

POLICY & PROCEDURE

Responsible Officer: Dean of Research
Approved By: President's Cabinet

Effective Date: July 27, 2015

See also: Procedure 527 Autoclave Safety

POLICY 527 AUTOCLAVE SAFETY

This policy applies to all research faculty, staff, students and visitors at KGI who make use of autoclaves for decontamination of laboratory waste and the sterilization of laboratory glassware, media, and reagents.

I. Purpose

An autoclave is a mechanical device that utilizes pressurized steam to destroy microorganisms. It is a common and familiar piece of equipment in research laboratories and it is easy to forget that it poses many hazards including physical hazards (e.g. heat, steam and pressure) and biological hazards (e.g. improperly autoclaved infectious materials).

Because hazardous conditions within autoclaves are so extreme, the chance for injury is high if not properly operated. This policy is intended to provide practical information that can be utilized by all researchers to safely operate the autoclaves at Keck Graduate Institute (KGI). Individual labs are encouraged to use this policy as a guide for training new personnel on the safe use of autoclaves.

II. Responsibilities

Keck Graduate Institute (KGI): It is KGI's duty to provide a safe and healthy workplace for the employee. KGI provides proper maintenance, testing and calibration on Autoclaves in service at KGI. To ensure quality maintenance the autoclave manufacture (Steris) provides all preventive maintenance, performance maintenance and calibration.

KGI ensures that each autoclave is monitored as follows:

- A Large Quantity Waste Generator license with onsite Treatment is maintained and certified annually by the California State Health Department.
- Heat Sensitive Tape Monitoring – KGI requires each operator to use heat sensitive sterilization indicator tape for each load to indicate that the load has undergone an effective steam sterilization process.
- Biological Indicators are used monthly to test to confirm the attainment of adequate sterilization conditions.
- KGI maintains records of all waste decontaminated at KGI by providing autoclave waste sign-up sheets.
- KGI maintains documentation records of autoclave preventive maintenance and repairs for 5 years.

Laboratory Safety Manager: It is the responsibility of Laboratory Safety Manager (Jasmine Yu) to provide autoclave safety online and hands-on training to new autoclave users. Laboratory Safety Manager should maintain training records in autoclave rooms. The laboratory safety manager is encouraged to use KGI Autoclave Safety Guideline as a guide to train new personnel.

Principal Investigator (PI) Supervisor: It is the responsibility of the PI/Supervisor to ensure that each person in the lab is appropriately trained by Jasmine Yu on the safe operation of the autoclave and for maintaining those training records in the lab with other safety training certificates.

Employees Who Use Autoclaves. It is the responsibility of the employee to follow KGI Autoclave Safety Guideline to operate autoclaves and call for help when operation fails. Users are required to adhere to Procedure 527.

See also POL 527 Autoclave Safety

PROCEDURE 527 AUTOCLAVE SAFETY

All autoclave users must be familiar with this procedure. Safety contacts are listed at the bottom of page 3.

1. **Do not autoclave items containing corrosives, solvents, volatiles or radioactive materials.**
2. Prior to Operation
 - a. Document specific hands-on operating instruction from the PI or Supervisor or work under the direct supervision of an experienced, trained person.
 - b. Before using the autoclave, check inside the autoclave chamber for any items left by the previous user that could pose a hazard.
 - c. Ensure that the drain strainer is clean before loading the autoclave.
 - d. Ensure that the door gaskets have not deteriorated, but are still intact and pliable.
 - e. Do not start load with empty readout tape! Contact Jasmine Yu or facility if the tape runs out.
 - f. Log all autoclave runs on the sign-up sheets next to the autoclave.
3. Selection of containers.
 - a. All biohazard waste must be placed into polypropylene bags (Red biohazard bags), which must be loosely closed so that the steam may get inside the bag. A stripe of indicator tape must be placed on the outside of red bag.
 - b. Use only borosilicate glass (Pyrex™ or Kimax™) which can withstand the high autoclave temperatures.
 - c. Use a heat resistant polypropylene or stainless steel “autoclave” trays.
4. Preparation and loading of materials.
 - d. Prior to placement in the autoclave, items should be placed into autoclave trays. Use a heat resistant polypropylene or stainless steel “autoclave” tray with a solid bottom and walls to contain the contents and catch spills.
 - e. Fill liquid containers only half full. If sterilizing glassware fill bottom of pan with ½ inch water to prevent cracking.
 - f. To prevent bottles from shattering during the pressurization, the caps of containers with liquids must be loosened before loading.
 - g. Load the autoclave as per the manufacturer’s recommendation. DO NOT overload the autoclave. Biohazard bags should be placed in a manner where the bags do not touch the sides or top of autoclave chamber.
 - h. Leave some space between items to allow steam circulation
5. Cycle selection (see Appendix A page 4 for more details)
 - a. Make sure that the door of the autoclave is fully closed and latched and ensure that the correct cycle for the items being autoclaved has been selected before starting the cycle.
 - b. **Use the slow exhaust cycle (liquid cycle) for liquids (to prevent contents from boiling over) and for decontamination of hazardous material.**
 - c. When running the liquids cycle the time is longer but uses lower temperatures to minimize evaporation of the liquids. Liquid cycles also have a longer depressurization time to avoid “boil-over” of liquids.
 - d. Select the Gravity “fast exhaust” cycle for glassware and for sterilizing wrapped items (e.g., surgical tools).
6. Time/Temperature selection

Take into account the size of the articles to be autoclaved in selecting time and temperature. For example, a 2 liter flask containing 1 liter of liquid takes longer to sterilize than four 500 ml flasks containing 250 mls of liquid each. Material with high insulating capacity (e.g. animal bedding, soil) increases the time needed for the load to reach sterilizing temperatures. Never exceed the manufacturer’s recommended pressures and temperatures during operation of the autoclave.

7. Removing the load after sterilization

- a. Most accidents occur during the final operations of opening and unloading the autoclave. When the pressure gauge reaches zero, wait 10 minutes and open the door slowly allowing the steam to gradually dissipate. Never open the autoclave before the pressure gauge reaches zero.
- b. Wear a lab coat/gown, eye protection, heat insulating gloves and close-toed shoes. Stand behind the door when opening and beware of a rush of steam from the door.
- c. Let the liquids stand for at least a full hour before touching with ungloved hands. Be sure to let others in the area know that a heat hazard is present.
- d. For dry loads, let the glassware cool for a minimum of 15 minutes before touching it with ungloved hands.

8. Quality Assurance Monitoring

KGI tests the autoclaves monthly to ensure proper function and effectiveness. A biological indicator is used which contains heat resistant spores that are killed by exposure to 121°C for Approximately 50 minutes. Results are recorded and maintained for State Health Department Inspections. If the systems fail the test, KGI will shut down the system and call for maintenance. Following maintenance another test will be run to verify the system is back online. The records of the log book will be kept on file for at least five (5) years.

9. Autoclave Failure

Discontinue use immediately if an autoclave is not working properly. Post a sign alerting others not to use the autoclave. Mechanical failures need to be attended to by a trained technician. Contact Laboratory Safety Manager for the maintenance or for further guidance. If an autoclave malfunctions during a run, the material inside must be retreated as though it is still contaminated/unsterilized.

10. Burn Emergency

If you are burned, you should seek medical treatment immediately. Burns to the face, third-degree burns or burns over large areas of the body should be treated as emergencies. (Call 911). Minor burns should be reported immediately to Laboratory Safety Manager and to your PI/Supervisor.

LAB SAFETY CONTACTS (current as of August, 2019):

Facility helpline: 909-607-0144 (70144)

Campus Safety: 909-607-8736 (78736)

Laboratory Safety Manager: Jasmine Yu at 909-607-8698 (78698)

APPENDIX A: AUTOCLAVE CYCLES

There are 2 basic autoclave cycles: Both cycles and the materials appropriate for each cycle are described below.

Cycle	Materials	Description
Gravity or "fast exhaust"	Dry goods, glassware, pipette tips, etc. wrapped goods (surgical tools, incubator racks, etc) , utensils	This cycle charges the chamber with steam and holds it at a set pressure and temperature for a set period of time. At the end of the cycle, a valve opens and the chamber rapidly returns to atmospheric pressure. Drying time may also be added to the cycle.
Liquid or "slow exhaust"	Liquids Media, LB broth, water/solutions, biohazard waste etc.	This cycle prevents sterilized liquids from boiling. Steam is exhausted slowly at the end of the cycle, allowing the liquids (which will be super-heated) to cool.

- **Bldg. 517 Autoclave:** Liquid or "slow exhaust". Our system has two (2) choices.
 - Liquid 1= 30min 121C,
 - Liquid 2= 60min at 121C
- **Bldg. 517 Autoclave:** Gravity or "fast exhaust" Our system has two (2) choices.
 - Gravity 3= 30min, dry 10min at 121C
 - Gravity 4 = 60 min, dry 15 min at 121C
- **Bldg. 535 Autoclave:** Liquid or "slow exhaust". Our system has two (2) choices.
 - liquid 1= 30min 121C,
 - liquid 2= 60min at 121C
- **Bldg. 535 Autoclave:** Gravity or "fast exhaust" Our system has two (2) choices.
 - Gravity 3= 30min, dry 10 min at 121C
 - Gravity 4= 60 min, dry 15 min at 121C
- **Bldg. 535 Autoclave:** Lab waste (slow exhaust) Our system has two (2) choices.
 - Lab waste 5= 30min at 121C
 - Lab waste 6= 60 min at 121C