

INTRODUCTION TO PLANT PATHOGENS

PRACTICALS

Practical No. 4-5

**ACQUAINTANCE WITH PLANT PATHOLOGY LABORATORY AND
EQUIPMENTS**

The student should to get acquainted with the chemicals glassware and equipments of the plant pathology laboratory listed below.

INSTRUMENTS

1. Ultraviolet lamps

U.V. 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.

2. pH meter

It is used to determine the pH of solutions of unknown pH as well as for setting of pH of various media, and testing biochemical activity of microorganisms. pH is expressed as a number from 0 to 14. The number is an expression of the concentration of H ion in the solution. The optimum range of pH for bacteria is 6.5 to 7.5 and for fungi it is 4-6. The measure of pH with pH meter is done Electrometrically. Measurement of pH depends upon the development of membrane potential by a glass electrodes. As an alternate, pH papers are used to measure the pH of the medium.

3. Water bath

It is an insulated metallic box fitted with an electric heating mechanism and a thermostat, which maintains the temperature at desired level. There are racks for holding test tubes. These are usually used for melting of media, testing enzymatic activities of various microorganisms, widal test etc.

4. 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. The microbes are arrested from sediments settled at the bottom of the tube after centrifugation. The centrifugal force is noted in rpm of angular speed. A centrifuge consists of head which is rapidly revolving on upright motors. Generally four 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.

5. Balance

Various media components for culture media preparation and samples etc. are weighed on an ordinary balance. Whenever precision is required an electronic mono-pan balance is recommended. As most of the media ingredients are highly hygroscopic, the balance should be cleaned immediately after use.

6. 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.

7. 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 - 0.02 mm deep) at the centre covering an area of 1 mm, the area of 1 mm is further divided into 400 small squares. To get the number of cells per ml of sample the following formula is used. The number of cells per ml = average number of cells in a small square x 400 x 10⁴ (factor).

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8. Filters

Heat sensitive materials like vitamin solutions are sterilized by filtration technique as they are destroyed by heating at temperature normally used for sterilization e.g. Seitz filter.

9. Refrigerator

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 and to serve as a repository for thermo-labile solutions, serums, antibiotics and biochemical reagents.

10. 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. They are 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.

11. Hot plate stirrer

It is useful to stir the chemicals in water without heat to make suspension. It is fitted with the stirrer and heat control. 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.

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switch with pen and counter. The counter bar is depressed and the number of colonies is instantly displayed on digital read out.

12. Inoculation chamber

Most of the aseptic transfers are made using inoculation chamber made of wood. Now-a-days laminar airflow system is used as inoculation chamber. It is used for reducing danger of infection while working with infective microorganisms and for preventing 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 at uniform velocity either in horizontal or vertical direction. It works on the principle of application of high efficiency particulate filters (HEPA)-or fibre glass filter which can retain all particles including bacteria whose diameter is more than 5 microns.