PLANT PATHOLOGY LABORATORY RULES AND REGULATIONS

1. Always wear apron before entering the laboratory for, protecting clothes from contamination or accidental discoloration by staining solutions
2. Before and after each laboratory period, clean your workbench with a disinfectant
3. Never eat or drink in the laboratory
4. Never place pencils, labels or any other material in your mouth.
5. If a live culture is spilled, cover the area with a disinfectant such as 0.1% mercuric chloride for 15 min and then clean it.
6. Be careful of lab burners and put them off when not in use.
7. The waste paper and contaminated glassware should be kept in waste basket trays.
8. Wash your hands with soap and water before leaving the lab.
9. Broth culture must never be pipetted with mouth.
10. Keep aseptic culture tubes in an upright position in a rack or basket.
11. Materials such as stains, pipettes must be returned to original location after use.
12. Familiarize yourself in advance with the exercise to be performed.
13. Label all plates, cultures and rubes before starting.
14. Before execution of work, read over the exercise to be done and calculate the requirement and plan.
15. Each lab class will begin with a short introduction period. Don't begin work until you have received instructions.
16. Ask questions when you don't understand any method.
17. Properly record all experimental details and observations and keep your note book up to date. Clean the bench with a clean cloth or a piece of cotton, after completion of exercise.

ACQUAINTANCE WITH PLANT PATHOLOGY LABORATORY AND EQUIPMENTS

INSTRUMENTS

Microscope : is a device, which can magnify a microbial cell or a group of microbial cells to enable the human eye (< 0.25 mm)to study its structures, morphology etc.

A. Simple microscope: Consists of a simple lens system

B. Compound microscope: It consists of 2 or more lens systems- Depending on source of illumination, they are of two kinds:

a. **Light microscope:** Specimen is illuminated by visible light or U.V. rays with a max magnification of 1000-2000 or more. These are used for observing stained and unstained specimens and counting of microbes. They include the bright field, dark field, U.V phase contrast and the fluorescent microscope.

b. **Electron microscope:** The images are formed on a fluorescent screen by electron beam focused by magnets instead of lens, with a magnification of 1- 3 lakh times. These are used for observation of viruses and ultra structures of cells.

Autoclave

- It is an apparatus in which saturated steam under pressure affects sterilization called autoclaving. The pressure increases boiling point of water and produces steam with a high temperature.
- Cells are destroyed by high temp and not by the pressure. Most of the organisms are killed at 121 °C and 151b pressure per sq. inch in 15 min.
- It is more efficient and common instrument used to sterilize solids and liquid media for microbial culture, heat resistant instruments and glasswares It is not recommended for oils, powders, heat sensitive fluids and plastics.
- Autoclave is a double walled cylindrical metallic vessel made of thick stainless steel copper, lid of which is opened to receive the material to be sterilized. The lid is provided with pressure gauge noting the pressure, steam clock for air exhaustion of the chamber. It is also provided with safety valve to avoid explosion.
- The materials to be sterilized are kept in a basket provided with holes all around for the free circulation of steam.
- Moist air has most penetrating power than dry heat and hence it is more efficient than dry- heat

Material	Pressure (lb/inch)	Temperature (°C)	Time in min.
PDA/water	10	115	30
do	15	121	15
do	20	126.0	10
Soil/grains	**21**	**126.6**	**60-90**

Pressure cooker: It is a suitable alternative to an autoclave. Some labs will have a big size pressure cookers implanted with a pressure gauge. In case of power failure materials are sterilized in pressure cooker.

Hot air oven: It is an electrically operated equipment with a thermostat (ambient Temp. to 300°C) used for sterilizing glassware, metals through dry heat. It requires longer duration for sterilization because heat conduction is slow and has less penetrating power

An oven consists of an insulated cabinet, which is held at a constant temp, by means of an electric thermostat. Some ovens are also fitted with fan to keep hot air uniformly circulated at constant temperature.

For proper circulation of hot air, the shelves are perforated. The scheduled temperature for sterilization with dry air is as follow

Temperature (°C)	Time in hrs
180	1/2
170	1
160	2
140	3
120	**8**

BOD Incubator:

- It is used for the growth and development of microorganism grown on culture medium at a constant and desired temp. It is similar to an oven in construction
- It consists of an insulated cabinet fitted with a heating and cooling element at the bottom with a thermostat . The temp, of the incubation is maintained at desired level (5- 50^0C) by an automatic device called thermostat.
- It is provided with double doors, made of glass so that the contents of incubator maybe viewed without admitting outside air.
- A beaker of water can be placed in Incubators to retard the dehydration of medium during growth of micro organisms.
- Some incubators are provided with fluorescent light that can be used to encourage sporulation.

Refrigerator

- It consists of an insulated cabinet fitted with a cooling element at the bottom with a thermostat. The temp. is maintained by an automatic device called thermostat
- It is a basic requirement in the microbiological laboratory and used for storing stock cultures of microorganism at 4°C to save sub-culturing every few days.
- The stored cultures at low temperature are fairly inactive and will not suffer damage due to evaporation of medium. It is also used to store sterilized media to prevent dehydration
- It also serve as a repository for thermo-labile solutions, serums, antibiotics and biochemical reagents.

Fermentor:

It provides a controlled environment and pH under aseptic conditions for the growth of microorganism to obtain a desired product eg. Antibiotics, secondary metabolites, toxin etc. in liquid cultures.

Microtome:

It is used for fine and uniform section cutting of the samples for the histopathological study

Temperature and humidity control chamber: In this one can adjust both temperature and humidity

Colony counter:

It is an electronic apparatus used to count the number of colonies on a Petri plate.

A Petridis fits into the recess in the platform.

The colonies on plates are counted on an illuminated screen, illuminated from beneath with a large magnifying lens which provides 1.5X magnification.

Some instruments are also fitted with electronic micro-switch with pen and counter. The counter bar is depressed and the number of colonies is instantly displayed on digital read out.

Micrometers: are used for the measure ment of size of microscopic structures by equipping the microscope with an occular micrometer which is caliberated against a stage micrometer.

Specimen press board: used for the dry preservation of diseased samples. It helps in pressing of diseased plant samples under the presence of blotter paper

Inoculation chamber/ Laminar flow

- It is used for the isolation and purification of microorganism in culture medium. It creates aseptic condition in the chamber while working with infective microorganisms/ during transferring of culture and prevents contamination of sterile materials.
- It is a hood like structure having germicidal ultraviolet lamp and Bunsen burner.
- It consists of mid table as working place onto which sterile air is pumped by the blower fitted at bottom at uniform velocity either in horizontal or vertical direction. It works on the (HEPA)- principle of application of high efficiency particulate filters (or fiber glass filter with particles of 0.3 microns which can retain all contaminanants including bacteria / ULPA (Ultra-Low Penetration Air) with particles 0.12 microns in diameter

Ultraviolet lamps: UV rays with 200-300 nm wave length is germicidal. The lamp which produces U.V. rays of near 200-300 nm wavelength kill or inactivate most of the virus and vegetative form of microorganism present in laboratory or on an inoculation chamber.

Centrifuge

It is an apparatus that rotates at high speed and separates substances as particles on the basis of mass and density by means of centrifugal force (rpm). The microbes are arrested from sediments settled at the bottom of the tube after centrifugation. The

A centrifuge consists of head which is rapidly revolving on upright motors. Generally metal caps are attached to the head for holding tubes or other container of the material from which particulate matters to be separated.

During centrifugation liquid containing particulate matter is kept in the tubes, run at a particular speed and when centrifugation is completed, the particulate matter gets settled at the bottom of the tubes.

The commonly used centrifuges are of low speed, high speed and ultracentrifuge with highest speed limit of 5000 rpm, 18000 rpm, and 20,000 to 60,000 rpm, respectively.

These are used for separation of virus particles, bacterial cells, and fungal spores, separation of mixtures of liquids varying in their density and concentrating microorganisms in various samples for enzymatic and other studies.

Spectrophotometer or colorimeter

It is an electrically operated simple instrument used for estimating population of bacteria, based on the principle of turbidity determination.

Turbidity is the cloudiness of the suspension. The more turbid a suspension, less light will be transmitted through it.

In other words, the amount of light absorbed and is scattered is proportional to the mass of cell. As bacteria grow in a broth, the clear broth becomes turbid.

Since turbidity increases as the number of cells increases, this is used as an indicator of bacterial density in broth.

The turbidity is expressed in unit of optical density (O.D.) which is expressed using Spectrophotometer.

It is used for measuring concentration of bacterial cells and for the quantification of compounds like sugar, phenol, enzymes ,proteins , NA, chlorophyll either in pure form or in mixtyre

Camera lucida:

is an attachment of microscope that enables to draw clear cut outline sketches of the objects seen under the microscope. Reflecting prism and a mirror are the two essential parts of a camera lucida

Haemocytometer or Petroff - Hausser counting chamber

The number of microorganisms present in a given liquid sample can be counted and morphology of bacteria can be observed by direct cell count method using haemocytometer.

It is a special glass slide with a depression (0.1 mm - mm deep) at the centre covering an area of 1 mm, the area of 1 mm is first divided in 25 medium squares and each medium square is further divided into 16 small squares means 1mm contains 400 small squares .

Sintered glass filters: Heat sensitive materials like vitamin solutions are sterilized by filtration technique as they are destroyed by heating at temperature normally used for sterilization

Bunsen Burner

- It is named after R.W. Bunsen. It is a type of gas burner with which a very hot particularly non-luminous flame is obtained by allowing air to enter at the base and mix with gas. In the absence of Bunsen burner, alcoholic lamp is used.
- It provide flame (800-1800^{o}C) which is used to sterilize inoculation needles / loops before they are inserted into culture.
- It is also used for flaming the mouth of test tubes, media containing flasks and other glass apparatus to avoid contamination by other microorganisms.
- It provides aseptic conditions near 2 cm zone which helps during transfer of microorganism.

Hot plate stirrer

- It is useful to stir the chemicals in water without heat to make suspension.
- Stirring is done by creating magnetic field, which causes the bar magnet kept in the container to spin resulting in the stirring of the medium

Electrophoresis:

Is the migration of charged particles or molecules in a medium under the influence of an applied electric field. It is used to determine the number, amount and mobility of components in a given sample or to separate them. Presently it is being used for the determination of molecular wt of protein and DNA sequencing

PH meter:

Is used to determine the pH of solutions of unknown pH as well as for setting of pH of various media used for the cultivation of microoganisms and for the testing of biochemical activeties

TOOLS

Inoculation loop or Inoculation needle: Used for aseptic transfer of culture.

- **It consists of an insulted handle provided with screw device at the tip which holds a heat resistant nichrome or platinum wire approximately 3 inches long. The end of wire is bent to form a loop.**
- **Inoculation needle is similar to loop, but the holder contains a straight piece of wire instead of a loop. They are sterilized by flaming in the blue portion of burner flame until it is red.**
- **The loop is mainly used to transfer culture of microorganism growing on liquid cultures.**
- **Inoculation needles are used to transfer cultures of microorganisms growing on solid medium in form of colonies.**

Glass spreader

- It is bent T or L shaped glass rod used for spreading of liquid culture and sample on sterile agar plate

Glass Ware

Test Tube: a) Test tubes of 18 x 150 mm size are used for preparation of broth, agar slants , b) 25 x 150 mm size are used for preparation of dilution blanks, c) Screw caps tubes with round bottom of size 15 x 125 mm are used for maintenance of culture.

Petri-dishes: Petri-dishes, a pair of circular glass containers named after Petri (Julius Richard Petri, 1887), are used for the preparation of agar plates. The common size is 90 mm in diameter

Pipettes, Flask and Beakers: Different sizes of pipette and conical flask are used for preparation of dilutions and plating.

Generally pipette of 10 ml and 1 ml are used for sterile transfer of known volumes of liquid.

For preparation of dilutions conical flasks of 250, 500 and 1000 ml are used for preparation of medium.

The volume of media should not exceed 2/3 of the volume of flask. Beakers of size 250, 500 and 1000 ml are used for preparation of medium.

Slides and cover slips:

Rectangular slides of 75 x 25 mm size made of glass with polished edge are used for observation of microorganisms.

Square or circular cover slips of size 18x18mm or 20 mm diameter are used for covering the specimen glass slide while observing under high power objective of a microscope.

The thickness of cover slip shouldn't exceed 0.016 mm.