

AIM OF THE EXPERIMENT

DATE - 3.11.14

To study the methods of sterilization.

THEORY

There are various methods of sterilization used for the destruction of micro-organisms or articles to be used for micro-biological experiments. The general way to sterilise is by heat, although other methods such as use of chemicals remove by filtration are also employed. In sterilization most of the culture media, heat is applied in some forms according to the nature of materials.

STERILIZATION OF HEAT

Sterilization may be done either with dry heat or moist heat. In the former, a higher temperature and longer time is required than the latter method.

(A) DRY HEATi) RED HEAT

Inoculation needles, tips of forceps and such appliances are sterilized by heating them over ordinary spirit lamp or burner until red heat.

ii) HOT AIR OVEN

The electric hot air oven is convenient apparatus for sterilizing things like culture tubes, petri-dish, flasks, pipettes and many other glass wares. Before placing in oven, the mouth of these material are plugged with cotton wool. In hot air oven, a temperature of 160°C or 180°C for one hour or half an hour is necessary for complete sterilization of this articles. A precaution to be taken that materials are introduced or taken out only when temperature has come down.

iii) FLAMING

This is the procedure of passing articles through a flame

without heating it to red hot. This is followed in sterilization of scalpels, needle or mouth of culture tubes or cotton plugs or cover slides. Needles, scalpels may also be sterilised by dipping them in dehydrated spirit and burning the spirit off.

B) MOIST HEAT

i) BOILING IN WATER BATH

A convenient type of sterilization is to boil the articles for about 5 minutes at 100°C . This will kill many vegetative forms of organism and some spores. But, this method is not sufficient to kill the bacteria. A fish bathes type of vessels may be used to this. This may be used in sterilizing both glass as well as metal articles. These articles are introduced while water is cool.

ii) STEAM AT 100°C

Arnold steam sterilization is the appropriate apparatus for the sterilization by steam at 100°C . This has an advantage that media are also sterilized in it. The exposure to steam at 100°C . For one hour or half an hour is usually enough for sterilization can be used for such media as meat nutrient agar. It however, cannot be used for nutrient dilution. Exposure at 100°C for 20 minutes and each of 3 successive days kill all vegetation form of bacteria. This method is also employed for sterilizing media containing sugars which may be composed by higher temperature or by heating.

iii) STEAM AT HIGH PRESSURE

The principle on which the autoclave works is that water boils when its vapour pressure is equal to the pressure of the atmosphere which surrounds it. If, therefore the pressure be increased in a closed vessel, the temperature at which the water boils, will rise above 100°C . The exact temperature depending upon the pressure employed. This is the most commonly used methods for sterilization media. But this high temperature

and pressure may deteriorate the nutrient value. Autoclaving should be avoided for sugar media or gelation.

STERILIZATION BY FILTRATION

This process can be carried out by the use of filtrate of unglazed protein or of diatomaceous earth. The pores of which are small that ordinary bacteria are prevent from passing through out to set filtrates in which asbestos dishes are used as filters which are reliable and commonly used.

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