University of Cincinnati
Animal Care and Use Program

Guidance on Sterilization of Animal Research-Related Supplies

The purpose of these guidelines is to provide guidance on commonly used methods for sterilization of surgical instruments and other materials for use in IACUC-approved animal protocols along with methods for monitoring the sterilization procedure.

Definitions

Autoclave maintenance - service/upkeep work performed as recommended by manufacturer.

Autoclave validation - the process of verifying that an autoclave is functioning correctly and achieving the desired level of sterilization.

Sterilization Pouch - Contains chemically impregnated indicators on the external and internal surfaces of the pouch that undergo a visual color change when exposed to appropriate temperature. (For example, Chex-All® Propper™ Sterilization Pouches, available from Fisher Scientific.)

Steam sterilizer Indicator Tape (a.k.a autoclave tape) - Impregnated with a chemical that undergoes a visual color change when exposed to a high temperature. The tape is placed on the outside of the item to be sterilized and confirms temperature has been reached, but not that sterilization has occurred.

Chemical Indicator Strips - Contain a chemical that undergoes a visual color/physical change when all sterilization parameters (time, temperature, and penetration) have been met. They are used to ensure that the steam has penetrated to the inner layers of the pack.

Biological Indicator - Highest order of indicators that monitor time, temperature, and penetration by confirming the killing of microbial spores of *Geobacillus stearothermophilus*, a thermophilic bacterium. This is the only method that verifies sterilization is occurring.

Surgical Pack Preparation and Storage

Packs to be autoclaved should be wrapped in porous, temperature-safe materials that will allow sterilant penetration. Appropriate materials include paper, cloth, and peel pouches. Paper and cloth packs should be double wrapped to avoid contamination of instruments once opened.

Each autoclaved pack or peel pouch containing instruments for survival surgical procedures must use external (autoclave tape) and internal (chemical indicator) indicators within each pack.

Internal indicators must be placed into the center of the pack to verify adequate steam penetration. BOTH EXTERNAL AND INTERNAL INDICATORS MUST CHANGE in order for the pack to be used for survival surgical procedures.

All sterilized items should be marked on the outside of the pack with the date of sterilization. Ideally, sterile items are autoclaved as close to the day of use as possible (e.g. the day before surgery). Items sterilized in plastic peel-down pouches and wrapped packs are considered sterile for 6 months unless the packaging is broken, wet, soiled or otherwise damaged.

If any of the following events occur the pack should be repackaged and sterilized prior to use:

- Any tears or perforations in the wrapping, whether instruments are directly exposed or not.
- Wetting of the surgical pack.
- Dropping of packs onto the floor or excessive accumulation of dust on the outer surface.

Autoclave Validation

This testing must be performed and documented for any autoclave used to sterilize instruments for survival surgery.

All autoclaves (laboratory/departmental) used for the purpose of survival surgery equipment/instrument sterilization at UC must be validated at a minimum frequency of every month using biological indicators.

Failure of any of these validation cycles indicates that autoclave settings must be adjusted, or the autoclave serviced. An autoclave that fails validation cannot be used again for sterilization of surgical instruments until a biological indicator passes. The results of all tests, pass or fail, must be documented and available for review upon request and at semi-annual IACUC inspections.

Chemical Indicators

Chemical indicators for autoclaves are typically designed to visibly change color after exposure to normal autoclave operating temperature (121°C at 15psi). These indicators are only used as a quick reference to show that the autoclave cycle reached the temperature that the indicator was rated for.

1. **Autoclave tape**

   Tape indicators are adhesive-backed paper tape with heat sensitive, chemical indicator markings and change color or display diagonal stripes, the words “sterile” or “autoclaved” when exposed to temperatures at or above 121°C. Some autoclave bags have chemical indicator markings that appear when exposed to temperatures at or above 121°C.

   Tape indicators are not designed nor intended to prove that organisms have been killed.

   Indicator tape is typically used on the outside of all containers that are set to be decontaminated (autoclave bags, autoclave pan, etc.)

   - Confirm autoclave tape has appropriately changed to indicate adequate sterilization after sterilization cycle completion

2. **Chemical sterilization indicator strips**

   Sterilization indicator strips are crucial components of surgical packs, placed inside to ensure proper sterilization. They confirm that the sterilant (usually steam) has penetrated to the center of the pack, reaching all instruments. Chemical indicator strips are placed inside the surgical pack, typically in the center, to verify steam penetration to the core of the pack.

   - Place one chemical indicator strip in each pack or pouch
   - Once opened, packs should not be used if the chemical indicator has not changed appropriately.

Biological indicators

Autoclaves shall be validated to ensure sterilization utilizing biological indicator assays. Chemical indicators DO NOT validate sterilization.

Biological indicators are designed to show that an autoclave cycle was successful at eliminating microorganisms. These indicators utilize bacterial spores such as *Geobacillus stearothermophilus* which are highly resistant to heat and steam. *G. stearothermophilus* spores become inactivated when exposed to 121.1°C temperature for more than 20 minutes. This strain can indicate that a cycle reached temperature and the minimum length of time the temperature was held. A biological indicator should be placed in an autoclave cycle on a monthly basis. After the autoclave cycle is complete the biological indicators are required to be incubated according to the manufacturer and compared to a control indicator (which was not included in the cycle). If the test indicator shows signs of microorganism growth such as that found in the control indicator (cloudy media, colony formation) then the validation has failed and the autoclave should be put out of service until it can pass validation. If there is no growth in the control indicator then the validation fails and must be repeated until it can pass validation. If an autoclave fails validation it may require servicing or a change in settings.

Autoclave validation procedure

Responsibility: all individuals (employees, students, volunteers, visiting faculty, etc.) who operate, are responsible for, or own an autoclave or biological sterilizer. This procedure shall be used in conjunction with the autoclave manufacturer's manual and other required departmental/ clinical procedures.

Perform validation as stated below per manufacturer recommendations:

1. Monthly - Biological indicator validation shall be completed. For autoclaves used less frequently, validation should be performed at each use.
2. Every autoclave load – use autoclave tape and chemical sterilization strips to verify appropriate temp has been reached.

Autoclave Documentation

An Autoclave Log Sheet should indicate biological indicator manufacturer information, type of load tested, the validation test date, name and phone number of autoclave user conducting validation test. Records shall be maintained either in the room with the autoclave or signage should be posted indicating the location of records. Autoclaves not meeting the autoclave validation requirement should be labeled with a sign stating, "Not to be used for animal research-related supplies".

References

Guide for the Care and Use of Laboratory Animals: Eighth Edition. 2011: pp. 70-73

CDC Guidelines for Disinfecting Agents

CDC Sterilizing Practices