



JABSOM KAKA'AKO STANDARDIZED AUTOCLAVE PROTOCOL

GENERAL INFORMATION

The autoclave uses high pressure and high temperature steam to effectively kill microorganisms or render a bio hazardous material inactive. For effective sterilization, the materials/load must be saturated with this steam. Air pockets or insufficient steam supply will prevent effective decontamination.

The potential risks of using an autoclave are heat and steam burns, hot fluid scalds, injuries to hands and arms from the door, or bodily injury in the event of an explosion. Additional injury or exposure to bio hazardous material may be incurred if the contents of the bio hazardous waste are packaged improperly. Onsite training on how to use the autoclave properly and safely is essential for all new employees to prevent injury. The use of heat-insulating gloves, lab coat and closed-toe shoes will help prevent burns and scalds during loading and unloading. If a load of bio hazardous waste is inadequately autoclaved, there is potential for human and environmental exposure to bio hazardous materials.

If you have never operated the autoclave you will be using, contact an experienced user in your laboratory for instruction on safe operation. For maintenance issues on the autoclaves, you may contact the JABSOM Facilities Office at 692-1880.

AUTOCLAVE SAFETY

Prevent injuries when using the autoclave by observing the following:

- Never put volatile chemicals or solvents (alcohols, chloroform), or corrosive chemicals (bleach, acetic acid, formalin, fixed tissues etc.), or radioactive materials in an autoclave. (See below.)
- Wear appropriate street clothing including closed-toe shoes and Personal Protective Equipment (PPE) including a lab coat, heat resistant gloves, and eye protection especially when unloading the autoclave.
- Don't autoclave sealed containers as they pose an explosion risk.
- Never open the door to the autoclave if there is water running out the bottom. Clogged steam lines, equipment malfunction or plugged drains may cause a buildup of scalding water. Call JABSOM Facilities at 692-1880.
- Prevent steam burns and shattered glassware by making sure that the cycle is completed and pressure in the autoclave chamber is zero before opening the door at the end of a cycle. Make sure that no personnel, including you, are directly in front of the door.
- Be especially careful with liquids due to superheating, a condition that occurs when liquids are at a temperature above their normal boiling point but do not appear to be boiling. In situations where personnel are in a hurry to remove flasks or bottles from the autoclave, the superheated liquids may boil out of their containers or explode.

Items that **should not be processed in the autoclave include:**



- **Hazardous chemicals** (including items associated with hazardous chemicals): In general, do not autoclave flammable, reactive, corrosive, toxic or radioactive materials. **Never autoclave dried bleach or bleach associated materials or nitrocellulose as both compounds pose a fire or explosion risk.** Lab coats that have been contaminated with chemicals should not be autoclaved but cleaned by an approved laundry service or disposed of as chemical waste. To schedule a chemical waste pick-up, fill out the Chemical Waste Disposal Request Form:
www.hawaii.edu/ehso/jabsom/Chemical%20Waste%20Disposal%20Request%20Form%20and%20Instructions.xls
- **Radioactive materials:** Contact the Manoa EHSO Radiation Safety Program for information on proper disposal of radioactive materials. To schedule a pickup, fill out a Radioactive Waste Pick-up Request Form available online at:
<http://www.hawaii.edu/ehso/radiation/reference.htm>
- **Pathological waste:** Includes animal carcasses, tissues and organs and human tissues and organs. JABSOM contracts pathological waste disposal to Hawaii BioWaste. Contact Kaka'ako EHSO to request a pick up date.
- **Mixed wastes:** If the biological waste also contains hazardous chemicals or radioactive materials, contact Kaka'ako EHSO for further assistance.
- **UH Kaka'ako Waste Disposal Guidelines:**
<http://www.hawaii.edu/ehso/kakaako/Kaka%27ako%20Waste%20Disposal%20Guidelines.pdf>

PREPARING MATERIALS FOR AUTOCLAVING

To ensure adequate steam penetration, pack solid materials loosely; don't intentionally compact waste or overfill biohazardous waste bags. Bags/containers should be placed in a large, leak-proof, polypropylene or stainless steel tray to avoid or contain spills. Before processing, open the bags/containers so that the steam can penetrate and effectively raise the temperature for adequate killing. A small amount of water may be added to ensure heat transfer inside the bag/container. If the bag is closed during autoclaving, the temperature of the contents may not be raised sufficiently for decontamination. If you are processing more than one tray make sure that there is ample room between the trays so steam circulation is not impaired. Place indicator tape on the bag.

Place containers of liquids (bottles, beakers, flasks etc.) covered with a cotton plug or steam-penetrable bung in a large, leak-proof, shallow pan. Inspect the glass to make sure there are no cracks. Do not fill containers to the top but leave plenty of head room. Bottles with narrow necks may boil over violently if filled too full of liquid. Avoid the use of bottles if possible, but if it is necessary, make sure that the screw-cap is nearly unscrewed allowing for pressure changes or it may explode. Water should be added to the pan to help prevent heat shock to the containers. Place the pan and contents in the autoclave and put indicator tape on the containers.

Generally recommended parameters for biological waste are sterilization time of 60-120 minutes (excluding exhaust time) and 250°F/121°C at 15 psi. Studies have shown that the processing time necessary to achieve decontamination of biological material depends on several loading factors. Load size, type of container, and moisture content all impact decontamination time. If the waste is packed too tightly or it is a very large load, the length of the sterilization time may need to be increased to allow the steam to penetrate the center of the load. The cycle



parameters may require different settings depending on the infectious materials you are trying to decontaminate. The tighter the autoclave is packed, the longer it will take to reach 121°C in the center of the load. To assure that the material you are autoclaving is properly decontaminated/treated (killed or inactivated) add 10-15 minutes to the estimated sterilization time for over-kill.

SIGN-UP SHEET/USER LOG – EXAMPLE ON PAGE 10

- It is **mandatory** for users to provide the following information on the sign-up sheet/user log while using the autoclave:
 - Your name (printed legibly; no initials)
 - Contact phone number (cubicle/lab or cell phone)
 - Principle Investigator's name
 - Start and End times
 - Cycle used (e.g. #1, #9, #10 - may vary depending on department's edits of cycle parameters)
- Usage is based on a "first come-first serve" basis per the sign-up sheet.
- Operating times for the boilers are from 7:30 am – 4:30 pm, Monday to Friday. Please note that the autoclaves take 30-45 minutes to reach operational pressure from when boilers are first turned on in the morning. Your cycle should also be completed by 4:30 pm depending on the cycle you select. Autoclaves are not operable during the weekends and holidays.
- "Blocking-off" or reserving time for more than one cycle is not permitted.
- Users with small individual loads may share autoclave cycles. Do not overcrowd the autoclave chamber, which can affect decontamination and proper functioning of the autoclave.

GENERAL PROCEDURE (Tuttnauer Model 5596 SP-1V)

1. **Turning Autoclave ON (Per JABSOM Facilities Management, the autoclaves should always remain ON)**
 - a. Flip green power switch from **STANDBY** to **ON**. Autoclave should turn on and computer screen will be visible.
 - b. If green power switch is already **ON** but computer screen is **BLANK**, press and hold power button (circle with line in it on the upper right corner of keypad) until it "beeps," computer screen will become visible. Autoclave is now on.
2. **Setting up for a RUN**
 - a. There are generally 3 programmed cycles for standard use (unless otherwise stated on the autoclave and these may vary depending on if departments have changed cycle parameters):
 - i. Cycle 1: 15 min sterilization, 10 min dry: **Sterilizing dry articles** such as clean glassware, tips, tubes, surgical instruments
 - ii. Cycle 9: 60 min sterilization, slow exhaust: **Decontamination Cycle** (Approximately 2 hrs long)
 - iii. Cycle 10: 15 min sterilization, slow exhaust: **Sterilizing Liquids**

Do not mix contaminated and clean items together during the same autoclave cycle. Clean items generally require shorter sterilization times while a bag of infectious waste typically requires a longer decontamination time to render all contents non-infectious.



Use autoclave tape with each load as an indicator that proper temperature was reached and check the print out after each load to ensure the correct cycle parameters and that the temperature was maintained for the specified time.

- b. **For Waste Decontamination and Liquid Cycles, an autoclavable secondary container must be used to catch any leakage or spillage.**
- c. Place all articles to be sterilized into the CART. DO NOT place anything directly onto the chamber floor.
 - i. Do not overcrowd the cart; keep all items within the confines of the rack. An overpacked autoclave chamber does not allow efficient steam distribution. Considerably longer sterilization times may be required to achieve decontamination if an autoclave is tightly packed.
 - ii. Biohazard waste bags must be opened to allow for steam to escape; do not seal openings tightly (i.e. don't "goose-neck" or tie bags closed). It may also be necessary to add a cup of water to the waste for steam generation.
 - iii. **Never seal a liquid container with a cork or stopper. This could cause an explosion inside the autoclave.**
 - iv. Do not overfill an autoclave bag. Steam and heat cannot penetrate as easily to the interior of a densely packed autoclaved bag, so the innermost areas may not get decontaminated.
 - v. Use caution when handling an infectious waste autoclave bag, in case sharp objects were inadvertently placed in the bag. Never lift a bag from the bottom to load it into the chamber. Handle the bag from the top.
- d. Select cycle by using the arrow keys to SCROLL UP or DOWN to the desired cycle.
- e. Press **CLOSE** button, door closes by moving UP.
- f. Wait for **READY** signal on screen and for the compressor to stop working (that is the LOUD noise you will hear during this step; this condition may vary between units). Check that the Chamber Pressure is near 20 PSI.
- g. Press **START**; RUN LIGHT should show and the cycle will begin.

3. End of Cycle

- a. Cycle is complete when the **READY** and **OPEN** indicators are displayed. Press **OPEN** and allow the door to completely open and vent before removing items. **Avoid burn and hot steam hazards; use heat resistant gloves, safety glasses, and a lab coat when operating an autoclave and handling all items.** Pull the CART out onto the trolley and push cart to counter or to "decontaminated waste collection" area.
- b. Record your TIME OUT on the log sheet. **Leave the autoclave door OPEN.**
- c. After every third cycle, the gasket (red or brown rubber around door opening) should be sprayed with the provided silicon spray (spray available at the JABSOM stock room).
- d. **Per JABSOM Facilities Management, the autoclaves must remain ON at all times.**
- e. For autoclaved, decontaminated solid waste, follow the guidelines below for proper disposal.

4. Problems or Questions



- a. If you need additional silicon spray, autoclave receipt paper rolls, or autoclave ink ribbon, visit the JABSOM stock room or contact JABSOM Facilities.
- b. For operational or mechanical issues, contact Edward Nagamine (Facilities), enagamin@hawaii.edu, phone# 692-1880 and submit a Work Order Form.
- c. If there is uncontrollable flooding, contact security 692-1911 or 692-0911.
- d. Do not leave an autoclave operating unattended for a long period of time. Stay in the vicinity while running an autoclave cycle in case there is a problem.

QUALITY CONTROL & AUTOCLAVE PERMITS

- **Quality control tests (biological indicators) for autoclaves used for decontamination** will be conducted monthly by Kaka'ako EHSO. Tests will be coordinated with the primary users of the subject autoclave.
- The test results will be documented on a **LOG** posted near the autoclave; copies will also be on file with Kaka'ako EHSO. If "clean sterilization only" designated autoclaves are at any point used for decontamination of biohazardous waste, Kaka'ako EHSO must be contacted and quality control tests must be performed prior to disposal of any "decontaminated" waste.
- **Autoclave permits** are issued by the Hawaii Department of Labor Boiler and Elevator Branch. Copies of the permit are posted at or near each autoclave. Renewal Permits shall be coordinated by JABSOM Facilities and EHSO.

AUTOCLAVE REPAIR

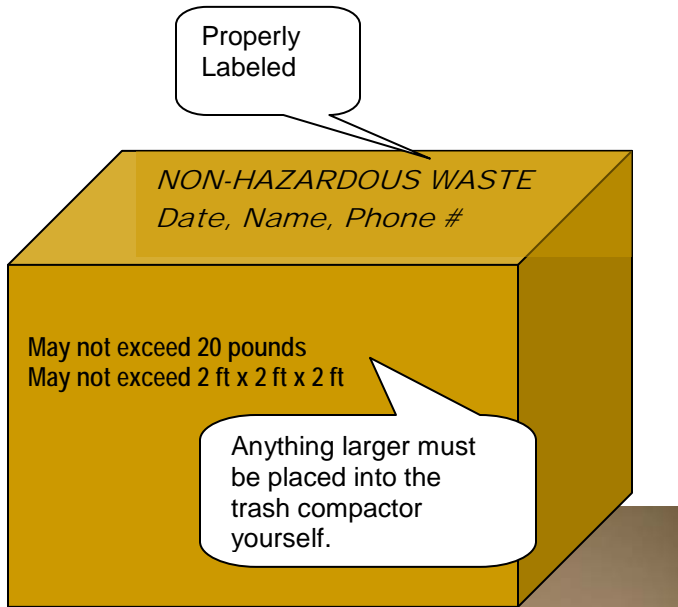
If an autoclave is not functioning correctly, it is important that you contact the JABSOM Facilities Office to report the issue as soon as possible. Complete the online work order request form at: <http://jabsom.hawaii.edu/JABSOM/departments/intro.php?departmentid=79> .



AUTOCLAVED WASTE DISPOSAL

OPTION 1: Required Packaging for autoclaved waste that will be collected by Custodial Staff.

- **NO LIQUIDS:** read the waste disposal guidelines for proper treatment and disposal of liquid biohazard waste.
- **NO METAL Sharps:** All metal sharps must be contained in puncture proof metal sharps collection containers; contact Kaka’ako EHSO for proper disposal.
- **EXCLUDED:** all waste from Vivarium, ABSL3, BSL3 facilities.



For Autoclaved Solid Waste, including pipet tips, pipets, tubes, plates, gloves, absorbent materials, flasks, etc.:

1. Waste must be properly decontaminated by autoclaving.
2. Waste must be allowed to cool to room temperature.
3. Be on the alert with handling pressurized containers.
4. Ensure any agar media has been allowed to re-solidify.
5. Drain any liquids (liquefied agar must never enter the drain system).
6. Place the autoclave bag or any autoclaved containers (no metal) into heavy duty black trash bag(s) (available in the decontamination autoclave rooms), secure closed and ensure no leakage.
7. Place into a cardboard box, tape the box closed and label *NON-HAZARDOUS WASTE, Date, Responsible Person’s Name and Phone Number.*
8. Place packaged autoclaved waste in designated areas ONLY:
 - IBR: outside of autoclave room, or outside of the labs with your trash cans
 - 204: on the designated cart outside of 204
 - 239A: on the designated cart outside of 240 (the dishwasher room)
 - 304: on the designated cart outside of 304
 - 334A: on the designated cart outside of 335 (the dishwasher room)
9. All leaks and spills shall be immediately cleaned up by the responsible individual; the custodial staff will not clean up any spills or leaks!!! Allow autoclave to cool before attempting to clean up a spill. If glass breaks in the autoclave, use tongs, forceps or other mechanical means to recover the fragments. Do not use bare or gloved hands to pick up broken glassware.
10. EHSO shall be notified of any improperly packaged waste, questionable waste, or leaking waste. If non-compliance becomes a problem, the custodial staff will be instructed to discontinue collection of autoclaved waste from your area and all those affected will have to place their autoclaved waste into the trash compactor.





OPTION 2: NOT BOXED & LABELED = NO CUSTODIAL PICK-UP

For all solid, non-metal sharps autoclaved waste that you choose not to box, you must take it to the trash compactor and place it inside yourself.

1. When the autoclaved waste has cooled to room temperature, place it into the heavy duty black trash bag(s), and place it into the trash compactor.
2. Do not allow your bags to leak any contents while transporting to the trash compactor. If there is a spill, immediately clean it up; do not leave any liquid on the floors as this will pose a slip hazard.
3. Do not leave any autoclaved waste sitting outside of the trash compactor; if the compactor is full, contact Security or Facilities.



NOTES

1. Tuttnauer Model 5596 SP-1V is the most commonly used autoclave in the BSB, therefore the protocol was tailored to this model.
2. All users must complete the required applicable safety trainings (contact Kaka'ako EHSO 692-1854).
3. In the event that a laboratory's primary autoclave is not operational the wastes to be autoclaved shall be autoclaved at the nearest secondary autoclave and transported in a closed-top, leak-proof, puncture-proof container. Communicate to all users that the autoclave is "not operational." Contact Facilities – Edward Nagamine at 692-1880 – and submit a work order.
4. In the event that primary and secondary autoclaves are not operational, store biological wastes in a laboratory refrigerator, freezer, or cold room until the autoclaves are repaired. If this cannot be done, please contact Kaka'ako EHSO (692-1854) to authorize an alternative, including chemical treatment, as per your biohazardous wastes written contingency plan.



5. All biohazardous wastes must be transported in a leak-proof, puncture-proof, secondary container to autoclave rooms. If transport through common areas, such as the lobbies, is required, waste must be transported in a closed-top, leak-proof, puncture-proof secondary container. (Exterior surfaces of secondary container must be decontaminated before and after transport). Gloves or dirty hands shall not be used to handle common areas such as door handles and elevators.
6. All autoclave bags must be red or clear Polypropylene (PP) bags. Bags must be slightly opened during autoclaving, they must not be sealed.
7. **Comply with the Kaka'ako BSB Waste Disposal Guidelines.** (See link at <http://www.hawaii.edu/ehso/kakaako/reference.htm>)
8. Biological items containing solvents, volatile or corrosive chemicals (phenol, trichloroacetic acid, chloroform, etc), chlorinated compounds (HCL, bleach), chemotherapeutic agents, or any radioactive materials must not be autoclaved; autoclaving of chemical-biological waste at 120 to 130 °C may result in the volatilization or release of the chemical constituent; autoclaving waste containing flammable liquids may result in a fire or explosion. Contact Kaka'ako EHSO (692-1854) about proper treatment of these "mixed" wastes.

FREQUENTLY ASKED QUESTIONS

What is an autoclave?

An autoclave, or steam sterilizer, is an insulated pressure chamber in which saturated steam is used to elevate the temperature. Autoclaves are found in research laboratories, healthcare centers and other places that require high-level disinfection.

How does an autoclave work?

An autoclave uses pressurized steam to decontaminate infectious waste. Decontamination autoclaves must operate at a temperature of 121°C, a pressure of 15 PSI, and a minimum cycle time of 60 minutes. The effectiveness of an autoclave depends on the time, temperature, and direct steam contact with infectious agents. Other factors that influence treatment efficiency include waste density, physical state and size, and organic content.

How do I use the autoclave?

Follow manufacturer's instructions and the Kaka'ako BSB Standardized Autoclave Procedures for the Tuttnauer autoclaves built into the BSB. A Tuttnauer training video is available for viewing, contact Kaka'ako EHSO for more information.

What wastes can be autoclaved?

Follow the guidelines set forth in your USDA, CDC, and HDOA permits for the decontamination of your study's biohazardous waste. Refer to the Kaka'ako BSB Disposal Guidelines for more information.

The following are examples of infectious waste products which may be autoclaved and then disposed as decontaminated biohazardous waste into the regular trash or into the sanitary sewer (sink drains):

- Cultures and stocks of infectious agents
- Laboratory wastes that were exposed to infectious agents
- Human blood waste and human blood-products
- Blood, biological waste, and discarded materials contaminated with excretion, exudates, or secretion from humans or animals
- Discarded equipment and parts in contact with infectious agents
- Preparations made from genetically altered living organisms and their products

What waste cannot be autoclaved?

Types of waste that must not be autoclaved include cancer therapeutic drugs, toxic and volatile chemicals, radioisotopes, hazardous chemical waste or any other harmful material that can be vaporized



and disseminated with heat. Do not autoclave flammable, reactive, corrosive, toxic or radioactive materials. Contact Kaka'ako EHSO for assistance with the proper treatment and disposal of preserved tissues or specimens, do not autoclave these items. Contact Kaka'ako EHSO for assistance with the proper treatment and disposal of animal tissues and body parts and human body parts or organs. When metal sharps are autoclaved, they must be submitted to Kaka'ako EHSO because metal sharps may never enter the regular trash.

Which autoclaves can be used?

There are two autoclaves designated for "clean" sterilization only and therefore may not be used to decontaminate waste, BSB 240 & BSB 335.

The following autoclaves may be used for both decontamination and clean sterilization and have been validated by monthly quality control spore testing. Be sure that autoclave users have entry clearance and all required trainings/certifications.

- IBR/Anatomy 166 (department owned, restricted access)
- CMB 204
- Shared: BSB 239A
- Medicine/Trop. Med. 304
- Trop. Med. 334A

How should I collect and dispose the waste?

Follow the guideline set forth in your permits or protocols.

In general, liquid infectious waste may be autoclaved or treated with hypochlorite solutions (by approved method) and disposed via the laboratory sink drain.

For solid infectious waste, follow the disposal procedures described in the Kaka'ako Waste Disposal Guidelines or contact Kaka'ako EHSO for more information.

Questions and Resources:

Contact the Kaka'ako Environmental Health & Safety Office with waste disposal questions.

<http://www.hawaii.edu/ehso/kakaako/>
692-1854; 692-1855; BSB 112



**JABSOM KAKA’AKO
 AUTOCLAVE LOG**

LOCATION (BUILDING NAME/ROOM NUMER): _____

Date	Name	Contact #	PI / Dept	Start Time	End Time	Cycle Used

- Usage is based on a first come-first serve basis per the sign up sheet.
- Blocking off or reserving time for more than one cycle is not permitted.
- If you sign in a highlighted slot, spray gasket with the silicon spray when cycle is completed.