# EXPERIMENT NO. 1

## Sterilization in Steam Autoclave

**Introduction**

Practice the procedures, especially the closing and opening of the autoclave lid, without switching on the power until the entire sequence can be performed without any difficulty. Use autoclave only after you demonstrate to the instructor your ability without referring back to this write-up.
The sterilization is carried out at $121^0$C. The time at which the material to be sterilized is to be held at $121^0$C in the autoclave (holding time) will depend upon volume of liquid and expected count of contaminants present. Usually, for upto 200 ml medium stored in 1L flask, a holding time of 15 minutes at $121^0$C is sufficient.

**Caution:** The steam causes severe burns than boiling water. The temperature and pressure relationship is shown in the below (Table-1). The heat of vaporization released upon the condensation of steam causes much severer damage than does the same quantity of boiling water. Always watch out for the hot metal parts on the autoclave. Wear heat-insulating (asbestos) gloves when handling hot autoclaved items.

Table:1 Steam Temperature and Pressure Relationship in Autoclave

| **Autoclave Pressure (psig)** | **Autoclave temperature (ºC)** | | | | |
|---|---|---|---|---|---|
| | **Head Space AIR DISCHARGE from autoclave** | | | | |
| | **100 %** | **66%** | **50%** | **33%** | **0%** |
| 0 | 100.0 (ºC) | | | | |
| 1 | 101.9 | | | | |
| 2 | 103.6 | | | | |
| 3 | 105.3 | | | | |
| 4 | 106.9 | | | | |
| 5 | 108.4 (ºC) | 100 (ºC) | 94 (ºC) | 90 (ºC) | 72 (ºC) |
| 6 | 109.8 | | | | |
| 7 | 111.3 | | | | |
| 8 | 112.6 | | | | |
| 9 | 113.9 | | | | |
| 10 | 115.2 | 109 | 105 | 100 | 90 |
| 11 | 116.4 | | | | |
| 12 | 117.6 | | | | |
| 13 | 118.8 | | | | |
| 14 | 119.9 | | | | |
| 15 (~ 1.01 Kg/cm$^2$) | 121.0 | 115 | 112 | 109 | 100 |

Around the working range of 15 psi, each psi raises the autoclave temperature by approximately 1ºC.

**Operating Procedures**

1. Check the water level in level gauge and add de-mineralized (DM) water, if necessary. The electrical heater will be damaged if it runs dry. The DM-water will minimize scale formation.

2. Place the material to be sterilized in autoclave. Make sure that all the components of each item can withstand the heat of autoclaving; many plastic materials will melt or deform. Leaving a few drops of water inside a closed container where the steam in the chamber cannot easily penetrate will enhance the sterilization effect. Vent all containers to avoid explosion. If bottles are capped, make sure that the caps are screwed on lightly so that any excess pressure can escape. The caps can be screwed tightly later as they are taken out from the autoclave. At the same time, make sure that everything is closed so that contaminants cannot enter after autoclaving. Note that the need for pressure relief and prevention of contaminant entry are not contradictory. For example, shaker flasks can be vented with cotton plugs; fermentor jars, and nutrient jars can be vented with in-line filter units. On the other hand, some items to be autoclaved cannot be conveniently vented; they must be placed in a protective steel casing. The glass pH electrodes are such examples. Cover the cotton plugs with aluminum foil to prevent their wetting by condensing steam. Now close the lid and engage the locking mechanism.

3. Open the exhaust valve. (Not required in autoclaves fitted with vacuum breaker valve)

4. Check that pressure controller is set at 15 Kg/cm$^2$. Now switch on the electrical heater.

5. With increase in temperature, discharge of air present in headspace will begin through the exhaust valve. Wait till all the air escapes and steam begins to come out. Now close the exhaust valve. (Not required in autoclaves fitted with vacuum breaker valve). If exhaust valve is closed before the complete air discharge, controller set at 15 Kg/cm$^2$ will maintain total pressure of steam & air in the autoclave but the temperature achieved will correspond approximately only to partial pressure of steam and not to the total pressure, and therefore, it will be less than 121$^0$C. Refer to Table-1 for actual temperature with respect to extent of air discharge at various total pressures in autoclave. Wait till the pressure in autoclave reaches 15 Kg/cm$^2$. Start the timer set for the duration of holding time. After completion of holding time (15-20 minutes), the timer will automatically switch off power supply to heater. This sets the autoclave in cooling mode.

6. The autoclave's steam pressure will now begin to fall. Allow the pressure to come down only by ambient cooling. Do not open the exhaust valve to accelerate fall of autoclave's pressure. This will cause unnecessary loss of water from media in flasks as well as in autoclave from due to rapid boiling. At best you could keep it crack opened so that steam flushes out very slowly. If exhaust valve is not kept crack-opened as per instruction at step-6, vacuum will be created during cooling and this may damage the autoclave.

7. Check the chamber pressure in Pressure gauge in the Fig-1 and if it is zero Kg/cm$^2$ Open the exhaust valve completely (not required for autoclave fitted with vacuum breaker valve). Do not attempt to open the lid unless the chamber pressure is at zero Kg/cm$^2$. Opening a chamber filled with pressurized steam can be very dangerous. Keep your face outside the headspace of autoclave. Watch out for a small puff of residual hot steam being released, as the door is first opened. With this caution open the lid of the autoclave.

8. Again, remember to wear gloves. Watch out for the boiling liquid and handle the hot autoclaved items with great care so as not to burn oneself. Do not touch the hot chamber wall. Now is the time to make sure that the sterile side of all the sterilized items are not open to invasion by contaminants. For example, plug the mouth of shaker flasks if the plugs have come off during autoclaving. Check all connections on a fermentor jar to make sure that they all remain sealed and that no tubing has been broken. Reconnect them quickly whenever possible. Remove all autoclaved items from the chamber.

9. Clean up any spills and close the autoclave door when done.

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## Fig: 1 Schematic set up of an autoclave