

## AUTOCLAVE USE

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<http://biology.clc.uc.edu/fankhauser/Labs/Microbiology/Autoclave.htm>

Pasteur showed that once a medium is sterilized, it will remain sterile until contaminated by microorganisms (i.e., they do not appear by spontaneous generation.). Heating is one of the most convenient and effective methods of sterilization. Simple boiling at 100°C, however, is not completely effective for sterilization because thermophilic spores can survive this temperature. However, the boiling point of water goes up when exposed to increased pressures. In a pressure cooker where the pressure is 15 pounds/sq inch (1 atmosphere) above standard pressure (760 mm Hg), water boils at 121°C. This temperature kills all life forms in 15 minutes or less. These conditions have become the standard for sterilizing apparatus or small volumes of liquid: i.e., treat over boiling water at 15 pounds pressure for 15 minutes (commonly called "15 pounds for 15 minutes").

An autoclave is a piece of laboratory apparatus which acts as an automated pressure cooker. The conditions inside are thermostatically controlled so that heat is applied until 121°C is achieved, at which time the timer starts, and the temperature is maintained for the selected time.

### ILLUSTRATE THE AUTOCLAVE, LABEL THESE PARTS:

drain valve *	door	<b>INSTRUMENT CLUSTER:</b>
drain pipe	door latch	slow/fast exhaust*
water level	(note caution to avoid steam burns)	timer*
		pressure indicator*
		temperature indicator*

- \* (Enter the appropriate settings or numbers on the dials for the beginning of the timing segment when autoclaving media)

### USING THE AUTOCLAVE:

1. Close drain valve.
  2. Add 4 L (1 gallon) of water into the bottom of autoclave (fill to lower lip of opening.)
  3. Load material to be autoclaved, prepared as follows:
    - a. **liquids in bottles** 3/4ths filled, *caps on slightly loosened.*
    - b. **clean petri dishes** top side up, in can if desired.
    - c. **apparatus** wrap in brown paper, fold over edges, staple to hold.
    - d. **contaminated petri dishes** agar side down, in pan if plastic.
- NOTE: Do not load tall bottles near the front of the autoclave, the door hits them.
4. Close door, latch securely by pressing down firmly.
  5. Select appropriate exhaust: slow for liquids (3.a.), fast for apparatus (3.b - 3.d.).
  6. Select the time of treatment to begin the process (15 minutes for small volumes. For containers containing volumes over 1 liter: 30 minutes, over a gallon: 60 minutes.)
  7. **CAUTION:** After sterilization, when pressure has returned to zero, open up the door with care--the steam will burn your wrists if they are above the door.
  8. Remove hot items with the aid of gloves. For media-containing used plates, pour out any melted contaminated agar immediately and rinse glassware in hot water.
  9. **EMPTY WATER:** Open drain valve to empty water in bottom of autoclave (make sure drain is in proper position). Failing To drain the used water will result in plugging the drain.