Autoclave

Sterilization: is the killing or removal of all microorganisms, including bacterial spores, which are highly resistant.

Advantages of sterilization:

1- Prevent transmission of diseases
2- Prevent contamination and growth of undesirable bacteria
3- Prevent spoilage of material by microorganisms.

Heat Sterilization

Heat sterilization is the most widely used and reliable method of sterilization, involving destruction of enzymes and other essential cell constituents. The process is more effective in hydrated state where under conditions of high humidity, hydrolysis and denaturation occur, thus lower heat input is required. Under dry state, oxidative changes take place, and higher heat input is required.

This method of sterilization can be applied only to the thermostable products, but it can be used for moisture-sensitive materials for which dry heat (160-180°C) sterilization, and for moisture-resistant materials for which moist heat (121-134°C) sterilization is used.

The efficiency with which heat is able to inactivate microorganisms is dependent upon the degree of heat, the exposure time and the presence of water. The action of heat will be due to induction of lethal chemical events mediated through the action of water and oxygen. In the presence of water much lower temperature time exposures are required to kill microbe than in the absence of water. In this processes both dry and moist heat are used for sterilization.

Dry Heat Sterilization: used for sterilizing different materials. Heated air or fire is used in this process. As compared to the moist heat sterilization, the temperature in this method is higher. The temperature is usually higher than 356° F or 180 °C.

Dry heat helps kill the organisms using the destructive oxidation method. This helps destroy large contaminating bio-molecules such as proteins. The essential cell constituents are destroyed and the organism dies. The temperature is maintained for almost an hour to kill the most difficult of the resistant spores. Hot air oven is the instrument in which this process is carried out

Moist Heat Sterilization

Water at high pressure level is used in moist heat sterilization. Autoclave is the instrument in which this process is carried out. The temperature of the steam in this method is lower when compared with dry heat sterilization, but the high pressure helps with effective sterilization to take place.

The structural proteins and the organism's enzymes are destroyed through moist heat. This results in the death of the organisms. Moist heat method is used for heat sensitive materials and materials through which steam is permeable. Culture media is also sterilized through moist heat sterilization.

Through moist heat sterilization, the most resistant of the spores require a temperature of 121°C for around half an hour. It is a more effective method when compared with dry heat sterilization. This can be supported by the fact that through moist heat, sterilization can be achieved at lower temperatures in a shorter duration.

These were the main difference between the dry and the moist heat sterilization methods. If you want to sterilize materials that are more heat sensitive when compared with both these methods, you should go for filter sterilization or chemical decontamination methods.

Definition of the Autoclave

An autoclave is a machine that provides a physical method of sterilization by killing bacteria, viruses, and even spores present in the material put inside of the vessel using steam under pressure. Autoclave sterilizes the materials by heating them up to a particular temperature for a specific period of time. The autoclave is also called a steam sterilizer that is commonly used in healthcare facilities and industries for various purposes. The autoclave is considered a more effective method of sterilization as it is based on moist heat sterilization.

Purpose of the Autoclave

The autoclave is equipment designed with the aim of reliably eliminating1 microorganisms, which would otherwise be present on objects used in diagnostic activities, in treatment or surveillance in health institutions (hospitals, laboratories). It is also widely used in the food processing and pharmaceutical industries.

In the laboratory, materials and objects are sterilized for the following purposes:

1. To prepare materials for bacteriological cell cultures (test tubes, pipettes, Petri dishes, etc.) in order to avoid their contamination.
2. Prepare elements used for taking samples. (All must be in sterile conditions: needles, tubes, containers).
3. Sterilize contaminated material.

Autoclaves are available in many sizes. The smallest are the table-top type and the largest are complex equipment that require a great amount of pre-installation for their operation. The volume of the sterilization chamber is taken as a reference and measured in cubic decimeters [dm^3] or in liters in order to measure the autoclave's size. Depending on how their operation is controlled, it is possible to find manual, semiautomatic or fully automatic models.

Autoclave components

The simplest form of the autoclave is the pressure cooker type or laboratory bench autoclaves. The following is the detailed description of different components/ parts of an autoclave:

1. Pressure Chamber

- The pressure chamber is the main component of a steam autoclave consisting of an inner chamber and an outer jacket.
- The inner chamber is made up of stainless steel or gunmetal, which is present inside the out chamber made up of an iron case.
- The autoclaves used in healthcare laboratories have an outer jacket that is filled with steam to reduce the time taken to reach the sterilization temperature.
- The inner chamber is the case where the materials to be sterilized are put.
- The size of the pressure chamber ranges from 100 L to 3000 L.

2. Lid/ Door

- The next important component of an autoclave is the lid or door of the autoclave.
- The purpose of the lid is to seal off the outside atmosphere and create a sterilized condition on ht inside of the autoclave.
- The lid is made airtight via the screw clamps and asbestos washer.
- The lid consists of various other components like:

Pressure gauge

- A pressure gauge is present on the lid of the autoclave to indicate the pressure created in the autoclave during sterilization.
- The pressure gauge is essential as it assures the safety of the autoclave and the working condition of the operation.

Pressure releasing unit/ Whistle

- A whistle is present on the lid of the autoclave is the same as that of the pressure cooker.
- The whistle controls the pressure inside the chamber by releasing a certain amount of vapor by lifting itself.

Safety valve

- A safety valve is present on the lid of the autoclave, which is crucial in cases where the autoclave fails to perform its action or the pressure inside increases uncontrollably.
- The valve has a thin layer of rubber that bursts itself to release the pressure and to avoid the danger of explosion.

3. Steam generator/ Electrical heater

- An electrical steam generator or boiler is present underneath the chamber that uses an electric heating system to heat the water and generate steam in the inner and the outer chamber.
- The level of water present in the inner chamber is vital as if the water is not sufficient; there are chances of the burning of the heating system.
- Similarly, if the water is more than necessary, it might interfere with the trays and other components present inside the chamber.

4. Vacuum generator (if applicable)

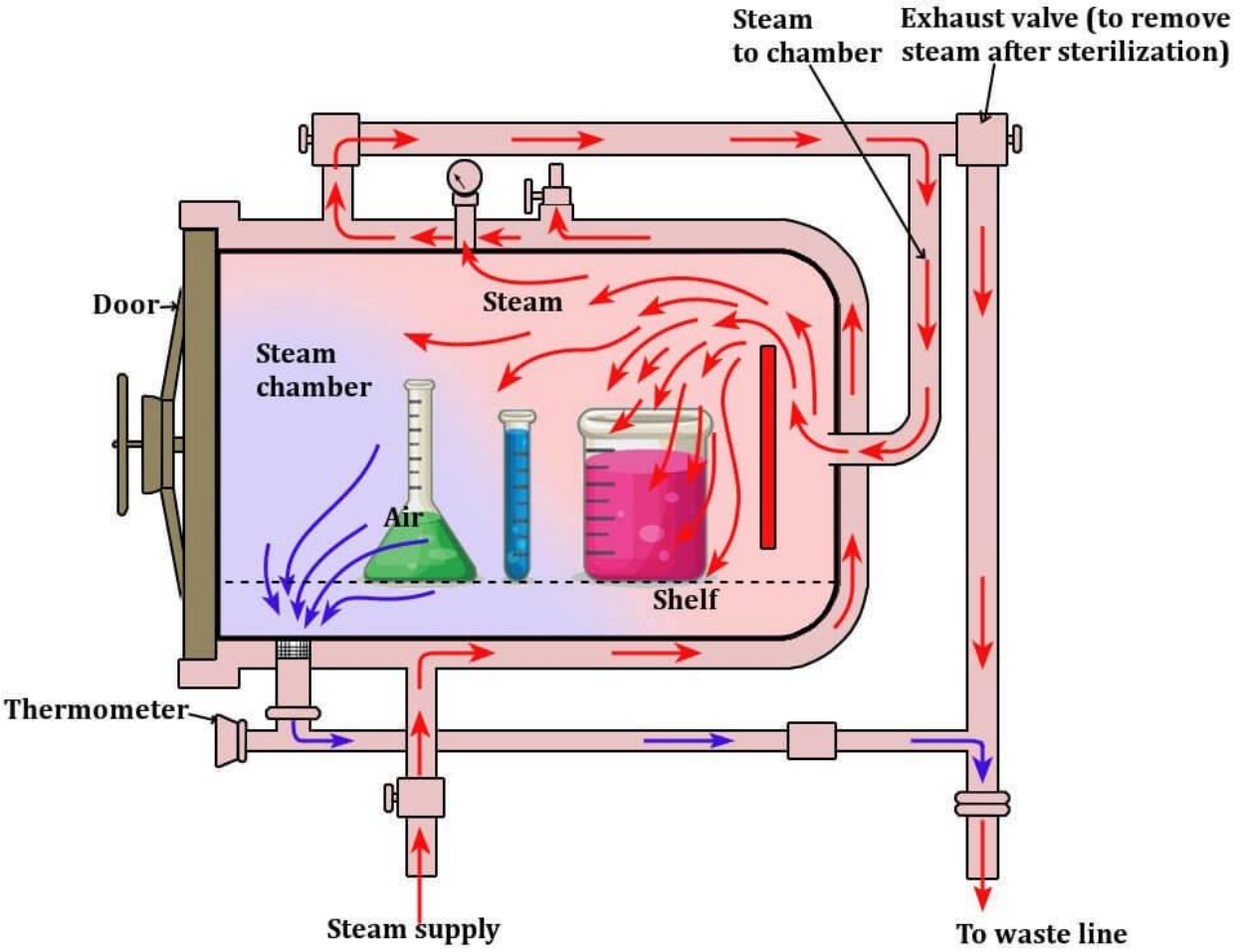
- In some types of autoclaves, a separate vacuum generator is present which pulls out the air from the inside of the chamber to create a vacuum inside the chamber.
- The presence of some air pockets inside the chamber might support the growth of different microorganisms. This is why the vacuum chamber is an important component of an autoclave.

5. Wastewater cooler

- Many autoclaves are provided with a system to cool the effluent before it enters the draining pipes.
- This system prevents any damage to the drainage pipe due to the boiling water being sent out of the autoclave.

Operation Principles of Autoclave

Autoclaves work by taking advantage of the thermodynamic properties of water which can be considered as a pure substance



1. The autoclave works on the principle of moist heat sterilization where steam under pressure is used to sterilize the material present inside the chamber.
2. The high pressure increases the boiling point of water and thus helps achieve a higher temperature for sterilization.
3. Water usually boils at 100°C under normal atmospheric pressure (760 mm of Hg); however, the boiling point of water increases if the pressure is to be increased.
4. Similarly, the high pressure also facilitates the rapid penetration of heat into deeper parts of the material, and moisture present in the steam causes the

coagulation of proteins causing an irreversible loss of function and activity of microbes.

5. This principle is employed in an autoclave where the water boils at 121°C at the pressure of 15 psi or 775 mm of Hg.
6. When this steam comes in contact with the surface, it kills the microbes by giving off latent heat.
7. The condensed liquid ensures the moist killing of the microbes.
8. Once the sterilization phase is completed (which depends on the level of contamination of material inside), the pressure is released from the inside of the chamber through the whistle.
9. The pressure inside the chamber is then restored back to the ambient pressure while the components inside remain hot for some time.

Uses of Autoclave

Autoclaves are important devices to ensure the sterilization of materials containing water as they cannot be sterilized by dry heat sterilization. Besides, autoclaves are used for various other purposes.

1. They are used to decontaminate specific biological waste and sterilize media, instruments, and lab ware.
2. Regulated medical waste that might contain bacteria, viruses, and other biological materials is recommended to be inactivated by autoclaving before disposal.
3. In medical labs, autoclaves are used to sterilize medical equipment, glassware, surgical equipment, and medical wastes.
4. Similarly, autoclaves are used for the sterilization of culture media, autoclave able containers, plastic tubes, and pipette tips.

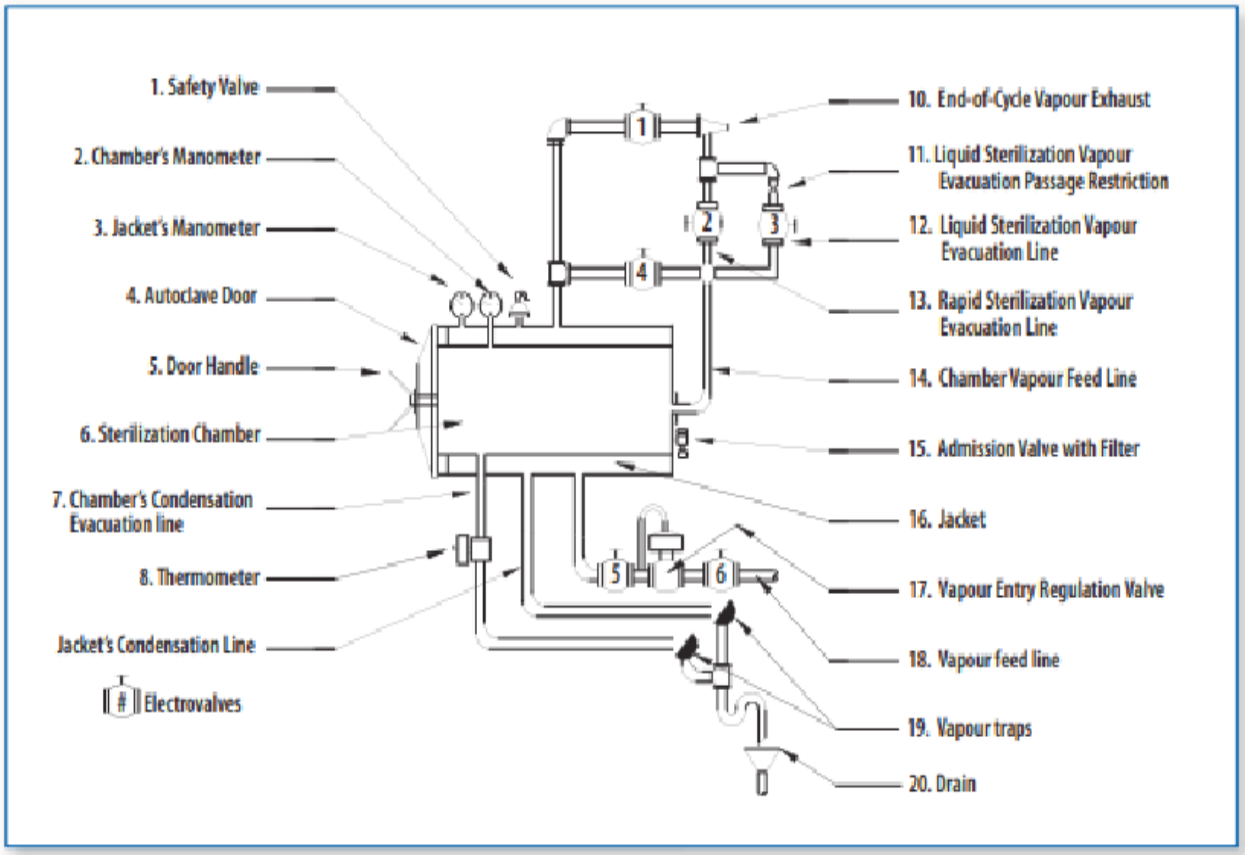
Cross-section diagram of the vapor autoclave

Figure 32 shows the main components of the vapor system of an autoclave. For clarity, parts normally located around the autoclave (their precise location depends on the manufacturer), have been included on top and at the bottom of the autoclave diagram.

Description of the components in the diagram

A brief description of the most common elements of the vapor circuit of an autoclave is given next. The same number identifying each component is used in Figure 32 and its description below. Note that the configurations vary depending on each manufacturer's design.

Figure 32. Vapour circuit of an autoclave



1. **Safety valve**: A device that impedes the vapor pressure from rising above a determined value. The manufacturers install these in the sterilization chamber as well as in the jacket.
2. **Chamber manometer**: A mechanical device that indicates the vapor pressure in the sterilization chamber.
3. Jacket manometer (pressure gauge). A mechanical device that indicates the vapor pressure inside the autoclave's jacket.
4. **Autoclave door**: A device which allows the sterilization chamber to be isolated from the outside environment. It normally has safety devices that

prevent it from opening when the chamber is pressurized. It also has seals for preventing vapor from leaving the chamber when the equipment is in operation. Autoclave doors can be manually or electromechanically operated.

5. **Door handle:** A device which in some equipment, allows the operator to open and close the door. The larger capacity equipment in general has motorized mechanisms for activating the door.
6. **Sterilization chamber**: The space where objects or materials to be sterilized are placed. When the door is closed, the chamber remains isolated from the exterior. When the sterilization process is in progress, it is fi lled and pressurized with vapor.
7. **Chamber condensation evacuation line**: A duct that allows the collecting of condensation formed in the sterilization chamber as a consequence of the heat transference processes between the vapor and objects being sterilized.
8. **Thermometer**: An instrument that indicates the temperature at which the sterilization processes in the autoclave chamber is done.
9. **The jacket's condensation evacuation line**: A duct that allows the extraction of condensation formed in the casing as a result of heat transference between the vapor and the jacket's walls.
10. **Vapor exit at the end of the cycle**: When a sterilization cycle is finished, vapor is extracted from the autoclave by controlled procedures.
11. **Vapor passage restriction for liquid sterilization cycle**: A mechanical device that restricts the passage of vapor during a liquid sterilization cycle to allow the temperature to decrease in a controlled manner and to prevent sterilized liquids from boiling
12. **Vapor evacuation duct for sterilization of liquid:** A path followed by vapor when a liquid sterilization process is being conducted and which passes through the restriction described above.
13. **Vapor evacuation line during the rapid sterilization cycle**: A path that follows vapor when a rapid sterilization cycle is being carried out.
14. **Vapor feed line**: A conduct that feeds the autoclave with vapor. This line has controls and accessories that enable vapor to reach the autoclave at the conditions stipulated for the sterilization cycle.
15. **Air admission valve with filter**: A device that allows the entry of filtered air upon finishing the sterilization cycle. The valve homogenizes the pressure of the sterilization chamber to that of the atmosphere.

16. **Jacket**: A space located around the sterilization chamber in which vapor circulates. Its purpose is to transfer heat to the chamber and lessen the formation of condensation. It is connected to the chamber and to the drainage through lines controlled by electro valves. Not all autoclaves have jackets. Some manufacturers substitute it by placing electrical resistors around the sterilization chamber.
17. **Vapor entry regulation valve:** It is a mechanical device which controls the pressure at which vapor enters the autoclave. Depending on the cycle selected, the pressure and the temperature will be different. The greater the pressure, the greater the temperature. The lesser pressure, the lesser the temperature.
18. **Vapor feed line**: A duct that brings vapor from the boiler or the vapor generator to the autoclave.

19. **Vapor trap**: A device designed to take maximum advantage of vapor's thermal energy. Its function is to prevent vapor from leaving the system. The trap only allows condensed liquid formed in the chamber, jacket and autoclave conducts to leave.
20. **Drain**: A collection line for the condensed liquid produced in the autoclave to exit.

Nowadays, autoclaves use microprocessor-controlled systems and each one of their valves and accessories work in accordance with pre-established programs stored in their memory. Operations remain recorded in a registering system, which allows the different stages of the sterilization to be checked. Each manufacturer has incorporated registering systems which are indispensable for quality control.

Vapor production. The vapor autoclaves use is generated in devices which transfer thermal energy to water using electrical energy or fossil combustible. These are called boilers or vapor generators and constitute a fundamental component of the autoclave. Depending on their size and the frequency of use, autoclaves have vapor feed systems that originate from a central system of boilers or from their own vapor generator. These generally function with electrical resistors and come already incorporated into the equipment or are supplied as an accessory by the manufacturers

Types of Autoclave

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

1. 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.

2. 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.

3. 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.

4. 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.

Autoclave operation

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:

1. Before beginning to use the autoclave, it should be checked for any items left from the previous cycle.
2. A sufficient amount of water is then put inside the chamber.
3. Now, the materials to be sterilized are placed inside the chamber.
4. The lid is then closed, and the screws are tightened to ensure an airtight condition, and the electric heater is switched on.
5. The safety valves are adjusted to maintain the required pressure in the chamber.
6. 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.
7. The drainage pipe is then closed, and the steam inside is allowed to reach the desired levels (15 lbs in most cases).
8. Once the pressure is reached, the whistle blows to remove excess pressure from the chamber.
9. After the whistle, the autoclave is run for a holding period, which is 15 minutes in most cases.
10. 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.
11. The discharge pipe is then opened to allow the entry of air from the outside into the autoclave.
12. Finally, the lid is opened, and the sterilized materials are taken out of the chamber.

Quality Control

In order for a product to be considered sterilized, it is necessary to verify that all the stages of the sterilization process have been carried out correctly. To verify that these have been fulfilled, a series of tests have been developed to evaluate the characteristics of the process and its influence on the activity of microorganisms. Evaluations of the temperature, pressure, time, humidity and general equipment behavior are carried out to certify that it complies with, and functions according to procedures that demonstrated its validity and reliability. There are also tests or indicators that allow the death of the microorganisms to be certified in order to guarantee the quality of the sterilization processes. Different categories of tests have been developed. Some are featured next:

1. Sterilization process indicators. These are designed for supervising the functioning of the autoclaves. They include instruments that control parameters like temperature, time and pressure (thermometers, manometers and chronometers) and register the development of the process.
2. Chemical indicators. These are typical chemical tests changing color or state when exposed to the different phases of the sterilization process.
3. Biological indicators. These are considered the best methods for controlling the quality of a sterilization process. They are made of live microorganisms which have a greater resistance to a determined sterilization process, or of chemical reagents which react in the presence of the specific proteins of this type of organism.

Precaution

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:

1. Autoclaves should not be used to sterilize water-proof or water-resistant substances like oil or powders.
2. The autoclave should not be overcrowded, and the materials should be loaded in a way that ensures sufficient penetration of articles by the steam.
3. The items to be autoclaved should always be placed in a secondary container.
4. Only autoclave able bags are to be used to autoclave packaged waste.

5. To ensure sufficient penetration, articles should be wrapped in something that allows penetration by steam, and materials like aluminum foils should not be used.
6. The items placed inside the chamber should not touch the sides or top of the chamber.
7. The wastes and clean items should be autoclaved separately.
8. Attempts to open the lid when the autoclave is working should never be made.
9. Liquid components should never be autoclaved in sealed containers.
10. The liquid inside the containers should only be filled 2/3rd of the total volume to prevent the spilling of the liquid.
11. Plastic or polyethylene trays or containers should not be used as they might melt and damage the autoclave.
12. Besides, never autoclave flammable, reactive, corrosive, toxic, or radioactive materials, household bleach, or paraffin-embedded tissue.
13. 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.

Advantages of Steam Sterilization Method

1. Nontoxic to patient, staff, environment
2. Cycle easy to control and monitor
3. Rapidly microbicide
4. Least affected by organic/inorganic soils among sterilization processes listed
5. Rapid cycle time

Disadvantages of Steam Sterilization Method

1. Deleterious for heat-sensitive instruments
2. Microsurgical instruments damaged by repeated exposure
3. May leave instruments wet, causing them to rust
4. Potential for burns