

INSTITUTIONAL ANIMAL CARE AND USE POLICY***Sanitization of Research Equipment***

Previous Review Date(s): 5/10/2021, 3/7/2022

Approval Date: 12/2/2024

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Purpose

This document outlines the procedures and responsibilities to assure proper sanitization of restraint, enrichment and other equipment used in animal studies that cannot be processed through a mechanical cage wash. This equipment is used and sanitized by investigators in non-centralized areas, laboratories, satellite spaces and does not include equipment maintained by the Division of Comparative Medicine (DCM).

Policy

Quality assurance of the proper sanitization of research equipment exposed to animals or carcasses including but not limited to restraint, enrichment and other devices must be quantitatively assessed on an annual basis.

Background

Cleaning and disinfection are necessary to prevent cross-transmission or exposure to microorganisms, excrement, biological fluids, and pheromones from one research subject to another and to remove these substances as well as allergens from work environments shared with humans. When accompanied by mechanical wiping or scrubbing to remove organics and other soilage, effective disinfection by definition eliminates vegetative microorganisms from inanimate objects. Sanitization of cages and equipment by hand with hot water and detergents or disinfectants can also be effective but requires considerable attention to detail. Whether the sanitization process is automated or manual, regular evaluation of sanitization effectiveness is recommended.

Responsibilities:

Principal Investigators are responsible for disinfection of all equipment and work surfaces in their laboratory and laboratory equipment in the vivarium that may have come in contact with animals prior to and after use with each animal. Principal Investigators are required to keep a log sheet documenting dates of equipment cleaning for all species. At least every three years the IACUC is required to monitor the effectiveness of sanitization of experimental equipment that is currently in use. In addition, IACUC will review (during routine lab inspections) lab logs to ensure that sanitization is regularly practiced. Principal Investigators are required to sanitize any equipment before it goes into storage and once it is removed from storage, prior to use.

General Sanitization Procedures

All portable and fixed equipment as well as surfaces that come in contact with animals must be cleaned and disinfected prior to and after each episode of use by the personnel

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using the equipment and/or procedural areas (e.g., rodent surgery, euthanasia chambers, behavioral testing apparatus, anesthetic chamber, animal restraint device, stereotactic surgical frame, countertop and work surface). Disinfection is made most effective when preceded by effective mechanical cleaning, where applicable. Any direct or indirect contact bedding used in chambers must be removed prior to sanitization and replaced with fresh, clean substrate following testing. Direct contact bedding changes and sanitization procedures are required between test subjects where a chamber may be used sequentially.

Options for Sanitization

1. Washing in a mechanical washer or, if not possible.
2. Hand-washing, generally exclusive for housing enclosures not conducive to mechanical washing.
3. Hand sanitizing, which is most appropriate for fixed surfaces, stationary equipment and delicate, heat- or moisture-sensitive apparatus.

Recommended Disinfectants – Follow manufacturer recommended contact times for each agent:

Agent	Examples	Comments
Alcohols	70-90% ethyl alcohol 85% isopropyl alcohol Hand gel sanitizers	Remove gross contamination before using. Inexpensive. May damage rubber and plastic items. Alcohols are only acceptable if no other agents can be used on a specific piece of equipment.
Quaternary Ammonium	Roccal®, Quatricide®, CONFLIKT	Safe, generally effective general environmental disinfectant. Rapidly inactivated by organic matter and other environmental materials. Compounds may support growth of Gram-negative bacteria. Quatricide 1:5 dilution



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Chlorine	Sodium hypochlorite (Clorox® 10% solution fresh-made), Chlorine dioxide (Clidox®, Alcide®, MB-10®), 15% calcium hypochlorite	Highly effective. Corrosive and oxidizing. Presence of organic matter reduces activity. Unstable – solutions must be fresh. Clidox 1:18 dilution, minimum contact time: 3 minutes at 20°C on pre-cleaned surfaces. MB-10 Prepare according to manufacturer recommendations, minimum contact time: 10 minutes. Strong Bleach Solution: ¼ cup of bleach to 1 gallon of cool water or 1 tablespoon of bleach to 1 quart of cool water (add the bleach to the water in either case). Weak Bleach Solution: 1 tablespoon bleach + 1 gallon of cool water
Phenolics	Lysol®, TBQ®, Vesphene™	Broad spectrum activity. Less affected by organic material than other disinfectants. Some may be corrosive, harsh, toxic with a pungent odor. Skin irritant.
Chlorhexidine salts	Nolvasan®, Hibiclens®	Presence of blood does not interfere with activity. Rapidly bactericidal and persistent, but bacterial spectrum is narrow. Effective against many viruses.
Hydrogen peroxide, per-oxygenated compounds	3% hydrogen peroxide, Virkon-S®	Broad spectrum of activity. Environmentally safe. Powder is corrosive. 7-day stability. Contact time is 10 minutes.

Peracetic acid	Minncare® (1% peracetic acid)	Broad spectrum activity. Corrosive in pure form. Biodegradable. Often requires special equipment and/or expertise. Most useful for medical and surgical devices. Strong, pungent odor. Respiratory irritant, and can only be used in DUCTED cabinets.
Hydrogen peroxide, acetic acid	Spor Klenz	

Cleaning Recommendations

Restrainers and Enrichment Devices:

1. Wash and rinse in a mechanical cage washer, or
2. Hand wash

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- Wash used restrainer or device to remove all soil
- Soak or spray equipment with a suitable sanitizer (see list above)
- Soak or let sit (for sprayed items) for at least the minimum contact time for the product used.
- Rinse with clean water, allow to dry.

Test Chambers

1. Remove all loose bedding, feces and other materials from chamber.
2. Wipe down or spray all surfaces with one of the recommended cleaning agents and allow to sit for the minimum contact time.
3. Wipe down surfaces with clean water

Stereotaxic and other Surgical, Technical, or Experimental Equipment

1. Wipe all surfaces with appropriate disinfectant.
2. Surgical instruments must be sterilized before use and maintained using aseptic technique (see Rodent Survival Surgery, Large Animal Surgery)

Sanitization Contraindications

It is not recommended that chlorine dioxide-based disinfectants/sterilant be used on stainless steel equipment and surfaces unless it is cleaned off thoroughly with water. If residual odors from the cleaning chemicals might affect study animals, or if chemicals may damage equipment, a special request for exemption from sanitizing equipment must be obtained from the Institutional Animal Care and Use Committee (IACUC) as part of an approved animal use protocol.

Quaternary ammonium compounds should be used with caution around breeding animals, as studies have demonstrated decreased fertility in rodents exposed to these chemicals.

Verification of Sanitization of Research Equipment and Non-centralized areas

Research equipment located in non-centralized areas, laboratories, and procedure spaces (caging, behavior, imaging, stereotaxic) are typically hand-washed; therefore, a system is needed to ensure proper sanitization of these items. All equipment (caging, behavior, imaging, stereo tax, etc.) coming in contact with animals must be sanitized with each use. It is essential to validate that the process used for disinfection is effective. This may be done in several ways including via ATP bioluminescence technology which assays for ATP (the "energy source" of living cells). It is advisable to consult with Division

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of Comparative Medicine (DCM) Veterinary Staff and Environmental Health and Safety (EH &S) with respect to methods for validation of disinfection efficacy. All research equipment must be tested to ensure adequate sanitization annually. Records of testing must be maintained by the Principal Investigator (PI) and DCM and available for during the IACUC semiannual inspections and/or upon request.

How Does Testing Work

- DCM will use a handheld swab testing device that detects Adenosine Triphosphate (ATP) the source of energy for use and storage at the cellular level.
- DCM Veterinary Service Staff, PI, or lab's designee will swab an area approximately 4"x4" on the piece of equipment.
- The swab is to be delivered to DCM and tested within 4 hours. Contact DCM [here](#)
- The fluid inside the swab will combine with any ATP left behind on the piece of equipment during swabbing and will cause a bioluminescent reaction
- The handheld device will measure the light emitted in Relative Light Units (RLUs)
- Results will be provided to PI and/or their designee. Records of disinfection and validation must be maintained and should detail the date and method of testing, and the results. These records must be available to the IACUC as requested.

REFERENCES

1. [Adapted from Emory University IACUC Policy, Guidelines and Standard Operating Procedures](#), (accessed 12/10/2020).
2. [Adapted from Purdue University IACUC Policy, ATP Testing of Hand-washed Equipment](#) (Accessed 11/26/2024)
3. Adapted from University of Kentucky IACUC Policy 137, Policy on Validation of Disinfection of Research Equipment Used on Animals and guidelines : Annual Validation of Equipment Cleaning (Accessed 11/26/2024) ,
4. [Guide for the Care and Use of Laboratory Animals, Environment, Housing, and Management, pp72-73](#)