

Autoclave: Good Practice Guide

Table 1: Items that CANNOT and CAN be autoclaved

ITEMS THAT <u>CANNOT</u> BE AUTOCLAVED	ITEMS THAT <u>CAN</u> BE AUTOCLAVED
- SEALED CONTAINERS	- CONTAINERS WITH LOOSENED CAPS OR LIDS (E.G. ALUMINUM FOIL CAP)
- OILS, WAXES	- CONTAMINATED SOLID ITEMS, SUCH AS: PETRI DISHES, EPPENDORF TIPS, PIPETTES, GLOVES, PAPER TOWELS
- FLAMMABLE MATERIALS	- ITEMS FOR STERILIZATION, SUCH AS: GLASSWARE, MEDIA, AQUEOUS SOLUTIONS, EQUIPMENT
- MATERIALS CONTAINING: SOLVENTS, OR CORROSIVE CHEMICALS (I.E. PHENOL, TRICHLOROACETIC ACID, ETHER, CHLOROFORM, ETHANOL)	- BIOHAZARD MATERIALS MUST BE LABELED AS SUCH AND SECURED IN CONTAINMENT VESSELS OR AUTOCLAVE BAGS
- MATERIALS CONTAINING: VOLATILE >3% SALINE AND CHLORINATE COMPOUNDS (I.E. HCL, NAOH OR BLEACH)	- MATERIALS CONTAINING: 1 -3% SALINE AND CHLORINATE COMPOUNDS (I.E. CaCl ₂ , NaCl, KCl, PBS)
- RADIOACTIVE MATERIALS	- STAINLESS STEEL
- SOME BUFFERS (MOPS) MAY DEGRADE IN THE AUTOCLAVE	- BOROSILICATE GLASS (PYREX)
	- LOOSE OR DRY MATERIALS WRAPPED OR BAGGED IN STEAM-PENETRABLE PAPER OR LOOSELY COVERED WITH ALUMINUM FOIL
- SOME PLASTICS (POLYSTYRENE (PS), POLYETHYLENE (PE), AND HIGH DENSITY POLYETHYLENE (HDPE)) CANNOT BE USED AS SECONDARY CONTAINER	- SOME PLASTICS (POLYPROPYLENE (PP) AND POLYCARBONATE (PC)) CAN BE USED AT SECONDARY CONTAINER

Table 2: STERIS Liquid Cycle recommendations

Volume of Liquid in One Container (ml)*	Recommended Sterilization Time at 121°C (minutes)**
75	25
250	30
500	40
1000	45
1500	50
2000	55
>2000	55+10 min/L

- *This time may vary due to viscosity of liquids and other parameters.
- **Listed times include the combined time required to reach 121°C and the time required to achieve sterilization at 121°C.

Table 3: Gravity Cycle Recommendations

Items	Recommended Sterilize Time at 121°C (minutes)	Dry Times (Minutes)
Glassware (empty, inverted, vented)	15	0
Instruments (metal combined with suture tubing or other porous materials (unwrapped))	20	0
Hard Goods (unwrapped)	15	0
Hard Goods (wrapped in muslin or equivalent)	30	30
Biological Waste	60 (unless exempt*)	0

*See Table 4 for Botany Approved List for exempt organisms

Table 4: The 'Botany Approved List' of organisms that can be autoclaved for less than the required 60 minutes (see BOTANYwasteSOP2019MarFINAL document for details)

transgenic <i>Arabidopsis thaliana</i> plants
transgenic <i>Helianthus annuus</i> plants
<i>Physcomitrella patens</i> moss plants
<i>Chlamydomonas reinhardtii</i> algae (plants)
<i>Nicotiana benthamiana</i> plants
non-pathogenic, non-hazardous <i>Agrobacterium tumefaciens</i> (microbe)
non-pathogenic, non-hazardous <i>Escherichia coli</i> bacterial strains (microbe)
<i>Saccharomyces cerevisiae</i> (microbe)

PPE

Often material to be loaded contains potentially infectious material or toxins, thus standard laboratory protective equipment must be worn.

- Nitrile or latex gloves
- Safety glasses or goggles; it is advisable to wear a full face shield if a splash hazard exists
- Laboratory coat
- Closed-toe and closed-heel shoes

In addition, since the interior of the autoclave is extremely hot, **heat resistant gloves** are required for loading and unloading autoclaved materials. Do not wear previously used nitrile gloves inside the heat resisting gloves, since you risk contaminating the inside of the heat resistant gloves. Always change nitrile or latex gloves beforehand (or do not use nitrile gloves).

Preparing Materials To Be Loaded

- Before preparing items for sterilization, or decontamination, ensure that each item can be autoclaved.
- Review the SDS and PSDS if you are unsure of the proper safety precautions and personal protective equipment required for the material to be autoclaved.
- Tape indicator (autoclave tape-heat sensitive) should be used on all loads.
- All items to be autoclaved should be placed inside a suitable **primary container** (*i.e.* flasks, tubes, beakers, biohazard bags, or wrapping paper or muslin for instruments), which in turn is put into a **secondary container** (*i.e.* autoclave pan).
 - Heat-resistant secondary containers must be large enough to contain any leaks

- in the primary containers.
- **Plastic secondary containers must be (polypropylene (PP) and polycarbonate (PC)) only.**
- Avoid crowding or stacking items.
- Do not overload secondary container; leave sufficient space between each item for steam circulation. For large loads, if the space is greater than 6 inches between each item, the run time can be set for the volume/weight of the largest item. If the space is smaller than 6 inches apart, items are considered to be one, and must be sterilized according to the mass of the two objects combined.
- Do not fill primary containers beyond 75% of their holding capacity.
- **All primary containers must be unsealed by loosening screw or vent caps**, capping open containers with aluminum foil or opening plastics bags slightly (no less than three fingers width)
 - **DO NOT AUTOCLAVE SEALED CONTAINERS OR BAGS.**

Loading Autoclave

- Stand away from the door and carefully open the door to release any leftover steam inside the autoclave.
- Check the autoclave's interior to make sure there is no potential hazard left from the previous user.
- Clean the drain before loading materials to allow good circulation.
- Make sure the bags do not touch the interior walls of the autoclave to avoid melting.

Unloading Autoclave

- Stand away from the door and carefully open the door to release any leftover steam inside the autoclave.
- If possible, allow the materials inside the autoclave to stand for 10 mins to release any steam or hot air trapped inside the material.
- Try not shake any liquid material while removing it from the autoclave. Use a cart to transport autoclaved materials from autoclave to lab.
 - Liquid may be extremely hot, sudden jarring may cause the liquid to boil over and is a potential burn hazard.

Autoclaving Procedures

- Check glass containers for cracks, replace any broken containers.
- Liquids and dry materials require different cycles and need to be autoclaved separately.
- Liquid cycles: time autoclaved for depends on the largest volume (See Table 1).
- Waste ,or Kill cycles, for RG1 or RG2 materials for a minimum of 60 minutes at 121°C and at 15 psi (this is a UBC requirement). The exception are those organisms that have been exempt and can be found on the 'Botany Approved List' (See Table 4), which can

be autoclaved for less time.

- When autoclaving soil to sterilize, or destroy plant material, soil must be **dry** and must be run on a **liquid cycle** for 30 (or more) minutes depending on size of load.
- Bags of soil or plant material must weigh less than 10 kg.
- Refer to the 'BOTANYwasteSOP2019MarFINAL' document for details regarding proper disposal of RG1, exempt and non-exempt, and RG2 Biological waste

Report any problems or error messages to Jessica Trat (Jessica.trat@botany.ubc.ca) or Joanne Denny (denny@zoology.ubc.ca)