Autoclave

An Autoclave is the most essential instrument used in microbiology laboratory for sterilization. It is based on the principle that saturated steam under pressure kills microorganisms. The water boils at 100°C and the steam accumulates in a closed container resulting in an increase in pressure. It is not the pressure that kills the organisms but the high temperature of the steam. The boiling point of water at 15psi pressure is 121°C. Most of the organisms are killed at 121°C (15psi) in 15 minutes including the heat-resistant spore-formers. Steam temperature increases with increase in steam pressure. The autoclave is used for sterilization of media both solid and liquid, heat stable liquids, heat resistant instruments glassware and rubber products. It is also used to sterilize glassware when required.

Design of autoclave

An autoclave is a double –walled metallic vessel. The body is usually made up of steel or aluminium. The lid is provided with a pressure guage for recording the pressure, steam cock (exhaust value) for air exhaustation, a safety value to avoid explosions. Both vertical and horizontal types of autoclaves are available, but for routine laboratory use vertical types are commonly used.

Operation:

1. In standard vertical autoclave, sufficient quantity of distilled water is added at the bottom of the autoclave till the mark so that the heating coil dips completely.
2. The objects to be sterilized are wrapped properly in a paper or aluminium foil and loaded in the basket provided and placed at the bottom of the autoclave.

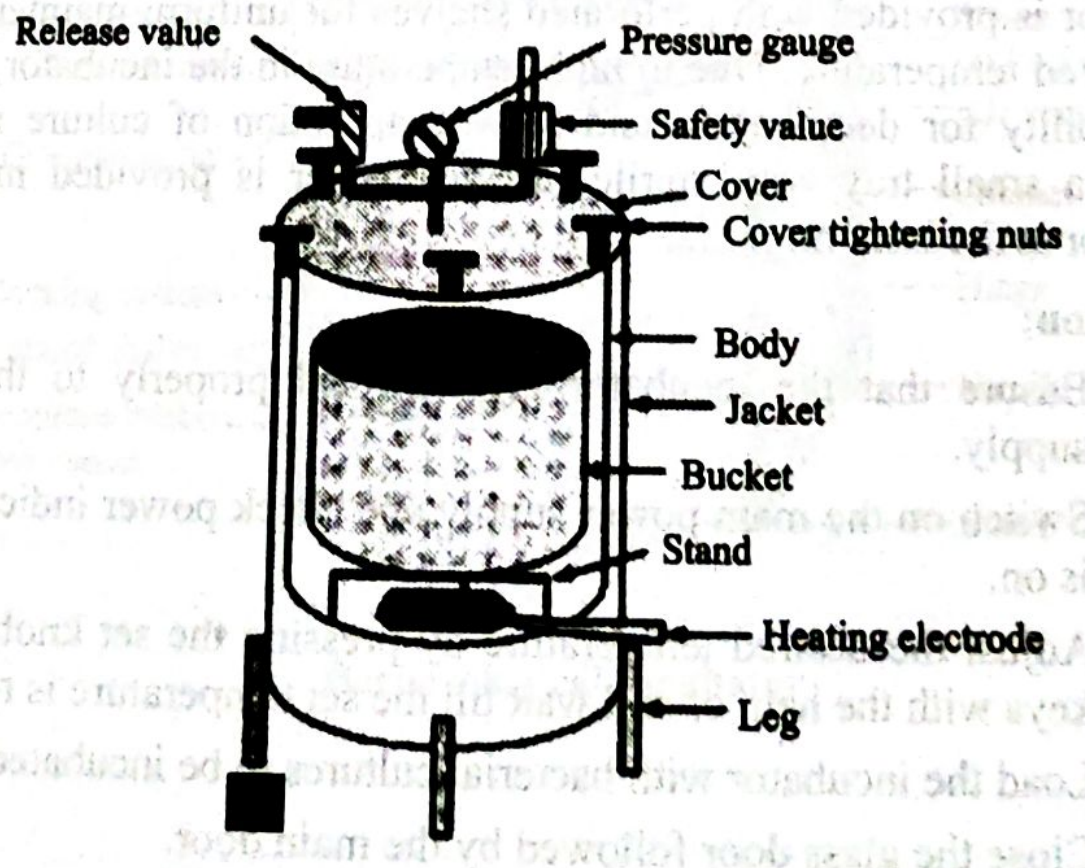
3. Objects are loaded in such a way that facilitates the steam to be directly in contact with the surface.
4. Now place the lid of the autoclave and tighten the opposite screws provided.
5. Switch on the autoclave by keeping the steam release valve open so that air inside the autoclave is allowed to escape completely through this valve.
6. Close the valve when water vapor is seen to escape through it.
7. Temperature and pressure inside goes on increasing. The pressure increase is observed in the pressure gauge.
8. Usually, sterilization is done at 121 °C (a pressure of 15 pounds per square inch i.e. 15 psi) for 15 minutes.
9. The required time is considered from the point, when the required temperature-pressure is attained. Once required temperature-pressure is attained, it is maintained.
10. After the specified time (15 minutes), switch off the autoclave and open slightly the steam release valve. If fully opened immediately, due to sudden fall in pressure, liquids may spill out from the containers.
11. Autoclave lid is opened only after the pressure drops back to normal atmospheric pressure (0 psi).
12. Unload the hot sterilized materials by holding them with a piece of clean cloth or asbestos- coated hand gloves.
13. Transfer the sterilized material immediately to laminar air flow.

Relationship between pressure and temperature in an autoclave:

Pressure in pounds per Square inch (psi)	Temperature in °C
5	109
10	115.6
15	121.0
20	126.0
25	130

Precautions:

1. The water level inside the autoclave should be well above the heating coil.
2. If water is less, the bottom of the autoclave gets dried during heating and burns the heating coil. On the other hand, if there is too much water, it takes a long time to reach the required temperature.
3. Always tighten the opposite screw while closing the lid on all sides.
4. The air in the chamber of autoclave must be completely replaced by pure steam. Keep open the steam outlet until pure steam starts going out.
5. The autoclave should never be opened, when there is pressure inside.
6. The required 15 psi pressure must be maintained constantly for 15 minutes
7. Overcooking of the medium will change the composition of the medium and may not support microbial growth, Agar may also lose the gelling (solidifying) property
8. Do not open the lid until the pressure gauge shows zero.
9. The Autoclave should get validated periodically.



A Schematic diagram of laboratory autoclave