GUIDELINES ON SANITIZING BEHAVIORAL OR LABORATORY EQUIPMENT

I. PURPOSE

To describe recommended sanitization procedures for behavioral or other equipment where animals come into contact during research procedures. Sanitization is the process of decreasing the bioload on a surface to a reasonable level (see below), using chemical agents. Adequate sanitization will prevent the spread of microbial agents that may cause sub-clinical or clinical diseases that could jeopardize the validity and reproducibility of research data, complicate its interpretation, or cause zoonotic concerns.

II. RESPONSIBILITY

The Principal investigator and their staff are responsible for routine cleaning and disinfection/sanitization of such equipment used in their approved protocols. In addition, they are responsible for assessing and documenting the efficacy of said sanitation, the results of which should be readily available during inspection procedures.

III. GENERAL DECONTAMINATION POLICY AND PROCEDURES

1. All portable and fixed equipment as well as surfaces that come in contact with animals must be sanitized prior to and after each episode of use as well as between procedures performed on animals from different cohorts (e.g., animals from different cages, from different rooms, from different rack sides, or animals of different health status but tested on the same day). The BRF staff can be contacted to arrange to sanitize any equipment that is heat stable.

2. Appropriate disinfectants include:

- Quaternary Ammonium Compounds (i.e., Cavicide, Quatricide)
- Peroxygen Compounds (i.e., Virkon)
- Chlorine Compounds (i.e., bleach, MB-10, Clidox)
- Alcohols are acceptable for some applications (i.e., isopropyl alcohol, Sani Cloth wipes). However, it is not effective against non-enveloped viruses or against bacterial spores. The surface should be saturated and allowed to air dry.
- Cleaning agents designed to mask animal odors should not be used, as they do not substitute for good sanitation practices. In addition, they may expose animals to volatile compounds that can alter basic physiologic and metabolic processes.

3. Safety Issues:

- Appropriate PPE must be worn when handling cleaning agents.
- Read the Safety Data Sheet and all literature that comes with the product, and follow all instructions (i.e., contact time required, safety precautions, etc).
- Ensure that any secondary containers are appropriately labeled.
- Cleaning Utensils used should be constructed of materials that resist corrosion, be maintained in good condition, routinely cleaned, and stored neatly to minimize contamination and promote drying.

4. Additional Comments:

- No disinfectant works effectively on soiled surfaces. Always clean the equipment of gross debris, excreta, etc prior to using a disinfectant.
- Always read and follow manufacturer's label directions for use and storage of any disinfectant product.
- Do not use expired disinfectants.
- Do not store disinfectants in secondary containers whenever possible; if secondary containers must be used, ensure that the container is appropriately and completely labeled.
- Behavioral equipment can be sent through the BRF cage washer for disinfection; however, equipment must be able to withstand high temperatures and water. Contact the BRF team to arrange the disinfection of your equipment.
- Studies have shown that animals (mice) do not react adversely to the scent of disinfectants commonly used on behavioral equipment. (*Effects of Various Cleaning Agents on the Performance of Mice in Behavioral Assays of Anxiety* .JD Hershey, JJ Gifford, et.al. J Am Assoc Lab Anim Sci. 2018 Jul; 57(4): 335–339.

5. Special Circumstances:

Water tanks used in behavioral testing (e.g., Morris water maze) should be drained, cleaned, and sanitized between studies of animal cohorts (e.g., animals from different cages, from different rooms, from different rack sides, or of different health status) at the discretion of the PI, but must be cleaned and sanitized at an interval that ensures an acceptable level of sanitation.

IV. CONFIRMATION OF SANITIZATION

According to the Guide for the Care and Use of Laboratory Animals, 8th edition: "Whether the sanitation process is automated or manual, regular evaluation of sanitation effectiveness is recommended" (Pg. 73). Equipment sanitized BRFs cage washer is routinely assessed for the

effectiveness of the sanitation process. Otherwise, a method for verifying the sanitation program must be implemented and documented.

Examples of ways to assess the efficacy of sanitation include:

- Plating and Counting: The classic methodology utilizes RODAC (Replicate Organism Detection and Count) plating.
- ATP Bioluminescence Technology: Bioluminescence technology uses adenosine triphosphate (ATP), the "energy source" for living cells.
- The efficacy of sanitation should be confirmed at least quarterly.
- IACUC recommends use of an ATP Luminometer ((SystemSURE Plus™ (Hygiena) or similar system), which provides immediate results in an easy-to-use process. In addition, a log should be maintained to indicate:
  a. Room number or area being cleaned/sanitized
  b. Item/equipment cleaned /sanitized
  c. Date cleaning/sanitation took place
  d. Initials of the individual responsible for performing the cleaning/sanitation
  e. Results of ATP test and any retesting results

V. ATP TESTING

Efficacy of sanitization is verified by documenting that the level of contamination has been minimized. This is done via the use of an ATP luminometer (e.g., EnSure™ or SystemSURE ™ (Hygiena) https://www.hygiena.com/other-products/systemsure-plus-other.html) and testing swabs (UltraSnap™ https://www.hygiena.com/other-products/ultrasnap-other.html).

This system measures adenosine triphosphate (ATP), the universal energy molecule found in all animal, plant, bacteria, yeast and mold cells. When ATP is brought into contact with the luciferase/luciferin reagent in the sampling device, light is emitted in direct proportion to the amount of ATP present. The system therefore measures the amount of light generated and provides information on the level of contamination in seconds. The higher the reading, the more contamination present. Follow the manufacturer's guidelines on using a luminometer; in general, the procedure includes:

- The sampling device is removed from its protective packaging, opened and swabbed across the surface of the equipment to be tested.
- Activate the sampling device.
- Insert the sampling device into the SystemSURE Plus™
- Record the results on the log sheets

Hygiena® luminometers come pre-programmed with a lower limit of 10 RLUs and an upper limit of 30 RLUs. The Pass, Caution, and Fail ranges are as follows:

PASS	0-10 RLU
CAUTION	11-30
FAIL	31+

If any results are outside this recommended acceptable range, sanitization procedures should be repeated until a passing result is obtained. If tests have to be repeated multiple times without a pass grade, equipment should be set aside, labeled as "do not use" and the veterinary staff should be consulted. Results should be reviewed by the PI on a regular basis.

SAMPLE LOG SHEET: SANITIZATION OF BEHAVIORAL OR LAB EQUIPMENT

DATE							
ROOM #							
ITEM							
AGENT USED							
ATP RESULTS							
INITIALS							
SUPERVISOR/PI REVIEW DATE							