IV. Common Procedure in the Laboratory

A. Maintaining a microbe-free environment

A microbe-free environment is essential for most of the work in a plant pathology laboratory. Contamination (growth of unwanted fungi, bacteria or any other microorganism) may come from various sources such as utensils, samples brought from a field and human interferences. All samples brought from the field must be considered as sources of inoculums and hence, must be treated with due care. In order to avoid false positive results, contaminants should be removed or maintained at its minimum level as possible. Common techniques used to maintain a microbe free environment are listed below.

1. Dry heat sterilization

Dry heat sterilization kills microbes by oxidation. It is used when the materials to be sterilized are not damaged by high temperature under dry conditions. This method is best for the sterilization of dry glassware such as test tubes, glass Petri dishes, flasks, pipettes, all glass syringes and instruments such as forceps, scalpels and scissors. An electric oven is a commonly used tool for heat sterilization. It can be adjusted to a wide range of temperature for specific duration of time.

Temperature (^{o}C)	Exposure time (Hrs)
180	1
170	2
150	4
120	12-16

2. Moist heat sterilization (**Autoclaving)**

Moist heat kills microorganisms, probably by coagulating and denaturing their enzymes and structural proteins, a process in which water participates. All culture media therefore are sterilized by moist heat. Autoclaving at temperatures greater than 100 °C is the most reliable method and widely used for the sterilization of culture media. Most autoclaves and pressure cookers operate at 121 °C, at which the minimum holding period for sterilization is 15 minutes. It is essential that all air is expelled from the autoclave; otherwise it will not reach the correct temperature.

3. Ultraviolet light radiation

Ultraviolet light is used for sterilization because it kills most microorganisms. While it is recommended for plastic materials, it cannot penetrate glass. Ultraviolet systems must be installed in a closed chamber to avoid exposure, since it is harmful to the eyes.

4. Sterilization of instruments

Forceps, inoculating needles and other instruments must be sterilized before contact with a culture to avoid cross-contamination. Inoculating needles are best sterilized by heating to red heat (incineration) in a flame. Needles must be allowed to cool to room temperature again before being used. Hot needles are the most common cause of failure of sub-culturing, hyphal tipping and single sporing. Forceps and scalpels are sterilized by dipping in alcohol. Before use, the alcohol is burnt off by passing the forceps through a flame to ignite it. Do not hold the instrument in the flame, since this will heat it up too much.

5. Sterilisation of work surfaces

Trays, benches and other surfaces may be sterilized with a liquid disinfectant. Alcohol is the most commonly used. Alcohol works best as a sterilant if it contains some water, and a solution of 70% ethyl alcohol is suitable.

B. Dilution

In a plant pathology laboratory, dilution techniques are often followed to lower the concentration of solutions.

Example: If one wants to dilute a 99.7% ethanol and make 500 ml of a 70% ethanol solution,

C_1= 99.7%

C_2= 70%

V_2= 500 ml

$$C_1V_1 = C_2V_2$$

$$V1 = \frac{C2V2}{C1}$$

$$V1 = \frac{(70\%) \text{ x } (500 \text{ ml})}{(99.7\%)}$$

V_1= 351 ml

Steps

1. Pour 351 ml from the 99.7 % labeled alcohol in to a jar
2. Fill the jar with dilute water to make the final volume of the solution 500 (add 149 ml water)
3. Shake the mixture.
4. Pour the new diluted solution into a new container.
5. Label it (70% Ethanol)

C. Microscopy

Microscopes and stereoscopes are essential for the identification, classification, and quantification of plant pathogens, as well as for observing symptoms and lesions and evaluating experiments.

1. Using a dissecting microscope (Stereoscopes)

A dissecting microscope is used to examine diseased materials for the presence of small fungal structures, such as pycnidia, acervuli, sporodochia and perithecia, under low magnification (up to approximately ×100). Using the dissecting microscope, such structures can be easily transferred to a slide preparation for examination under a compound microscope, at higher magnification (up to ×400).

A dissecting microscope is also used for fine work such as the transfer of germinated single spores or hyphal tips during purification of fungal cultures, and the examination of nematodes, which can also be transferred to a slide for examination under a compound microscope. Developing colonies are best examined under a dissecting microscope.

2. Adjusting the compound microscope

It is very important that objective lenses are not scratched or touched by agar, fungus or stained preparations. Cover slips are very thin and, if broken, can cut fingers.

1. Place a slide containing a specimen on the stage of the microscope.
2. Turn the lamp on and adjust the transformer to approximately 50% brightness.
3. Bring the specimen into focus with the ×10 objective.
4. Close the field iris diaphragm so that it becomes small.
5. Adjust the condenser height to bring the field iris diaphragm into focus.

6. Turn the two condenser centering knobs until the image of the field iris diaphragm is centered.

7. Open the field iris diaphragm until it just disappears from view (i.e. until it is slightly larger than the field of view).

8. Adjust the aperture iris diaphragm to clearly show the specimen. The setting number (numerical aperture) on the aperture iris diaphragm should be approximately 75% of the numerical aperture on the objective being used.

9. Examine the specimen.

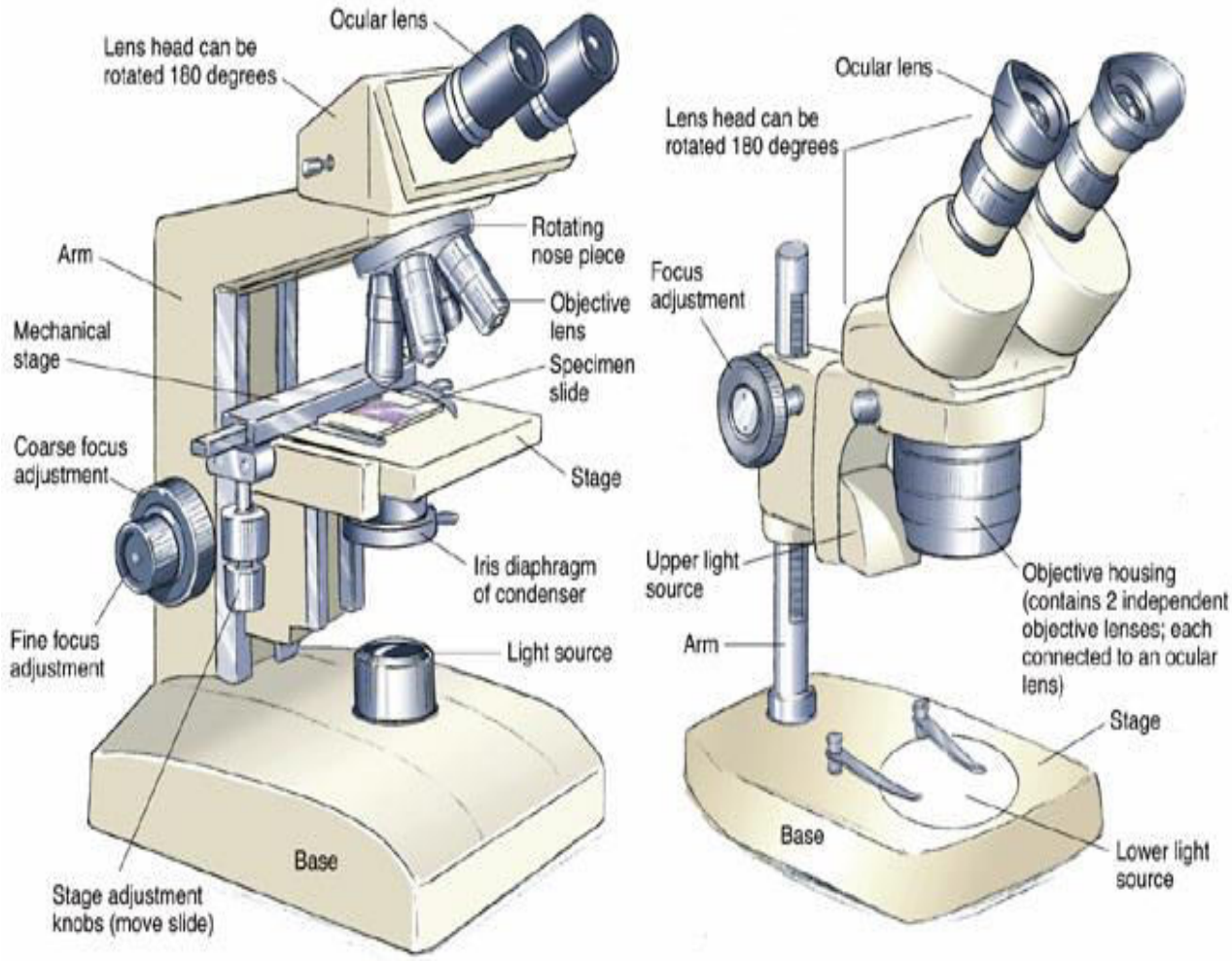

Figure 2. Compound and Stereo Microscopes

3. Mounting specimen

Specimen such as fungal spores or spore forming structures such as pycnidia, perithecia or cleistothecia can be mounted on slides in water or lacto phenol cotton blue solution. Spores from