

Quadrant II – Transcript and Related Materials

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Paper Title: Plant Tissue Culture

Unit: 01 (Introduction to Plant Tissue Culture)

Module Name: Sterilization Practices in Laboratory

Module No: 05

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Notes:

Concept of Sterilization:

Contamination is the presence of a constituent, impurity, or some other undesirable element that spoils, corrupts, infects, makes unfit, or makes inferior a material, physical body, natural environment, workplace, etc.

Three main sources of contamination:

1. Through culture vessels and culture medium.
2. Through explants.
3. During inoculation.

Sterilization is the method by which all microbes, spores and acellular entities (e.g. viruses, viroids, prions) are destroyed or removed from an object or habitat.

What is the need for sterilization?

Sterilization eliminates microorganisms and thus avoids contamination by bacteria and fungi. To maintain an aseptic environment, all culture vessels, media and instruments used in handling tissue, as well as the explant itself should be surface sterilized.

How can microorganisms be killed?

1. By denaturation of proteins
2. By interruption of DNA synthesis/repair
3. By disruption of cell membranes.

Care to be taken during inoculation procedure:

1. Ensure that the laboratory is dust free to avoid contamination.
2. Wash your hands before handling any cultures or reagents.
3. Use proper clothing (aprons) and protective head gear.
4. Check for contamination in media bottles before using them for experiment.
5. Do not leave culture bottles open after inoculation.

General methods of sterilization:

1. Physical methods:

Heat- Dry heat and moist heat.

- ✓ Heat sterilization is the most effective and widely used method of sterilization, where the bactericidal activity results through the destruction of enzymes and other essential cell constituents.
- ✓ The effects of heat sterilization occur more rapidly in a fully hydrated state, as it requires a lower heat input, with low temperature and less time, under high humidity conditions where the denaturation and hydrolysis reactions are predominant, rather than in the dry state where oxidative changes take place.
- ✓ Under circumstances where thermal degradation of a product is possible, it can usually be minimized by adopting a higher temperature range, as the shorter exposure times generally results in a lower partial degradation.
- ✓ This method of sterilization is applicable to thermostable products. Still, it can be applied to both moisture-sensitive and moisture-resistant products, for which dry (160–180°C) and moist (121–134°C) heat sterilization procedures are respectively used.

Moist heat

- ✓ Moist heat sterilization is one of the most effective methods of sterilization where the steam under pressure acts as a bactericidal agent.
- ✓ Moist heat sterilization usually involves the use of steam at temperatures in the range 121–134°C.
- ✓ High pressure increases the boiling point of water and thus helps achieve a higher temperature for sterilization.

- ✓ High pressure also facilitates the rapid penetration of heat into deeper parts of material and moisture present in the steam causes the coagulation of proteins causing an irreversible loss of function and activity of microbes.
- ✓ The high temperature-short time cycles not only often result in lower fractional degradation, but they also provide the advantage of achieving higher levels of sterility assurance due to more significant inactivation factors.
- ✓ The most commonly used standard temperature-time cycles for clinical porous specimens (e.g. surgical dressings) and bottled fluids are 134°C for 3 minutes and 121°C for 15 minutes, respectively.
- ✓ An autoclave is a device that works on the principle of moist heat sterilization through the generation of steam under pressure.

Dry Heat

- ✓ Dry sterilization is the process of removing microorganisms by applying moisture-free heat which is appropriate for moisture-sensitive substances.
- ✓ The dry heat sterilization process is based on the principle of conduction; that is the heat is absorbed by the outer surface of an item and then passed onward to the next layer. Ultimately, the entire item reaches the proper temperature needed to achieve sterilization.
- ✓ Dry moisture-less heat destroys microorganisms by causing denaturation of proteins and also lyses the proteins in many organisms, causes oxidative free radical damage, causes drying of cells, and can even burn them to ashes, as in incineration.
- ✓ Dry heat sterilization is used for the sterilization of materials which are difficult to sterilize by moist heat sterilization for several reasons.
- ✓ Substances like oil, powder, and related products cannot be sterilized by moist heat because moisture cannot penetrate into deeper parts of oily materials, and powders are destroyed by moisture.

Filtration

- ✓ The process of filtration is unique among sterilization techniques in that it removes, rather than destroys, microorganisms.
- ✓ Further, it is capable of preventing the passage of both viable and nonviable particles and can thus be used for both the clarification and sterilization of liquids and gases.
- ✓ The primary mechanisms involved in filtration are sieving, adsorption, and trapping within the matrix of the filter material.
- ✓ Filtration uses membranous filters that have tiny pores that let the liquid pass through but prevent bigger particles such as bacteria from passing

through the filter. Therefore, the smaller the pore, the more likely the filter is to stop more things from going through it.

- ✓ Certain types of filter (membrane filters) also have an essential role in sterility testing, where they can be employed to trap and concentrate contaminating organisms from solutions under test.
- ✓ These filters are then placed in a liquid nutrient medium and incubated to encourage growth and turbidity.
- ✓ The principal application of sterilizing-grade filters is the treatment of heat-sensitive injections and ophthalmic solutions, biological products, air, and other gases for supply to aseptic areas.
- ✓ They may also be required in industrial applications where they become part of venting systems on fermenters, centrifuges, autoclaves, and freeze dryers.

Radiation

- ✓ Gamma radiation sterilization is the most popular form of radiation sterilization. [1,4] Co-60 and, to a lesser extent, Cs-137 serve as radiation sources and undergo decomposition to release high energy gamma rays.
- ✓ The produced electromagnetic radiation is highly penetrating and can kill contaminating microorganisms.

2. Chemical methods:

- ✓ Often chemical sterilizers function by using low temperature, highly reactive gases that come into direct contact with the test article (often through a semi-porous membrane or package.)
- ✓ Liquids – for example, bleach – are also used for sterilization. e.g. ethyl alcohol, sodium hypochlorite.

Methods of sterilization of plant tissue culture laboratory:

1. Using disinfectants

- ✓ Chemical agents used to inactivate or destroy microorganisms on surfaces.
- ✓ Various types of chemical disinfectants used are:
Alcohol, Formaldehyde, Hydrogen peroxide, Phenolics, Sodium hypochlorite, Ethanol (70% vol/vol), Ammonium compounds etc.

2. Fumigation technique

- ✓ Fumigation is the method of gaseous sterilization which is used for killing microorganisms and prevention of microbial growth in air and on surfaces.
- ✓ This method completely fills an area with gaseous fumigants to suffocate or poison the microbes and pests within.
- ✓ Two methods for fumigation are:
Method 1: Using Electrical evaporator with formaldehyde.
Method 2: Using formaldehyde with Potassium Permanganate.

Advantages of fumigation:

- ✓ Fumigants are toxic to all forms of life; therefore it is possible to control all life stages of microorganisms.
- ✓ It is the quickest way to sterilize the given area.
- ✓ It reduces residue problems.

3. Using Sterile Cabinets with HEPA filters

- ✓ High Efficiency Particulate Air (HEPA) filters are designed to control the number of particles entering a clean area by filtration.