#### Practical Microbiology (Lab 1)

### Laboratory safety (Biosafety):

Microbiologists doing diagnostic work in microbiology lab. are in exposure to danger of infection from clinical sample and culture, as well as noninfectious danger such as fire, toxic substances, radioactive materials and others. A microbiology lab. is a unique environment that requires special practices and containment facilities in order to protect persons working with microorganisms.

**Biosafety** is the application of laboratory practices and procedures, laboratory facilities and safety instruments when working with potentially infectious M.O.

\*\*What is the importance of biosafety?

It is important for the protection of students, workers, co-workers, lab support personnel and environment.

#### General instructions and precautions in microbiology lab.:

1- To prevent contamination of your clothes, laboratory coats must be worn and buttoned while in the lab. Laboratory coats should not be worn outside the laboratory.

2- Sterilize the bench before and after the work in the lab.

- 3- Use rubber gloves and nose mask for all works with infective material.
- 4- Keep your nails in short forms.
- 5- Never eat, smoke or drink in the lab.
- 6- Don't touch your eyes, mouth, nose, during the work.
- 7- Don't use your mouth for sucking solution, always use pipetting device.
- 8- Never take any culture or equipment from lab. to the outside.
- 9- Avoid pouring any of biohazardous fluids and chemicals in the lab. sink.

10- Dispose the contaminated material and all cultures to their appropriate places (see Biohazard Waste Disposal below).

11- Flame transfer loops, wires, or needles before and immediately after use to transfer biological material

12- Do not walk about the laboratory with transfer loops, wires, needles, or pipettes containing infectious material.

13- Label all materials with your name, date, and any other applicable information (e.g., media, organism, etc.).

14- Wash your hands with detergent and water before leaving the lab.

# Some of Important instruments and tools of Microbiological Lab.:

1- **Electric oven**: used for sterilizing the glassware and tools that need dry heating with (160  $^{\circ}$ ) for (2 hrs) approximately.

2- **Autoclave**: to sterile the culture media and some of chemical solutions which need wet heating with (121 C<sup>o</sup>) and pressure (15 bar / Inch<sup>2</sup>) for suitable time.

3- **Incubator**: For provide the suitable temperature to grow M.O. such as bacteria & fungi, most bacterial species grow under (37  $^{\circ}$ ) for (18-48 hr).

4- **Compound Microscope**: is a vital instrument in microbiological Lab. to identification the bacteria by it is magnification ability.

5- **Refrigerator**: Used for storage the culture media, bacteria growth culture and other material that need low temperature.

6- **Distiller**: it's providing sterile distilled water.

7- **Biological safety cabinet**: for availing an uncontamination environment during the laboratory work.

8- Gas (Bunsen) Burner: used to sterile the mineral tools such as loop, needle and nozzle bottles or flasks.

9- Sensitive Balance: for weighting the powders.

10- Water bath: is a device that maintains water at a constant temperature to regulate the temperature of substances subjected to heat.

11- **Centrifuge**: it's used to generate extremely high rotating speeds, creating a centrifugal force that forces materials of different densities to separate.

12- **pH meter**: it's used to measure the pH of a substance.

13- Ultra Violate light source: to sterile the atmospheric laboratory.

14- Flask, Cylinders, Pipettes, Tubes, Slides and other glassware.

15- **Millipore Filter Unit**: used for sterilization of solutions by filtration especially the solutions are damage by heating sterilization.

16- Loop & Needle: platinum wires used for transporting, culturing and inoculating the bacterial growth (loop) or for stabbing cultures (Needle).

17- Petridish: these dishes (plates) used as container of culture media.

18- **Biohazard Waste Disposal**: it is important to dispose of materials in proper containers to avoid potentially serious injury or illness to individuals coming in contact with these materials.

19- Washing brush: for bottle washing.

20- Hot plate with magnetic stirrer: used for the heating and agitation of solutions (Magnetic beads are used for the agitation).

### **Bright-field microscope**

### **Introduction:**

A microscope is an optical instrument that is used to observe tiny objects, often objects that cannot be seen at all with the unaided human eye (the "naked eye"), the word of microscope is combination of two words; "*micro*" meaning small and "*scope*" meaning view. The following terms present the three most important properties of lenses and their images:

**Magnification**: The amount that the image of an object is enlarged (e.g., 100X)

**Resolving power**: The extent to which object detail in an image is preserved during the magnifying process.

**Contrast**: The degree to which image details stand out against their background.

**Simple microscope**: A simple microscope is defined as a microscope containing only one magnifying lens. Actually, a magnifying glass could be considered a simple microscope.

**Compound microscope:** A compound microscope is a microscope that contains more than one magnifying lens.



Types of microscopes:

\* The compound light microscope (Bright-field microscope): It's used to observe morphology of microorganisms such as bacteria, protozoa, fungi, and algae in living (unstained) and nonliving (stained) state. Objects are observed against a bright background cannot observe microbes less than 0.2  $\mu$ m in diameter or thickness, such as spirochetes and viruses. The light microscope is called a compound microscope because it contains two types of lenses that function to magnify an object. The lens closest to the eye is called the ocular, while the lens closest to the object is called the objective.



(a) Principal parts and functions

#### \*The light microscope components:

1- Ocular lens (eyepiece): it is use for look through to see image of the specimen.

2- **Body tube:** the long tube that holds the eyepiece and connect it to the objectives.

3- **Nosepiece:** the rotation part of the microscope at the bottom of the body tube and it holds the objectives.

4-**Objective lenses:** A compound light microscope often contains four, the scanning lens (4X), the low-power lens (10X), the high-power lens (40 X), and the oil-immersion lens (100 X). With an ocular lens, that magnifies 10 times.

5- Arm: part of the microscope is attach to the foot and supports the body tube.

6- **Coarse adjustment knob:** large, round knob on the side of the microscope used for focusing the specimen; it may move either the stage or the upper part of the microscope.

7- **Fine adjustment knob:** small, round knob on the side of the microscope used to clear the focus of the specimen after using the coarse adjustment knob.

8- **Stage:** large, flat area under the objectives; it has a hole in it that allows light through; the specimen slide is placed on the stage for viewing.

9- **Stage clips:** shiny, clips on the top of the stage which hold the slide in place.

10- **Aperture:** the hole in the stage that allows light through for better viewing of the specimen.

11- **Condenser**: It is a vital part of the microscope, act on collect, control and concentrate light from the lamp into the specimen.

12- **Condenser height control knob:** This knob is use to precisely regulate the vertical height of the condenser

13- **Iris diaphragm control:** regulate amount of light going through the aperture.

14- **Light or lamp:** source of light usually found near the base of the microscope: the light source makes the specimen easier to see.

## \*How to use a microscope:

- 1. Put the microscope on flat table where the handle toward you.
- 2. Clean the microscope lenses by special papers and don't touch lenses by hands.

- 3. Put the slide on the stage carefully and make sure the cover glass on the slide.
- 4. Examine the sample by low objective lens  $(10\times)$  then by high objective lens  $(40\times)$  with fine adjustment but without using coarse adjustment to avoid the breaking of slide.
- 5. Avoid the use of immersion oil lens without oil and directly clean the lens and slide from oil after the use.
- 6. Don't use one eye during the examination of sample.
- 7. After the finish of examining rise the slide from stage carefully then clean it and clean the microscope lenses then cover the microscope to keep out it from dust.

Note: The oil-immersion objective (x100) must be used to study bacteria, because they are so tiny. To use the oil-immersion objective, a drop of immersion oil must first be placed between the specimen and the objective; the immersion oil reduces the scattering of light and ensures that the light will enter the oil-immersion lens.

**Electron microscopes** use an electron beam as a source of illumination. electron microscopes have a much greater magnification than compound light microscopes (x200,000). There are two types of electron microscopes: transmission electron microscopes (TEMs) and scanning electron microscopes (SEMs).