BOTANY [Hons.]
Sixth Semester
DSE3P (Practical)

Industrial and Environmental Microbiology

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Study 1:

Principles and functioning of instruments in microbiology laboratory

Instrument 4: Digital Analytical Mass Balance

Working Principle:

The digital analytical mass balances used in the Microbiology laboratories are very sensitive instruments which are used for weighing substances upto the milligram (0.001 g) level. Balances measure the force/weight that acts downward on the balance pan. Most analytical balances are electromagnetic and hence they measure this weight by using an electromagnet. The electrical current which can generate this force is directly proportional to the weight and is used to calculate the mass after appropriate calibration. This mass is subsequently displayed on the screen of the instrument.

Description:

Most analytical balances have either automatic internal motorized calibration or calibration with external weights or both. Analytical balances have a weighing chamber enclosing the weighing pan with glass doors [also called draft shield] to prevent interference from vibration and air currents. The shield also prevents the accumulation of dust.

The doors on the right and left slide open to allow access to the weighing pan. The front panel of the balance has a display monitor. Most balances are switched on by pressing a circular red coloured on button. After few seconds the digital display monitor shows "0.000 g". To weigh any substance, one of the draft shield doors is slide on and the substance placed in the center of the pan. The door is closed and after a few second, the digital monitor displays a constant mass.

There are two common methods used in weighing: i) weighing by difference or ii) taring the balance. In weighing by difference, the mass of the substance is calculated by subtracting the weight of an empty container from the total weight of the container and the substance. In taring the balance, the balance is first set to disregard the mass of the container. Then the mass of the added substance is measured directly.

Precautions:

1. **Tongs, clamps, or a tissue should always be used to handle solid objects or liquid containers.**
2. **Containers to be used when weighing chemicals.**
3. **The area around the weighing pan should always be cleaned with a soft brush after use.**
4. **No chemicals should be directly placed onto the balance weighing pan.**
5. **Butter paper, plastic weighing boats, small beakers, watch glasses, small vials etc. can be used for weighing chemicals.**

Students please Note:

Diagram of Digital Analytical Balance will be sent to all of your email-IDs.

Instrument 5: Laminar Air Flow Cabinet / Hood

Working Principle:

Laminar airflow is used to exclude volumes of air and prevent airborne contaminants [dust/microbes] from entering a sterile area. A laminar flow unit creates dust free microbe free air environment.

Air from the room passes through the HEPA (High Efficiency Particulate Absorbing) filters. It is then inserted to the working chamber by a unidirectional vertical descending flow. From the working area the air is moved back to the outer environment by the perforation in the bottom rear area of the cabinet, or through the space between the working surface of the table and the protecting glass.

Description:

A laminar flow cabinet is an enclosed workbench. It creates a contamination free work environment with the help of HEPA filters. These filters capture all the particles entering the cabinet. Those substances which are not hazardous for the personnel health should only be used inside a laminar flow hood . The other basic components of a laminar flow hood include UV light, glass shield, an air intake fan, a protection plate, windows, etc.

The type of cell culture hood required depends on the requirements of the laboratory, the kind of airflow needed, working principle, and the type of operation. There are two main types of laminar flow hoods: horizontal airflow hood and vertical airflow hood.

Precautions:

1. Large objects should never be placed near the back of the hood. These objects contaminate everything downstream and disrupt the laminar flow pattern of air too, which normally suspends the contaminants and removes them from the area.
2. Waste and other items should never enter the hood. All calculations should be done before entering the hood. And clutter should be less.
3. Hands should be cleaned by ethanol.Do not touch your hair, face or clothing while working.
4. Excess dust should be removed from items before introducing them into the hood.

Students please Note:

Diagram of Laminar Air flow Hood will be sent to all of your email-IDs.

Instrument 6: Centrifuge

Working Principle:

Centrifuge are designed to separate solids from liquids in suspension or separate two liquids with different density and non-homogenous liquids by using centrifugal force. It applies centrifugal force to separate the useful component in mixtures of liquids and solids or liquids and liquids depending on different density and particle size of solid particles in the liquid. The sedimentation centrifuge can also categorize solids according to different density and particle size. It is generally used in chemical, oil, food, pharmaceutical, coal, water treatment and shipping etc. as well as in laboratories.

Description:

Centrifuge has a rotor rotating its axle called bowl with the help of a motor. The rotor itself has adapters which can hold containers of specific capacity. Suspension or emulsion inside the specific containers are put in the rotor and it is rotated with the same desired speed for a desired time interval at a particular temperature.

Precautions:

(1) The centrifuge should be placed on a firm base.
(2) The centrifuge tubes should be balanced properly before the centrifugation.
(3) The chamber of the rotor should be kept clean.
(4) All the spills should be cleaned immediately.
(5) O-rings and rotor threads should be lubricated weekly.
(6) The rotor should be disinfected weekly on a regular basis

Students please Note:

Diagram of Centrifuge will be sent to all of your email-IDs.

Instrument 7: Microscope

Working Principle:

Magnification: **Modern microscopes differ significantly in both appearance and complexity. In the light microscope, light rays which have passed through the specimen are transmitted through two sets of lenses, the objective (which is nearest to the specimen), and the eyepiece (which is further away from the specimen but nearest to the observant eye). The objective produces the magnified image of the specimen first. It is known as the primary image. The eyepiece subsequently magnifies this primary image into the final one which is seen by the observer. The total magnification obtainable by the microscope is the product of the magnification of the objective and that of the eyepiece.**

Resolving power of an objective: **The increase in magnifying power of the objective is proportional to its resolving power. This is the minimum distance by which two points needs to be separated in order for them to be distinguished as two distinct points rather than as a single fused image. As such, the higher the resolving power of an objective, the closer can be the minute details in the specimen which it can separate in the image.**

Description:

The conventional microscope comprises of a base from which arises a short upright pillar, and, attached to this pillar, a body which carries the illuminating mechanism, the stage, the tube, the arm with its coarse and fine adjustments screws, and the eyepiece and objective lenses.

The illuminating mechanism: **It consists of a mirror having plane and concave sides, a condenser below the stage, and an iris diaphragm . The iris diaphragm regulates the amount of light passing up from the mirror. The condenser lens can be moved up and down by rotating a knob located on the side of the arm of the microscope. The condenser increases the illumination and gathers the rays of light from a wide angle to fall on the object.**

The stage: **The stage is a broad flat rectangular surface with a central aperture fixed to the body. The objects mounted on slides, which are to be examined are placed on the stage. The aperture allows light from the condenser lens to the objective lens. The mechanical stage has clamps/ clips and spring lever attached to it. The clips on both sides of the stage holds the slide in place. The mechanical stage is equipped with two additional knobs. One knob moves the slide either to the left and right. On the other hand, the other knob helps to move in the backward and forward directions.**

Description:

The tube: **The tube is either monocular or binocular. It is attached to the body above the stage and supports the eyepiece and objective lenses at a known distance and angle. The eyepieces, at the upper end of the tube, can easily be pulled out and replaced. The tube is fitted with a nosepiece into which the objectives are screwed at the lower end. The tube carrying the two sets of lenses is lowered and raised by the coarse and fine adjustments screws in order to focus the specimen on the slide. The knobs for focussing are located near the base or the upper arm depending on the type of microscope.**

The objective: **There are three objectives in general use, usually known as the low power, high power and oil immersion objectives. These respectively have focal lengths of 16mm, 4mm and 2mm, and magnifications of about 10X, 40X and 100X respectively. Objectives are engraved with their magnifications.**

The eyepiece: **Two eyepiece sets are required for ordinary work, for example, a low power, 5X eyepiece set and, a higher power, 10X eyepiece set.**

Precautions:

1. Any dust and dirt from the stage and other surfaces of the microscope should be wiped with a clean cloth or a small soft brush.
2. The knobs i.e focusing controls, mechanical stage controls, etc., are delicate and should never be forced.
3. The objective must be move to one side when inserting and removing specimens to prevent scratching of the front lens of the objective.
4. The lamp brightness should be turned to its lowest setting, switched off the microscope, and the plug removed from the mains socket after use.
5. The microscope should be covered with its dust protector.
6. The microscope should be always carried by using both hands. One hand should hold the arm of the microscope while the other hand supports the base.
7. If the microscope requires to be stored for a number of days in hot humid conditions, it should be sealed in an airtight plastic bag. Placing sachets of dry silica gel which absorbs moisture from the bag is also recommended.

Students please Note:

Diagram of Microscope will be sent to all of your email-IDs.

References/sources used:

1. https://andyjconnelly.wordpress.com/2017/02/12/the-laboratory-balance-a-practical-guide/
2. http://www.acmasindia.com/blog/what-is-laminar-hoodlaminar-air-flow/
3. https://www.lamsys.com/products/vertical_laminar_flow/

Further reading:

1. https://www.shimadzu.com/an/hplc/support/lib/lctalk/66/66lab.html
2. https://www.grainger.com/know-how/equipment-information/kh-laboratory-balance-scale-types-care-terms
3. https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/laminar-airflow