

	Title: Autoclave Use and Quality Assurance	SOP #	AV 60-103
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Standard Operating Procedure

1. Purpose

Autoclaves (steam sterilizers) are ineffective and potentially very hazardous if operated incorrectly. This Standard Operating Procedure (SOP) outlines the proper use and maintenance procedures for autoclaves.

2. Scope

All animal units using autoclaves for steam sterilization of media, instruments, lab ware, or decontamination of biological waste.


3. Responsibilities

Principal Investigators must ensure personnel are properly trained in use and maintenance of the autoclave prior to use.

4. Procedure

Effective steam sterilization depends on the interaction of temperature, pressure, and time, but additional conditions inside the autoclave chamber such as materials, containers, container placement, and total volume of the materials also influence sterilization success. Each of these factors must be controlled within a narrow range of values or condition:

- **Pressure/temperature relationship:** Pressurization to 15 psi typically “superheats” steam to about 121°C (250°F), which is adequate to kill all microorganisms and to decontaminate or sterilize in a reasonable time.
- **Volume:** “Dense” materials such as media in bottles to be treated in the autoclave should occupy no more than half of the autoclave chamber volume, so that steam can circulate completely around and into the load. Less dense materials such as bagged waste can occupy somewhat more space but should never contact the autoclave chamber wall.
- **Contact:** To sterilize or decontaminate uniformly, superheated steam must contact all areas of the load.
- **Time:** Other factors being equal, autoclave loads up to about 2.0 ft³ in volume require 30-60 minutes to sterilize at 15 psi and 121°C. Larger loads and tightly packed materials may require much more time. After loading and starting the autoclave, processing time

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starts after the autoclave reaches normal operating conditions of 121°C (250°F) and 15 psi pressure.

Primary Containers:


- **Autoclave waste bags** – Autoclave waste bags are used for bulk dry disposable materials such as paper towels, plastic centrifuge tubes, bundled serological pipettes, and plastic petri dishes (with or without agar-based culture media). Autoclave bags are made of translucent, colorless, steam resistant plastic film, usually with a pre-applied “sterilization indicator” and often printed with the universal biohazard symbol. If the waste being autoclaved is not contaminated with animal infectious agents or certain high hazard plant pathogenic agents, the biohazard symbol is inappropriate and should be defaced before use. **Autoclave bags should be loosely taped or tied during decontamination to allow steam to penetrate into the bag. Air pockets in tightly sealed bags may cause localized sterilization failure.**
- **Bottles** – Always loosen the screw top so that it barely engages the threads on the neck of the bottle.
- **Steam and heat-resistant pouches** – Commonly used to sterilize surgical instruments.

Secondary Containers:

- Autoclave bag contents may spill out or liquefy, block internal ports, and seriously damage the autoclave during the autoclave cycle, so it is critically important to place autoclave bags in secondary containers during decontamination cycles.
- Liquid media bottles must always be autoclaved in leakproof tubs.
- Polypropylene plastic or stainless steel tubs with 6-12 inch sides are commonly used to contain material in the autoclave.
 - Do not use polyethylene or any other type of plastic tub because many other plastics will melt or distort permanently in the autoclave.
 - Repeated autoclaving will eventually crack polypropylene – watch for this and replace these tubs at the first sign of “crazing” or other deterioration.
 - Stainless steel containers with leakproof seams are durable and good conductors of heat, and may be good alternatives to polypropylene tubs.

Indicators are used to validate the sterilization/decontamination process.

- **Chemical indicators** change color after being exposed to 121°C (250°F), but most provide no information on how long they remained at that temperature.
- **Tape indicators** can only be used to verify that the autoclave reached normal operating temperatures for decontamination. Like most chemical indicators, they provide no information on time at that temperature.

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- **Biological indicators** including spore strips or ampules are the “gold standard” – they verify that the autoclave is performing correctly and is capable of killing microorganisms. **A load test using *Geobacillus stearothermophilus* for steam cycles should be performed at least bimonthly, and more often for autoclaves that are routinely used to inactivate infectious agents.** Spore strips typically require incubation for up to seven days after the run, but many ampule tests only require 48 hours.
 - **Procedure:** Place the spore strip or ampule in the center of the load and retrieve it for completion of the test after the load is processed in the autoclave. If the load is a single bag, the strip should be tied or autoclave-taped to a length of cotton string or a wire and placed in the center of the bag contents with the string or wire extending out of the bag opening (some indicators are manufactured with an attached string). The string or wire is used to retrieve the spore strip after the run. When using biological indicators, be sure to follow all instructions meticulously for use and post-run treatment. Avoid using spore strips with disinfectant-soaked materials because contact with the disinfectant may kill the strip agents and yield a potentially false negative test.

Exhaust:

- Always use slow exhaust when autoclaving liquids. If you use fast exhaust, the media will boil out of the bottles as the steam exits the chamber. Fast exhaust may be used for bagged or other dry material. If slow exhaust or a specific cycle intended for liquids is not available on your autoclave, do not attempt to treat liquids in that unit.
- At the end of a cycle, verify that the chamber pressure is less than 1.0 psi before opening the door. Open a hinged autoclave door just 2-3 inches and allow the steam to escape from within the autoclave before opening the door all the way. Stand well back from the autoclave door. Always open the door as slowly as possible.
- Allow materials inside the autoclave to cool for at least 10 minutes with the door open before unloading the autoclave. Removing contents too soon may heat stress the component material.

Service:

- A professionally trained service provider should inspect the autoclave according to the autoclave manufacturer’s recommendations for inspection intervals and service. Most such recommendations are based on cumulative hours of use rather than specific calendar intervals.
- Autoclave gauges should be calibrated at least annually.

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- If an autoclave fails to function correctly or a user finds a problem between scheduled inspections, the unit must be professionally serviced. **Do not resume operation of an autoclave until it has been inspected and repaired.**

Recordkeeping:

- Records of maintenance, logs, calibration results and biological indicator load tests should be kept for at least three years.

Training:

- All individuals who use autoclaves must be trained. Autoclave users should understand the time, temperature, and pressure requirements and should also understand the correct loading procedure, the appropriate type of exhaust for the materials, and the correct method for opening the autoclave after a run. Users and facility managers must also be able to verify that the autoclave is functioning correctly, by using indicators or by monitoring the run parameters.

5. References

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5. The Federation of Animal Science Societies: Guide for the Care and Use of Agricultural Animals in Research and Teaching, 3rd Edition, 2010
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6. Definitions

1. AV – Attending Veterinarian – responsible for the health and well-being of all laboratory animals used at the institution



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2. AVMA – American Veterinary Medical Association – a not-for-profit organization for advancing the science and practice of veterinary medicine to improve animal and human health
3. SOP – Standard Operating Procedure – established or prescribed methods to be followed routinely for the performance of designated operations or in designated situations
4. IACUC – Institutional Animal Care and Use Committee – responsible for assessment and oversight of the institution’s animal care program components and facilities
5. Chair – Chairperson of the IACUC
6. PI – Principal Investigator – a Central Oregon Community College employee having the background and training in scientific and administrative oversight necessary to conduct and manage the proposed study
7. AUP – Animal Care and Use Protocol – protocol created by the principal investigator of the proposed research, testing, or educational study
8. Protocol – Animal Care and Use Protocol – protocol created by the principal investigator of the proposed research, testing, or educational study
9. COCC – Central Oregon Community College
10. Guide – the *Guide for the Care and use of Laboratory Animals*
11. AWA – Animal Welfare Act – requires that minimum standards of care and treatment be provided for certain animals bred for commercial sale, used in research, transported commercially, or exhibited to the public
12. PHS – Public Health Service – establishes guidelines for the proper care of animals to be used in research
13. Clinical veterinarian – the Attending Veterinarian or designee

Revision	Author	Revisions Made	Effective Date	Approval
1	Cindy Elston	<ul style="list-style-type: none"> • New SOP 	11/4/16	Cindy Elston (AV)
2	Cindy Elston	<ul style="list-style-type: none"> • Changed biological indicator should be tested at least ‘monthly’ to at least ‘bimonthly’ 	3/16/17	Cindy Elston (AV)