Practical Work N° 1

Introduction to the microbiology laboratory

1. Introduction

In vitro study of microorganisms requires specific equipment and apparatus, as well as a laboratory with a somewhat specialized structure. Microbiological manipulations often involve pathogenic microorganisms. Therefore, it is essential to protect against contamination while adhering to aseptic conditions and safety guidelines.

2. Objective of the practical Work

The aim of this practical work is to:

- Gain familiarity with a microbiology laboratory, including its equipment and operational procedures.
- Develop hands-on experience with fundamental microbiological techniques, such as sterilization, handling of laboratory tools, and safe disposal of contaminated materials.

3. Materials used in Microbiology

1. Glassware and small instruments

A variety of tools are used in microbiological manipulations. Some are specific (limited to microbiology), while others are commonly used across multiple experimental sciences. There are single-use instruments and reusable ones (which can be used multiple times after sterilization).

Single-use items (disposable)

- **Pasteur pipettes**: it is a pipette with a tapered tip, one end of which is plugged with carded cotton. The cotton prevents microorganisms from passing through while allowing liquid to be aspirated. After use, the Pasteur pipette is disinfected with bleach and then discarded.
- **Microscope slides**: A small glass plate used to hold and support a prepared sample for microscopic observation. After use, they are placed in a crystallizing dish containing bleach before being discarded.
- **Coverslips**: A small, thin piece of glass used to cover a sample placed on a slide for microscopic observation. They follow the same disposal process as slides.
- **Plastic Petri dishes**: Circular containers widely used in microbiology as a support for culturing microorganisms. After use, they are immediately incinerated.

Reusable items (multiple uses)

- **Inoculation loop**: A straight wire with a looped end, made of platinum (a non-corrosive metal) or a nickel-chrome alloy. It must be protected from twisting and kept clean. It is used for picking up microbial samples.
- **Glass Petri dishes**: A support for culturing microorganisms.
- **Magnifying glass**: Useful for macroscopic examination of microbial colonies.
- **Forceps**: A metal or wooden tool used to hold slides during staining and can also be used to plug graduated pipettes before sterilization.
- **Test tubes**: Used for preparing dilutions, as well as for containing culture media (solid slants, butt media, or liquid) or reagents.
- **Various glassware**: There are several types of glass instruments used in microbiology, including beakers, volumetric flasks, Erlenmeyer flasks, graduated pipettes, and counting chambers (e.g., Thoma and Malassez cells, used for cell counting).

Other items

Products (culture media, alcohol, dyes, reagents, etc.), carded cotton, cones, and more.

2. Equipment

2.1. The optical microscope

The organisms studied in microbiology have a unique characteristic: their extremely small size (on the order of microns) requires the use of a range of techniques to study them, such as microscopy. The optical microscope is the primary tool in microbiology. It allows for the separation of details in the image of a small object, thereby magnifying the image so that it can be observed by the human eye. It consists of two main parts:

A) Mechanical part: Comprising the following components:

a) **Base**: Serves as the foot of the microscope.
b) **Arm**: Supports the eyepiece holder and the objective holder.
c) **Revolving nosepiece:** Holds 3 or 4 objectives; its allows for switching between objectives.
d) **Stage**: A fixed platform used to hold the sample being examined.
e) **Stage clips**: A system for securing the slide in place.
f) **Eyepiece holder**: Holds 1 or 2 eyepieces and includes a sliding mechanism to adjust the eyepieces according to the user.
g) **Diaphragm**: A adjustment system used to regulate light intensity.
h) **Coarse and Fine focus knobs**: Used for focusing.

B) Optical part: Comprising:

a) A **lamp** and a **condenser**, which consists of several lenses to enhance the illumination of the specimen.
b) **Objectives**: Optical systems made of one or more lenses that produce a real image of the object. There are two types of objectives:

- **Dry objective**: The lens of this type does not touch the object; it is separated from the specimen by air.
- **Immersion objective**: The lens of this type touches the object through a medium, typically immersion oil, which has a refractive index very close to that of glass. This oil is used to achieve total reflection of light rays exiting the slide. The most commonly used oil is "cedarwood oil."

c) **Eyepieces**: Composed of two lenses—a lower lens to illuminate the real image produced by the objective, and an upper lens that magnifies the real image by 10x to produce a virtual image.

2.2. Incubation equipment

- **Incubator**: A heated chamber equipped with an electrical heating system and a temperature regulator. It is used for incubating microbial cultures.
- **Water bath**: A metal tank containing distilled water and a heating element. The temperature is controlled by a thermostat. It is used for incubation requiring constant additions.

2.3. Sterilization Equipment

- **Bunsen burner**: Suitable for sterilizing the air in the work area (15-20 cm around the burner), platinum wires, the necks of tubes and flasks, and the exterior of Pasteur pipettes. This provides temporary sterilization.
- **Hot air oven (Pasteur oven)**: Heating is provided by an electrical resistance or gas burners. The oven is used for sterilizing glassware, metal, and porcelain.
- **Autoclave**: A metal cylinder with a heating element immersed in water. It generates steam under pressure, reaching high temperatures (above 110°C). It is particularly suitable for sterilizing non-heat-sensitive culture media, all liquids, and plastic or rubber materials.
- **Filters**: Membrane filters are commonly used for sterilizing heat-sensitive, non-viscous solutions.

2.4. Cold storage equipment

- **Refrigerator (4°C)**: Used for storing culture media, microbial strains, and reagents.
- **Freezer (-20 to -80°C)**: Used for long-term storage of sera, bacteria, and fungi, typically in glycerol to protect them from the lethal effects of freezing.
- **Cooler**: Used for transporting samples sensitive to temperature changes.

4. Safety and asepsis guidelines in the microbiology laboratory

4.1. Presentation of a workstation

- All work must be carried out on a clean bench equipped with waste bins, wash bottles (containing distilled water, bleach for decontamination and disinfection, ethanol, bactericide, etc.).

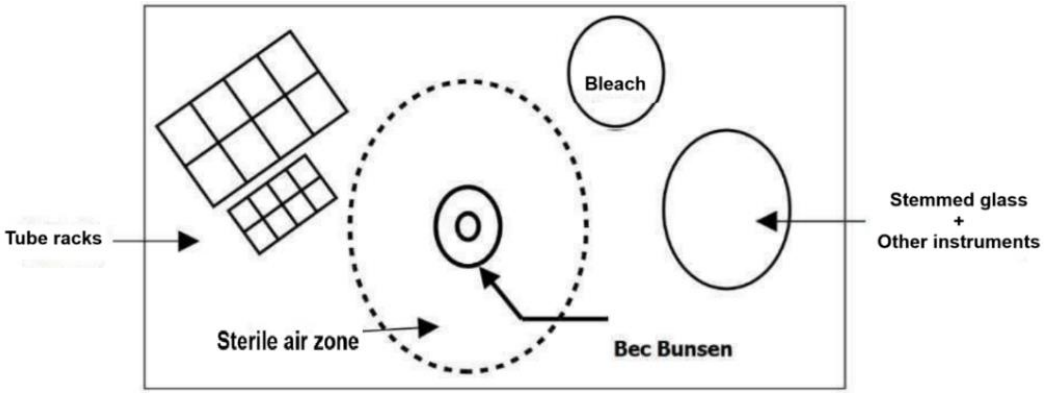

Figure: Organization of microbiology workstation

- To disinfect the bench before lighting the Bunsen burner, apply a few drops of bleach to the surface and spread it with an absorbent sponge (located at the end of the bench) until the bench is dry.
- At the end of the session, it is essential to leave the benches clean, meaning they should be wiped down with bactericide.
- Other items found on the bench include white tape (to avoid writing on glass bottles), a marker, scissors, Parafilm, and a matchbox.

4.2. Biosafety recommendations:

- Wear a long-sleeved lab coat that is fully closed.
- Tie back long hair and trim nails.
- Remove jewelry (rings and bracelets).
- Eating, drinking, and smoking are strictly prohibited in the laboratory.
- Clean the bench with diluted bleach.
- Light the Bunsen burner to create a sterile zone.
- Wash hands thoroughly before and after handling materials, and before leaving the laboratory, even temporarily.
- Avoid opening windows during manipulations.
- Work in a **seated** position.
- Work under aseptic conditions near the Bunsen burner, following the instructor's demonstrations (e.g., how to hold tubes, caps, inoculation loops, etc.).
- Shake all suspensions before use.
- Open containers with microbial cultures carefully to avoid splashing.
- Avoid touching your mouth or face during manipulations.
- Flame metal loops before and after use for sampling. Start by heating the middle part of the tool to dry any remaining culture before flaming the tip to avoid splattering.
- Be cautious with alcohol near flames (it is flammable).
- All materials used for culturing (swabs, Pasteur pipettes, slides) must be placed in a crystallizing dish containing bleach.
- Sterilize all contaminated materials at the end of the session.
- Take all necessary precautions to safely store or destroy microbial strains to prevent contamination.
- Immediately inform the lab supervisor in case of breakage of a culture container, accidental contamination of a handler, or any incident involving the dispersal of microbial material.

Work to do: Basic microbiological techniques

Materials

- Bunsen burner
- Inoculation loop
- Pasteur pipette
- Test tubes and racks
- Distilled water
- Beaker containing bleach (for contaminated waste)
- Diluted bleach

Protocol

1. **Preparation of the work area**

- Clean the workbench by applying a few drops of diluted bleach and spreading it evenly.
- Light the Bunsen burner by slightly opening the gas valve and igniting the gas with a match or lighter. Adjust the flame to obtain a blue flame.

2. **Sterilization of the inoculation loop**

- Hold the inoculation loop by the handle and place the loop in the flame. Keep it there until it becomes **red-hot** (approximately 5 to 10 seconds). Allow the loop to cool for a few seconds before using it to collect a sample. After use, sterilize the loop again before placing it down or storing it.

3. **Sterilization of the Pasteur pipette**

- Hold the Pasteur pipette by the end containing the cotton and quickly pass the glass tip through the Bunsen burner flame. Use quick, repeated movements (1 to 2 seconds) to avoid melting the glass.
- Allow the glass tip to cool for a few seconds before using it to aspirate a liquid.
- After use, immediately place the Pasteur pipette in a beaker containing bleach for decontamination.

4. **Handling a test tube**

- Hold the test tube in one hand and the cap in the other. Open it near the Bunsen burner flame to maintain a sterile zone, and quickly pass the mouth of the tube through the flame to sterilize the opening.
- For adding or removing liquid, use a sterile Pasteur pipette or an inoculation loop (to collect a droplet).
- Sterilize the mouth of the tube again and immediately close it after manipulation.

5. **Disposal of contaminated waste**

- After use, place all contaminated instruments (Pasteur pipettes, inoculation loops, etc.) in a beaker containing bleach or another appropriate disinfectant.
- Never leave contaminated waste on the workbench.

6. **Turning off the Bunsen burner and final cleaning**

- Close the gas valve to turn off the Bunsen burner. Ensure the flame is completely extinguished.
- Clean the workbench again with diluted bleach.
- Ensure all contaminated waste is properly disposed of.
- Return all clean instruments to their designated places.
- Check that the gas and water taps are closed before leaving the laboratory.