

Experiment-9: Sterilization Techniques

Aim: Effective sterilization techniques are essential for working with isolated cell lines for obvious reasons you don't want bugs from the environment growing in your nice culture medium, and equally, cultures must be sterilised before disposal.

Wet Heat (Autoclaving)

The method of choice for sterilisation in most labs is autoclaving; using pressurised steam to heat the material to be sterilised. This is a very effective method that kills all microbes, spores and viruses, although for some specific bugs, especially high temperatures or incubation times are required. Autoclaving kills microbes by hydrolysis and coagulation of cellular proteins, which is efficiently achieved by intense heat in the presence of water. The intense heat comes from the steam. Pressurised steam has a high latent heat; at 100 °C it holds 7 times more heat than water at the same temperature. This heat is liberated upon contact with the cooler surface of the material to be sterilised, allowing rapid delivery of heat and good penetration of dense materials. At these temperatures, water does a great job of hydrolyzing proteins so that those bugs don't stand a chance.

Dry Heat (Flaming, baking)

Dry heating has one crucial difference from autoclaving. You've guessed it there's no water, so protein hydrolysis can't take place. Instead, dry heat tends to kill microbes by oxidation of cellular components. This requires more energy than protein hydrolysis so higher temperatures are required for efficient sterilization by dry heat. For example sterilization can normally be achieved in 15 minutes by autoclaving at 121°C, whereas dry heating would generally need a temperature of 160 °C to sterilize in a similar amount of time.

Filtration

Filtration is a great way of quickly sterilizing solutions without heating. Filters, of course, work by passing the solution through a filter with a pore diameter that is too small for microbes to pass through. Filters can be scintered glass funnels made from heat-fused glass particles or, more commonly these days, membrane filters made from cellulose esters. For removal of bacteria, filters with an average pore diameter of 0.2µm are normally used. But remember, viruses and phage can pass through these filters so filtration is not a good option if these are a concern.

Solvents

Ethanol is commonly used as a disinfectant, although since isopropanol is a better solvent for fat it is probably a better option. Both work by denaturing proteins through a process that requires water, so they must be diluted to 60-90% in water to be effective. Again, it's important to remember that although ethanol and IPA are good at killing microbial cells, they have no effect on spores.

Radiation

UV, x-rays and γ rays are all types of electromagnetic radiation that have profoundly damaging effects on DNA, so make excellent tools for sterilization. The main difference between them, in terms of their effectiveness, is their penetration. UV has limited penetration in air so sterilisation only occurs in a fairly small area around the lamp. However, it is relatively safe and is quite useful for sterilizing small areas, like laminar flow hoods. X-rays and gamma rays are far more penetrating, which makes them more dangerous but very effective for large scale cold sterilization of plastic items (e.g. syringes) during manufacturing.

Result:

Reference:

- 1) Sadasivam S and Balasubramanian T (1985). Practical Manual (Undergraduate), Tamil Nadu Agriculture University, Coimbatore, p.2.
- 2) Sadasivam S, Manickam A (2008). Biochemical Methods, New Age International Publishers, 3rd Edition, ISBN: 978-81-224-2140-8.