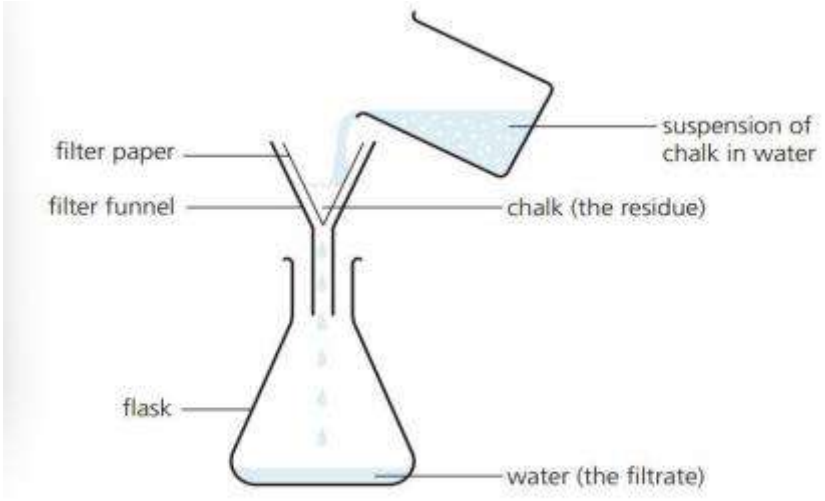
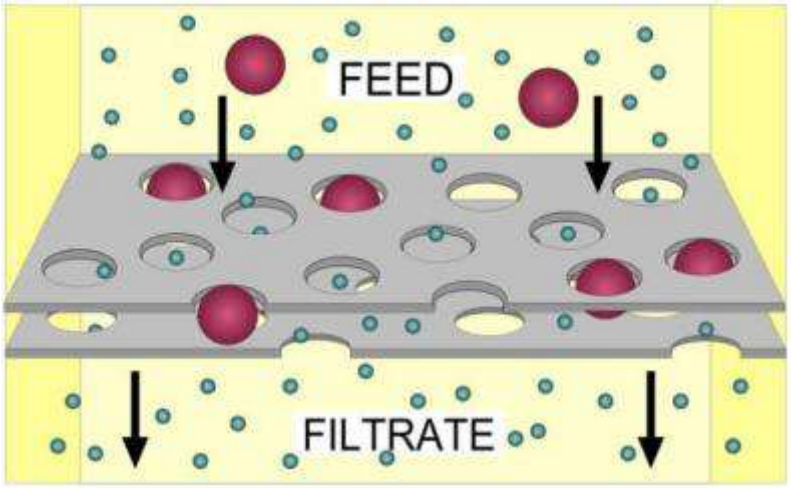
Filtration apparatus

What is Filtration?

- Filtration, the technique used to separate solids from liquids, is the act of pouring a mixture onto a membrane (filter paper) that allows the passage of liquid (the filtrate) and results in the collection of the solid. Two filtration techniques are generally used in chemical separations in general chemistry lab: "gravity" filtration and "vacuum" filtration.
- The most convenient ways of filtration are either through a filter paper or through a filter crucible. it is widely used in industry and daily base uses for the separation of solids from a suspension or colloidal solution. the most advanced example of using filtration for blood during dialysis.



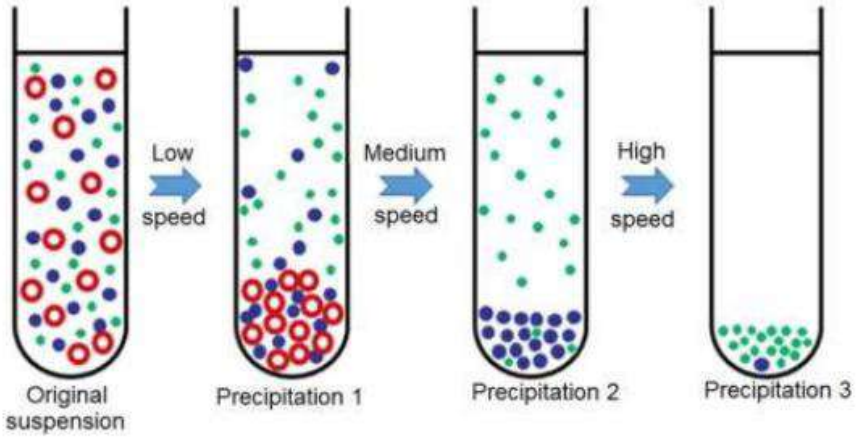


Types of Filtration

1- Gravity Filtration: This is where the mixture is poured from a higher point to a lower one. It is commonly done through simple filtration, using filter paper in a glass funnel, where the insoluble solid particles are captured by the filter paper and the liquid goes right through by gravity's pull. Depending on the volume of the substance at hand, filter cones, fluted filters, or filtering pipets can be used.

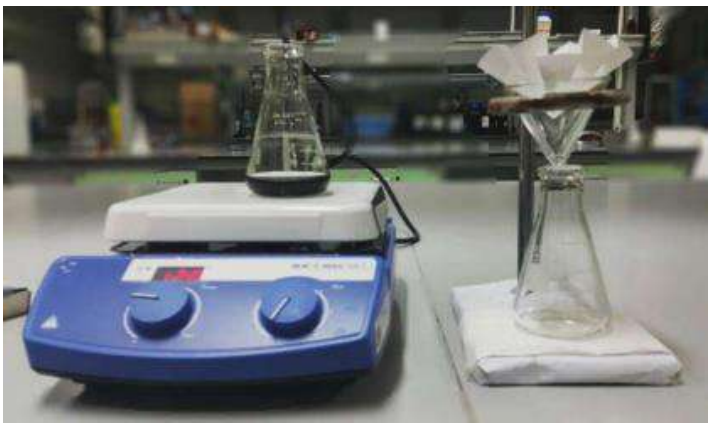
2- Vacuum Filtration: In vacuum filtration, a vacuum pump is used to rapidly draw the fluid through a filter. Hirsch funnels and Buchner funnels, which are the same kind of funnel in two different sizes, are used along with filter paper. The funnels have a plate with holes in it, as we can see below, and they are usually used when the substance to be filtered is small in volume.

3- Centrifugal Filtration: This kind of filtration is done by rotating the substance to be filtered at very high speed. Due to the horizontal rotation, the more dense matter is separated from the less dense matter.



4- Cold Filtration: Cold filtration makes use of very low temperatures, often by using an ice bath. Some substances, such as fatty acid particles, become suspended in the mixture as they cool down, which then allows us to filter them out more easily.

5- Hot Filtration: This is often used for crystalline compounds that contain impurities. The way this filtration is done is by melting down the crystalline compound, removing the impurities as the substance is still in liquid form, and finally recrystallizing the now pure substance. Often, it is recommended that the apparatus used in this filtration be heated up so that the filtered substance doesn't crystallize in the funnel and block the flow.



6- Multilayer Filtration: This can refer to multiple layers of different material, including sand, gravel, or charcoal, where the different layers contain different particle sizes of that material. In this type of filtration, a mixture of liquid and insoluble solid particles is poured over the layers, and the solid particles are caught throughout, resulting in a filtered liquid.

Difference between Filtration and sieving.

- Filtration differs from sieving, where separation occurs at a single perforated layer (a sieve). In sieving, particles that are too big to pass through the holes of the sieve are retained. In filtration, a multilayer lattice retains those particles that are unable to follow the tortuous channels of the filter.

Filtration Sieving

Applications of Filtration

1. Industrial air cleaning.
2. Filtration, as a physical operation is very important in chemistry for the separation of materials of different chemical composition.
3. Pharmaceutical industry applications.

4. Nuclear facility filters.
5. Cleaning of water, like river water, from impurities.
6. Used for sterilization without the use of heat, as long as the filter's pores are small enough to catch the microorganisms.

Polymerase chain reaction (PCR)

What is PCR?

- Polymerase chain reaction (PCR) is one of the most important biotechnological tools developed. It refers to a biological technique that helps to produce several copies of DNA outside of any living cell. DNA polymerase is the key enzyme that is present behind the whole process. The enzyme involved in the synthesis of new DNA strands by binding with a single DNA strand.
- This technique was developed by Kary Mullis in 1983 who was awarded the Nobel Prize in 1993 for this achievement. The development of recombinant DNA technology is mostly dependent on this technique.
- PCR is also valuable in a number of laboratory and clinical techniques, including DNA fingerprinting, detection of bacteria or viruses, and diagnosis of genetic disorders.
- PCR is used in many areas of biology and medicine, including molecular biology research, medical diagnostics, and even some branches of ecology.
- PCR is very simple, inexpensive technique for characterization, analysis and synthesis of specific fragments of DNA or RNA from virtually any living organisms.
- PCR is a fast technique used to "amplify" - copy - small segments of DNA. Because significant amounts of a sample of DNA are necessary for molecular and genetic analyses, studies of isolated pieces of DNA are nearly impossible without PCR amplification.

Principle and Working Mechanism of PCR

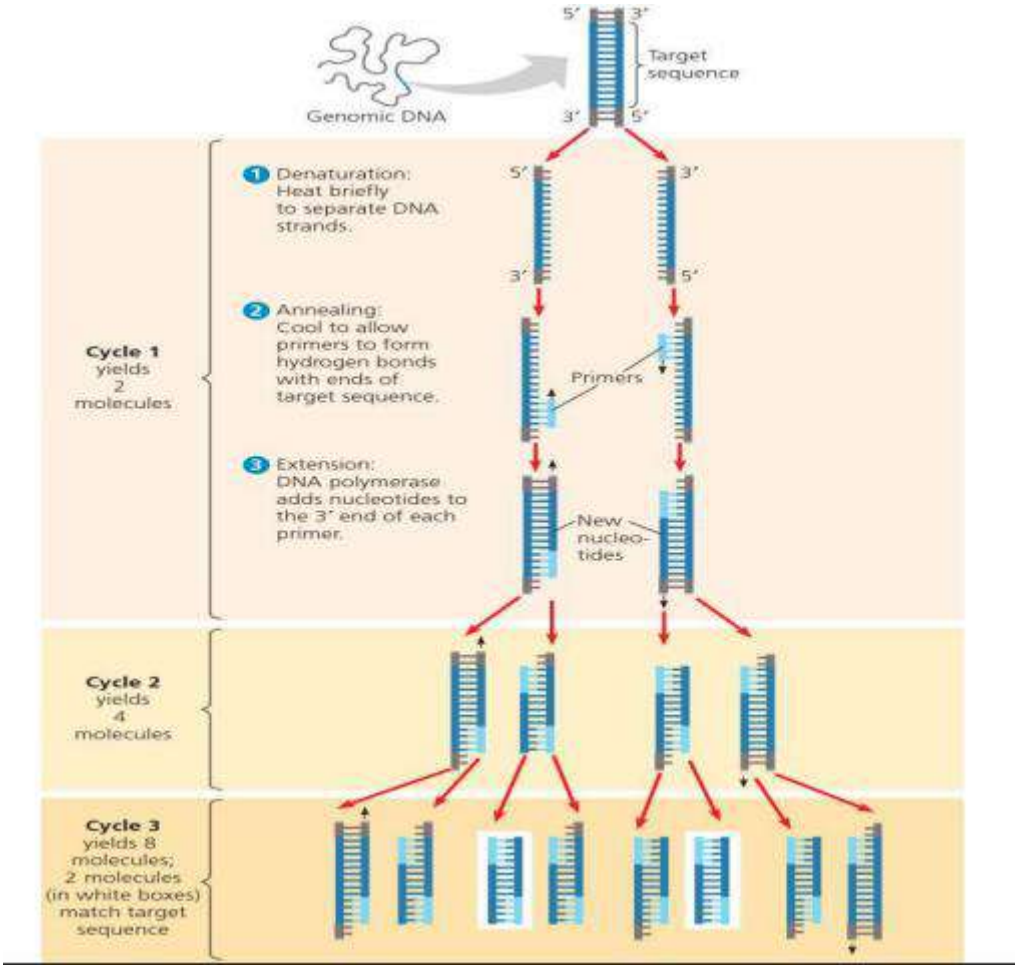
- To amplify a segment of DNA using PCR, the sample is first heated so the DNA denatures, or separates into two pieces of single-stranded DNA. Next, an enzyme called "Taq polymerase" synthesizes - builds - two new strands of DNA, using the original strands as templates. This process results in the duplication of the original DNA, with each of the new molecules containing one old and one new strand of DNA. Then each of these strands can be used to create two new copies, and so on, and so on. The cycle of denaturing and synthesizing new DNA is repeated as many as 30 or 40 times, leading to more than one billion exact copies of the original DNA segment.
- The entire cycling process of PCR is automated and can be completed in just a few hours. It is directed by a machine called a thermocycler, which is programmed to alter the temperature of the reaction every few minutes to allow DNA denaturing and synthesis.

The major steps of PCR can be divided into three parts:

Denaturation : This step involves heating the reaction mixture to 94°C for 15-30 seconds. During this, the double stranded DNA is denatured to single strands due to breakage in weak hydrogen bonds.

Annealing : The reaction temperature is rapidly lowered to 54-60°C for 20-40 seconds. This allows the primers to bind (anneal) to their complementary sequence in the template DNA.

Elongation : Also known at extension, this step usually occurs at 72-80°C (most commonly 72°C). In this step, the polymerase enzyme sequentially adds bases to the 3′ each primer, extending the DNA sequence in the 5′ to 3′ direction. Under optimal conditions.



A basic PCR set-up requires several components and reagents, including:

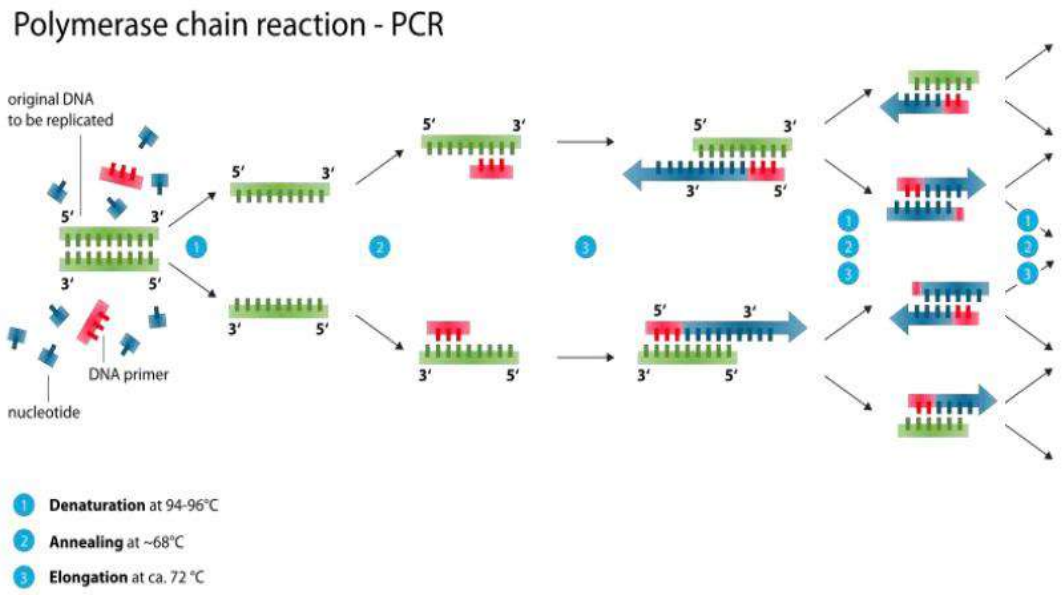
1- **DNA Template** : The double stranded DNA (dsDNA) of interest, separated from the sample.

2- **DNA Polymerase** : Usually a thermostable Taq polymerase that does not rapidly denature at high temperatures (98°), and can function at a temperature optimum of about 70°C.

3- **Oligonucleotide primers** : Short pieces of single stranded DNA (often 20-30 base pairs) which are complementary to the 3' ends of the sense and anti-sense strands of the target sequence.

4- **Deoxynucleotide triphosphates** : Single units of the bases A, T, G, and C (dATP, dTTP, dGTP, dCTP) provide the energy for polymerization and the building blocks for DNA synthesis.

5- **Buffer system** : Includes magnesium and potassium to provide the optimal conditions for DNA denaturation and renaturation; also important for polymerase activity, stability and fidelity.



Types of PCR :

1- Standard PCR.

2- Reverse transcriptase Polymerase chain reaction (RT-PCT): for RNA

- One step RT-PCR
- Two step RT-PCR

3- Real time PCR: for DNA or RNA

- Dye binding to ds DNA
- Fluorescent probes

Applications of PCR

1. Forensic science: DNA finger printing, paternity testing and criminal identification
2. Diagnosis: Molecular identification of microorganisms
3. Gene cloning and expression
4. Gene sequencing
5. Vaccine production by recombinant DNA technology
6. Drug discovery
7. Mutation study
8. Human genome project

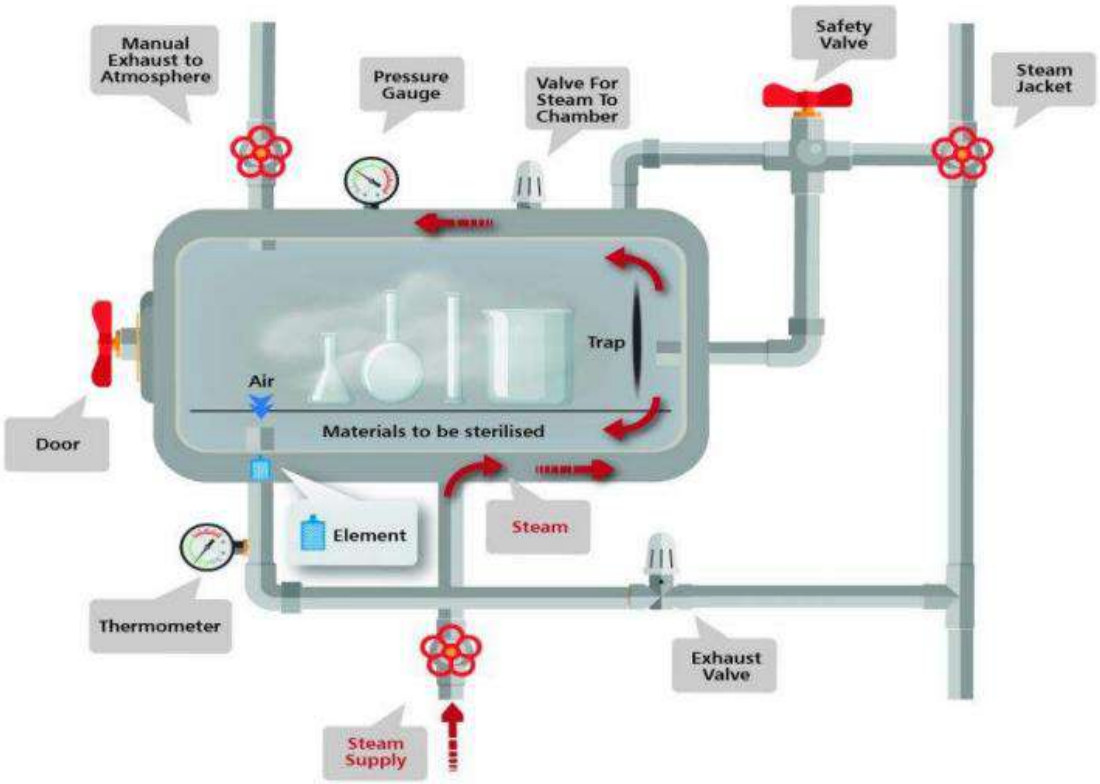
Autoclave

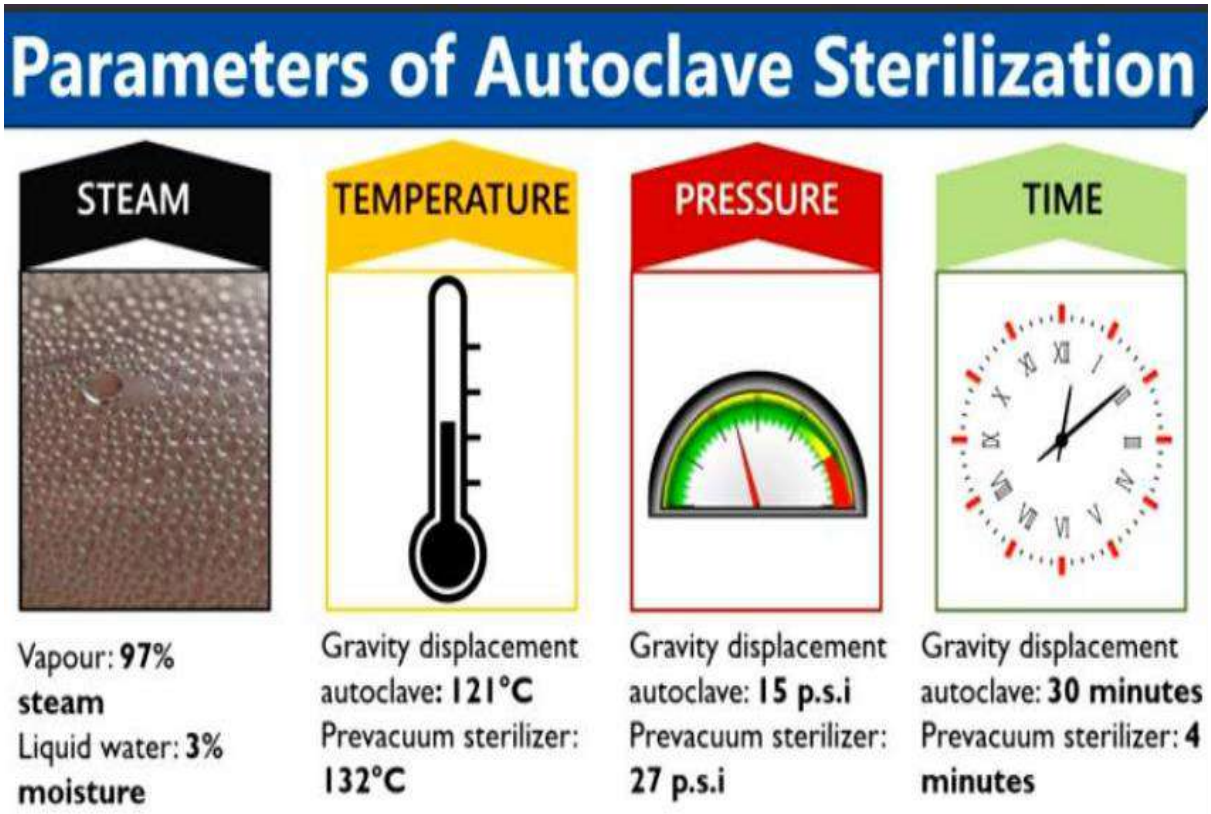
What is Autoclave ?

- Autoclaves are also known as steam sterilizers, and are typically used for healthcare or industrial applications.
- An autoclave is a machine that uses steam under pressure to kill harmful bacteria, viruses, fungi, and spores on items that are placed inside a pressure vessel. The items are heated to an appropriate sterilization temperature for a given amount of time.
- The moisture in the steam efficiently transfers heat to the items to destroy the protein structure of the bacteria and spores.
- It is performed in a machine known as the Autoclave where high pressure is applied with a recommended temperature of 121°C for 15-20 minutes to sterilize the equipment. Autoclaving or steam sterilizer is used in several industries including medicine, dentistry, microbiology and veterinary science.
- The Steam Digester, now commonly known as a pressure cooker, was invented by French physician Denis Papin around 1681 for food preparation. The autoclave was re-invented for medical and scientific use by Charges Chamberland in 1879.

Principle of Autoclave

- It depends upon the principle of moist heat sterilization, in which goods are exposed to direct steam under controlled temperature and pressure for a specific time. Thus, autoclaving depends on steam quality, pressure, temperature, and time duration. The autoclave uses moist heat, which kills microbial cells, intracellular structures and spores via absolute denaturation. Moist heat coagulates enzymes and structural proteins.
- If bulky objects are being sterilized, heat transfer to the interior will be slow, and the heating time must be sufficiently long so that the object is at 121°C for 15 min. Extended times are also required when large volumes of liquids are being autoclaved because large volumes take longer to reach sterilization temperature.


Manual Exhaust to Atmosphere
Pressure Gauge
Valve For Steam To Chamber
Safety Valve
Steam Jacket
Trap
Air
Materials to be sterilised
Door
Element
Steam
Thermometer
Exhaust Valve
Steam Supply


Parameters of Autoclave Sterilization
STEAM
TEMPERATURE
PRESSURE
TIME
Vapour: 97% steam
Liquid water: 3% moisture
Gravity displacement autoclave: 121°C
Prevacuum sterilizer: 132°C
Gravity displacement autoclave: 15 p.s.i
Prevacuum sterilizer: 27 p.s.i
Gravity displacement autoclave: 30 minutes
Prevacuum sterilizer: 4 minutes

Autoclave Sterilization Cycle

Purge Phase

It involve displacement of the air inside the pressure vessel. Different autoclaves use different means of air displacement.

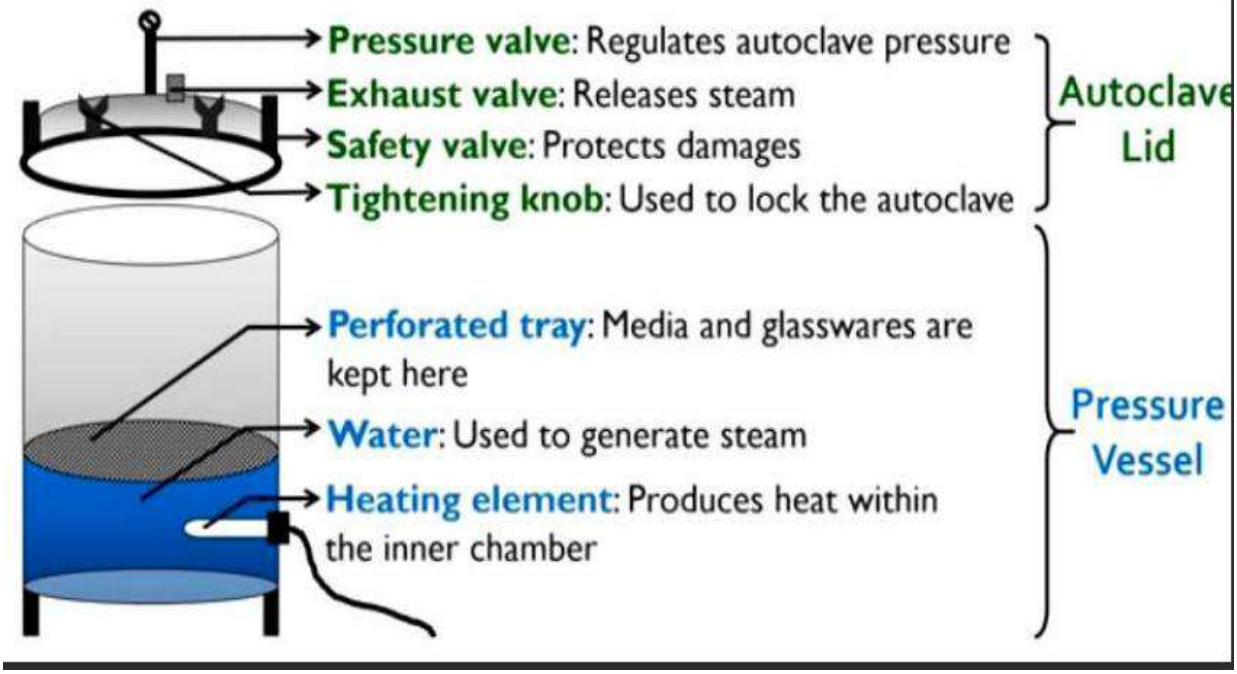
Exposure (Sterilization) Phase

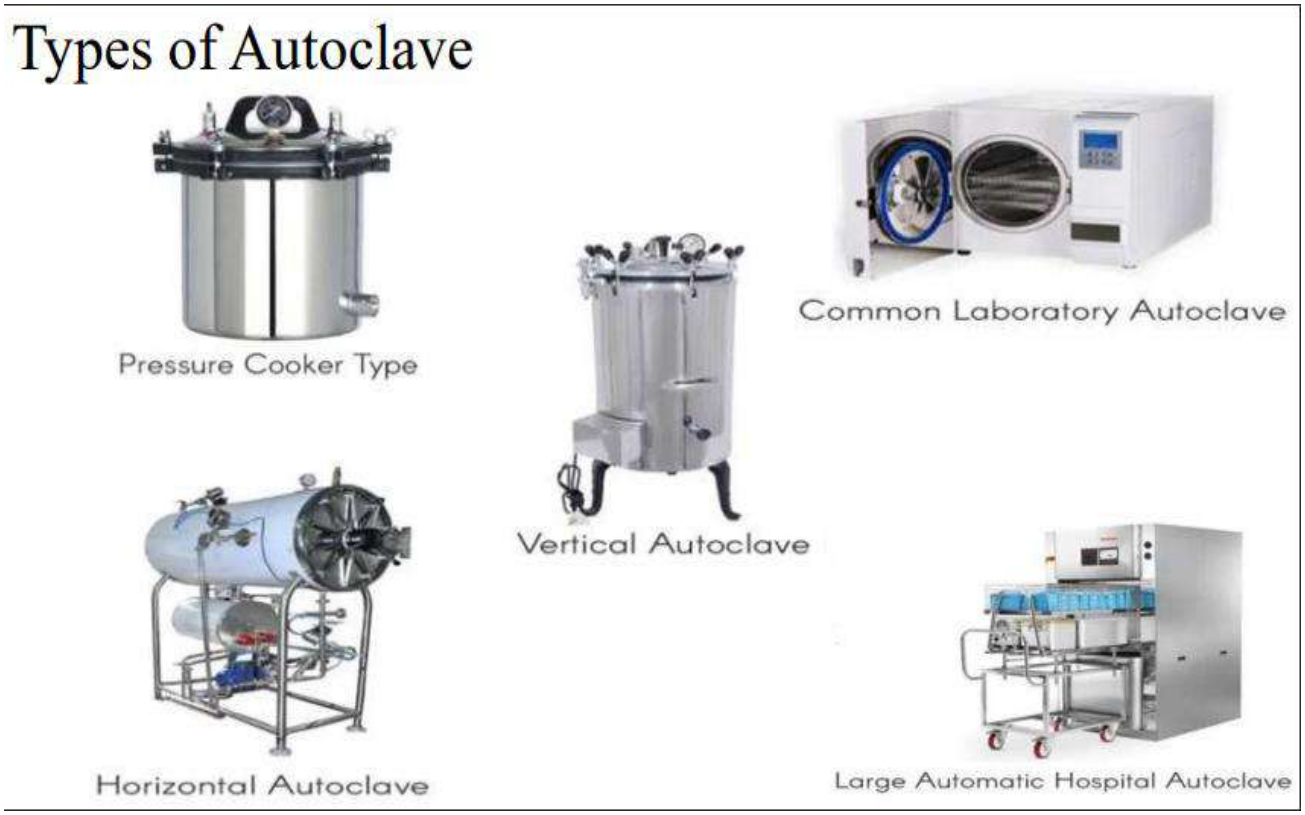
It supplies **continues steam** in to the pressure vessel. Items inside the chamber meet the exposure phase under the desired time and temperature.

Exhaust or final Phase

It is the stage of **depressurizing** the chamber. As the steam completely evaporates, take out the items.

Parts of Autoclave





Advantages & Limitations of an Autoclave

Advantages

1. Nontoxic and **inexpensive** method to conduct.
2. Rapid **microbicidal** and **sporicidal effect**.
3. **Penetrate** fabrics more effectively than the dry heat.
4. Helps in hardening the rubber and hydrothermal method.

Limitations

1. Might cause **corrosion** and combustion of lubricants.
2. Some plasticware might **melt** under high heat.
3. Many chemicals **break down** during the sterilization process.
4. **Time taking** process.

Uses of Autoclave

Autoclave is the method of choice for sterilizing the following:

1. Surgical instruments.
2. Culture media.
3. Autoclavable plastic containers.
4. Plastic tubes and pipette tips.
5. Biohazardous waste.
6. Glassware (autoclave resistible)