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Chapter 1

Fundamentals of laboratory practices: Principles, techniques, and safety protocols

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1. Introduction:

This chapter aims to highlight the importance of using appropriate handling and sterilization procedures in microbiological practices. It emphasizes how crucial it is to comprehend microbes and use aseptic methods in order to avoid contamination.

Following recommended laboratory procedures is essential when handling live microorganisms for a number of reasons:

a) To avoid contaminating the laboratory environment, oneself, or others.
b) To prevent contaminating the cultures under study in the experiments.
c) To avoid unintentionally releasing microbes from the lab.

2. Cleaning of Glassware:

Before being sterilized, all glassware used in microbiological research needs to be thoroughly cleaned to get rid of dirt and debris.

2.1. Cleaning of New Glassware:

Conical flasks, test tubes, and petriplates should all be cleaned with new glassware by submerging them in a 1% trisodium phosphate solution in an appropriate container. Prior to drying and sterilizing, boil, rinse with tap water, neutralize with 1% HCl, rinse again with tap water, and then finish with distilled water.

2.2. Cleaning of Used Glassware:

It is recommended to empty and rinse used glassware using both warm and cold water before cleaning it with a 1% detergent or a solution of trisodium phosphate. To guarantee cleanliness, rinse with tap water, then with distilled water, dry, and finally sterilize. This methodical technique reduces the possibility of contamination during laboratory procedures, ensuring the validity and integrity of microbiological research.

2.3. Cleaning of Pipettes and Microscopic Slides:

Used pipettes should be kept, preferably overnight, in a tall glass or non-reactive container with a solution of chromic acid. This procedure successfully eliminates any solid film that is stuck to the glass's surface. Microscopic slides must be cleaned with extreme care to make sure they are spotless and prepared for usage.

2.4. Preparation of Chromic Acid:

Dissolve 80 g of potassium dichromate in 300 mL of water to make chromic acid. Slowly pour in 460 mL of pure H_2SO_4, stirring all the time. Glass bottles can be used to hold the reactivity of the resultant chromic acid solution. An alternative is to perform the first cleaning using a detergent solution or a 1% KMnO4 solution. Glassware and pipettes should be thoroughly dried after cleaning; this can be done by first placing them in a hot air oven set to 100 °C before sealing them with cotton plugs. After that, sterilization is carried out in the hot air oven for one to two hours at temperatures between 160 and 180 °C (Boyer, 2006).

3. Sterilization:

Aseptic technique refers to any methods used when handling germs to avoid contamination. Sterilization is the principal technique used to do this. Any procedure that successfully gets rid of transmissible agents like bacteria, viruses, fungi, and prions from foods, surfaces, drugs, equipment, or biological culture media is called sterilization. This is accomplished by subjecting the object to physical or chemical agents for a predetermined amount of time.

Methods of Sterilization: Various methods of sterilization include:

3.1. Heat Sterilization:

The most popular and dependable technique, heat sterilization, involves destroying vital cell components and enzymes. The degree of hydration of the substance affects this method's efficacy. Moisture-resistant materials are sterilized by wet heat at 121 °C, while moisture-sensitive materials are sterilized by dry heat at 160–180 °C (Skoog et al., 2017).

3.1.1. Dry Heat Sterilization:

Higher temperatures are employed in dry heat sterilization; these temperatures normally range from 160 to 180 °C, and depending on the particular temperature used, exposure lengths may reach up to two hours. This approach makes use of multiple strategies:

a. **Incineration**: Complete burning of materials.

b. **Red Heat**: Heating until red-hot.

c. **Flaming**: Passing materials through a flame.
d. **Hot Air Oven**: A hot air oven is used for sterilization. Because dry heat sterilization is non-corrosive and has great penetrability, it is useful for sterilizing metallic tools and glassware. It successfully eliminates bacterial endotoxins, also known as "pyrogens," which are immune to conventional sterilizing techniques.
Dry heat sterilization is typically carried out in a hot air oven.

3.1.2 Moist Heat Sterilization:

Moist heat sterilization utilizes steam in various forms to effectively inactivate microorganisms:

a. Saturated Steam (Autoclaving): Steam under pressure at 121 °C.
b. Boiling Water/Steam at Atmospheric Pressure: Sterilization achieved through boiling.
c. Hot Water below Boiling Point: Sterilization using water heated just below its boiling point.

Saturated steam is a highly effective sterilizing agent, making moist heat sterilization one of the most successful methods. In autoclaves, common laboratory media, liquid materials, used and discarded culture media, glassware, bacterial filters, and rubber caps can all be sterilized. Typically, sterilization is carried out for 15-20 minutes at 121 °C and 15 psi pressure. The contents remain safely preserved and can be stored until needed again.

3.2. Gaseous Sterilization:

Chemically reactive gases with biocidal qualities, such as formaldehyde (methanol, HCHO) and ethylene oxide $(CH_2)_2O$, are used in gaseous sterilization. As an alkylating agent, ethylene oxide is a colorless, odorless, and combustible gas; nonetheless, both gasses have the potential to cause cancer and mutagenesis. They also cause cutaneous, ocular, and nasal mucosa discomfort through acute poisoning.

3.3. Radiation Sterilization:

Radiation sterilization uses a variety of radiation, including particle radiation (accelerated electrons) and electromagnetic radiation (gamma rays and UV light). These radiations mainly target microbial DNA; UV light causes excitation, while gamma rays and electrons cause ionization and the generation of free radicals. For dry items, radiation sterilization is usually used. UV radiation is used in air sterilization, surface sterilization of aseptic work spaces, and water treatment for manufacturing because of its lower energy and restricted penetration. 260 nm is the ideal wavelength for UV sterilization, and mercury lamps that generate light at 254 nm are appropriate UV light sources in this spectrum.

3.4. Filtration Sterilization:

Filtration sterilization operates by physically removing microorganisms instead of destroying them. It is commonly used for both clarifying and sterilizing liquids and gases, efficiently blocking the passage of both viable and non-viable particles (Tripathi et al 2013 a.b).

3.4.1. Applications of Filtration in Sterilization:

3.4.1.1 Sterilization of Gases:

High Efficiency Particulate Air (HEPA) filters are capable of removing up to 99.97% of airborne particles with diameters greater than 0.3 micrometers. Before reaching the HEPA filter, air typically undergoes a preliminary filtration step to eliminate larger contaminants (Wilson and Walker).

3.4.1.2. Types of Filters Used in Filtration Sterilization:

a. **Depth Filters:** Composed of fibrous or granular materials packed to form intricate channels. Materials include diatomaceous earth, unglazed porcelain, sintered glass, or asbestos.
b. **Membrane Filters:** These 0.1 mm thick porous membranes are composed of polycarbonate, polyvinylidene fluoride, cellulose acetate, and/or cellulose nitrate, among other synthetic materials. They are kept in specific holders and supported by frames. Centrifugation or positive or negative pressure allow fluids to pass through the membranes.

3.4.1.3 Implementing Filtration to Sterilize Liquids: Membrane filters with a nominal pore diameter of 0.22 micrometers are commonly used**.** For organic solvents, viscous liquids, and corrosive liquids, sintered filters are recommended (Plummer, 1988).

4. Good Laboratory Practices:

1. As a safeguard against contamination or staining agents, use an apron or lab coat at all times.
2. Clean bench tops thoroughly using suitable disinfectants—such as Lysol (1:500), phenol (1:100), spirit, or 90% ethanol—both before and after each laboratory session.
3. Keep your lab bench clear of extraneous stuff, leaving only room for your notebook and necessary equipment.
4. To prevent contamination, refrain from eating, drinking, or smoking within the lab.

5. Never remove bacterial cultures, equipment, or media from the laboratory.
6. Refrain from putting contaminated tools on bench surfaces, such as pipettes, needles, or inoculation loops.
7. To avoid cross-contamination, make sure loops and needles are sterilized by burning them after use.
8. Discard used pipettes and cultures in the appropriate containers by following the guidelines for disposal.
9. Handle all microbial cultures as though they were possible diseases.
10. Use liquid detergent or soap to give yourself a complete hand wash before entering and after you leave the laboratory.
11. Pull back long hair to lessen the risk of cultural contamination and fire.
12. Always use a test tube rack to handle cultures, and place them securely on a test tube stand or in a basket on the lab bench during transfers.
13. Immediately cover the contaminated area with filter paper dipped in disinfectant in the event of spilled cultures or damaged culture tubes. Thoroughly clean the area after fifteen minutes.
14. Tell the instructor right once if you sustain any unintentional burns or wounds.
15. Never use your mouth to pipette chemical reagents or broth cultures. When pipetting, always use mechanical equipment.
16. Consistently follow aseptic procedures at all times.
17. Ensure you thoroughly understand the laboratory exercise before beginning any procedures.
18. Before beginning any experiment, make sure all plates, tubes, and cultures are appropriately labeled.
19. For experimental purposes, only self-stick labels should be used.

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