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Module's Name: Introduction to Laboratory Tools and Instruments

Content Creator

Dr. Amanulla Khan
Department of Botany
Anjuman Islam Janjira,
Degree College of Science,
Murud-Janjira, Raigad Maharashtra-402401 India
E-mail ID: dramanullak@gmail.com

Module 1: Introduction to Laboratory Tools and Instruments

1.1 Study of Basic Laboratory Instruments (Microscope, Colorimeter, Autoclave, Oven, Incubator, Laminar Air Chamber, Tilak Air Sampler).

Aim: To study of Basic Laboratory Instruments w. r. t. Microscope, Colorimeter, Autoclave, Oven, Incubator, Laminar Air Chamber, Tilak Air Sampler.

01: Microscope:

The microscope is a crucial instrument used to view small objects or details that are invisible to the naked eye by magnifying their images. It's particularly useful for observing the shapes of bacteria, fungi, parasites, and host cells in both stained and unstained samples.

a. Types of Microscopes

I. **Simple Microscope:** Utilizes a concave mirror and contains a single adjustment screw for focusing.

II. **Compound Microscope:** Uses a plane mirror on one side and a concave mirror on the other, featuring a coarse adjustment for rapid focusing. It's the most common type used in microbiology and consists of two lens systems for magnification. These microscopes can be monocular (single eyepiece) or binocular (two eyepieces).

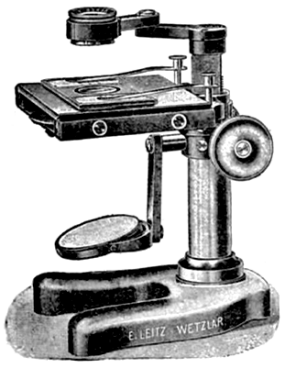

Fig.1: Simple Microscope

Fig.2: Compound Microscope

b. Parts of the Microscope:

The main parts of the microscope are the eye-pieces, microscope tube, nosepiece, objective, mechanical stage, condenser, coarse and fine focusing knobs, and light source.

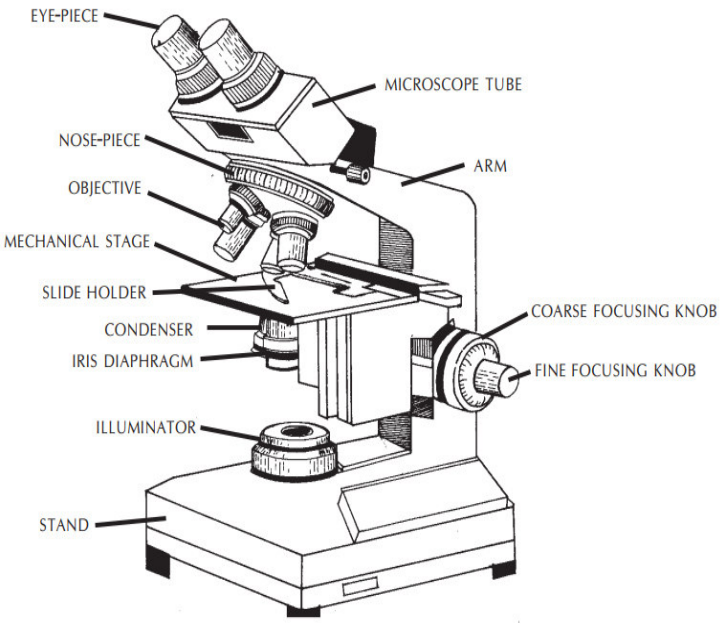

Fig.3: Parts of the Microscope

i. **Eye-pieces:** The specimen is viewed through the eyepiece, which magnifies the image formed by the objective lens. The magnification power ranges from 5x to 20x. Binocular microscopes allow for the adjustment of the distance between eyepieces to fit the user's eyes.

Fig.4: Eye-piece of the Microscope

ii. **Microscope Tube:** Attached to the top of the arm, this tube can be monocular or binocular and supports the eyepiece.

iii. **Mechanical Tube Length:** The distance between the objective lens insertion point and the top of the draw-tube for eyepieces.

iv. **Nose-piece:** Located under the arm, it holds and rotates the objectives, arranging them in order of increasing magnifying power to prevent contamination with immersion oil.

Fig.5: Nose-piece and Objectives of the Microscope

v. **Objectives:** These lenses, typically with magnifications of 4x, 10x, 40x, and 100x, form the initial image of the specimen. The numerical aperture (NA) indicates the lens's light-gathering ability, correlating with its resolving power. Immersion oil, which has the same refractive index as glass, is used with the 100x objective to enhance image clarity. The 100x objective is for oil immersion.

The numerical aperture (NA) is the measure of light-gathering power of a lens. The NA corresponding to the various magnifying powers of the objective is:

Magnification	Numerical aperture

10x	0.25
40x	0.65
100x	1.25

A high NA indicates a high resolving power and thus useful magnification

To provide the best image at high magnification, immersion oil is placed between the slide and the oil immersion objective (100x). Unlike air, immersion oil has the same refractive index as glass. Therefore, it improves the quality of the image. If immersion oil is not used, the image appears blurred or hazy

vi. **Condenser:** Illuminates the specimen and controls light intensity and contrast. Some condensers can be adjusted vertically using a rack-and-pinion mechanism.

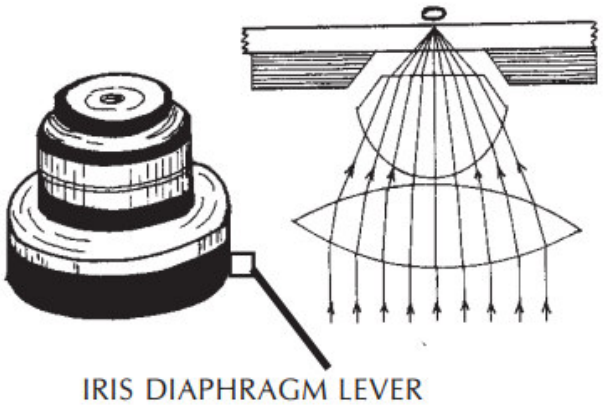

Fig.6: Condenser of the Microscope

vii. **Two-sided Mirror:** Reflects light to illuminate the specimen, with one plain side for artificial light and a concave side for natural light. It can rotate freely and is usually mounted at the base of the microscope.

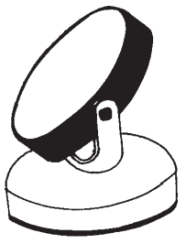

Fig.7: Two-sided Mirror of the Microscope

c. Functioning of the Microscope

A compound light microscope relies on three primary optical components to produce a sharp and clear image: the condenser, the objectives, and the eyepieces. Each part plays a crucial role in the functionality of the microscope.

1. **Condenser:** The condenser is responsible for illuminating the specimen. It does this by converging a parallel beam of light onto the specimen from either a built-in or natural light source. This concentrated light enhances the visibility and contrast of the specimen, making it easier to observe fine details.
2. **Objectives:** The objective lenses are located close to the specimen and are the first to magnify the image. The objectives form a magnified, inverted (upside-down) image of the specimen. These lenses come in various magnifying powers, such as 4x, 10x, 40x, and 100x (oil immersion), allowing for different levels of detail to be observed.
3. **Eyepieces:** The eyepiece, or ocular lens, is where the viewer looks through to see the magnified image. It further magnifies the image created by the objective lens. The final image, which is observed by the viewer, is formed below the plane of the slide. Eyepieces typically have magnifications ranging from 5x to 20x.

The total magnification of the compound light microscope is determined by multiplying the magnifying powers of the objective lens and the eyepiece. For example,

if the eyepiece has a magnification of 10x and the objective lens has a magnification of 100x, the total magnification is calculated as follows:

Total Magnification=Magnification of Eyepiece × Magnification of Objective

Total Magnification=10*x*×100*x*=1000x

This means the specimen will be magnified 1000 times its actual size, allowing for detailed observation of microscopic structures.

02 Colorimeter: A colorimeter is an instrumental device used in colorimetry to measure the absorbance of a specific wavelength of light by a sample solution. Invented by Louis J. Duboscq in 1870, this device is fundamental in determining the concentration of solutes in a solution by comparing the color intensity of the sample to that of a reference solution with a known concentration.

Instrumentation of Colorimeter

1. **Light Source:**
   - Provides energy across the visible spectrum (380-780 nm). Tungsten lamps are typical for visible and near-infrared ranges, while halogen deuterium lamps are used for the UV range (200-900 nm).
2. **Slit:**
   - Reduces stray light by allowing a precise beam to pass through.
3. **Condensing Lens:**
   - Produces a parallel beam of light after it passes through the slit.
4. **Monochromator:**
   - Filters monochromatic light from polychromatic light, allowing only the desired wavelength to pass through. Types include prism, glass, and gratings.
   - **Prism:** Refracts light when it passes through.
   - **Glass:** Selectively transmits specific wavelength ranges.
   - **Gratings:** Made of graphite, separates light into different wavelengths.
5. **Cuvette (Sample Cell):**

- Holds the sample solution. Sizes vary (square, rectangle, round) and typically have a 1 cm diameter. Types include glass, quartz, and plastic.
- **Glass Cuvettes:** Absorb light at 340 nm, cost-effective.
- **Quartz Cuvettes:** Allow both UV and visible light.
- **Plastic Cuvettes:** Inexpensive, easily scratched, shorter lifespan.

6. **Photocell (Photodetector):**
   - Converts light energy into electrical energy, measuring light intensity.
7. **Galvanometer:**
   - Detects and measures the electrical signal from the photocell, displaying optical density (OD) and percentage transmission.

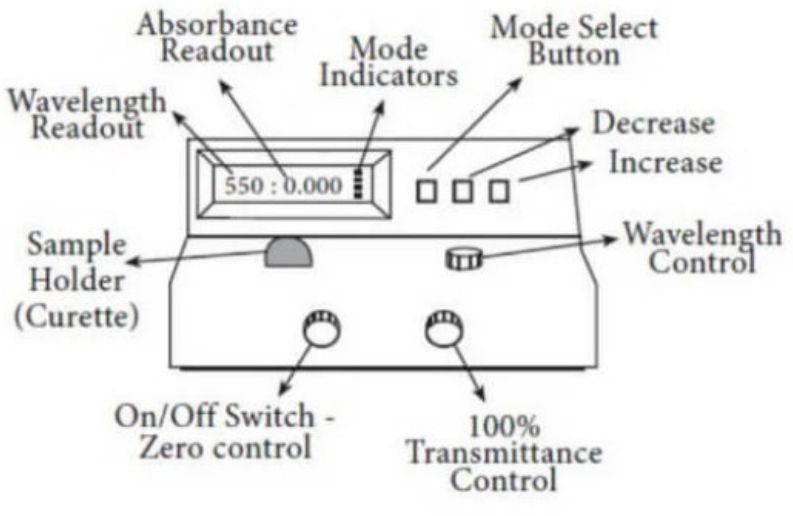

Fig.8: Colorimeter

Principle of Colorimeter

When an incident light beam (I_0) passes through a solution, part of it is reflected (I_r), absorbed (Ia), and the remainder is transmitted (I_t). The relationship is:

$$I_0 = I_r + I_a + I_t$$

By measuring the initial intensity (I_0) and the transmitted intensity (I_t), the absorbance (I_a) can be determined, as the reflected light (I_r) is kept constant using cuvettes with identical characteristics.

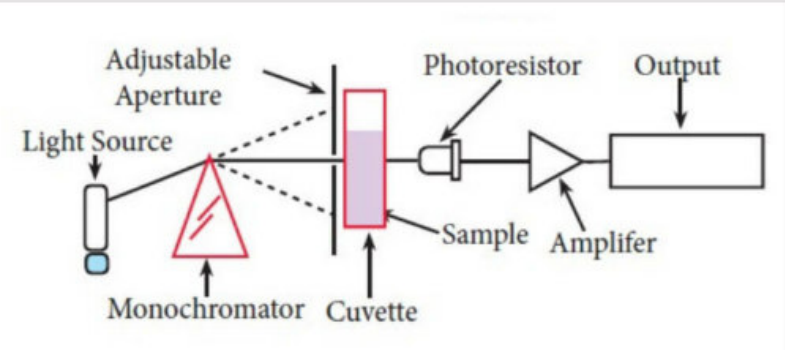

Fig.9: Principle of Colorimeter

Colorimeter Operating Procedure

i. **Power On:** Rotate the Power Switch knob clockwise. Allow the device to warm up for 15 minutes to stabilize the light source and detector.

ii. **Set Wavelength:** Adjust the Wavelength Control knob to select the desired wavelength for the measurement.

iii. **Switch to Transmittance Mode:** Press the MODE control key until the "Transmittance" indicator lights up.

iv. **Zero Adjustment:** Use the Zero Control knob to set the display's T-factor to 0.0% with the sample chamber empty and the cover closed.

v. **Insert Blank Solution:** Fill a cuvette with the blank solution. Clean the exterior to remove any contaminants, then place it in the sample chamber, aligning and securing it properly.

vi. **Calibrate Transmittance:** Adjust the Transmittance/Absorbance control knob to set the display to 100.0% transmittance.

vii. **Switch to Absorbance Mode:** Press the MODE control key to switch to Absorbance mode. The display should read 0.0. Adjust if necessary.

viii. **Insert Sample Solution:** Place the sample solution in another cuvette, clean the exterior, and insert it into the sample chamber.

ix. **Read Measurements:** Read the %T value directly from the display. Switch to Absorbance mode to read the A value.

x. **Remove Cuvette:** - Reverse the insertion process to remove the cuvette from the sample chamber.

Applications of Colorimeter

i. **Printing Industry:** Evaluates the quality of ink and paper by measuring color consistency and accuracy.
ii. **Food Industry:** Measures the composition of food products, including color additives and concentration of ingredients.
iii. **Medical Labs:** Analyzes biochemical samples such as blood, urine, and cerebrospinal fluid for various diagnostic purposes.
iv. **Textile and Paint Industries:** Assesses the quality and color of textile and paint products to ensure consistency and quality control.

03 Autoclave:

An autoclave is a device that uses steam under pressure to sterilize materials by killing bacteria, viruses, and spores. It is widely used in healthcare and various industries for its effective sterilization method, which leverages moist heat.

Fig.10: Autoclave

Autoclave Parts/Components

1. Pressure Chamber:

- The core component consisting of an inner chamber and an outer jacket.
- Inner chamber: Made of stainless steel or gunmetal.
- Outer jacket: Made of iron, filled with steam to accelerate the sterilization process.
- Sizes range from 100 to 3000 liters.

2. Lid/Door:

- Seals off the external atmosphere to create a sterile environment.
- Made airtight with scrsew clamps and an asbestos washer.
- Includes several key components:
    - **Pressure Gauge:** Indicates the pressure inside the autoclave.
    - **Pressure Releasing Unit/Whistle:** Controls pressure by releasing excess vapor.
    - **Safety Valve:** Releases pressure if it becomes dangerously high to prevent explosions.

3. Steam Generator/Electrical Heater:

- Located beneath the chamber, it heats water to generate steam.
- Water levels must be monitored to avoid damage to the heating system or interference with the contents.

4. Vacuum Generator (if applicable):

- Removes air from the chamber to prevent air pockets that could harbor microorganisms.

5. Wastewater Cooler:

- Cools the effluent before it enters the drainage system to prevent damage from boiling water.

Principle of Autoclave Operation

Autoclaves operate on the principle of moist heat sterilization. Steam under pressure increases the boiling point of water, allowing higher temperatures to be reached for effective sterilization. The steam penetrates materials, coagulating proteins and irreversibly inactivating microbes. The standard conditions are 121°C at 15 psi for 15 minutes, but this can vary based on the material's contamination level.

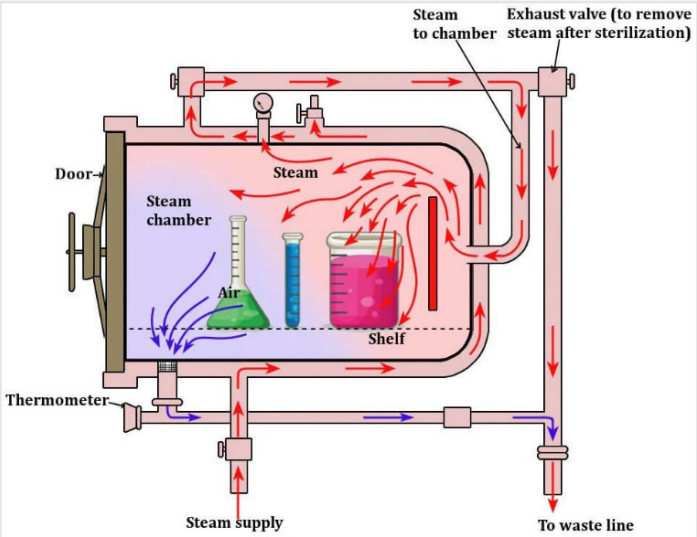

Fig.11: Principle of Autoclave

Autoclave Operating Procedure: Autoclaving typically involves running the machine at 121°C for at least 30 minutes using saturated steam at a minimum of 15 psi of pressure. Below is a step-by-step guide for running an autoclave:

1. **Pre-Check:** Ensure the autoclave is free of any items from the previous cycle.
2. **Add Water:** Fill the chamber with a sufficient amount of water.
3. **Load Materials:** Place the items to be sterilized inside the chamber.
4. **Seal the Chamber:** Close the lid securely and tighten the screws to create an airtight seal. Turn on the electric heater.
5. **Adjust Safety Valves:** Set the safety valves to maintain the required pressure.
6. **Boil Water:** Allow the water to boil. The air-water mixture should escape through the discharge tube to displace all the air inside. Complete displacement is indicated when water bubbles cease to emerge from the pipe.
7. **Close Drainage Pipe:** Once air displacement is complete, close the drainage pipe.

8. **Reach Desired Pressure:** Allow the steam to build up to the desired pressure level (typically 15 psi). The whistle will blow to release excess pressure once the desired level is reached.
9. **Holding Period:** Maintain the autoclave at the desired pressure and temperature for the holding period, usually 15 minutes.
10. **Cool Down:** Turn off the electric heater and let the autoclave cool until the pressure gauge shows that the pressure has dropped to atmospheric levels.
11. **Release Pressure:** Open the discharge pipe to allow air to enter and equalize the pressure inside the autoclave.
12. **Unload Materials:** Finally, open the lid and remove the sterilized items from the chamber.

Uses of Autoclave

1. **Healthcare and Laboratories:** Sterilizes medical equipment, glassware, surgical instruments, and medical waste.
2. **Research and Development:** Sterilizes culture media, autoclavable containers, plastic tubes, and pipette tips.
3. **Waste Management:** Inactivates regulated medical waste containing biological materials before disposal.
4. **Industry:** Used in pharmaceuticals, food processing, and other industries to ensure sterile conditions.

04 Hot Air Oven:

A hot air oven is essential laboratory equipment used for sterilizing objects and samples through dry heat. This method, known as dry heat sterilization, was introduced by French scientist Louis Pasteur in the late 1800s. Pasteur used dry heat briefly to eliminate harmful microorganisms in wine without altering its taste.

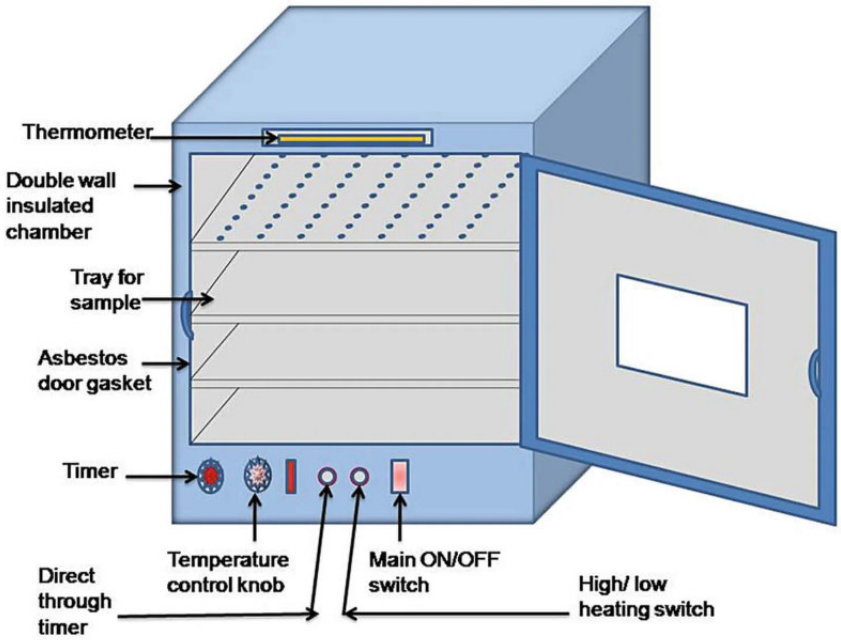

Fig.12: Hot Air Oven

Hot air ovens are ideal for sterilizing heat-resistant items that do not melt, change form, or catch fire at high temperatures. They effectively kill microorganisms and bacterial spores at high temperatures over several hours. Effective sterilization requires selecting the appropriate temperature and holding time based on the type of microorganism and material. Common settings are 170°C for 30 minutes, 160°C for 60 minutes, and 150°C for 150 minutes.

Principle of Hot Air Oven

Hot air ovens operate on the principle of dry heat sterilization using convection, conduction, and radiation. Heating elements heat the air inside the chamber, which is evenly circulated with the help of fans. This hot, dry air exposure heats the external surfaces of items, transferring heat inward. In microorganisms, this heat evaporates water, causing oxidative damage to cellular components, protein denaturation, and ultimately cell death.

Components of a Hot Air Oven

Mechanical Parts:

1. **Coat/Cabinet:** Made of aluminum or stainless steel, providing insulation and preventing heat loss.
2. **Fibreglass:** Insulates the space between the outer cabinet and inner chamber, using brown or yellow fiberglass.
3. **Chamber:** Rectangular chamber made of aluminum or stainless steel with ribs for shelves.
4. **Shelves (Mesh):** Aluminum plates that hold items, allowing air movement and aeration.
5. **Motorized Fans/Blower:** Distributes hot air evenly inside the chamber.
6. **Door:** Single door with asbestos gasket to reduce heat loss.

Electrical Parts:

1. **Power Supply:** 220V-50Hz transformer and rectifier.
2. **Heater:** Generates heat through electrical current, with various heater types operating from 50 to 300°C.
3. **Thermostat:** Heat sensor directly connected to the heater, preventing temperature overshoot.
4. **Temperature Indicator:** Thermometer or thermocouple to measure internal temperature.
5. **Timer:** Electrical or mechanical timers for setting sterilization duration.
6. **Fuse:** Prevents electrical damage during short circuits or high loads.
7. **Control Panel:** Allows control of temperature, time, with indicator lamps and switch knobs.

Hot Air Oven Operating Procedure

1. **Power On:** Plug in and switch on the oven.
2. **Preheat:** Preheat the oven for 30 minutes before loading items.
3. **Set Temperature and Time:** Adjust the temperature gauge based on the content volume.
4. **Load Items:** Place items on shelves with adequate spacing for heat circulation.
5. **Close Door:** Fasten screws and let the temperature rise.
6. **Monitor Temperature:** Check the thermometer to ensure the desired temperature is reached.
7. **Sterilization Period:** Once the holding period is achieved, switch off the oven and allow it to cool.

8. **Unload Items:** Remove items using oven mitts or tongs, then close the door.

Applications of Hot Air Oven

1. **Laboratory Sterilization:** Sterilizes glassware, metal items, culture media, and non-volatile compounds.
2. **Quality Testing:** Tests food items, pharmaceuticals, and consumables for temperature stability.
3. **Research Use:** Used in biology, chemistry, and material science research.
4. **Sample Treatment:** Heat treatment and drying of metals, alloys, soil, and other materials.

05 Incubator:

An incubator in microbiology is an insulated, enclosed device that maintains optimal temperature, humidity, and other environmental conditions necessary for the growth of organisms. It is crucial for cultivating both unicellular and multicellular organisms under controlled conditions.

Components of an Incubator

Cabinet: The main body of the incubator, a double-walled cuboidal structure, with a capacity ranging from 20 to 800L. The outer wall is made of stainless steel, while the inner wall is aluminum, with glass wool insulation in between to prevent heat loss and reduce energy consumption. Inward projections on the inner wall support shelves inside the incubator.

Door: The insulated door includes a glass panel for observing the interior without opening it, and a handle for easy maneuvering.

Control Panel: Located on the outer wall, the control panel contains switches and indicators for controlling the incubator's parameters, including a switch for the thermostat.

Thermostat: Used to set and maintain the desired temperature inside the incubator.

Perforated Shelves: Shelves with perforations to allow hot air circulation, which can be removable for cleaning.

Asbestos Door Gasket: Provides an airtight seal between the door and cabinet to maintain the internal environment.

L-shaped Thermometer: Positioned on the outer wall with one end inside the chamber to read the internal temperature.

HEPA Filters: Advanced incubators may include HEPA filters to reduce contamination from airflow.

Humidity and Gas Control: CO2 incubators have a water reservoir for maintaining humidity and gas chambers for CO2 concentration control.

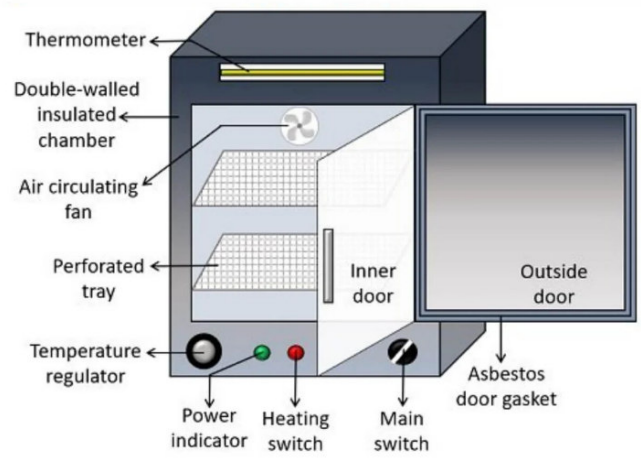

Fig.13: Incubator

Principle and Working of an Incubator

An incubator operates on the principle that microorganisms require specific environmental conditions for growth. It maintains optimal conditions of temperature, humidity, oxygen, and CO2 levels.

- **Temperature Control:** The thermostat regulates temperature using heating and no-heating cycles. The thermometer allows external temperature monitoring.

- **Insulation:** Insulation maintains an isolated environment inside the cabinet, promoting effective microbial growth.
- **Humidity and Airflow:** Controlled through various mechanisms to simulate natural growth conditions.
- **CO2 Concentration:** Adjustable to balance pH and humidity for optimal organism growth.
- **Shaking Incubators:** Variations include shaking incubators for continuous culture movement, aiding cell aeration and solubility studies.

Operating Procedure

1. **Preparation:** Ensure no leftover items from previous cycles are in the incubator. If cultivating multiple organisms with the same requirements, they can be placed together.
2. **Preheating:** Close the door, switch on the incubator, and heat to the desired temperature. Use the thermometer to confirm the temperature.
3. **Parameter Setting:** Set required CO2 concentration and humidity levels if needed.
4. **Loading Cultures:** Place petri dish cultures upside down on perforated shelves to prevent condensation on the medium surface.
5. **Sealing:** For long incubation periods, seal plates with adhesive tape or place them in plastic containers.
6. **Incubation:** Lock the door and maintain the cultures inside for the required period.

Applications of Incubators

1. **Microbial and Cell Culture:** Growing and maintaining microbial or cell cultures.
2. **Growth Rate Acceleration:** Enhancing the growth rate of slow-growing organisms.
3. **Biochemical Oxygen Demand:** Supporting microbial colony reproduction for biochemical oxygen demand tests.
4. **Breeding and Hatching:** Used in zoology for breeding insects and hatching eggs.
5. **Controlled Storage:** Providing a controlled environment for sample storage before laboratory processing

06 Laminar Air Chamber (Laminar Air Flow):

A laminar flow hood, also known as a laminar air flow cabinet, is an enclosed workstation designed to create a contamination-free environment through the use of filters that capture all particles entering the cabinet. It is particularly useful for aseptic media distribution and plate pouring.

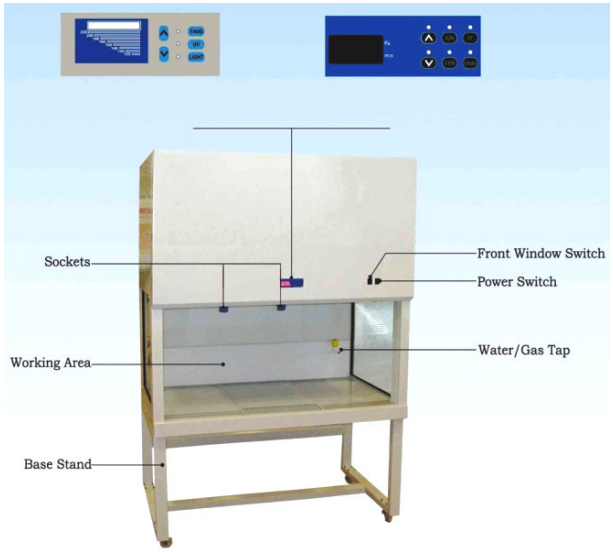

Fig.14: Laminar Air Flow

Components:

i. Cabinet:

- Constructed from stainless steel to prevent spore collection, with minimal gaps or joints.
- Insulated to maintain an uncontaminated environment inside.
- Features a glass shield on the front, which may either fully open or have openings for hand access.

ii. Working Station:

- A flat surface inside the cabinet where processes occur.
- Made of stainless steel to prevent rusting and facilitate easy cleaning.

- Used to place culture plates, burners, loops, and other necessary tools.

iii. Filter Pad/Pre-filter:

- Located at the top, it traps dust particles and some microbes before the air enters the cabinet.

iv. Fan/Blower:

- Positioned below the filter pad, it sucks in and circulates air within the cabinet.
- Directs air towards the HEPA filter to trap remaining microbes.

v. UV Lamp:

- Sterilizes the interior of the cabinet and its contents before use.
- Should be turned on 15 minutes before operation to avoid exposure to UV rays.

vi. Fluorescent Lamp:

- Provides adequate lighting inside the cabinet during operation.

vii. HEPA Filter:

- Ensures a sterile environment by trapping fungi, bacteria, and dust particles.
- Provides particulate-free air by filtering pre-cleaned air.

Principle/Working of Laminar Flow Hood

The laminar flow hood operates based on the principle of maintaining a laminar flow of air through the cabinet.

- Air is drawn in through one or more HEPA filters, creating a particulate-free environment.
- The filtration system processes the air, which then flows uniformly across the work surface.

- Air first passes through the pre-filter, ensuring streamlined flow into the cabinet.
- The blower directs air towards the HEPA filters, which trap contaminants, allowing only clean air to flow out.
- Effluent air exits through perforations at the bottom rear of the cabinet and over the workbench towards the operator's face.
- The sides of the hood are enclosed, and positive air pressure is maintained to prevent external contaminated air from entering.

Procedure for Running the Laminar Flow Cabinet:

1. **Preparation:** Ensure no items sensitive to UV rays are inside the cabinet.
2. **Sterilization:** Close the glass shield and switch on the UV light for about 15 minutes to sterilize the workbench.
3. **Airflow:** Switch off the UV light and wait for about 10 minutes before turning on the airflow.
4. **Operation:** Start the airflow approximately 5 minutes before beginning work. Open the glass shield and switch on the fluorescent light. Optionally, sterilize the workbench with disinfectants like 70% alcohol for additional protection.
5. **Completion:** After completing the work, turn off the airflow and fluorescent lamp, and close the glass shield.

Uses of Laminar Flow Cabinet

1. **Laboratory Applications:** Used for contamination-sensitive processes like plant tissue culture. Ideal for media plate preparation and organism culture.
2. **Pharmaceutical Industry:** Used in drug preparation techniques to ensure a contamination-free environment.
3. **Specialized and General Lab Techniques:** Can be custom-made for specific tasks or used for general microbiological and industrial lab procedures.

07: Tilak's Continuous Air Sampler:

Tilak's Continuous Air Sampler is an innovative and indigenous device designed by Prof. S.T. Tilak and Kulkami in 1970. This air sampler earned Prof. Tilak the President's Medal awarded by the Invention Promotion Board, New Delhi, in 1972.

Equipment Description and Working

Technical Specifications:

- **Dimensions:** 10.4" x 10.4" x 8"
- **Power Supply:** AC 230V
- **Sampling Rate:** 5 Litres/minute (0.17 Ft^3/minute)
- **Sampling Duration:** Continuous for 7 days

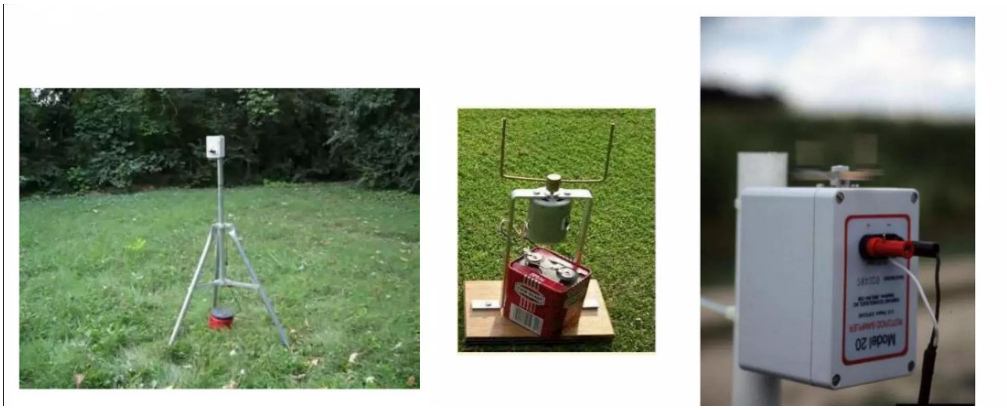

Fig.15: Tilak's Continuous Air Sampler

Components:

- **Cubical Tin Box:** The main body of the sampler.
- **Elevated Round Cap:** Placed over the closing lid at the top.
- **Electric Clock:** Synchronizes with the rotating drum.
- **Projecting Tube:** Sucks air through an orifice at 5 Litres/minute.

- **Rotating Drum:** Coated with a thin layer of petroleum jelly on cellotape, it captures bio components from the air. The drum completes one full rotation in 7 days.
- **Fan:** A small fan with three prongs fixed in the cover creates negative pressure by forcing air out of the collection chamber.
- **Exhaust Area:** Measures 6 x 2.7 cm, located in the lid.

Working Principle:

- Air is drawn into the sampler through the projecting tube, impinging on the cellotape coated with petroleum jelly on the rotating drum.
- This setup entraps biocomponents as the drum rotates, providing a continuous trace of air samples over a week.
- The collected samples are then mounted on slides using glycerine jelly for microscopic examination.

Sampling Method

Procedure:

1. **Location:** Sampler placed at 1 meter above ground in the field.
2. **Sampling Rate:** 5 Litres/minute.
3. **Sampling Duration:** Every 7 days, tapes are changed at about 12-hour intervals.
4. **Preparation of Slides:** The cellotape is cut into 14 equal parts, each representing 12-hour sampling periods, and mounted on slides with glycerine jelly.

Preparation of Glycerine Jelly:

- **Ingredients:**
    - Gelatin: 40g
    - Glycerine: 120ml
    - Distilled Water: 140ml
    - Phenol Crystals: 0.5g
- **Procedure:** Mix glycerine and distilled water, heat in a water bath, add gelatin while stirring, then add phenol crystals as a preservative.

Calculations for Conversion Factor

1. **Sampled Area:** 8.4 cm^2 (84,000,000 sq. microns)
2. **Scanned Area:** 9600 sq. microns
3. **Volume of Air Sampled:** 5 Litres/minute; 7200 Litres/24 hours
4. **Conversion to Cubic Meters:** 7200 Litres x 0.001000028 = 7.3 m^3
5. **Volume in Scanned Area (24 Hours):** 69.12 Litres (or 14.2 m^3 with conversion factor 14)

The conversion factor of 14 is used to estimate the number of spores per cubic meter of air based on the spores counted on the cellotape.

Scanning and Identification

- **Microscopic Examination:** Under 10x and 45x eyepiece objectives.
- **Identification Basis:**
  - Microscopic characters
  - Comparison with reference fungal materials
  - Cultural characters

The number of spores per unit volume of air is computed using the conversion factor of 14, assuming a trapping efficiency of 75%

Applications:

- **Agricultural Research:** Used for monitoring airborne spores in crop fields, aiding in disease management and optimizing fungicide applications.
- **Environmental Monitoring:** Assesses air quality and bioaerosols in various ecosystems.
- **Public Health:** Studies allergenic spores and pollen, contributing to allergy and respiratory disease management.
- **Industrial Use:** Ensures clean air environments in pharmaceutical and manufacturing settings.
- **Academic Research:** Supports studies in aerobiology and aeromycology.

Module 1: Introduction to Laboratory Tools and Instruments

1.2 Study of stains and staining techniques.

Aim: To study Stains and Staining techniques.

Stains: A stain is a substance used in microscopy and various biological applications to enhance the visibility of cells, tissues, and their components. Stains can highlight specific cell types, organelles, or molecular processes, making it easier to study the morphology, physiology, and biochemical properties of biological specimens.

Staining: Staining is a crucial technique in microscopy used to enhance contrast in microscopic images. It involves using stains and dyes to view biological tissues more clearly, often with the aid of different microscopes.

Purposes of Staining

- **Enhance Visualization:** The primary reason for staining cells is to enhance the visualization of the cell or its components under a microscope.
- **Highlight Metabolic Processes:** Stains can be used to highlight metabolic processes or to differentiate between live and dead cells in a sample.
- **Enumeration of Cells:** Staining helps in enumerating cells to determine biomass in an environment of interest.
- **Examine Bulk Tissues and Cell Populations:** Stains may be used to define and examine bulk tissues (e.g., Vascular bundle), cell populations (e.g., different blood cells), or organelles within individual cells.
- **Flow Cytometry and Gel Electrophoresis:** Biological staining is also used to mark cells in flow cytometry and to flag proteins or nucleic acids in gel electrophoresis.

Fixation: Fixation involves several steps aimed at preserving the shape of cells or tissues as accurately in its original stage as much as possible. Heat fixation is sometimes used to kill the cells, adhere them to a slide, and make them permeable to stains.

Mordant: A mordant is a chemical that enhances the binding of a dye to a substance, such as cells or tissues, by forming an insoluble compound with the dye.

Accentuators: Accentuators are substances that enhance the selective staining power, leading to more intense and vivid staining.

Mounting: Mounting is the process of preparing samples for microscopic observation by attaching them to a glass microscope slide.

Staining Techniques

- **Direct (Positive) Staining:** In this technique, the organism itself is stained while the background remains unstained.
- **Indirect (Negative) Staining:** Here, the background is stained, leaving the organism unaltered and visible against the stained background.

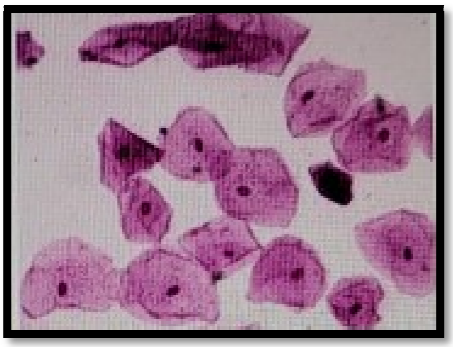

Fig.16: Direct (Positive) Staining

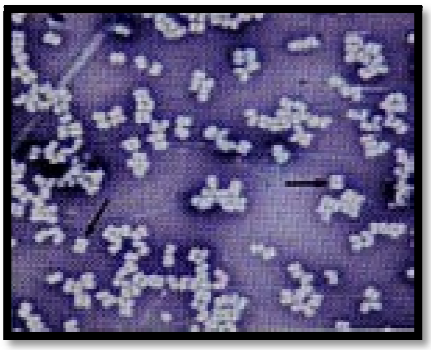

Fig.17: Indirect (Negative) Staining

Kinds/Types of Staining Technique:

Stains are categorized into three main types:

1. **Simple Stain**
2. **Differential Stain**
3. **Acid-Fast Staining (Ziehl–Neelsen stain)**

1. Simple Staining:

Simple staining involves immersing a sample in a dye solution, followed by rinsing and observation. Some dyes require a mordant, a chemical that reacts with the dye to form an insoluble, colored precipitate, which helps the stain remain fixed even after washing away excess dye. This method typically uses **only one dye** and can be performed with *basic dyes in direct*

staining or acidic dyes in negative staining. Simple staining is useful for studying cellular morphology, examining the nature of cellular contents, and determining the intracellular location of cell.

Commonly Used Simple Stains:

- **Methylene Blue:** Applied by flooding the smear with the stain for 2 minutes, then allowing it to air dry. This stain clarifies the morphology of organisms such as **Fungi,** *H. influenzae* and *Gonococci*.. Stains acidic cell parts (like nucleus) blue. Used on animal, bacteria and blood specimens. Can be used as a substitute for Janis B green.
- **Dilute Carbol Fuchsin:** Prepared by diluting carbol fuchsin with distilled water to a ratio of 1/15. The smear is stained for 30 seconds, then washed and blotted dry. This stain is used for *throat swabs*, as a counterstain in Gram staining, and to highlight the morphology of *Vibrio cholerae*.
- **Polychrome Methylene Blue:** Produced by allowing Loeffler's Methylene Blue to ripen slowly in half-filled bottles, which is aerated periodically. The stain develops a polychrome property through slow oxidation, enhanced by the addition of potassium carbonate. This stain is used to demonstrate the McFadyean reaction of *B. anthracis*, showing **blue bacilli** surrounded by purple capsular material.

2. Differential Stains:

Differential stains utilize multiple dyes to categorize cells into different groups or types based on their structural and chemical characteristics. Unlike simple stains, which mainly reveal cell morphology, differential staining provides more detailed information about cell wall properties, such as thickness.

Gram Staining is a key differential staining technique used to differentiate bacterial species into two main categories: Gram-positive and Gram-negative. This method, developed by Hans Christian Gram, involves using crystal violet as the primary dye, iodine as a mordant, and a counterstain like safranin or fuchsin.

Principles of Gram Staining:

- **Crystal Violet:** Stains all bacterial cell walls.

- **Gram's Iodine:** Acts as a mordant to fix the primary dye.
- **Decolorizer:** Removes crystal violet from Gram-negative bacteria, which have a thinner peptidoglycan layer and an outer membrane.
- **Counterstain:** Safranin or fuchsin stains the decolorized Gram-negative bacteria, making them appear red or pink.

Gram Reaction:

- **Gram-Positive Bacteria:** Retain the crystal violet stain due to their thick peptidoglycan layer and appear dark blue or violet.
- **Gram-Negative Bacteria:** Lose the crystal violet stain during decolorization and take up the counterstain, appearing red or pink. Their cell walls are thinner with an additional outer membrane.

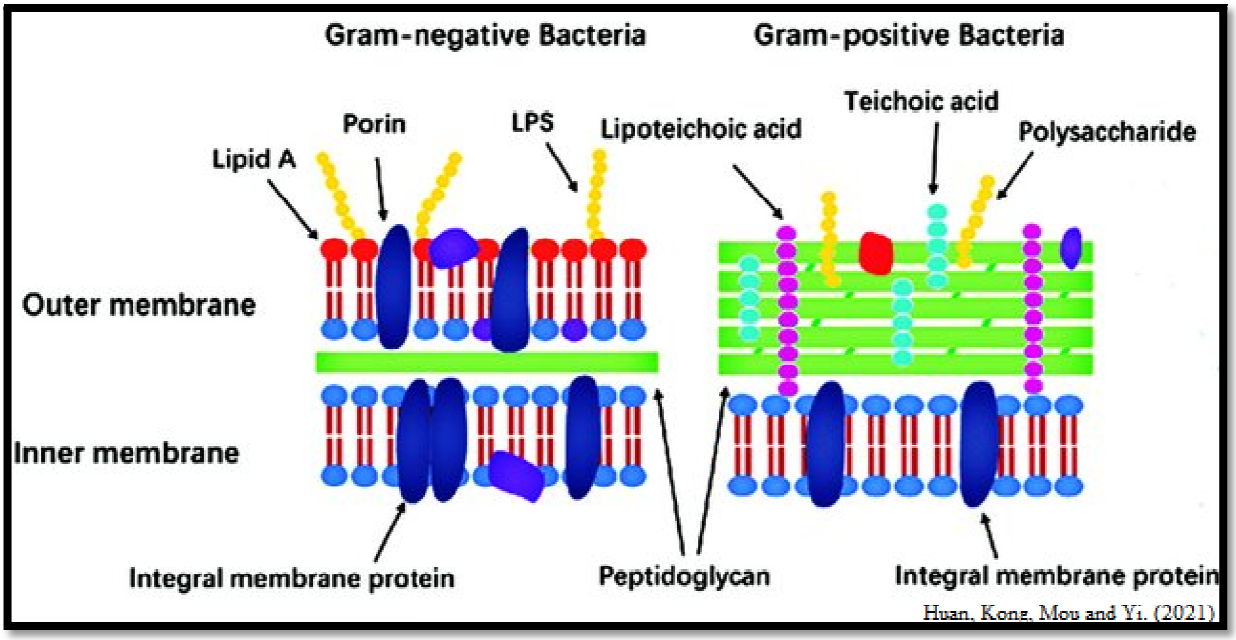

Fig.18: **Gram-Positive and Gram-Negative Bacteria**

Gram Stain Procedure

1. **Prepare the Slide:** Place a drop of NaCl solution onto a glass microscope slide.
2. **Sample Collection:** Using a sterilized and cooled inoculation loop, collect a small amount of bacterial colony.
3. **Mixing:** Gently mix the bacteria into the NaCl drop on the slide.
4. **Air Drying:** Allow the bacterial sample to air dry completely.

5. **Heat Fixing:** Using a slide holder, pass the dried slide through the flame of a Bunsen burner 3 to 4 times, with the smear side facing up.
6. **Primary Stain:** Flood the slide with crystal violet stain and let it sit for 1 minute.
7. **Rinse:** Rinse the slide gently with water to remove excess stain.
8. **Mordant Application:** Apply iodine to the slide, covering it completely, and let it sit for 1 minute.
9. **Rinse:** Rinse the slide with water to remove excess iodine.
10. **Decolorization:** Flood the slide with acetone alcohol. After 10 to 15 seconds, rinse with water. Avoid overexposure to prevent removing the crystal violet from Gram-positive cells.
11. **Secondary Stain (Counterstain):** Flood the slide with safranin and let it sit for 1 minute.
12. **Rinse:** Rinse the slide with water to remove excess safranin.
13. **Drying:** Allow the slide to air dry completely.
14. **Microscopy:** Place the stained smear on the microscope stage, smear side up. Apply a drop of immersion oil directly to the smear and examine using the 100X objective lens.

3. Acid-Fast Staining:

The Ziehl–Neelsen stain, commonly known as the acid-fast stain, is a widely used differential staining technique. This method was first described by two German doctors, bacteriologist Franz Ziehl (1859-1926) and pathologist Friedrich Neelsen (1854-1894). Some bacteria resist decolorization by both acid and alcohol, classifying them as acid-fast organisms. This technique divides **bacteria** into two groups: acid-fast and non-acid-fast, and is extensively used in diagnosing tuberculosis and leprosy. Mycobacterium tuberculosis, the causative agent of tuberculosis (TB), is the most significant member of this group.

Acid-Fast Staining (Ziehl-Neelsen) Procedure:

1. **Prepare the Smear:** Make a smear, allow it to air dry, and then heat fix it.
2. **Primary Stain:** Flood the smear with Carbol Fuchsin stain, a lipid-soluble, phenolic compound that can penetrate the cell wall.
3. **Filter Paper:** Cover the flooded smear with filter paper.
4. **Steam:** Steam the slide for 10 minutes, adding more Carbol Fuchsin as needed.
5. **Cool and Rinse:** Allow the slide to cool and rinse with deionized (DI) water.

6. **Decolorize:** Flood the slide with acid alcohol (3% HCl and 95% ethanol) or 20% H_2SO_4 for 15 seconds. Tilt the slide at a 45-degree angle over the sink and add acid alcohol dropwise until the red color stops streaming from the smear.
7. **Rinse:** Rinse the slide with DI water.
8. **Counter Stain:** Add Loeffler's Methylene Blue stain to the smear, which will color non-acid-fast cells blue. Leave the stain on for 1 minute.
9. **Final Rinse and Dry:** Rinse the slide and blot it dry.
10. **Microscopy:** Use an oil immersion objective to view the stained slide.

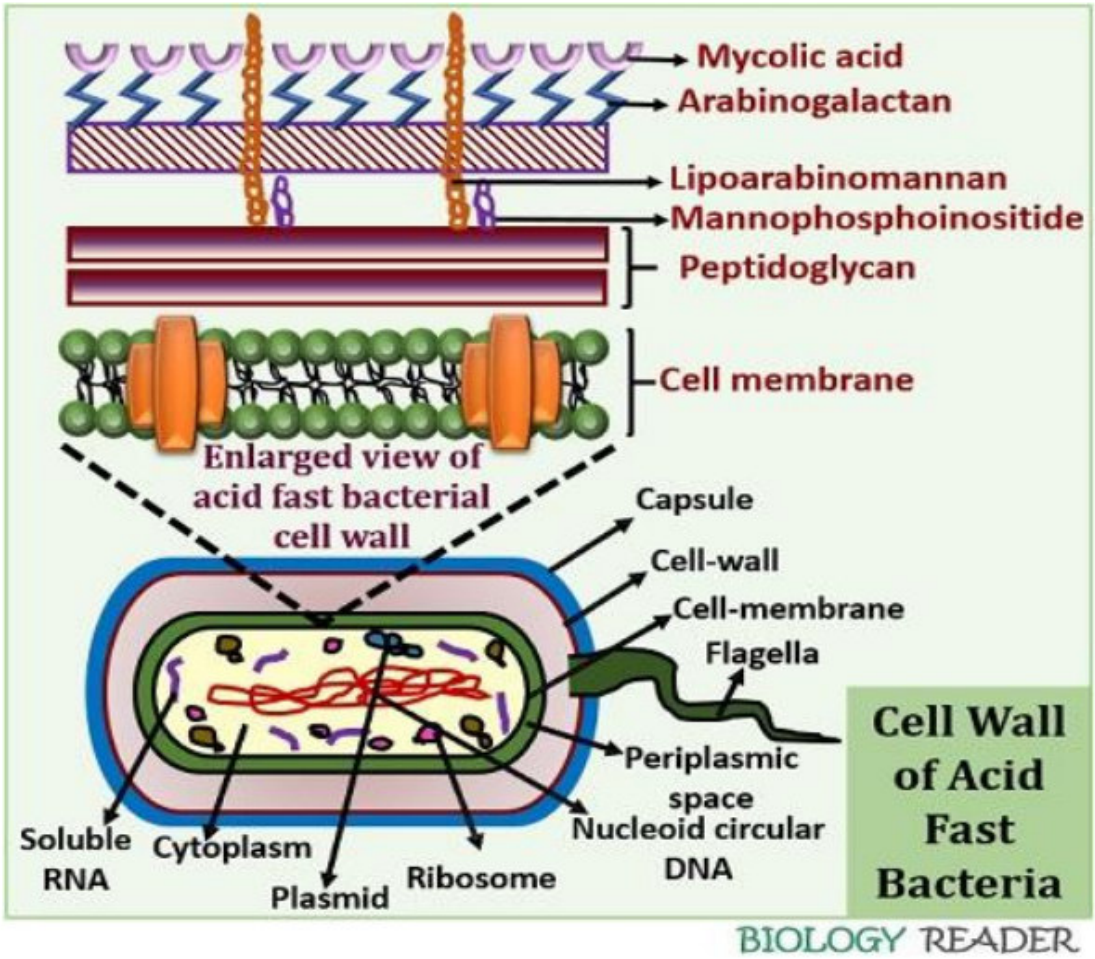

Fig.19: **Acid-Fast Staining**

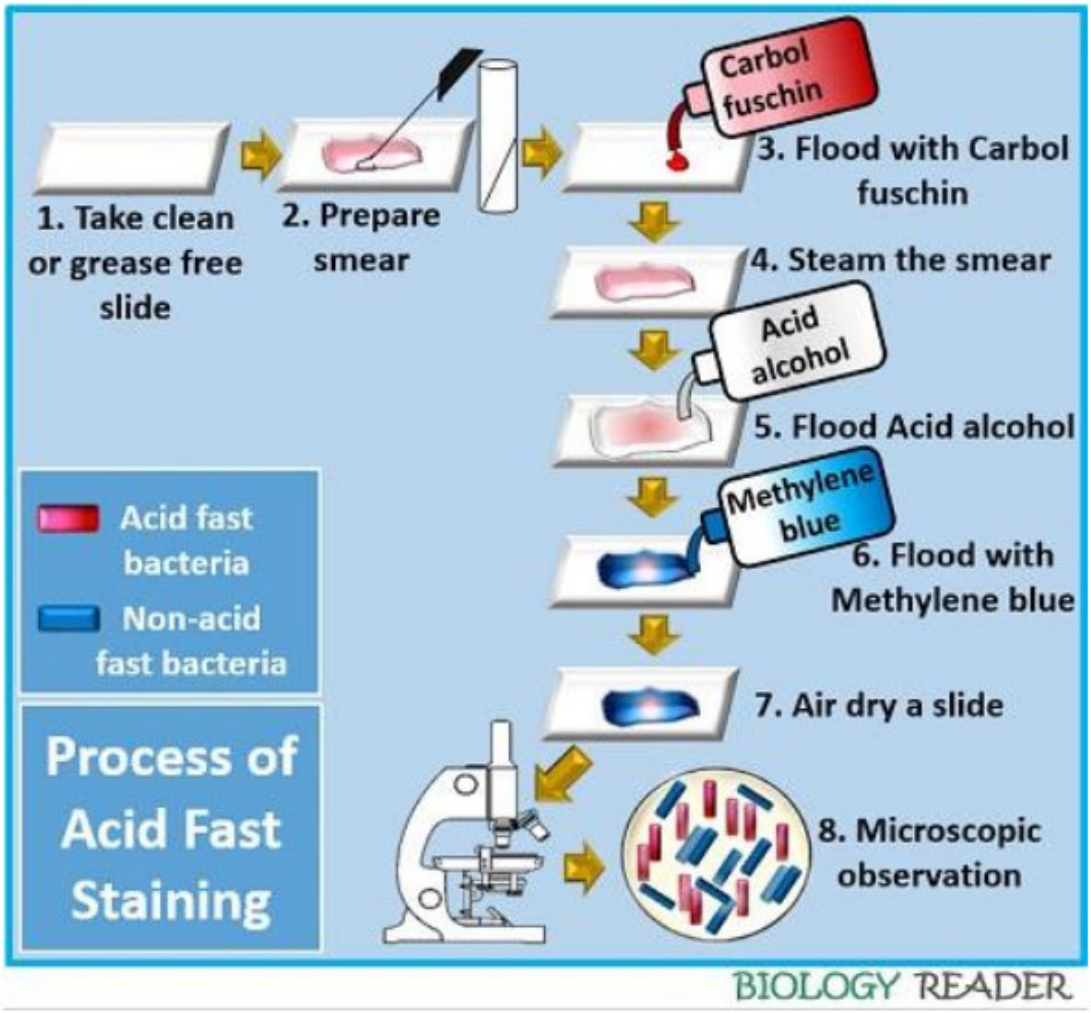

Fig.20: Process of Acid Fast Staining

Common Stains in Botany:

In plant science, specific stains are commonly used to highlight different tissues and structures. These stains should be handled with care, as they can be hazardous to health and can stain clothing and skin. Proper safety equipment such as gloves and lab coats should always be worn. Here are some commonly used stains in botanical experiments:

1. Safranin O (1%)

Formulation:

- 50 mg safranin powder
- 50 ml distilled water

Dissolve safranin powder in water while stirring, then filter. Safranin O stains **xylem and fibers** bright red, and nuclei and plastids pink. Mainly used for sections of plant tissues, stains red.

2. Light Green

Formulation:

- Light Green powder
- Distilled water or alcohol

Light Green stains plant tissues green, often used in conjunction with other stains to highlight different **collagen fibers**.

3. Fast Green

Formulation:

- Fast Green powder
- Distilled water or alcohol

Fast Green stains plant **cell walls and connective tissues**, often used in combination with other stains for detailed tissue analysis.

4. Acetocarmine

Formulation:

- Acetocarmine powder
- Acetic acid

Acetocarmine is used to stain **chromosomes** in dividing cells, highlighting them in a reddish color, making it useful for studying mitosis and meiosis.

5. Iodine Potassium Iodide (IKI, Lugol Solution)

Formulation:

- 2 g potassium iodide (KI)
- 100 ml distilled water
- 0.2 g iodine Dissolve potassium iodide in water, then add iodine. This solution stains **starches** blue to black.

Iodine Stains carbohydrates in plant and animal specimens brown or blue-black. Stains **glycogen** red.

Caution: Iodine vapors are toxic. Avoid inhalation and handle with care.

6. Safranin O (1%)

Formulation:

- 50 mg safranin powder
- 50 ml distilled water Dissolve safranin powder in water while stirring, then filter. Safranin O stains **xylem and fibers** bright red, and nuclei and plastids pink.
- Mainly used for sections of plant tissues, stains red.

2. Toluidine Blue O (TBO)

Formulation:

- 50 mg Toluidine Blue O
- 100 ml water Dissolve the powder in water and adjust the pH to 6.8. TBO is a metachromatic stain: **lignin** appears blue to blue-green, **pectins** pink, and **nuclei** blue to greenish-blue.
- Stains **acidic cell** parts (like **nucleus**) dark blue. Used to show **mitosis in plant cells**.

7. Aniline Blue

Formulation:

- 0.5% (w/v) aniline blue in 0.2 M phosphate buffer (pH 6.5) or deionized water Filter through Whatman no. 1 paper. Aniline blue stains **callose tissue**.

8. Phloroglucinol HCl

Formulation:

- 2 g Phloroglucinol
- 80 ml of 20% ethanol
- 20 ml concentrated HCl (12 M) Dissolve Phloroglucinol in ethanol, then add HCl. Cover with aluminum foil to prevent degradation. This stain highlights **lignin.** **Caution:** Mix in a fume hood as concentrated HCl is very corrosive. Handle with care.

9. Sudan VI, Sudan Black, Oil Red O, and Nile Blue:

These are Lipid staining involves using dyes soluble in lipids to visualize **intracellular lipids** in tissue sections. These dyes can be substituted for one another, as they all work on the principle that the dye is more soluble in the lipid than in the solvent.

Procedure:

1. Cut the sample into 8-10 micron sections and air dry.
2. Rinse with 60% isopropanol.
3. Stain with Oil Red O solution for 15 minutes.
4. Rinse with 60% isopropanol.
5. Dip in alum hematoxylin to stain the nuclei.
6. Rinse with distilled water.
7. Mount in water or glycerin jelly.

10 Coomassie Brilliant Blue (CBB)

Formulation:

- 0.1% Coomassie Brilliant Blue R-250 powder
- 50% methanol
- 10% acetic acid
- 40% distilled water

Preparation:

1. Dissolve 0.1 g of Coomassie Brilliant Blue R-250 in 50 ml of methanol.
2. Add 10 ml of acetic acid.
3. Add 40 ml of distilled water.
4. Mix well until the dye is completely dissolved.

Usage: Coomassie Brilliant Blue (CBB) stain is a widely used method for routine visualization of **proteins** separated on **polyacrylamide gels**. It is an organic dye that forms complexes with basic amino acids, making proteins visible as blue bands on the gel. This stain is particularly useful in **SDS-PAGE** (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) for protein analysis and quantification.

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University of Mumbai's
NEP 2020 Based Curriculum
Vertical 04: Skill Enhancement Courses (SEC)
Name of the Course: Tools and Techniques in Plant Science (Practical)

60 Hours Credits: 02

Subject:	Botany
Class:	F. Y. B.Sc.
Semester:	I

Paper: Tools and Techniques in Plant Science (Practical)

Module Number: 01

Module's Name: Introduction to Laboratory Tools and Instruments

Summary

Content Creator

Dr. Amanulla Khan
Department of Botany
Anjuman Islam Janjira,
Degree College of Science,
Murud-Janjira, Raigad Maharashtra-402401 India
E-mail ID: dramanullak@gmail.com

Summary:

Module 1: Introduction to Laboratory Tools and Instruments

1.1 Study of Basic Laboratory Instruments (Microscope, Colorimeter, Autoclave, Oven, Incubator, Laminar Air Chamber, Tilak Air Sampler).

Aim: To study of Basic Laboratory Instruments w. r. t. Microscope, Colorimeter, Autoclave, Oven, Incubator, Laminar Air Chamber, Tilak Air Sampler.

1. **Microscope:**

   The microscope is a fundamental tool for magnifying tiny objects, such as bacteria and fungi, invisible to the naked eye. There are two main types:

   - *Simple Microscope*: Uses a concave mirror and a single adjustment screw for focusing.
   - *Compound Microscope*: Utilizes a plane and concave mirror with a coarse adjustment. It offers high magnification and is widely used in microbiology.

   Key parts include the eyepieces, objectives (4x, 10x, 40x, 100x), condenser, and light source. The total magnification is calculated by multiplying the magnifications of the eyepiece and objective.

2. **Colorimeter:**

   A colorimeter measures the absorbance of light at specific wavelengths to determine solute concentration in solutions. It has several components, such as a light source, slit, condensing lens, monochromator, cuvette, photocell, and galvanometer. By measuring the light passing through a solution, the device helps in quantitative analysis in fields like the printing, food, medical, and textile industries.

3. **Autoclave:**

   The autoclave sterilizes equipment using steam under pressure, typically at 121°C and 15 psi. It consists of a pressure chamber, lid with safety valves, steam generator, and vacuum generator. The process effectively kills bacteria, viruses, and spores, making it essential in healthcare, labs, and industries for sterilizing materials.

4. **Hot Air Oven:**

   A hot air oven is used for dry heat sterilization, primarily for items that can withstand

high temperatures. It works by circulating hot air to kill microorganisms through dehydration and oxidative damage. Common settings include 170°C for 30 minutes, 160°C for 60 minutes, or 150°C for 150 minutes. It is used for sterilizing glassware, metal items, and heat-resistant materials.

5. **Incubator:** An incubator is an insulated device designed to maintain optimal environmental conditions (temperature, humidity, CO2 levels) for the growth of microorganisms or cells.

   Components: Cabinet, door, thermostat, perforated shelves, L-shaped thermometer, and humidity/gas control (for CO2 incubators).

   Working Principle: Regulates temperature, humidity, and gas levels, essential for microbial growth or cell culture. Incubators create a sterile and controlled environment using insulation and air circulation.

   Applications: Used in microbial and cell culture, growth acceleration, biochemical oxygen demand (BOD) tests, breeding, hatching, and controlled storage.

6. **Laminar Air Chamber:** A laminar flow cabinet is a contamination-free workstation that filters particles and microbes using a HEPA filter. It is mainly used in laboratories for aseptic processes.

   Components: Stainless steel cabinet, work station, pre-filter, fan, UV lamp, fluorescent lamp, HEPA filter.

   Working Principle: Maintains a laminar flow of air, filtered through HEPA to ensure a sterile working environment. Positive air pressure prevents external contamination.

   Applications: Used for media preparation, plate pouring, plant tissue culture, and drug preparation in the pharmaceutical industry.

7. **Tilak's Continuous Air Sampler:** An indigenous device designed by Prof. S.T. Tilak for continuous air sampling. It captures airborne particles like fungal spores and pollen on a rotating drums coated with petroleum jelly.

Components: Tin box, elevated cap, electric clock, projecting tube, rotating drum, fan, exhaust area.

Working Principle: Draws air through a tube and captures particles on a rotating drum, providing a continuous air sample trace over seven days.

Applications: Widely used in agriculture, environmental monitoring, public health,industrial settings, and academic research for tracking airborne bio-components.

Summary:

Module 1: Introduction to Laboratory Tools and Instruments

1.2 Study of stains and staining techniques.

Aim: To study Stains and Staining techniques.

Stains and Staining

Stains are chemical compounds used to enhance contrast in microscopy, making biological specimens like cells, tissues, or specific components more visible and distinguishable. Staining techniques are crucial for studying the structure, function, and biochemical processes of biological samples.

Purposes of Staining:

1. **Enhance Visualization**: Stains make cellular structures more visible under a microscope.
2. **Highlight Metabolic Processes**: Used to differentiate between live and dead cells.
3. **Enumerate Cells**: Assist in counting cells in environmental or biological samples.
4. **Examine Tissues and Cell Populations**: Stains define specific tissues or organelles in cells.
5. **Flow Cytometry and Gel Electrophoresis**: Used for marking cells or proteins in research techniques.

Key Staining Techniques:

1. **Simple Staining**:
    - Uses one dye to color the organism, highlighting basic cellular morphology.
    - Common stains: Methylene Blue, Dilute Carbol Fuchsin, Polychrome Methylene Blue.
    - **Application**: Useful for visualizing cell shapes and structures.
2. **Differential Staining**:
    - Involves using multiple stains to differentiate between cell types.
    - **Gram Staining** is a crucial differential stain for bacteria.
    - **Application**: Differentiates Gram-positive (purple) and Gram-negative (pink) bacteria based on their cell wall structure.

- **Gram Staining Procedure**: Includes steps such as heat fixing, applying crystal violet, using iodine as a mordant, decolorizing, and counterstaining with safranin.

3. **Acid-Fast Staining**:
   - Ziehl–Neelsen staining is used to identify acid-fast bacteria such as *Mycobacterium tuberculosis*.
   - **Application**: Acid-fast bacteria retain the primary stain (Carbol Fuchsin) even after decolorization with acid alcohol, appearing red. Non-acid-fast bacteria take up the counterstain (Methylene Blue), appearing blue.
   - **Procedure**: Smear preparation, Carbol Fuchsin staining, steaming, decolorization with acid alcohol, and counterstaining with Methylene Blue.

Special Stains Used in Botany:

1. **Safranin O**: Stains plant xylem and fibers red, used in plant tissue sections.
2. **Fast Green**: Highlights plant cell walls and connective tissues.
3. **Toluidine Blue O (TBO)**: Metachromatic stain for lignin (blue), pectins (pink), and nuclei (blue-green).
4. **Iodine-Potassium Iodide (IKI)**: Stains starches blue to black, commonly used in plant specimens.
5. **Phloroglucinol HCl**: Stains lignin, commonly used in wood sections.
6. **Sudan Dyes**: Stains lipids in plant and animal tissues, useful for visualizing intracellular lipids.

Staining and staining techniques are fundamental tools in microscopy, allowing researchers to distinguish between different types of cells, tissues, and cellular structures. Each staining method, whether simple or differential, provides insights into the morphology, physiology, and chemical properties of the specimens being studied. These techniques are essential in biological research, clinical diagnostics, and plant science.

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Web Resource

Content Creator

Dr. Amanulla Khan
Department of Botany
Anjuman Islam Janjira,
Degree College of Science,
Murud-Janjira, Raigad Maharashtra-402401 India
E-mail ID: dramanullak@gmail.com

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Module 1.1: Study of Basic Laboratory Instruments (Microscope, Colorimeter, Autoclave, Oven, Incubator, Laminar Air Chamber, Tilak Air Sampler)

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Module 1.2: Study of Stains and Staining Techniques

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