ZOO515 – BIOLOGICAL TECHNIQUES

FINAL TERM MOST IMPORTANT QUESTIONS

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GROUP LINK: https://chat.whatsapp.com/BjC6W0jZx8o9yaC2ISh1Gu

1. What is Saw and Laser Microtome used for?

Saw and Laser Microtome is used to cut extremely thin sections of hard biological samples (like bones and teeth) and composite materials for microscopic analysis. This tool is mainly used in biomedical research, materials science, forensics, and histology where structural detail at the microscopic level is essential. It combines two technologies:

- Saw Microtome uses a diamond saw to cut hard tissues precisely without damaging their structure.
- Laser Microtome uses a focused laser beam to make non-contact, high-precision cuts, often useful for delicate or highly brittle samples.

2. List the components of TLC (Thin Layer Chromatography).

The main components of Thin Layer Chromatography (TLC) are:

1. ***Stationary Phase:*** A thin layer of adsorbent material (usually silica gel, alumina, or cellulose) coated on a solid support like a glass, plastic, or aluminum plate.
2. ***Mobile Phase (Solvent):*** A liquid solvent or solvent mixture that moves up the plate by capillary action, carrying the sample components with it.
3. ***Sample:*** The mixture to be separated, usually applied as a small spot near the bottom of the TLC plate.
4. ***TLC Plate:*** The flat surface on which the stationary phase is coated and the separation occurs.

5. ***Developing Chamber:*** A closed container (e.g., beaker with a lid) that holds the mobile phase and maintains solvent saturation to ensure uniform development.
6. ***Visualizing Agent:*** Used to detect colorless spots after development. Common methods include UV light or chemical sprays like iodine or ninhydrin.
7. ***Rf Value (Retention factor):*** A calculated value from the experiment, representing the ratio of the distance moved by a compound to the distance moved by the solvent front.

3. What is random sample?

A random sample is a subset of individuals chosen from a larger population in such a way that every member of the population has an equal chance of being selected. This method ensures that the sample is unbiased and representative, which makes the results of statistical analysis more reliable and generalizable to the whole population. Random sampling is commonly used in research, surveys, and experiments to avoid selection bias.

4. Explain Bacterial Transformation.

Bacterial transformation is a process by which bacteria take up foreign DNA from their environment and incorporate it into their own genetic material. This can naturally occur in some bacteria or be induced artificially in the lab.

Steps in Bacterial Transformation:

1. ***Preparation of Competent Cells:***
   Bacteria (like *E. coli*) are treated to make their cell membranes more permeable to DNA, often using calcium chloride or electroporation.
2. ***Introduction of Foreign DNA:***
   A plasmid (a small circular DNA molecule) carrying desired genes is added to the competent cells.
3. ***Uptake of DNA:***
   The bacteria take in the plasmid through their cell membrane.
4. ***Recovery and Expression:***
   The transformed bacteria are allowed to grow and express the new gene, such as one for antibiotic resistance or production of a protein.

5. List the units of concentration.

1. ***Molarity (M)*** – moles of solute per liter of solution
2. ***Molality (m)*** – moles of solute per kilogram of solvent
3. ***Normality (N)*** – equivalents of solute per liter of solution
4. ***Percent concentration:***
   - **% w/w** – grams of solute per 100 grams of solution
   - **% w/v** – grams of solute per 100 mL of solution
   - **% v/v** – milliliters of solute per 100 mL of solution
5. ***Parts per million (ppm)*** – mg of solute per liter or kg of solution
6. ***Parts per billion (ppb)*** – µg of solute per liter or kg of solution

7. ***Mole fraction (χ)*** – ratio of moles of one component to total moles in the mixture
8. ***Mass/volume ratio*** – e.g., g/L (grams per liter), mg/mL, µg/mL

6. Define qPCR and PCR. Why qPCR is preferred over PCR?

PCR (Polymerase Chain Reaction):

PCR is a technique used to amplify specific DNA sequences by repeatedly copying a target DNA region using DNA polymerase, primers, and thermal cycling. It helps detect or produce large quantities of DNA for analysis.

qPCR (Quantitative PCR or Real-Time PCR):

qPCR is an advanced form of PCR that quantifies DNA in real time during amplification by using fluorescent dyes or probes. It not only amplifies DNA but also measures the amount of DNA after each cycle.

Why qPCR is preferred over PCR:

1. ***Quantification:*** qPCR measures how much DNA is present, while traditional PCR only shows whether DNA is present or not.
2. ***Sensitivity:*** qPCR detects even low levels of DNA or RNA with high accuracy.
3. ***Speed:*** Results are obtained faster since there's no need for post-PCR gel electrophoresis.
4. ***Real-Time Monitoring:*** qPCR tracks amplification as it happens, reducing contamination risks.
5. ***Multiplexing:*** qPCR can detect multiple targets in one reaction using different fluorescent probes.

7. Write applications of PCR in medical diagnosis.

Applications of PCR in Medical Diagnosis:

1. ***Infectious Disease Detection:***
   PCR identifies pathogens like viruses (HIV, COVID-19, HPV) and bacteria (TB, Chlamydia) by detecting their genetic material.
2. ***Genetic Disorder Screening:***
   Used to detect mutations responsible for inherited diseases like cystic fibrosis**,** sickle cell anemia, and thalassemia.
3. ***Cancer Diagnosis:***
   Detects gene mutations or rearrangements linked to cancers (e.g., BRCA1/2 in breast cancer, BCR-ABL in leukemia).
4. ***Prenatal Diagnosis:***
   Screens fetal DNA for genetic abnormalities through non-invasive prenatal testing (NIPT)**.**
5. ***Forensic and Identity Testing:***
   Identifies individuals using unique DNA profiles, useful in paternity tests and criminal investigations.
6. ***Organ Transplantation:***
   PCR helps monitor organ rejection by detecting donor DNA in the recipient's blood.
7. ***Viral Load Monitoring:***
   Quantifies virus levels (e.g., in HIV or Hepatitis) to assess treatment effectiveness.

8. What instrument will you use for extraction of lipids? And Why?

To extract lipids, the most commonly used instrument is a Soxhlet extractor.

Why Soxhlet extractor?

1. ***Efficient Lipid Extraction:*** It allows continuous extraction of lipids from solid samples (like tissues or food) using organic solvents such as hexane or ether.
2. ***Solvent Recycling:*** The solvent is repeatedly vaporized and condensed, maximizing lipid yield without needing large volumes of solvent.
3. ***High Recovery Rate:*** Soxhlet ensures thorough extraction, ideal for quantitative analysis of lipids.

9. Write a detailed note on Northern Blotting.

Northern Blotting – Detailed Note

Northern blotting is a molecular biology technique used to detect and analyze specific RNA molecules (especially mRNA) within a complex mixture of RNA. It helps study gene expression patterns in different cells or tissues.

Principle:

Northern blotting works on the principle of nucleic acid hybridization, where a labeled DNA or RNA probe binds to its complementary RNA sequence. This allows identification and quantification of specific RNA transcripts.

Steps Involved:

1. ***RNA Extraction:*** Total RNA is isolated from cells or tissues using chemical or enzymatic methods.
2. ***Gel Electrophoresis:*** The RNA samples are separated by size using **agarose gel electrophoresis** under **denaturing conditions** (e.g., formaldehyde) to prevent secondary structures.
3. ***Transfer to Membrane:*** The separated RNA is transferred (blotted) onto a **nylon or nitrocellulose membrane**, usually by capillary action or vacuum transfer.
4. ***Fixation:*** RNA is **immobilized** on the membrane using UV cross-linking or heat.
5. ***Hybridization with Probe:*** A labeled **single-stranded DNA or RNA probe** (complementary to the target RNA) is applied. This probe binds specifically to the RNA of interest.
6. ***Washing and Detection:*** Excess probe is washed away, and the membrane is exposed to **X-ray film** or detected using **chemiluminescence** or **radioactivity** to visualize the RNA bands.

Applications of Northern Blotting:

- ***Gene Expression Analysis:*** To measure the expression level of specific mRNA across different tissues, time points, or conditions.
- ***Validation of Microarray/qPCR Data:*** Confirms gene expression results obtained from high-throughput techniques.
- ***RNA Size Determination:*** Estimates the length of RNA transcripts, helping identify alternative splicing or processing events.
- ***Detection of Viral RNA:*** Used to detect RNA viruses or viral gene expression in infected cells.

- ***Studying Tissue-Specific Expression:*** Helps determine which tissues express a gene of interest.

10. What is real time PCR?

Real-time PCR (also called qPCR, quantitative PCR) is a technique that amplifies DNA like regular PCR but simultaneously measures the amount of DNA produced during each cycle in real time using fluorescent dyes or probes.

Key features:

- ***Quantification:*** It provides quantitative data on how much DNA is present at each cycle, allowing measurement of initial DNA or RNA levels.
- ***Fluorescence detection:*** Fluorescent signals increase proportionally with the amount of PCR product.
- ***Faster results:*** No need for gel electrophoresis after PCR.
- ***High sensitivity and specificity.***

Uses:

- Gene expression analysis
- Pathogen detection
- Genetic mutation quantification
- Viral load measurement in clinical samples

11. Write a detailed note on Western Blotting.

Western Blotting

Western blotting (also called immunoblotting) is a widely used analytical technique in molecular biology and biochemistry to detect and quantify specific proteins in a complex mixture, such as cell or tissue extracts.

Principle:

Western blotting relies on the specific binding between an antibody and its target protein. Proteins are first separated by size using gel electrophoresis, then transferred onto a membrane, and finally detected using antibodies.

Steps Involved:

1. ***Protein Extraction:*** Proteins are extracted from cells or tissues using lysis buffers containing detergents and protease inhibitors to preserve protein integrity.
2. ***Protein Quantification (Optional):*** Protein concentration is measured to ensure equal loading.
3. ***SDS-PAGE (Gel Electrophoresis):*** Proteins are denatured with SDS and separated by size on a polyacrylamide gel using an electric current.
4. ***Transfer to Membrane:*** Separated proteins are transferred from the gel onto a nitrocellulose or PVDF membrane by electric current (electroblotting).
5. ***Blocking:*** The membrane is incubated with a blocking solution (e.g., BSA or non-fat dry milk) to prevent non-specific antibody binding.

6. ***Incubation with Primary Antibody:*** The membrane is incubated with a primary antibody specific to the target protein.
7. ***Incubation with Secondary Antibody:*** After washing, a secondary antibody that recognizes the primary antibody and is conjugated to a reporter enzyme (like HRP or alkaline phosphatase) is applied.
8. ***Detection:*** A substrate is added that reacts with the enzyme on the secondary antibody to produce a detectable signal (chemiluminescence, fluorescence, or colorimetric). The signal intensity correlates with the protein amount.

Applications:

- Protein identification and confirmation
- Quantification of protein expression levels
- Detection of post-translational modifications (phosphorylation, glycosylation)
- Diagnosis of diseases by detecting specific proteins (e.g., HIV proteins)
- Validation of gene expression studies at the protein level

12. What is Gas Chromatography?

Gas Chromatography (GC) is an analytical technique used to separate and analyze volatile compounds in a mixture. It works by vaporizing the sample and passing it through a column with a stationary phase, carried by an inert gas (mobile phase).

How it works:

- The sample is injected and vaporized in the injection port.
- An inert carrier gas (like helium or nitrogen) moves the vaporized sample through a column coated with a stationary phase.
- Different components separate based on their volatility and interaction with the stationary phase.
- Separated compounds exit the column and are detected by a detector (e.g., flame ionization detector, mass spectrometer).

Uses:

- Identifying and quantifying components in complex mixtures (e.g., pollutants, flavors, drugs).
- Quality control in pharmaceuticals, food, and petrochemical industries.
- Environmental analysis and forensic investigations.

13. Write principle of paper chromatography.

The principle of paper chromatography is based on the differential partitioning of components between a stationary phase (water trapped in the cellulose fibers of the paper) and a mobile phase (solvent). When the solvent moves up the paper by capillary action, different substances in the mixture travel at different rates depending on their solubility in the solvent and affinity for the paper. This causes the components to separate along the paper.

14. Write applications of vibrotome.

Applications of Vibrotome:

1. ***Preparation of Thick Tissue Sections:***
   Used to cut fresh or fixed biological tissues into thin sections (typically 30–300 µm) without freezing or embedding, preserving cell structure and function.
2. ***Neuroscience Research:***
   For slicing brain tissues to study neural circuits, brain anatomy, and electrophysiology.
3. ***Immunohistochemistry and Histology:***
   Produces sections suitable for antibody staining and microscopic examination.
4. ***Live Tissue Studies:***
   Enables preparation of viable slices for experiments requiring living cells, like electrophysiological recordings.
5. ***Botanical Research:***
   Used to section plant tissues for anatomical and physiological studies.
6. ***Material Science:***
   Cutting soft or delicate materials that require precise, vibration-assisted slicing.

15. Define Molarity, Molality, Normality and Concentration.

- Molarity is the concentration of a solute measured as the number of moles of solute dissolved in one liter of the entire solution.
- Molality refers to the concentration of a solute expressed as the number of moles of solute per kilogram of the solvent alone.
- Normality is the concentration based on the number of reactive units or equivalents of a solute per liter of solution, often used in acid-base or redox reactions.
- Concentration is a general term that indicates how much of a substance is present in a certain volume or mass of mixture or solution.

16. What are limitations of Electron Microscope?

Limitations of Electron Microscope:

1. ***High Cost:*** Expensive to purchase, operate, and maintain.
2. ***Complex Sample Preparation:*** Requires extensive and often time-consuming preparation like dehydration, coating, or freezing.
3. ***Vacuum Requirement:*** Samples must withstand a vacuum, so living organisms cannot be observed directly.
4. ***Non-Color Images:*** Produces black-and-white images; color must be added artificially.
5. ***Sample Damage:*** Electron beams can damage or alter delicate samples.
6. ***Size Limitations:*** Large samples often cannot fit inside the microscope chamber.
7. ***Technical Expertise:*** Requires skilled operators to prepare samples and interpret results.
8. ***Limited Field of View:*** Can only image small areas at very high resolution.

17. Write a note on contact freeze dryers.

Contact Freeze Dryers

Contact freeze dryers are a type of freeze-drying equipment used primarily to dry heat-sensitive materials by sublimation, preserving their structure and bioactivity.

Working Principle:

- The material to be dried is frozen on a chilled surface (usually a metal drum or plate).
- Heat is applied indirectly through this contact surface, gently supplying energy to sublimate ice directly into vapor without passing through the liquid phase.
- The water vapor is then removed under vacuum.

Features:

- Direct contact between the frozen sample and the cooled surface ensures efficient heat transfer.
- Suitable for drying thin layers or films of material.
- Provides uniform drying and preserves delicate structures.

Applications:

- Drying pharmaceuticals, enzymes, vaccines, and food products.
- Used in industries requiring high-quality dried products with minimal loss of activity.

18. Explain mechanism of Fixation.

Mechanism of Fixation:

Fixation is the process of preserving biological tissues by stopping all biochemical reactions and preventing degradation. The mechanism involves:

1. ***Denaturation and Cross-linking of Proteins:***
   Fixatives chemically react with tissue proteins, causing them to denature (lose their natural structure) and form cross-links between protein molecules. This stabilizes the tissue structure.
2. ***Prevention of Autolysis:***
   By inactivating enzymes within the cells, fixation stops self-digestion (autolysis) that would otherwise break down the tissue.
3. ***Inhibition of Microbial Growth:***
   Fixatives kill or inhibit bacteria and fungi, preventing decomposition.
4. ***Preservation of Cellular and Tissue Morphology:***
   The chemical cross-linking maintains the shape and location of cellular components, making tissues ready for further study under a microscope.

19. Enlist components of Rotary evaporator.

Components of a Rotary Evaporator:

1. ***Rotary Motor*** – Rotates the flask containing the sample to increase surface area for evaporation.
2. ***Evaporating Flask*** – Holds the sample to be evaporated.
3. ***Heating Bath*** – Usually a water or oil bath that gently heats the sample to promote evaporation.

4. ***Condenser*** – Cools the vapor back into liquid form for collection.
5. ***Receiving Flask*** – Collects the condensed solvent after evaporation.
6. ***Vacuum System*** – Reduces pressure to lower the boiling point of the solvent, speeding evaporation.
7. ***Control Panel*** – Allows adjustment of rotation speed, bath temperature, and vacuum pressure.
8. ***Support Stand/Frame*** – Holds all components securely.

20. Enlist two most commonly used air-free techniques used in laboratory.

Two most commonly used air-free techniques in the laboratory are:

1. Schlenk Line Technique
2. Glove Box Technique

21. What are diagnostic applications of PCR?

Diagnostic applications of PCR include:

1. ***Detection of Infectious Agents:*** Identifying viruses (e.g., HIV, COVID-19), bacteria (e.g., tuberculosis), and fungi by detecting their DNA or RNA.
2. ***Genetic Disorder Screening:*** Diagnosing inherited diseases by detecting specific gene mutations.
3. ***Cancer Diagnosis:*** Detecting cancer-related genetic mutations or markers.
4. ***Prenatal Diagnosis:*** Screening fetal genetic abnormalities non-invasively.
5. ***Forensic Medicine:*** Identifying individuals through DNA profiling.
6. ***Monitoring Viral Load:*** Measuring the amount of virus in patients for diseases like HIV or hepatitis.

22. Write applications of Real Time PCR.

Applications of Real-Time PCR (qPCR):

1. ***Gene Expression Analysis*** – Measuring the level of specific mRNA to study gene activity.
2. ***Pathogen Detection*** – Rapidly identifying viruses, bacteria, and other pathogens in clinical samples.
3. ***Quantification of Viral Load*** – Monitoring the amount of virus (e.g., HIV, Hepatitis) in patients.
4. ***Genetic Mutation Detection*** – Identifying mutations or single nucleotide polymorphisms (SNPs).
5. ***Drug Resistance Testing*** – Detecting genes responsible for resistance in pathogens.
6. ***Food Safety and Quality Control*** – Detecting contaminants or genetically modified organisms (GMOs).
7. ***Cancer Research*** – Quantifying oncogenes or tumor suppressor genes expression.

23. What are uses or applications of Western Blotting?

Applications of Western Blotting:

1. ***Protein Identification:*** Detects specific proteins in complex mixtures.
2. ***Protein Quantification:*** Measures relative levels of proteins under different conditions.
3. ***Verification of Gene Expression:*** Confirms whether genes are translated into proteins.
4. ***Detection of Post-Translational Modifications:*** Identifies modifications like phosphorylation or glycosylation.
5. ***Disease Diagnosis:*** Used in detecting disease markers, e.g., HIV infection confirmation.

6. ***Research on Signal Transduction:*** Studies protein interactions and signaling pathways.
7. ***Validation of Antibodies:*** Tests antibody specificity.

24. Explain sample preparation for TEM.

Sample Preparation for Transmission Electron Microscopy (TEM):

1. ***Fixation:*** The sample is chemically fixed using agents like glutaraldehyde and osmium tetroxide to preserve cellular structures and prevent degradation.
2. ***Dehydration:*** The fixed sample is gradually dehydrated through a series of increasing concentrations of alcohol or acetone to remove water.
3. ***Embedding:*** The dehydrated sample is infiltrated with a resin (e.g., epoxy) and polymerized to form a hard block, which supports the tissue for thin sectioning.
4. ***Sectioning:*** Ultrathin sections (about 50–100 nm thick) are cut from the resin block using an ultramicrotome with a diamond or glass knife.
5. ***Staining:*** Sections are stained with heavy metals like lead citrate and uranyl acetate to increase electron density and contrast.
6. ***Mounting:*** The stained sections are placed on copper grids for examination under the TEM.

25. Mention factors that affect the rate of migration of DNA.

Factors that affect the rate of migration of DNA during gel electrophoresis include:

1. ***Size of DNA fragments:*** Smaller fragments move faster through the gel than larger ones.
2. ***Gel concentration:*** Higher agarose or polyacrylamide concentration slows DNA migration by creating a tighter matrix.
3. ***Voltage applied:*** Higher voltage increases migration speed but may cause band distortion if too high.
4. ***Buffer composition and ionic strength:*** Affects the conductivity and stability of the electric field.
5. ***Shape of DNA:*** Supercoiled DNA migrates faster than linear or relaxed forms.
6. ***Temperature:*** Higher temperatures can increase migration rate but may also melt the gel or affect DNA integrity.
7. ***Conformation of DNA:*** Circular, linear, and nicked forms move at different rates.

26. Write a note on Bright Field Microscopy.

Bright Field Microscopy is a basic light microscopy technique where light passes through a specimen, making it appear darker against a bright background. It's best for stained or pigmented samples, providing true-color images. It's easy to use and affordable but has limited contrast for unstained, transparent specimens and lower resolution than advanced microscopes.

27. What is resolution in microscopy?

Resolution in microscopy refers to the microscope's ability to clearly distinguish two points that are very close together as separate entities. It determines the level of detail visible in the image—better resolution means you can see finer structural details. Resolution depends on factors like the wavelength of light or electrons used and the quality of the microscope's lenses or optics.

28. What are types of Soxhlet extractor?

Types of Soxhlet Extractors:

1. ***Standard Soxhlet Extractor*** – The common, classic design used for continuous solvent extraction of solid samples.
2. ***Semi-automatic Soxhlet Extractor*** – Includes modifications to allow easier solvent recovery or shorter extraction times.
3. ***Microwave-assisted Soxhlet Extractor*** – Combines microwave heating with Soxhlet extraction to speed up the process.
4. ***Ultrasound-assisted Soxhlet Extractor*** – Uses ultrasonic waves to enhance solvent penetration and extraction efficiency.

29. Write a note on Lyophilization.

Lyophilization, or freeze drying, is a dehydration technique used to preserve heat-sensitive materials by freezing them and then removing water through sublimation under vacuum. This process involves three main steps: freezing the sample, primary drying where ice turns directly into vapor, and secondary drying to eliminate remaining moisture. Lyophilization is widely used in pharmaceuticals, food preservation, and biological research because it helps maintain the structure, activity, and stability of sensitive products better than conventional drying methods.

30. What is Gel Electrophoresis?

Gel electrophoresis is a laboratory technique used to separate molecules like DNA, RNA, or proteins based on their size and charge. The sample is loaded into a gel matrix and an electric current is applied, causing the molecules to move through the gel. Smaller molecules move faster and travel farther than larger ones, allowing for their separation and analysis. This method is commonly used in genetic research, molecular biology, and diagnostics.

31. Define Cationic and Anionic exchange.

Cationic exchange is a process where positively charged ions (cations) in a solution are swapped with cations attached to a solid material (ion-exchange resin). **Anionic exchange** is a similar process but involves the exchange of negatively charged ions (anions) between the solution and anion-exchange resin. Both are used to purify, separate, or remove specific ions from mixtures.

32. Compare Light and Compound Microscope.

Light Microscope:

A light microscope uses visible light passed through or reflected from a specimen to produce an image. It can be as simple as a single lens magnifying glass or more complex with a few lenses. Light microscopes allow viewing of specimens at low to moderate magnification, typically up to around 400x. They are useful for observing larger cells, tissues, and some microorganisms, but their resolution is limited by the wavelength of visible light. Light microscopes are generally easy to use, portable, and inexpensive, making them common in educational settings.

Compound Microscope:

A compound microscope is a more advanced type of light microscope that uses two or more lenses—an objective lens close to the specimen and an eyepiece lens through which the image is viewed. This combination provides much higher magnification, often between 40x and 1000x, allowing detailed observation of cellular structures and microorganisms. The compound microscope also typically has a built-in light source, adjustable focus, and mechanical stage for precise sample handling. It is widely used in laboratories for research, clinical diagnosis, and detailed biological studies due to its improved resolution and clarity compared to simple light microscopes.

33. Write methods of preservation for insects.

Methods of Preservation for Insects:

1. ***Pinning:*** Insects are carefully mounted on pins through the thorax or other body parts, allowing easy handling and display. Used mainly for larger, hard-bodied insects.
2. ***Alcohol Preservation:*** Soft-bodied insects are stored in vials filled with 70-95% ethanol to prevent decay and maintain flexibility.
3. ***Freezing:*** Insects are preserved by freezing at low temperatures, which slows decomposition and is often used for temporary storage.
4. ***Drying:*** Insects are dried naturally or using drying ovens or silica gel to remove moisture and prevent fungal growth.
5. ***Slide Mounting:*** Tiny insects or insect parts are mounted on microscope slides using mounting media for detailed microscopic examination.

34. Define homogenous and heterogeneous mixtures.

Homogeneous Mixture:

A homogeneous mixture has a uniform composition throughout, meaning the different components are mixed so thoroughly that you cannot see or separate them easily. The particles are evenly distributed at the molecular or ionic level. Examples include solutions like sugar dissolved in water, saltwater, or air. These mixtures look the same no matter where you sample them from.

Heterogeneous Mixture:

A heterogeneous mixture has an uneven composition where the different components remain physically separate and can often be seen or separated easily. The properties and appearance can vary from one part of the mixture to another. Examples include mixtures like soil, salad, or oil and water, where different substances are clearly distinguishable.

35. What is collapse in lyophilization process? Give two reasons of collapse.

Collapse in lyophilization refers to the loss of the dried product's structure during the freeze-drying process, where the frozen matrix partially melts or shrinks, causing the final product to lose its porous, solid form and become sticky or dense. Two reasons for collapse are**:**

1. ***Exceeding the critical temperature:*** If the product temperature rises above its collapse temperature during primary drying, the structure softens and collapses.

2. ***Improper freezing:*** Inadequate freezing can cause large ice crystals or uneven ice distribution, leading to weak structure that collapses during drying.

36. Define confocal microscopy. How it is different from fluorescence microscopy?

Confocal Microscopy is an advanced optical imaging technique that uses a focused laser beam and a pinhole to eliminate out-of-focus light, producing sharp, high-resolution, and three-dimensional images of specimens, especially fluorescently labeled samples.

Difference from Fluorescence Microscopy:

- Fluorescence Microscopy illuminates the whole sample and collects all emitted light, including out-of-focus light, which can blur the image.
- Confocal Microscopy uses point illumination and spatial filtering (pinhole) to exclude out-of-focus light, resulting in clearer, more detailed images with better depth resolution.

37. Name commonly used fixatives.

Commonly used fixatives include:

1. **Formaldehyde (Formalin)**
2. **Glutaraldehyde**
3. **Alcohols (Ethanol, Methanol)**
4. **Acetic Acid**
5. **Osmium Tetroxide**
6. **Mercuric Chloride**
7. **Picric Acid**

38. What is purpose of fractionation?

The purpose of fractionation is to separate a complex mixture into smaller, simpler parts or fractions based on differences in physical or chemical properties. This helps isolate specific components for further analysis, purification, or study. Fractionation is widely used in biology, chemistry, and medicine to simplify mixtures like blood, cell extracts, or chemical compounds.

39. Define hot and cold fixation.

Hot Fixation:

A fixation method where the specimen is exposed to heat (usually by passing through a flame or placing on a hot plate) to quickly kill and preserve cells. It helps in fixing cells by coagulating proteins, often used in preparing bacterial smears.

Cold Fixation:

A method of fixation performed at low temperatures (using cold chemicals or chilled conditions) to preserve delicate structures and prevent damage. It is gentler than heat fixation and commonly used for preserving tissue morphology and enzyme activity.

40. What are requirements for fluorescence microscopy?

Requirements for Fluorescence Microscopy:

1. ***Fluorophores (Fluorescent Dyes):*** Molecules that absorb light at a specific wavelength and emit light at a longer wavelength.
2. ***Excitation Light Source:*** Usually a mercury or xenon arc lamp, or lasers, that provide the specific wavelength to excite the fluorophores.
3. ***Filters:***
    - Excitation filter to select the correct wavelength of light to excite the fluorophore.
    - Dichroic mirror to reflect excitation light toward the sample and allow emitted light to pass through.
    - Emission filter to isolate the fluorescent light emitted by the sample.
4. ***Objective Lens:*** High-quality lenses that collect emitted light efficiently.
5. ***Detector or Eyepiece:*** Camera or viewer to observe or capture the fluorescent image.

41. Write uses of explosive PCR.

1. ***Rapid DNA Amplification:*** Directly amplifies DNA from crude samples without prior DNA extraction, saving time.
2. ***Forensic Analysis:*** Quickly analyzes trace or degraded samples like hair, blood stains, or tissues.
3. ***Clinical Diagnostics:*** Detects pathogens directly from patient samples like blood or swabs.
4. ***Food Safety Testing:*** Identifies contamination or genetically modified organisms in food without complex sample prep.
5. ***Environmental Monitoring:*** Amplifies DNA from soil, water, or air samples quickly.

42. Write physical methods of tissue fixation.

Physical Methods of Tissue Fixation:

1. ***Heat Fixation:*** Using direct heat (flame or hot plate) to kill and fix cells, commonly used for bacterial smears.
2. ***Freezing:*** Rapidly freezing tissues to preserve structure and enzymes, often used before cryosectioning.
3. ***Microwave Fixation:*** Applying microwave energy to speed up the fixation process by accelerating chemical penetration and cross-linking.
4. ***Desiccation (Drying):*** Removing water by air drying or freeze-drying to preserve tissue morphology.

43. How DNA is visualized in Gel Electrophoresis?

DNA is visualized in gel electrophoresis by staining the gel with a dye that binds to DNA and fluoresces under UV light. Common dyes include ethidium bromide and safer alternatives like SYBR Green. When the gel is exposed to UV light, the dye-DNA complexes emit bright bands, allowing the DNA fragments to be seen and analyzed based on their size and position in the gel.

44. Define resolution and path of light in microscope.

Resolution:

Resolution is the ability of a microscope to distinguish two closely spaced points as separate and distinct. It determines the level of detail and clarity in the observed image—higher resolution means finer details can be seen clearly.

Path of Light in a Microscope:

Light from the illumination source passes through the condenser lens, which focuses it onto the specimen. The light then travels through the specimen and into the objective lens, which magnifies the image. The magnified light continues through the body tube to the eyepiece lens, where the image is further magnified and viewed by the observer's eye.

45. When bacterial transformation term was used in biology?

The term bacterial transformation was first used in biology in 1928 by Frederick Griffith. He discovered that non-virulent bacteria could acquire virulence by taking up genetic material from dead virulent bacteria, demonstrating the transfer of genetic traits. This experiment laid the foundation for understanding bacterial transformation.

46. What is transportation of Bacteria?

Transportation of bacteria refers to the process of safely collecting, preserving, and moving bacterial samples from one place to another—such as from a clinical site to a laboratory—for examination or testing. Proper transportation ensures the bacteria remain viable and uncontaminated, preserving their original characteristics for accurate identification and analysis. This often involves using specialized transport media and maintaining appropriate temperature conditions.

Disclaimer:

For best results, it is advised NOT to rely on this file only. Go through video lectures, handouts and other sources as well.

Regards,
Sir Mughal,
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