**Guidelines for Sanitization of Hand Washed Items**
The University of Texas at Austin
Institutional Animal Care and Use Committee

*These guidelines have been written to assist faculty, staff, and students in performing vertebrate animal procedures in a humane manner and complying with pertinent regulatory requirements*

This document provides information to be used when hand washing items that come in contact with animals at The University of Texas at Austin. It is organized into six sections:

Section A – Background
Section B – Definitions
Section C – Requirements
Section D – Sanitization of hand washed items
Section E – Validation of sanitization
Section F – References and Acknowledgements

## Section A- Background

Equipment that comes in direct contact with animals must be sanitized at appropriate intervals to prevent the spread of microbial agents that may cause clinical or subclinical disease and to minimize animal odors or residues that may influence research outcomes (e.g. behavioral testing). When possible, the use of mechanical washers is recommended for enclosures, chambers, and other moveable equipment. If equipment cannot be processed through cage washing equipment (for example, due to conformation, size, material, or electronic components), then the equipment must be sanitized by hand.

The recommendations in this guideline are sufficient to sanitize items and surfaces that come into contact with intact skin. The recommendations are not intended for non-intact skin, sterile tissue, or aseptic procedures. When working with infectious agents, researchers must use the disinfectants approved in their IBC protocol.

If an investigator wishes to deviate from these guidelines, a detailed SOP must be attached to the protocol and approved by the IACUC. The SOP should at a minimum include the agent and concentration to be used, the frequency of use, the surface contact time, if the agent needs to be rinsed or wiped away, the period of expiration after the solution has been prepared, the PPE necessary to safely use the disinfectant, and the sanitization procedures to be followed.

## Section B - Definitions

**Cleaning:** Removal of visible dirt and debris.

**Sanitization:** Reduction of the number of bacteria to a safe level.

**Disinfection:** Use of chemicals to kill bacteria and viruses.

## Section C - Requirements

All equipment used in *in vivo* experimental activities that comes in direct contact with the animals must be sanitized at appropriate intervals. Equipment includes but is not limited to: behavioral testing equipment (such as mazes, swim tanks, and fluid delivery systems), transport boxes, anesthesia induction chambers, and specialized housing systems.

All equipment is subject to visual assessment during IACUC semiannual inspections and must minimally meet the following standards at all times: free of dander, excrement, bedding, and disagreeable odors.

## Section D - Sanitization of Hand Washed Items

**General information:**

- Cleaning agents designed to mask animal odors should not be used, as they do not substitute for good sanitization practices. In addition, they may expose animals to volatile compounds that may alter physiologic and metabolic processes.
- If chemical solutions are prepared in a separate container from the original packaging, the secondary container must be labeled with the following information:
    - Name of chemical
    - Concentration
    - Date of expiration
        - If the product has no expiration date, the container must instead be labeled with the date mixed or decanted.
        - Labels should clearly state whether the date is an "expiration", "mixed", or "filled" date.
- Ensure all cleaning supplies (e.g. scrub pads, brushes) are in good working order. If not, replace them with new items.

**Steps for cleaning:**

- If possible, place an absorbent material (e.g., paper towel, rodent bedding) in the bottom of the equipment to minimize soiling of the equipment when animals are present.
- Between animals in a single day:
    1. Change the absorbent material.
    2. Remove grossly visible debris, such as urine and feces. Use soap and water as necessary so that the item is visibly clean (see below).
- In between experimental cohorts or at the end of the day:
    1. Remove grossly visible debris, such as urine and feces.
    2. Sanitize the equipment with soap and water.
    3. Disinfect the equipment. See the chart below for suggested disinfectants.
- For non-standard housing requiring hand sanitization, the maximum interval for complete sanitization is 2 weeks.

**Soapy water used for sanitization:**

- Appropriate detergents for soapy water cleaning include laboratory detergents (e.g. Alconox®, Liquinox®) and unscented dish detergent.
- Ensure all surfaces come into contact with the soap mixture.
- Steps for cleaning with soapy water:

1. Dampen your sponge, brush, towel, or equivalent with hot water.
2. To premix soapy water, mix 5 mL of liquid detergent or 5 grams of powdered detergent per liter of warm water.
3. Soak your sponge in premixed soapy water solution or apply a few drops of concentrated detergent directly to the sponge or cleaning implement.
4. Disperse soap throughout the sponge to create soapsuds.
5. Scrub the equipment surface until no visible debris remains, generally for 10 seconds or more.
6. Rinse off the soap with clean water.
7. Wipe the surface dry with clean paper towels or allow it to air dry.

- If items cannot be submersed in water, soapy water can be applied by sponge or via a spray bottle of soapy water, followed by a spray bottle of clean rinse water.

**Disinfection:**

- Disinfection is most effective when preceded by effective manual cleaning, such as washing with soapy water.
- Recommended contact times must be adhered to in order to achieve disinfection. If the surface dries before the contact time is complete, the surface must be rewetted with the disinfectant.
- Surfaces must be thoroughly saturated with the disinfectant solution.
- Following the disinfection contact time, wipe away any remaining disinfectant with clean paper towels or allow it to air dry.
- Recommended disinfectants:

| Disinfectant | Concentration | Contact Time | Expiration |
|---|---|---|---|
| Peroxigard™ (hydrogen peroxide) | Prepared by the manufacturer | 1 minute | See commercial bottle |
| Ethanol[a, b] | 70% | 10 minutes | No expiration date |
| Chlorhexidine diacetate | 1 ounce of 2% stock solution per gallon of water (or 8 mL stock solution per liter of water) | 10 minutes | 14 days |
| Bleach (sodium hypochlorite)[c] | 10% of stock solution | 10 minutes | 30 days[d] |

Footnotes:

a – Ethanol is not recommended for use on items made of plexiglass or acrylic as it can cause cloudiness or a shattered appearance.

b – Under most conditions, ethanol evaporates before the 10 minute contact time has elapsed. Ethanol must be reapplied as necessary to keep the surface wet for 10 minutes.

c – Following the contact time, bleach should be rinsed off with clean water.

d – If dilute bleach is exposed to sunlight, it should be freshly mixed daily.

**Maintenance of cleaning equipment:**

- Cleaning utensils (e.g. sponges, brushes) must be:
  - Assigned to and kept in a specific area
  - Maintained in good condition
  - Constructed of materials that resist corrosion
  - Routinely cleaned

    - Stored neatly to minimize contamination and promote drying
- To clean sponges:
    1. Soak the sponge with detergent
    2. Rinse the sponge with copious amounts of water
    3. Squeeze out the excess moisture
    4. Air dry

## Section E – Validation 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" (p.73).
- Sanitation validation is first performed using ATP (adenosine triphosphate) bioluminescence swab testing.
- Researchers are responsible for initial ATP testing by picking up a swab kit with instructions from the ARC, collecting the samples in accordance with the ARC instructions, then returning the swab(s) to the ARC. ARC personnel will process the swab(s) and report results to the lab. The ARC will maintain logs of ATP test results.
- One of each type of different equipment to which animals are exposed at any given time is tested at the frequency described below. Equipment should be swabbed, cleaned per the SOP, and then swabbed again with a different swab.
- A 90% reduction in relative light units is considered passing.
- For items with unacceptable levels of ATP the first time:
    - ARC personnel will meet with the lab to review the sanitation products and methods.
    - ARC personnel will retest the equipment.
- For items with unacceptable levels of ATP the second time:
    - An ARC veterinarian will meet with the lab to review test results and processes.
    - Microbiological contact testing may be performed as directed by the veterinarian.
- Re-validation must be performed annually, or sooner if any of the following factors occur: change in the sanitation process, different disinfectant used, new experimental equipment is obtained, or at the request of the IACUC.
- A copy of the report will be sent to the laboratory and should be kept and available for inspectors for at least 3 years.

## Section F – References and Acknowledgements


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| Approval Date | Major Change(s) Approved |
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