AUTOCLAVE

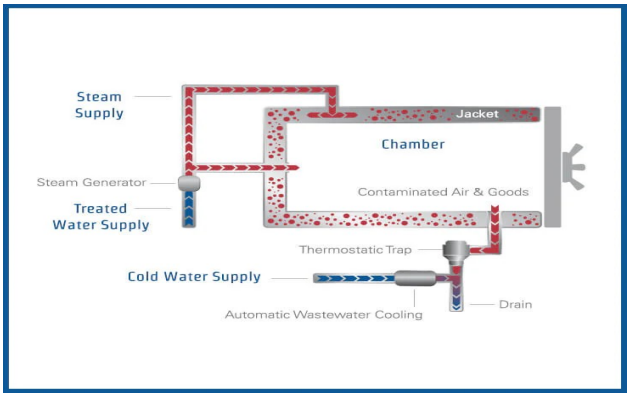
Autoclave: Working Principle and user manual

The maintenance of aseptic conditions is an absolute necessity in a tissue culture lab. several sterilization techniques used in labs, their purposes, and the machines used to perform the process.

Two most commonly used instruments used in plant tissue culture labs for the sterilization of equipment and materials are autoclave and microwave. They both work on two different principle of moist heat sterilization (Autoclave) and dry heat sterilization (microwave).

PRINCIPLE OF AUTOCLAVE

The autoclave works on the principle of moist heat sterilization. The high pressure inside the chamber increases the boiling point of water for the sterilization of equipment, while ensuring the rapid penetration of heat into the deeper parts of equipment. The moisture present in the steam causes coagulation of proteins of microbes causing irreversible loss of their activity and functions. Thus, killing them and sterilizing the equipment.



Working principle of Autoclave:

All size of autoclaves work on a single principle, that involves three cyclic phases of sterilization that are given below:

1. Purge phase: Air present in the sealed chamber is displaced with steam that moves in through the sterilizer.
2. Exposure phase: In this phase, the exhaust valve is closed and the temperature and pressure inside the sealed chamber are increased to the desired set point. The temperature is maintained for the set duration of time.
3. Exhaust phase: The exhaust valve is opened, steam is removed, and the chamber is restored to normal temperature.

Components of Autoclave and Their Functions

1. Pressure chamber

The pressure chamber is the main body of the autoclave. It consists of an inner chamber and an outer jacket. Generally, the inner chamber is made of stainless steel/gunmetal and the outer chamber is made of the iron case.

The autoclave in the labs and hospital comes with a jacketed chamber that is filled with steam and designed to reduce the time and cycle of sterilization. These autoclaves can range from sizes anywhere from 100L to 3000L. So, you can buy the machine according to your requirements.

2. Lid/Door

The lid, disconnect the chamber from the outside atmosphere and seal it to create the desired temperature and pressure inside the autoclave. Its consists of three other parts: Pressure gauge, whistle, and safety valve.

The pressure gauge shows the pressure build-up inside the autoclave and assures the safety of the machine and working conditions. The whistle present in the autoclave is the same as that of the domestic pressure cooker. It controls the pressure inside the chamber by releasing a certain amount of vapor.

The other crucial part of the autoclave is the safety valve. It has a thin layer of rubber, which bursts itself to release the pressure inside the chamber if the autoclave fails to perform its operations. It ensures your safety from any kind of autoclave explosion.

3. Steam generator

A steam generator is present underneath the chamber. It has an electric heating system that heats water to generate steam inside the chamber. Always, ensure the right volume of water is available in the generator to run the process smoothly and avoid burning or heating of autoclave parts.

4. Vacuum generator

This removes air from the chamber as the presence of any air pockets in the chamber might support the growth of any organism and your equipment will not be sterilized.

5. Waste-water cooler

Autoclaves are equipped with a waste-water cooler that cools the effluent (air, steam, and condensate) before it enters the draining pipes. It avoids damage of draining pipes that can be caused by extremely heated water.

Autoclave: Calibration

- Autoclave is one of the crucial machines of a tissue culture lab. It's required to sterilize media, culture vessels, tools, and other materials and equipment. However, you need to be alert (see precautionary points in the next section of this blog) while using the equipment.
- Here's the step-wise procedure of using an autoclave in your lab:
- Cover the top of the flask containing media with foil and place a piece of an autoclave tape over the foil. An autoclave tape is a normal looking tape that turns into a strip containing black diagonal lines when exposed to high temperature. This helps you to be aware of the sterilized containers. If you've prepared media in bottles, do not tighten the cap, just keep it loose about half way and place the autoclave tape on top of it.
- Turn on the power and ensure the drain valve of the autoclave is closed.
- Add deionized water to the level-indicator line.
- Now place the flask of culture media, tools, and other materials into the basket. Make sure there's enough space between each item and nothing is touching the wall and bottom floor of the autoclave.
- Place the basket in the autoclave and close the lid. Turn the handle to create an

air tight seal.

- Use the control panel to set the temperature to 121 degree Celsius and the pressure to 15 psi.
- Run autoclave for at least 15-20 minutes.
- Once the cycle ends and pressure gauge show 0 psi, slowly open the lid of the autoclave while wearing heat-resistant gloves.
- Take out your materials from the autoclave and let them cool before use.
- And, done! Your media and tools are ready to use. Just ensure opening them inside the laminar flow hood for use.
- Be Precautious while using Autoclaves
- Do not sterilize waterproof or water-resistant materials like oil or powders.
- Do not overcrowd the autoclave with the vessel and equipment. If possible sterilize your equipment in a shift-wise manner.
- Only use autoclavable bags to autoclave packages wastes.
- Use autoclavable bags to sterilize your equipment. Do not use aluminum foils.
- Do not fill the autoclave chamber up to the lid.
- Never attempt to open the autoclave while it’s operating.
- Tightly close the lid to ensure the completely closed condition of the autoclave for proper sterilization.
- Do not use regular plastics or trays in the autoclave.
- Never autoclave flammable, reactive, corrosive, toxic, or radioactive materials, household bleach, or paraffin-embedded tissue.
- Fill the water in the steam generator up to the volume where it touches the end of the vessel or chamber of the autoclave.