



Sanitation of Research Equipment Used with Animals

313.1 Purpose: This policy describes the requirement and procedures to clean and sanitize research equipment that is used with animals and to prevent the spread of microbial agents that may cause sub-clinical and clinical diseases that could jeopardize the validity and reproducibility of research data, or complicate its interpretation. The “Guide for the Care and Use of Laboratory Animals”, 8th edition recommends that “Sanitation of cages and equipment by hand with hot water and detergents or disinfectants can also be effective but requires considerable attention to detail” (p.71) and also stipulates: “Whether the sanitation process is automated or manual, regular evaluation of sanitation effectiveness is recommended” (p. 73).

The goal of this policy is to ensure that animal activity areas are cleaned and disinfected prior to and after use. 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.

313.2 Scope: This procedure applies to all faculty, students, and technicians who work with live animals in their labs or testing rooms as well as those maintaining equipment or devices in the animal research facility. It also applies to common use durable equipment provided by SOM/Yerkes DAR in support of animal research use (e.g., euthanasia chambers).

Note: The recommendations provided herein are sufficient to disinfect items and surfaces that come into contact with intact skin, but will not provide a degree of effect sufficient to protect mucous membranes, non-intact skin, sterile tissue, or the vascular system and are not acceptable for aseptic procedures. If sterilization is the goal through the use of chemical agents, then contact times of 12-24 hours, depending upon the agent, are necessary and are not the subject of this policy.

313.3 Responsibility

313.3.1 It is the responsibility of the individual(s) using a common procedural area or core facility to disinfect all equipment and work surfaces that may come in contact with animals prior to and after each use as described below.

313.3.2 It is the responsibility of the individual(s) using a procedural area or equipment in their own lab to disinfect all equipment and work surfaces that may come in contact with animals prior to and after each use as described below.

313.4 General Sanitization Procedures

313.4.1 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 using the equipment and/or procedural areas (e.g., ultrasound, PET/CT, bioluminescence, MRI, gamma irradiator, X-ray, behavioral testing apparatus, anesthetic chamber, irradiation pie cage, animal restraint device, stereotactic surgical frame, countertop and work surface, etc.).

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 sanitation and replaced with fresh, clean substrate following testing, unless the equipment will not be in use. Direct contact bedding changes and sanitation procedures are required between test subjects where a chamber may be used sequentially.

313.4.2 Options for Sanitization

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

313.4.3 Agents

The agents and procedures described here are not sterilizing and are not substitutes for sterilization methods. While most Environmental Protection Agency (EPA)-registered disinfectants have a 10-minute label claim, multiple investigations have demonstrated the effectiveness of these disinfectants against vegetative bacteria, yeasts, mycobacteria, and some viruses at exposure times of 30–60 seconds (Rutala, et al, 2008).

If high level disinfection is the goal (i.e., complete elimination of all microorganisms in or on an instrument, except for small numbers of bacterial spores), then 12 minute contact times by soaking or continuous wetting are necessary for previously cleaned items (Rutala, et al, 2008).

Where sanitation methods are accomplished within the confines of the animal research facilities, the chemical agent and technique approved and provided by the animal resources program are to be used, unless an exception is approved in the applicable protocol(s). In general, the chemical agents used to sanitize equipment kept in the animal facilities are those approved and provided by the animal resources program. Greater latitude in selection and use of agents may be accorded where research or sanitation needs justify a departure and in laboratory environments where there may be agents more compatible with those situations. Other procedures normally deployed by the animal resources program, such as vacuuming where applicable, may also be necessary.

Where hand sanitizing methods are used outside of the animal research facilities or hand washing is to be done, the SOP detailing the process must be attached appropriately to the protocol for IACUC review. The minimum information to be provided in the SOP is the sanitizing agent, its concentration, contact time to achieve sanitization, periodicity of fresh preparation (based upon shelf life or expiration date), whether or not it must be rinsed or wiped from surfaces, minimum PPE to be worn, instructions on when sanitation procedures must be done, and cleaning supplies needed (e.g., sponges, paper towels, scrub brushes, etc). The SOP, once approved by the IACUC, must be kept in close proximity to the equipment so that it can serve as a proper reference.

The chemical disinfectants with potential applicability, in order of appropriateness of use, are hydrogen peroxide and derivatives, quaternary ammonium compounds, alcohols, chlorine, and phenolics. Glutaraldehyde, formaldehyde, ortho-phthalaldehyde, and peracetic acid may have utility in specific circumstances.

Attachment 1 provides a list of suitable agents for hard surface disinfection. Attachment 2 describes an IACUC-validated protocol for the use of Virkon-S to sanitize materials in lieu of washing them. Attachment 3 describes an optional procedure for treadmill sanitization that some may find useful.

313.5 Special Procedures for Nonhuman Primate-associated Equipment

313.5.1 Joysticks, computer carts, and other equipment that cannot go through the cage washer:

- Sanitize with a Yerkes Facility-approved disinfectant after each nonhuman primate testing session.
- If feces/urine is present, equipment is scrubbed with soap and water before applying disinfectant.
- Touch-screen computer monitors are cleaned with glass cleaner solution and dried after each testing session.

313.5.2. Miscellaneous test objects or toys used during test sessions:

- Test objects are placed in a cage or transfer box and sanitized in the cage washer as described in Yerkes Facility SOP 4.11 Cagewashing.
- Alternatively, these objects can be soaked in a Center-approved disinfectant solution per product instructions and rinsed thoroughly.
- Jackets and other cloth objects may be sanitized in the washing machine using hot water, detergent and bleach.

313.5.3. Testing cages and transfer boxes:

- A Yerkes Facility-approved disinfectant is used to sanitize between animals if no feces/urine is present.
- If feces or urine is present, equipment is scrubbed with soap and water before applying disinfectant.
- Nonhuman primate testing cages and transfer boxes are the responsibility of the person using the equipment, such as research staff.

313.5.4. Primate restraint chairs:

- Wash with soap and water and wipe clean or sprayed with a Center-approved disinfectant and wipe clean every time an animal is removed from the chair.
- Mobile restraint chairs that can go through the cage-washer are cage-washed at least every two weeks when in regular use.

313.6 Decontamination Validation/Quality Control

An important component of any sanitation program is quality assurance testing to ensure the effectiveness of sanitation. The laboratory is required to participate in the QA program for laboratory-based devices per the standard operating procedures of either the Emory DAR or Yerkes, whichever applies.

313.7 References

Rutala WA, Weber DJ, et al. Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008. Accessed via the www at: http://www.cdc.gov/hicpac/pdf/guidelines/disinfection_nov_2008.pdf.

313.8 Attachments

Attachment 1: Recommended Hard Surface Disinfectants
Attachment 2: Manual Washing Using Virkon-S or 70% Ethanol
Attachment 3: An Optional Method for Cleaning of Treadmills

313.8 Document Properties

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Attachment 1: Recommended Hard Surface Disinfectants (e.g., table tops, equipment)

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.
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.
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
Aldehydes	Glutaraldehydes (Cidex®, Cetylcide®, Cide Wipes®), formaldehyde, ortho-phthalaldehyde	Rapidly disinfects surfaces. Sporicidal. Fungicidal. Harsh. Toxic. Irritating. Asthma and carcinogen risk. Not generally recommended except for surgical instruments and medical devices.
Phenolics	Lysol®, TBQ® Vesphene™	Broad spectrum activity. Less affected by organic material than other disinfectants. 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/peroxygenated 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	Minnicare® (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.
Hydrogen peroxide/peracetic acid/acetic acid	Spor Klenz	

Attachment 2: Manual Washing Using Virkon-S or 70% Ethanol:

- 1) Manually scrub the equipment using warm to hot tap water (+/- dish soap) and a scouring sponge or equivalent.
- 2) Spray Virkon-S or 70% ethanol on the enclosure surfaces and leave for 30-60 seconds (2).
- 3) Rinse afterwards with copious amounts of tap water.
- 4) Air dry or dry with paper towels.

The sponge should be specifically dedicated to washing only one piece of equipment to avoid cross contamination. Once washing is complete, rinse the sponge, soak it with Virkon-S spray or 70% alcohol, rinse the sponge with copious amounts of water after 1-10 minutes of the Virkon-S or 70% ethanol soak, squeeze out the excess moisture, and air dry. Virkon-S must be used fresh as once prepared it only has a 7-day shelf-life (3).

1. Guidelines for disinfection and sterilization in healthcare facilities, 2008 (CDC), p. 55. Available on the www at: http://www.cdc.gov/hicpac/pdf/guidelines/Disinfection_Nov_2008.pdf
2. IBID, p. 31.
3. Pharmacal Research Laboratories, Inc. Disinfectants: Virkon-S Powder & Virkon-S Tablets. Information available on the www at: <http://www.pharmacal.com/Virkon.htm>.

This manual hand-washing procedure can be used for small, manageable devices such as metabolism cages, small euthanasia chambers, anesthesia induction chambers, cages used for telemetry and behavioral purposes, restraint devices, irradiator pie plates, swim test cylinders.

Fixed or too large to handle mazes, water mazes, treadmills and the like should be cleaned using hand sanitization involving a "thorough" wipe down with "approved" disinfectant. Likewise, surfaces, platforms and stages associated with imaging devices, stereotactic surgery apparatus, microscopes, and irradiators not amenable to washing should be hand sanitized.

Attachment 3: An Optional Method for Cleaning of Treadmills

- 1) Turn off the treadmill and reset the shock settings at the end of the run.
- 2) Remove all animals from the treadmills and return to their home cages. If the lids for the treadmill are dirty, spray them with Virkon-S and wipe them down with paper towels.
- 3) Remove the lane dividers and clean by spraying with Virkon-S from the spray bottle and wipe dry with paper towels. These can be set aside on the shelf under the table.
- 4) Vacuum the treadmill belts using a HEPA-filtered vacuum with the edge cleaning attachment to remove any loose debris. This is particularly important for inactive treadmills.
- 5) Turn the treadmills back on at 10 m/min.
- 6) Using a carpet cleaning machine accessorized for spraying and suction and with Virkon-S in proper dilution as the liquid, spray the treadmill belts with 2 coats of Virkon S.
- 7) Scrub the treadmill belts with a scrub brush while the treadmills are running.
- 8) Vacuum the treadmills with the carpet cleaning machine while the treadmills are running.
- 9) Replace the Virkon-S solution in the reservoir with fresh water.
- 10) Spray the treadmill belts with 2 coats of water using the spraying device.
- 11) Vacuum the water off the treadmills with the carpet cleaning machine.
- 12) Wipe the treadmills down with paper towel to remove any overspray.
- 13) Tip the treadmills back to stand upright on their backs and transfer excrement collection trays to a cart.
- 14) Spray the counters with Virkon-S and wipe down with paper towel.
- 15) Vacuum the floor of the treadmill room using the HEPA-filtered vacuum with the floor tool attachment.
- 16) Check the floor for any remaining debris. If there is any debris remaining, wash the floor with a mop and bucket from the clean area and return the dirty mop and bucket to the cage wash (this should be done weekly after cage change outs).
- 17) Tie the garbage bag in the room and place in the waste receptacle in the hall. Put a new bag in the trash can in the treadmill room.
- 18) Rinse the excrement collection trays and waste collection bottle from the carpet cleaning machine and air or towel dry the trays.
- 19) Return the excrement collection trays and waste collection bottle to the treadmill room once they are dry.