Autoclave

1. Definition

Autoclave, also known as steam sterilizer, is the most effective machine used for the sterilization of lab equipment, water, or media. The machine uses steam under pressure to kill bacteria, viruses, and spores present in/on the equipment or culture media. It is used in scientific labs, healthcare facilities, and industrial operations for the sterilization of a range of tools, vessels, solutions or media, and materials.

The first autoclave was invented by Charles Chamberland in 1879. But, the concept of disinfection and sterilization was introduced in 1881 by Robert Koch. Then in 1933, the modern autoclave, the first pressure steam sterilizer with control performance, was developed.

2. 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.

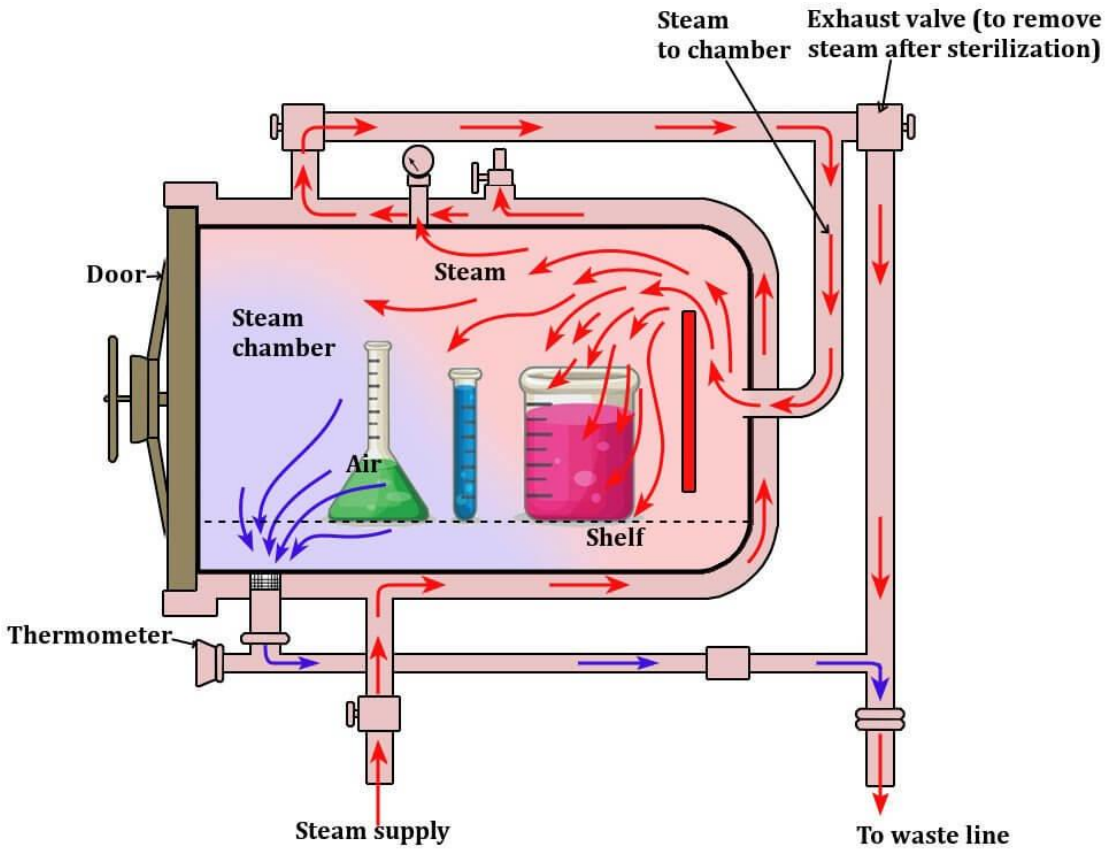

Fig 1: Working or principle of autoclaves. Source: https://microbenotes.com/

3. HOW DOES AUTOCLAVE WORK?

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

- Purge phase: Air present in the sealed chamber is displaced with steam that moves in through the sterilizer.
- 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.
- Exhaust phase: The exhaust valve is opened, steam is removed, and the chamber is restored to normal temperature.

Procedure for running an autoclave

In general, an autoclave is run at a temperature of 121° C for at least 30 minutes by using saturated steam under at least 15 psi of pressure. The following are the steps to be followed while running an autoclave:

- Before beginning to use the autoclave, it should be checked for any items left from the previous cycle.
- A sufficient amount of water is then put inside the chamber.
- Now, the materials to be sterilized are placed inside the chamber.
- The lid is then closed, and the screws are tightened to ensure an airtight condition, and the electric heater is switched on.
- The safety valves are adjusted to maintain the required pressure in the chamber.
- Once the water inside the chamber boils, the air-water mixture is allowed to escape through the discharge tube to let all the air inside to be displaced. The complete displacement can be ensured once the water bubbles cease to come out from the pipe.
- The drainage pipe is then closed, and the steam inside is allowed to reach the desired levels (15 lbs in most cases).
- Once the pressure is reached, the whistle blows to remove excess pressure from the chamber.
- After the whistle, the autoclave is run for a holding period, which is 15 minutes in most cases.
- Now, the electric heater is switched off, and the autoclave is allowed to cool until the pressure gauge indicates the pressure inside has lowered down to that of the atmospheric pressure.
- The discharge pipe is then opened to allow the entry of air from the outside into the autoclave.
- Finally, the lid is opened, and the sterilized materials are taken out of the chamber.

4. Components of Autoclave and Their Functions

A. 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.

B. 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.

C. Steam generator (if applicable)

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.

D. Vacuum generator (if applicable)

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.

E. 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.

Fig 2: A schematic diagram of autoclaves with their different labeled parts. Source: Microbenotes

5. Types of Autoclave

There are different types of autoclaves present in the market, some of which are:

- **Pressure cooker type/ Laboratory bench autoclaves (N-type)**

These, as domestic pressure cookers, are still in use in many parts of the world. The more modern type has a metal chamber with a secure metal lid that can be fastened and sealed with a rubber gasket. It has an air and steam discharge tap, pressure gauge, and safety valve. There is an electric immersion heater at the bottom of the chamber.

- **Gravity displacement type autoclave**

This is the common type of autoclave used in laboratories. In this type of autoclave, the steam is created inside the chamber via the heating unit, which then moves around the chamber for sterilization. This type of autoclave is comparatively cheaper than other types.

- **Positive pressure displacement type (B-type)**

In this type of autoclave, the steam is generated in a separate steam generator which is then passed into the autoclave. This autoclave is faster as the steam can be generated within seconds. This type of autoclave is an improvement over the gravity displacement type.

6. **Negative pressure displacement type (S-type)**

This is another type of autoclave that contains both the steam generator as well as a vacuum generator. Here, the vacuum generator pulls out all the air from inside the autoclave while the steam generator creates steam. The steam is then passed into the autoclave. This is the most recommended type of autoclave as it is very accurate and achieves a high sterility assurance level. This is also the most expensive type of autoclave.

Fig 3: Types of autoclave used in different settings. Source: Microbeonline (https://plantcelltechnology.com/)

7. **How To Use An Autoclave?**

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.

8. autoclaves precautions

Although autoclaves are pretty simple to use, there are certain rules of precautions to be followed while operating an autoclave. Some of the important precautions to be followed while running an autoclave are:

- Autoclaves should not be used to sterilize water-proof or water-resistant substances like oil or powders.
- The autoclave should not be overcrowded, and the materials should be loaded in a way that ensures sufficient penetration of articles by the steam.
- TheThe items to be autoclaved should always be placed in a secondary container.
- Only autoclavable bags are to be used to autoclave packaged waste.
- To ensure sufficient penetration, articles should be wrapped in something that allows penetration by steam, and materials like aluminum foils should not be used.

- The items placed inside the chamber should not touch the sides or top of the chamber.
- The wastes and clean items should be autoclaved separately.
- Attempts to open the lid when the autoclave is working should never be made.
- Liquid components should never be autoclaved in sealed containers.
- The liquid inside the containers should only be filled 2/3rd of the total volume to prevent the spilling of the liquid.
- Plastic or polyethylene trays or containers should not be used as they might melt and damage the autoclave.
- Besides, never autoclave flammable, reactive, corrosive, toxic, or radioactive materials, household bleach, or paraffin-embedded tissue.
- The paper should not be placed directly inside an autoclave as it is a combustible substance. It should be autoclaved in a waste bag on a bio bag setting to prevent fire.

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.

It's short and crisp information that you should know about autoclaves. It's always better to understand the use and principle of equipment you are working with for a better understanding of the science behind it, how it works, and if anything goes wrong, you know what it is!!