Guidelines for Sterilization of Equipment and Supplies for Sterile Procedures and Surgery

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. Under some circumstances deviations from these procedures may be indicated but such variances must be approved in advance by the IACUC.

Any invasive survival procedure performed on animals, including rodents, requires the use of surgical instruments, scalpels, needles, cannulas and other materials and supplies that have been fully sterilized. Sterilization is defined as the killing of all living microbial organisms including bacterial spores. This document provides information regarding the appropriate verification methods and shelf life (expiration) of sterile surgical instruments and materials. The Investigator is responsible for ensuring that each individual sterilizing materials for surgical procedures follows these guidelines. This guideline is applicable to all locations where vertebrate animals are maintained for biomedical research as part of University research and/or teaching projects.

Section A – Requirements

All instruments, supplies, or devices that will need to be handled by the surgeon or will have contact with the tissues of the animal must be sterilized before the surgery and must be handled and placed so that they remain uncontaminated until the surgery is completed. This includes all small-bore instruments, catheters, needles and items used in any surgery. Instruments that become contaminated by touching a non-prepped part of the animal, being dropped or placed outside of the sterile field, touched by a non-prepped assistant, etc. must be re-sterilized before they can be reused.

If lab made (pulled) pipettes are utilized for surgical procedures, sterility must be ensured. Due to the temperature and time components required for sterility, the heat used for pulling the glass is not sufficient for surgical sterilization. As such, after heat-pulling takes place, pipettes must be autoclaved or cold sterilized as described below. Alternatively (if those procedures risk damaging the pulled instrument), pipettes may be autoclaved in advanced and then pulled under heat as long as they are handled with sterile gloves and stored in a sterile container following the pulling procedure.

Section B – Methods of sterilization

Steam Autoclave – An effective cycle (pressure/temperature and time) must be utilized, for example 30 minutes at 121°C (250°F) to autoclave a pack in a typical gravity displacement sterilizer. Autoclave must be monitored and serviced to ensure proper function as described in Section E.

Dry Heat (Oven) Sterilization – A rarely used method that may be appropriate for some specialty items. Specific approval to use this method must be granted by the IACUC in the protocol. See Section F for more information.

Gas Sterilization (Ethylene oxide) – For use on materials damaged by heat and steam such as certain types of plastics and cannulas. Proper safety precautions must be used, and the manufacturer's directions must be followed.

Cold Chemical Sterilization – For use on materials damaged by heat and steam such as plastics and cannulas. See section G.

Hot Bead Sterilization – Can be used for multiple surgeries done in one session, however the instruments must first be sterilized completely by one of the methods listed above. Hot bead sterilization may then be used before the second, third, and fourth procedures after which the instrument set must again be re-sterilized. Ensure the tips of the instruments are cool before they come in contact with the animal.

Section C- Surgical pack preparation

- Packs to be autoclaved should be wrapped in porous, temperature safe materials that will allow steam penetration. Appropriate materials include paper, cloth, and peel pouches. Foil is only appropriate for dry heat sterilization and cannot be used when steam autoclaving.
- Each autoclaved pack used in survival surgical procedures must have an indicator(s) that confirm minimum temperatures were achieved during the cycle.
- For substantial packs that are double- or triple-wrapped, both external (autoclave tape) and internal (chemical integrator) indicators should be used to ensure steam penetration to the center items. Internal methods must be placed into the center of the pack to verify adequate steam penetration.
- Small paper/plastic sealed pouches used for packing single/few items (that aren't as difficult for steam penetration) require the use of external indicators on the pack.
- All indicators utilized must change in order for the pack to be used for survival surgical procedures.
- The date of sterilization and expiration date (see Section D) should be clearly marked on the outer wrapper.

Section D – Storage of Sterile Items

- Sterile packs prepared by research or technical staff by either autoclaving or ethylene oxide maintain sterility for one year when stored under dry conditions in a closed cabinet or drawer. Handling of stored packs must be minimized to avoid compromise.
- When not stored in a closed cabinet or drawer, the safe shelf life of staff-prepared sterile packs is reduced to 1 month.
- If sterilely packaged medical instruments or supplies (including needles, syringes, sutures, etc.) have an expiration date placed by the manufacturer, that shelf-life dating must be followed.
- In all cases packaging must remain dry and intact to maintain sterility. If packs have tears, perforations or gaps in the wrapping, are dropped on the floor, are grossly soiled with stains or dust, or show signs of having been wetted, they must be considered contaminated and not used for surgery without re-wrapping and re-sterilization.

Section E – Monitoring Autoclave Sterility

- Validation uses biological indicators to evaluate the functional effectiveness of an autoclave. This testing should be performed at least yearly and documented for any autoclave used to sterilize instruments for survival surgery.
- Failure of validation cycles indicates that autoclave settings need to be adjusted or the autoclave serviced. An autoclave that fails validation should not be used again for sterilization of surgical instruments until it has passed a biological indicator test.
- The campus-wide autoclave testing program is coordinated through EHS (https://ehs.utexas.edu/working-safely/waste-handling-autoclaves/autoclaving). This is primarily meant to validate autoclave function when used to decontaminate waste materials, but it does verify the basic operation of the unit. Supplemental testing beyond this may be indicated if the cycle settings for surgical instruments or supplies are significantly different than those use for waste decontamination.
- Anyone using an autoclave for the purpose of sterilization of equipment for survival surgery is responsible for ensuring that validation is occurring for the unit being utilized. If you have questions about whether this is being performed for units you are utilizing to sterilize surgical equipment, you can contact EHS for further information.
- This validation is more robust than the integrator strips, chemical indicators, and autoclave tape, as it monitors time, temperature, and steam penetration by confirming the killing of microbial spores of *Geobacillus stearothermophilus*, a thermophilic bacteria. This is the only method that actually verifies sterilization is occurring

Section F – Dry Heat Sterilization

- Dry heat sterilization is not as effective and efficient as wet heat (steam) sterilization and should be used only for materials that might be damaged by moist heat or that are impenetrable to moist heat. IACUC approval is required.
- The advantages for dry heat sterilization are that it is nontoxic, it penetrates materials, and it is noncorrosive for metal devices and sharp instruments. It may be acceptable for certain glass items that could be damaged by the autoclaving process.
- The disadvantages for dry heat are the slow rate of heat penetration and microbial killing that makes this a time-consuming method. It must also be done in a laboratory oven designed to accurately maintain temperatures over an extended cycle.
- Dry heat is not generally regarded as being suitable for plastics due to the low thermal transmission properties of plastics and the fact that most plastics will either warp or degrade during prolonged dry heat sterilization.
- Higher temperatures and longer times compared to steam sterilization are generally required. The most common time-temperature parameters for dry heat sterilization are:
  - -170°C (340°F) for 60 min
  - -160°C (320°F) for 120 min
  - -150°C (300°F) for 150 min.

Section G – Methods for Cold Sterilization

Investigators using heat sensitive, small-bore tubing (or other heat-sensitive products) during surgical procedures must have a sterilization method that is effective, is low risk for toxicity to animal tissue, and is not destructive to the items being sterilized. Cold sterilization as described in this section should be utilized. This guidance has been formulated to promote adequate sterilization of such equipment. However, if the product you are using is available for purchase pre-sterilized from a manufacturer, that should be the default option. These sterilization options should only be used when the product is not available in a sterile form.

1. If a sterile option mentioned above is not available for your item, the IACUC recommends the cold sterilization options listed below (see table).
2. Commonly used disinfectants such as alcohol, iodophors, quaternary ammonium and phenolic compounds are NOT effective sterilants and are not acceptable for use on items (e.g., catheters) intended to be sterile.
3. Cold sterilants must be labeled with the date mixed and the expiration date (as described below, or according to the manufacturer instructions).
4. Cold sterilization requires extended contact to be effective. Sterilization times vary between manufacturers. The following table lists the CDC recommended contact times for sterilization and should be followed.

Disinfectant	Hydrogen Peroxide	Peracetic Acid and Hydrogen Peroxide	Glutaraldehyde
Sterilization claim*	6 hours @ 20°C	3 hours @ 20°C	10 hours @ 20°C
Reuse Life	21 days	14 days	14 days
Effective Concentration	7.5%	7.35% / 0.23%	2%
Processing	manual	manual	Manual or automated
Organic material resistance	Yes	Yes	Yes
Product Names	Sporox II®	Spor-Klenz® Ygiene® Compliance Cold Sterile®	Cetylcide-G® Cidex Plus®

5. Follow manufacturers' instructions for dilution and use. Disposal should follow UT hazardous waste procedures.
6. Items placed into sterilization solutions must be free of organic material including blood. Cold sterilization chemicals are ineffective in penetrating organic material.
7. Items must be completely immersed in the solution.
8. Prior to use, items that have been cold-sterilized should be rinsed copiously (inside and outside) with sterile saline to remove residue, but they must be handled using aseptic techniques to remain sterile until use.

Section H – References

1. Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008 William A. Rutala, Ph.D., M.P.H, David J. Weber, M.D., M.P.H., and the Healthcare Infection Control Practices Advisory Committee (HICPAC), https://www.cdc.gov/hicpac/pdf/guidelines/Disinfection_Nov_2008.pdf
2. FDA-Cleared Sterilants and High-Level Disinfectants with General Claims for Processing Reusable Medical and Dental Devices, https://www.fda.gov/MedicalDevices/
3. https://freimann.nd.edu/assets/262663/fullsize/iacuc_cold_sterilization_pol17_1_.pdf
4. https://animal.research.uiowa.edu/oar-informational-sheet-accepted-sterilization-methods

Approval Date	Change(s) Approved
11-13-2023	• Adding clarification to the requirements section regarding small bore instruments.
	•