

1. Understand the principles and applications of microscopy techniques, including bright field microscopy and electron microscopy (SEM and TEM), as well as staining techniques.
2. Know various sterilization and disinfection techniques, including physical methods (dry heat, moist heat, filtration, radiation) and chemical methods (disinfectants, alcohols, aldehydes, fumigants, phenols, halogens, heavy metals).
3. Perform pure culture isolation, maintenance and preservation of cultures, cultivation of anaerobic bacteria, and accessing viable non-culturable bacteria (VNBC).
4. Understand the principles and applications of spectrophotometry and chromatography techniques, including UV-visible spectrophotometry, colorimetry, turbidometry, paper chromatography, and column chromatography.
5. Gain knowledge of centrifugation principles and applications, electrophoretic techniques (agarose and SDS polyacrylamide gel), and the principles and applications of radioisotopes.

Unit -1: Microscopy

No. of Hours: 9hrs

- 1 Microscopy: Principle, mechanism and applications of Bright field microscope.
- 2 Principle, mechanism and applications of electron microscope (SEM and TEM). Micrometry.
- 3 Staining Techniques – Simple, negative and Differential staining techniques (Gram staining, spore staining, Acid fast staining).

Unit-2: Sterilization and disinfection techniques No. of Hours: 9hrs

1. Sterilization, Disinfection, Antiseptic, Germicide, Sanitizer, Fungicide, Virucide, Bacteriostatic and Bactericidal agent.

Physical methods of microbial control: Dry heat-Incineration, Hot air oven; Moist heat- Pressure cooker, autoclave; Filter sterilization- laminar air flow, Membrane filter; Radiation methods – UV rays, Gamma rays.

2 Chemical methods of microbial control: disinfectants, types and mode of action- alcohols, aldehydes, fumigants, phenols, halogens and heavy metals.

Unit -3: Microbiological techniques

No. of Hours:

9hrs 1 Pure culture isolation: Streaking, serial dilution and plating methods, micromanipulator; cultivation.

2 Maintenance and preservation/stocking of pure cultures: sub culturing, overlaying cultures with mineral oils, lyophilization, sand cultures, storage at low temperature, Culture collection centers (MTCC, ATCC, DSMZ);

3 Cultivation of anaerobic bacteria; Accessing Viable non-culturable bacteria (VNBC). Buffers in culture medium. Cultivation of fungi, Actinomycetes, yeasts.

Unit-4: Spectrophotometry & Chromatography

No. of Hours: 9

- 1 Spectroscopy – Principles, laws of light absorption, Instrumentation and applications of UV- visible spectrophotometer. Colorimetry and turbidometry.
- 2 Chromatography: Principles and applications of paper chromatography (Ascending, Descending and 2-D), Thin layer chromatography.
- 3 Principle and applications of column chromatography (Partition, adsorption, ion exchange, exclusion and affinity chromatography). Column packing and fraction collection.

Unit - 5: Centrifugation, Electrophoresis & Radio isotopes No. of Hours:9

- 1 Centrifugation-Principles, types and applications.
- 2 Electrophoretic technique (agarose and SDS polyacrylamide gel) its Components, working principle and applications
- 3 Radioisotopes– characters and applications of radioisotopes, principle of autoradiography.

II. Skill Outcomes:

On successful completion of the course, the students will be able to

1. Recognize different microscopy techniques, identify microbial cell structures, interpret micrograph images, and understanding the principles of image contrast.
2. Prepare stained slides, differentiate stained and unstained structures, recognizing staining techniques, and describing the staining characteristics of microbial cells.
3. Perform the staining procedure, distinguishing between Gram-positive and Gram-negative bacteria, recognizing the importance of Gram's staining in bacterial classification, and interpreting Gram-stained slides.
4. Understand sterilization principles, operate autoclave and hot air oven, implement proper sterilization protocols, ensure sterility of media and glassware, and recognize the importance of sterile techniques in microbiology.
5. Understand streaking techniques, perform streak plate method, obtain isolated colonies, recognize contamination, and demonstrate proficiency in maintaining pure cultures for further study.

III SEMESTER
COURSE 7: MICROBIAL AND ANALYTICAL TECHNIQUES
credits - _1

1. Study of bright field, dark field and phase contrast, Electron microscope micrographs to visualize microbial cells.
2. Simple staining & Negative staining.
3. Gram's staining.
4. Sterilization of medium using Autoclave, Sterilization of glassware using Hot Air Oven.
5. Isolation of pure cultures of bacteria by streaking method.
6. Isolation of bacteria from natural habitat by spread and pour plate method (using serial dilution method)
7. Separation of monosaccharides/amino acids by paper/thin layer chromatography.
8. Demonstration of column packing in gel filtration chromatography.
9. Determination of absorption max for an aromatic amino acid.
10. Separation of bacterial cells (cell pellet) from broth culture by using a laboratory scale centrifuge.
11. Separation of DNA fragments by Agarose gel electrophoresis.

V References:

1. Pelczar M., Chan E.C.S. and Krieg, N.R. Microbiology. Tata Mc Graw Hill Publishing Co. Ltd., New Delhi.
2. Stainier R.V., Ingraham, J.L., Wheelis, M.L. and Painter P.R. The Microbial World. Printice-Hall of India (Pvt.) Ltd., New Delhi
3. Wilson & Walker. Principles and Techniques in Practical B i o c h e m i s t r y . 5th Edition Cambridge University Press (2000).
4. Murphy D.B. Fundamental of Light Microscopy & Electron Imaging. 1st Edition. Wiley Liss. (2001).
5. K L Ghatak. Techniques and Methods In Biology PHI Publication (2011)
6. Pranav Kumar. Fundamentals and Techniques of Biophysics and Molecular Biology (2016)
7. Aurora Blair. Laboratory Techniques & Experiments in Biology. Intelliz Press
8. D.T Plummer. An Introduction to Practical Biochemistry. McGraw Hill Publication 1987
9. Beckner, W.M., Kleinsmith L.J and Hardin J. The world of cell. IV edition Benjamin /Cummings (2000)

VI. Co-Curricular Activities:

1. Competition in performing laboratory techniques like staining
2. Artwork with bacteria or fungi in petridish
3. Quiz in identifying microscopic technique in various micrographs

III SEMESTER