B.Sc. III Year (Theory)

Semester –VI

Paper XX (C)

Microbiology and Disease Management

Unit-1

D. Sterilization of glassware's and media

Dr. S. S. Patale

Associate Professor

Department of Botany

Smt. S. K. Gandhi Arts, Amolak Science and P.H. Gandhi Commerce College, Kada Dist. Beed

Email- sspatale@rediffmail.com Ph. 9823937501

D) METHODS OF STERILIZATION:

Sterilization is the complete destruction or removal of all living organisms from the object being sterilized. Experiment designed to prove or to disprove spontaneous generation depended upon two general principles:

1) The complete sterilization of a suitable growth medium so that no living organisms exist at the start of the experiment.

2) The design of the vessel of a type that it is impossible for microbes to enter from outside.

If these two principles are strictly followed and conditions are otherwise suitable for multiplication of microbes, any growth occurring must be the result of spontaneous generation.

The attainment of sterility:

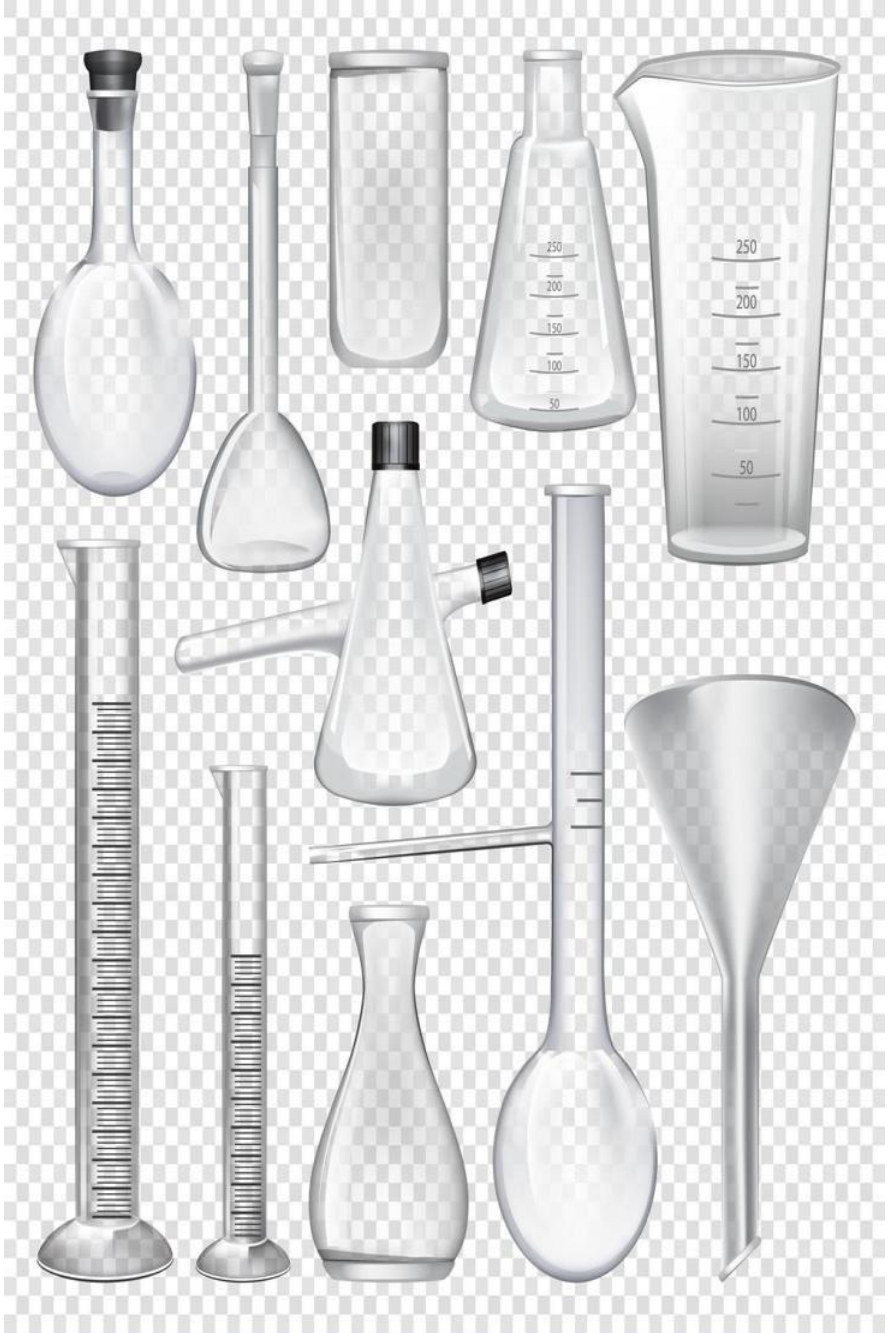
The usual method depends upon heat treatment. However, it was soon realized that microorganisms vary widely in their resistance of heating. Bacteria require higher temperature and some also produce heat stable spores. Thus boiling at normal pressure was insufficient to kill these spores and therefore autoclave was designed to increase the pressure and temperature. Sealing of flask was not proper as oxygen could no longer enter the vessel. It is necessary to include some kind of filter to prevent the entry of microbes but not of air. This led to the development of the cotton wool plug that was soon adopted by microbiologists. Some of the currently used sterilization methods are summarized below:

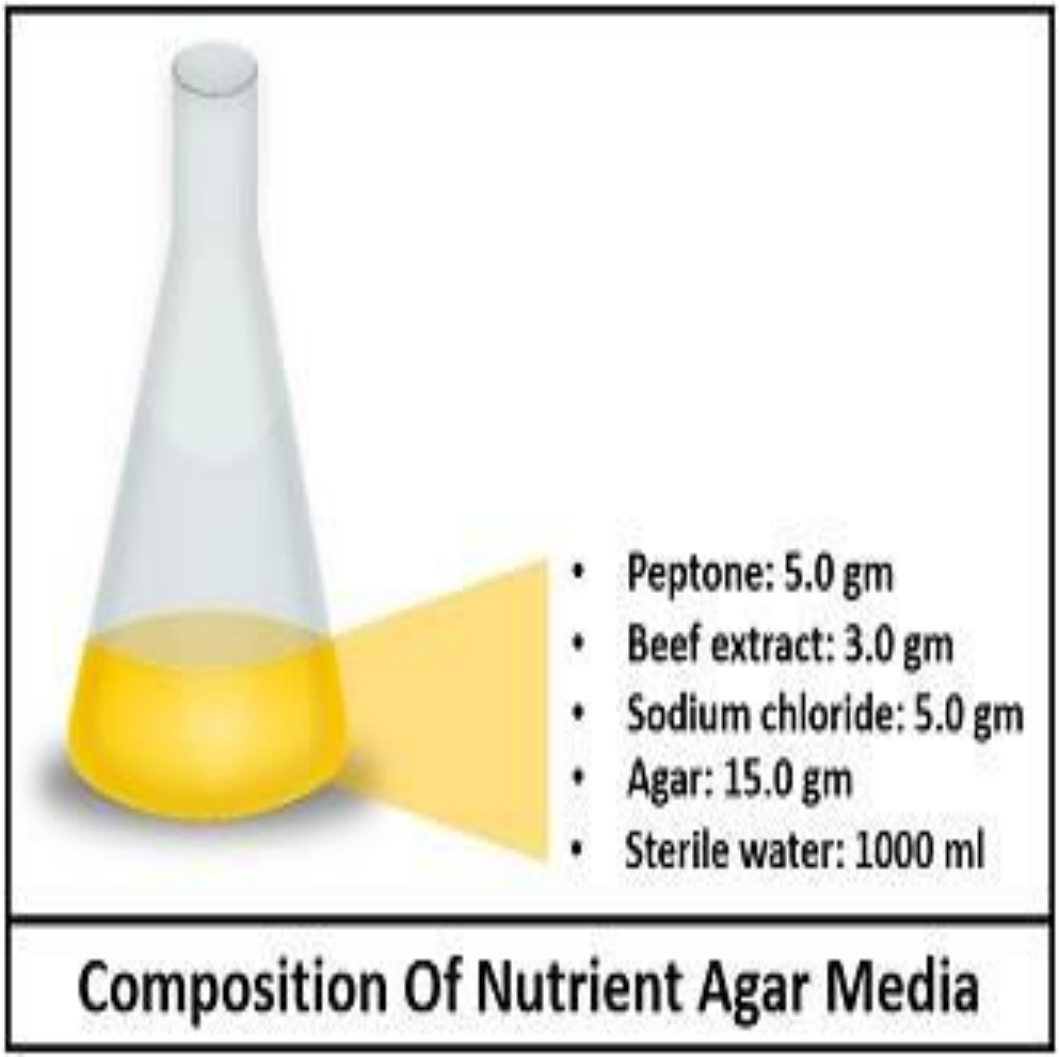
1) Heat: For general sterilisation a time and temperature that kill organisms including heat-resistant spores is used. The methods generally adopted are as follows:

a) Wet heat in autoclave: the usual method is a time of 30 min. at a pressure of 1.05kg/cm^2 that will give a temperature of 121^0C. this is the best practical method.

b) Tyndallisation: This is a course of three periods of boiling at 100^0C for 30 min. at daily intervals.

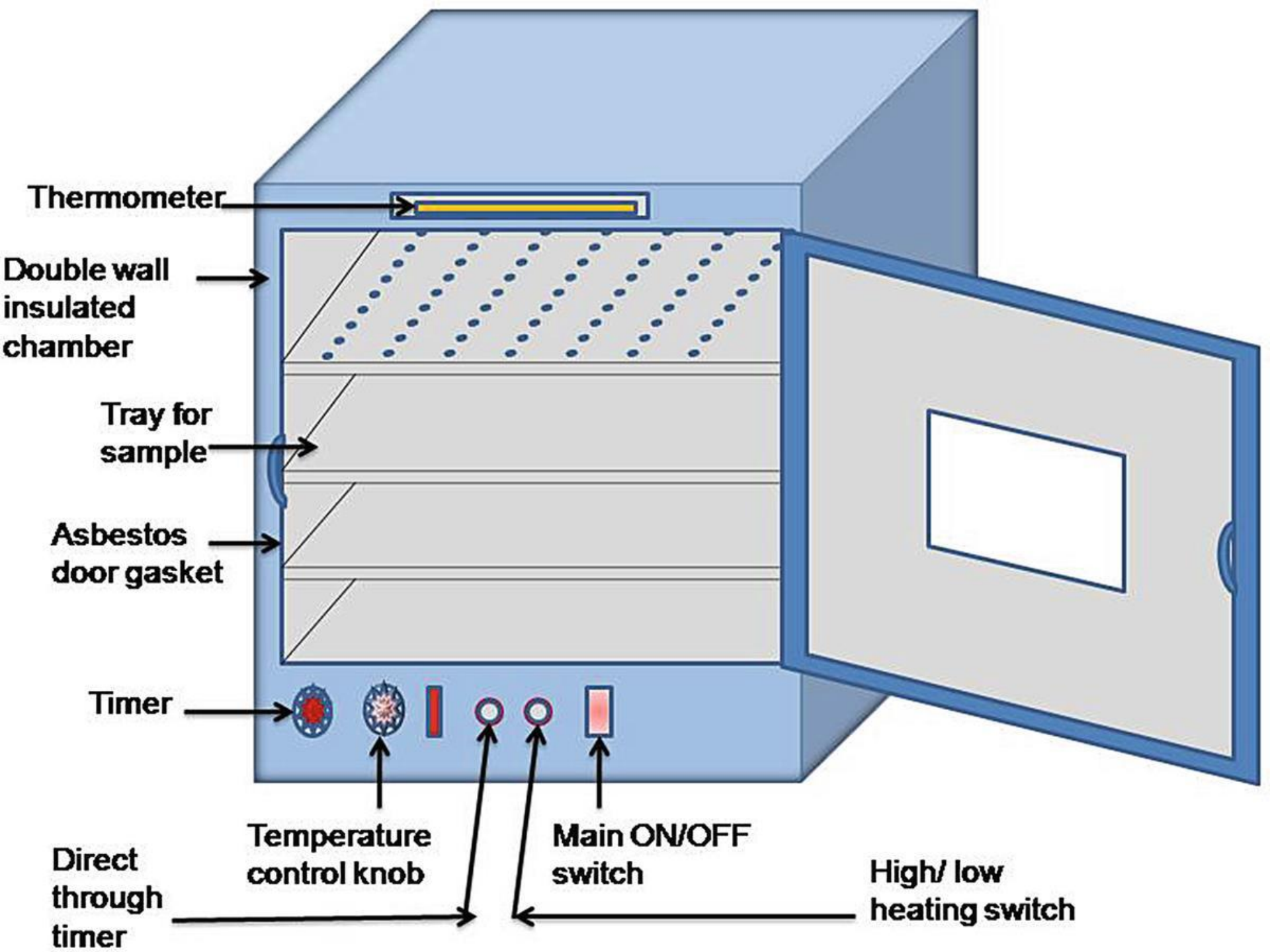
c**) Dry heat:** This is done in a dry oven, where a temperature of 160^0C for two hours is required.

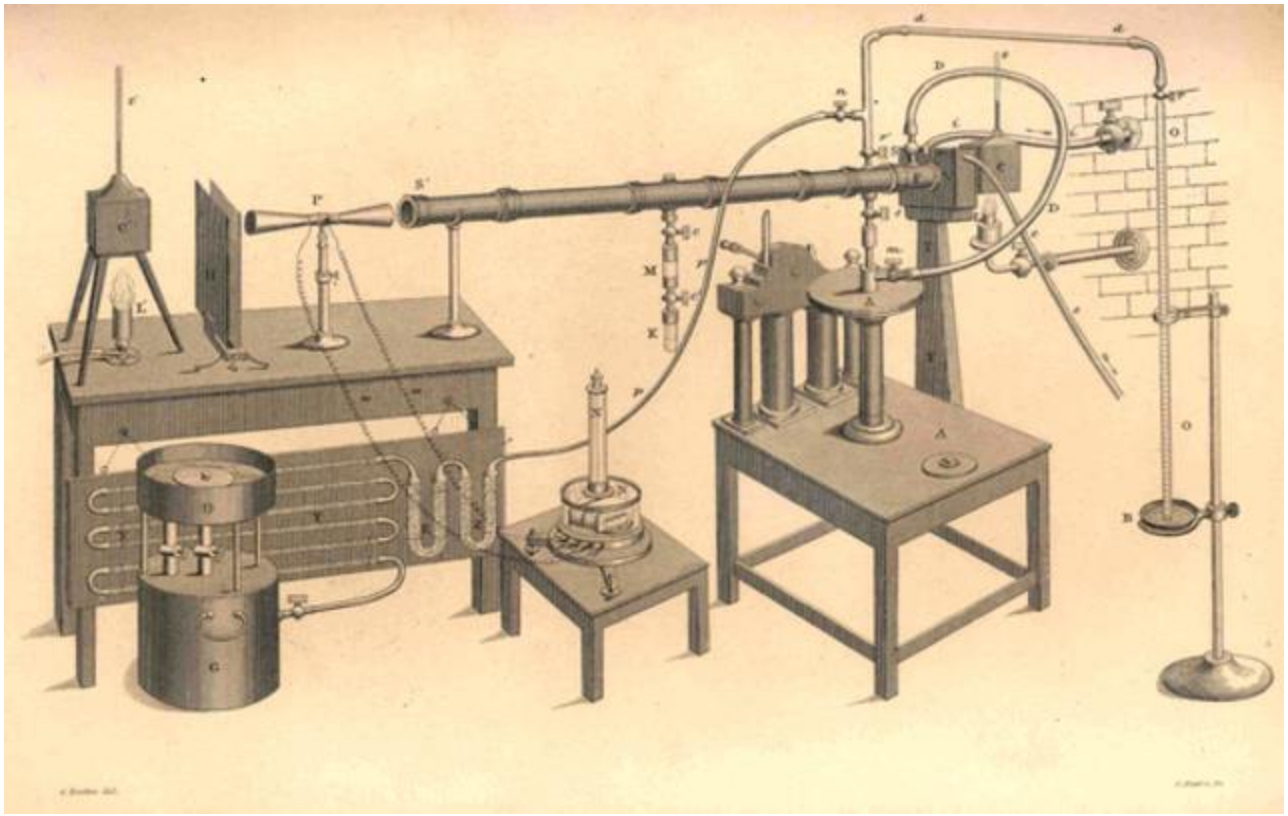



• Peptone: 5.0 gm
• Beef extract: 3.0 gm
• Sodium chloride: 5.0 gm
• Agar: 15.0 gm
• Sterile water: 1000 ml
Composition Of Nutrient Agar Media


Pressure Regulating Device
Pressure Gauge
Safety Valve
Autoclave Lid
Handles
Autoclave Body
Steam Release Valve
Vacuum Release Valve
Outer Stand


HOT AIR OVEN


Thermometer
Double wall insulated chamber
Tray for sample
Asbestos door gasket
Timer
Temperature control knob
Main ON/OFF switch
Direct through timer
High/ low heating switch



Tyndallisation

2) Filtration: The liquid or gas to be sterilized is passed through a filter with porosity sufficient to remove any microorganism in suspension. The cotton wool is used for gases. For liquids, a variety of filters are available made of materials such as cellulose nitrate. This method is very useful for sterilization of liquids containing heat liable components.

3) Radiation: Ultra violet light is very effective in sterilization of air. For solids, gamma rays or X-rays from radioactive cobalt are used. Ionizing radiation is often used to sterilize plastics and other heat liable materials.

4) Chemicals: Many chemicals are lethal to microbes. Hypochloride solution and phenolic derivatives are used as general laboratory disinfectants. Similar chemical is gaseous ethylene oxide. However, these may not cause sterilization under some conditions.

Thank You