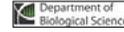


DO NOT use an autoclave UNLESS you: have received training from a **knowledgeable** and **competent** person have read the **Hazards** and **Emergency Procedures** before you start

Amsco Autoclave Instructions

BG McCashin Feb 10, 2006



1. Hazards: Safety Concerns

Hazard:	Example:	Control Measure:
Burn	hot metal: cart, door	always use PPE *
	hot liquid: glass breakage boil over	use only Pyrex glass for liquids vessels less than half full of liquid cool liquids 5' before moving; avoid jostling superheated liquids (boil over) all liquids in a tray; load/unload using the rack
	steam: when open door	use PPE, open slowly; stand back
Pressure release	steam leak around door	close door tightly before start program; tighten further to stop leak
	steam on before door is closed	NEVER try to close and seal door while steam is entering chamber (see 2A below)

* PPE – Personal Protective Equipment = lab coat + thermal gloves + eye protection + shoes (no sandals).

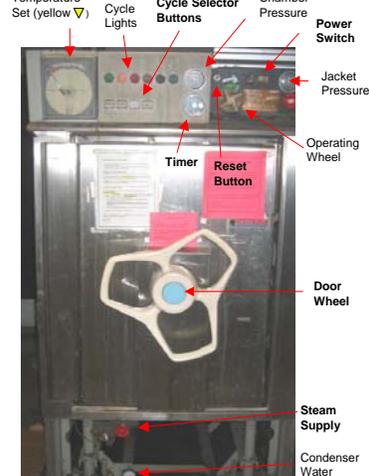
2. Emergency Procedures:

- 2A. Steam entering chamber while door is open:
Do not try to close door.
 You can:
 1. leave the autoclave door open, **walk away and contact Biotron staff** (2-5169) or the **Control Center** (2-5555) to shut off the steam.
 2. if you can do so safely, **turn off the steam supply valve** (below chamber at the front) or, if accessible, at a valve on the steam line behind the autoclave. Then post a warning sign and call the Biotron staff (2-5169) to check the autoclave.
- 2B. Small steam leak after sealing door: (not generally serious)
 you can **hand tighten** the wheel further (or get someone stronger to do it).
 Do not use any 'tool' to tighten the door.

3. Autoclave Controls:

There are several AMSCO models in the Department, not all controls exist on all autoclaves.

NOTE: only controls in **Bold** should be adjusted by the user



Startup Check:
 Normally the steam supply to jacket and chamber is ON
 You should not adjust any other controls than those indicated in bold.
 Contact the Biotron staff (**2-5916**) if you think controls are not set correctly.

Order	Control:	Desired Position:	If not correct:
1	Operating Wheel	OFF, green background color showing (no red color)	notify Biotron staff
2	Power Switch	ON	turn ON
3	Steam Supply	OPEN	turn open (CCW ☺)
4	Jacket supply	OPEN (jacket pressure 18-22 psi)	notify Biotron staff
5	Chamber Trap Bypass	normally closed	notify Biotron staff
6	Condenser Water	normally closed	notify Biotron staff
7	Jacket Pressure	18 - 22 psi	notify Biotron staff
8	Chamber Pressure	0 psi (needle is in white zone)	autoclave is in use; come back later
9	Yellow temperature set point	121 C	notify Biotron staff
10	Timer	15 - 75 minutes	set 15 - 75 min

For any assistance, call the Biotron Staff at 492-5916

4. Load Preparation:

4A. Biohazardous Waste:

- Solid bagged waste:** (e.g. Petri dishes containing agar, paper towels, latex gloves, genetically modified organisms (plants, flies))
- use a clear autoclave bag (rated to 125C or higher), not orange Biohazard, not clear polyethylene; not a green garbage bag
 - labelled "Biohazard" until goes into autoclave
 - open top of bag to allow air out and steam in
 - add a cup of water to each bag to help expel air
 - time >60 minutes to ensure sterility
 - don't overload autoclave; steam must reach all parts of load
- NOTE: to avoid bag meltdown, remove material within 2h of completing cycle.

Liquid waste: (e.g. bacterial, algal or cell cultures)

- in Pyrex™ or Kimax™ glassware only, no flint glass for liquids
- flasks less than half full
- top not sealed tight with cap or rubber stopper (use foam plug or tin foil cap)
- loosen threaded caps if present
- time >60 minutes

4B. Non-Waste Materials:

- Liquid media / solutions:**
- as above for liquid waste; for time, see section 9
- Solid material:** (e.g. utensils in trays or wrapped in paper, glassware)
- arrange items vertically on rack to promote contact with steam (like loading plates in a dishwasher; avoid stacking horizontally)
 - time >20 minutes

5. Loading autoclave:

- wear PPE: lab coat, thermal gloves, eye protection during loading/unloading
- *** ALWAYS use metal pans to hold all wet/plastic items (to contain leaks, boil over, bag meltdown). Use rolling rack to avoid having to lean into chamber
- do not overload autoclave; need space between packages for steam to circulate
- seal door CW ☹ **Hand tight only** (NEVER use any 'tool' to tighten or loosen door wheel)

6. Starting Autoclave Cycle:

- set timer:** minimum 15 minutes (use at least 60' for waste)
- 121C + >15 minutes duration to kill most organisms
 - larger volumes require longer time (see chart section 9)
 - for a kill run, set time at >60 minutes

select cycle you want according to the load:

Cycle	Use for:	Exhaust Rate:		Vacuum Dry
		Slow	Fast	
Unwrapped	solids only; glassware, instruments, bags of solid waste/Petri dishes with agar	no	✓	no
Wrapped	solids in envelopes or cans	no	✓	✓
Liquid	flasks of liquids (± agar)	✓	no	no

Press the cycle selector button to start the steam.

WAIT a few minutes for chamber pressure to increase >15 psi and check that:

- there is no steam leak around the door seal (see #2B above)
- the temperature indicator is rising (the timer will not start until the temperature indicator reaches the set point)

After the cycle is complete and the steam is exhausted, buzzer will sound when the pressure is at zero. **Press Reset** to silence buzzer.

Before attempting to open the door, check that the Chamber Pressure is Zero (needle in the white zone). The jacket pressure will still be at 18-22 psi (normal)

!! Very dangerous to open door if even a few pounds of pressure is in chamber !!

Never use any tool to release or tighten the door wheel.

For liquids: open door 2 cm and **wait 5 min** before removing the load to avoid boiling over of superheated liquids (temperature >100C).

7. Unloading Autoclave:

- wear PPE: lab coat + thermal gloves + eye protection + shoes/boots during unloading
- allow steam to vent for 30 seconds after the door is fully open before unloading
- autoclaved solid waste must be deposited **by you** into the green dumpster at the loading dock (Room G204)
- remove or deface any biohazard labels on the clear bag before disposal
- clean out tray
- close the autoclave door and spin wheel to loosely engage teeth with the frame but do not seal it tightly

8. Reporting Autoclave Problems:

- post a note that autoclave is not working and that service has been requested.
- contact the Biotron Staff for any problems/questions concerning autoclaves in the BioSci Dept. Phone 492-5916 or visit Room B212 or B214 (knock if doors are closed).
- if there is an autoclave emergency after hours, call the UofA Control Center at 2-5555 and request a plumber to shut off the steam.

9. Timing Guide for 121C:

Solids:	Time (min)
glassware (empty)	15
metal instruments (unwrapped)	15
metal instruments (in covered tray or wrapped in paper)	20
bulky items (paper towels, Kleenex)	30
Liquids:	
75 mL	20
500 mL	25
1000 mL	30
2000 mL	40
Bagged Biohazardous Waste	
Bag should be OPEN at the top to allow steam to enter Add a cup of water before autoclaving to help displace the air from the bag Do Not pack chamber too tightly; steam must contact all parts of the load	> 60

Further Information on how to use autoclaves is on the Safety web pages at the Department of Biological Sciences.