that water in automatic water systems meets the specifications of the local public drinking water sanitary standards.

2. RESPONSIBILITY

Facility supervisor/manager, Diagnostic and Research Support Service (DRSS) staff, veterinarian

3. MATERIALS

3.1. Sterile cotton-tipped applicators
3.2. Cooked-meat broth tubes
3.1. Replicate Organism Detection and Counting (RODAC) plates
3.2. Forceps
3.3. Scalpel
3.4. 70% ethanol
3.5. Masking tape

4. PROCEDURES

4.1. Items to be sampled:

   4.1.1. Water:
           4.1.1.1. From taps where bottles are filled
           4.1.1.2. From automatic watering systems (sipper tubes)
           4.1.1.3. From autoclaved bottles

   4.1.2. Sipper tubes:
           4.1.2.1. After being washed or after autoclaving (if applicable)

   4.1.3. Water bottles:
           4.1.3.1. After being washed or after autoclaving (if applicable)

   4.1.4. Cages or pens:
           4.1.4.1. After being washed or after autoclaving (if applicable)

   4.1.5. Bedding:
           4.1.5.1. From storage bin and from an autoclaved cage (if applicable)

   4.1.6. Feed
4.2. Sample collection:

4.2.1. Sample items immediately after they are sanitized and dried. Items must air dry, do not wipe dry.
4.2.2. Sample autoclaved items in a Biological Safety Cabinet.
4.2.3. Identify all samples with the description of the item sampled and sampling date.
4.2.4. Water:
   4.2.4.1. At the water filling station or tap, collect approximately 1ml of water directly into a cooked meat broth tube.
   4.2.4.2. From automatic watering system, wipe the sipper tube with alcohol and wait until it dries before collecting approximately 1ml of water directly into a cooked meat broth tube.

4.2.4.3. Sipper tubes:
   4.2.4.1. Moisten a sterile swab with broth.
   4.2.4.2. Swab the inside of several sipper tubes.
   4.2.4.3. Place the swab in the cooked meat tube and snap off the top part of the swab shaft.

4.2.5. Bottles:
   4.2.5.1. Moisten a sterile swab with broth.
   4.2.5.2. Swab the inside of several bottles.
   4.2.5.3. Place the swab in the cooked meat tube and snap off the top part of the swab shaft.

4.2.6. Cages (rodents):
   4.2.6.1. Moisten a sterile swab with broth.
   4.2.6.2. Swab the inside corners of several cages.
   4.2.6.3. Place the swab in the cooked meat tube and snap off the top part of the swab shaft.

4.2.7. Cages (large animals):
   4.2.7.1. Wait until the cage is cleaned, disinfected and completely dry.
   4.2.7.2. Remove the lid of a RODAC plate and contact the clean floor with the agar bed.
   4.2.7.3. Close the RODAC plate lid and identify sample.

4.2.8. Pens (large animals):
   4.2.8.1. Wait until the cage is cleaned, disinfected and completely dry.
   4.2.8.2. Remove the lid of a RODAC plate and contact the clean floor with the agar bed.
   4.2.8.3. Close the RODAC plate lid and identify sample.

4.2.9. Bedding:
   4.2.9.1. Using sanitized forceps (dipped in alcohol), collect a sample of chips and place in broth.

4.2.10. Feed:
   4.2.10.1. Open a new bag by making a small incision on the top of the bag with a sanitized scalpel (dipped in alcohol) and take a sample with sanitized forceps.
   4.2.10.2. Place the sample in the broth.
   4.2.10.3. Close the chow bag with tape. Record the milling date and lot number of the feed.

4.3. Evaluation of bacterial culture results:

4.3.1. Results will be interpreted by a veterinarian.

4.3.2. Consideration is given to:
   4.3.2.1. The method of sanitation
4.3.2.2. The species concerned
4.3.2.3. The intent of the sanitation process

4.4. Automatic Cage Washers:
4.4.1. Monitor water temperature of automatic cage washers daily using a chemical indicator strip. If the cage washer is used less than once a week, use a chemical indicator strip for each run. Rinse water temperature should reach 180°F (82°C). Record results and keep on file in the facility.
4.4.2. Record all service and routine maintenance. Maintain records at the facility.
4.4.3. Monitor efficacy of cagewasher sanitation on a quarterly basis. Sample items as described in Section 4.2 immediately after they are washed and (dried) dry.

4.5. Hand Washed Areas:
4.5.1. Anything that has direct contact with animals but is not sanitized in a mechanical cage washer or autoclave once every 14 days will instead be washed by hand. Monitor efficacy of hand washing sanitation on a quarterly basis.

4.6. Autoclaves:
4.6.1. Monitor autoclaves used to process items that come into direct contact with animals. Perform quarterly monitoring as per SOP.

4.7. Automatic Water Systems:
4.7.1. Flush automatic water systems at least once a week. Document each time the system is flushed. Sample the systems described in Section 4.2 to verify the automatic water system meets the specifications of the local public drinking water standards.

4.8. Exceptions to Testing Standards:
4.8.1. Research animals housed in an agricultural setting and those animals whose husbandry does not require environmental sanitation, such as fish, may or may not be included in the microbiological monitoring program at the discretion of the veterinarians and the Facility Animal Care Committee (FACC).