RAMA UNIVERSITY

www.ramauniversity.ac.in

FACULTY OF ENGINEERING &TECHNOLOGY

DEPARTMENT OF BIOTECHNOLOGY

Methods of inoculation

Using a wire loop

Wire loops are sterilised using red heat in a Bunsen flame before and after use. They must be heated to red hot to make sure that any contaminating bacterial spores are destroyed. The handle of the wire loop is held close to the top, as you would a pen, at an angle that is almost vertical. This leaves the little finger free to take hold of the cotton wool plug/screw cap of a test tube/bottle.

Using a pipette

Sterile graduated or dropping (Pasteur) pipettes are used to transfer cultures, sterile media and sterile solutions

Micropippete

Nowdays, inoculation of micro organism are also done by graduated micropippete. Micro pipette of several volume ranges are available.

Preparation of culture media

Culture media are frequently prepared from dehydrated media powder. Dehydrated media are hygroscopic and are sensitive to moisture, heat and light. They are adversely affected by drastic changes in temperature e.g. hot/cold cycling temperatures which may occur between day and night laboratory temperatures in winter. Culture media must be stored at the specified temperature, under specified conditions and not longer than the shelf-life periods appropriate to each product

Reconstitution of dehydrated media

❖Complete instructions for the preparation of culture media are given on the label of each bottle. As a general rule it is wise to prepare one week's requirement only.

❖Always use freshly prepared distilled or deionised water. Use warm (50°C) water to hasten the solution of the medium. Rinse all glassware with the distilled/deionised water and make sure that the vessels are clean and free from toxic chemicals which may be absorbed on to the surface of the glass e.g. bile salts, tellurite, selenite etc.

❖Prepare the medium in a vessel about twice the final volume of the medium to allow adequate mixing. Follow the instructions given on the label of each product.

❖Open the culture medium container away from draughts and moisture. Avoid inhaling the powder and prolonged skin contact.

❖Weigh the powder quickly, accurately and without creating 'clouds of dust'. Reclose the container as soon as possible.

❖Pour half the required volume of distilled water in the vessel, then the weighed quantity of medium and agitate briskly for a few minutes.

❖Pour the rest of the distilled water down the sides of the vessel to wash any adherent medium back into solution.

❖This is an important step because dry culture media powder above the level of the water may not be sterilized in the autoclave and may be a source of contamination.

❖Agar-free media will usually dissolve on gentle agitation. Media containing agar should be **heated** to dissolve the agar **before** autoclaving.

❖Bring the medium to the boil without scorching or burning.

❖Most culture media will require final sterilization in an autoclave at 121°C for 20 minutes.

❖The pH of the dehydrated medium must be adjusted by the manufacturer so that the final pH of the prepared medium conforms with the label specification when the medium has been cooled to 25°C.

❖Do not adjust the pH of dehydrated media prior to sterilization.

Sterilization of culture media

❖Although sterilization of culture media is best carried out in a steam autoclave at temperatures between 121°C it has to be recognised that damage is caused to the medium by the heating process.

❖Heat-treatment of complex culture media which contain peptides, sugars, minerals and metals results in nutrient destruction, either by direct thermal degradation or by reaction between the medium components.

❖Toxic products caused by chemooxidation can also be formed during heat-treatment.

❖It is important, therefore, to optimise the heating process so that a medium is sterile after heating but minimal damage is caused to the ingredients of the medium.

❖As a general rule it is accepted that short-duration, high-temperature processes are more lethal to organisms and less chemically damaging than are longer, lower temperature processes e.g. 3 minutes at 134°C is preferable to 20 minutes at 115°C.

❖A general instruction for sterilizing culture media in volumes up to one litre at 121°C for 20 minutes is given on each label.

❖Autoclaves vary in performance, however, and thermocouple tests using different volumes of media should be carried out to determine the 'heat-up and 'cool-down' times.

❖ It will be essential to do this when volumes of media greater than two litres are prepared.

❖ In order to avoid overheating large volume units of media, the 'heat-up' and 'cool-down' periods are normally integrated into the 121°C holding time.