

**Vocational Higher Secondary  
Education (VHSE)**

**Second Year**

**MEDICAL LABORATORY TECHNOLOGY**

*Reference Book - Teachers' Version*



Government of Kerala  
Department of Education

State Council of Educational Research and Training (SCERT),  
KERALA  
2016

## **Foreword**

Dear Teachers

This reference book (**Teachers' Version**) is intended to serve as a transactional aid to facilitate classroom transaction and as a ready reference for teachers of Vocational Higher Secondary Schools. It offers some guidelines for the transaction of the course content and for undertaking the practical work listed in the course content. As the curriculum is activity based, process oriented and rooted in constructivism focusing on the realisation of learning outcomes, it demands higher level proficiency and dedication on the part of teachers for effective transaction.

In the context of the Right- based approach, quality education has to be ensured for all learners. The learner community of Vocational Higher Secondary Education in Kerala should be empowered by providing them with the best education that strengthens their competences to become innovative entrepreneurs who contribute to the knowledge society. The change of course names, modular approach adopted for the organisation of course content, work-based pedagogy and the outcome focused assessment approach paved the way for achieving the vision of Vocational Higher Secondary Education in Kerala. The revised curriculum helps to equip the learners with multiple skills matching technological advancements and to produce skilled workforce for meeting the demands of the emerging industries and service sectors with national and global orientation. The revised curriculum attempts to enhance knowledge, skills and attitudes by giving higher priority and space for the learners to make discussions in small groups, and activities requiring hands-on experience.

The SCERT appreciates the hard work and sincere co-operation of the contributors of this book that includes subject experts, industrialists and the teachers of Vocational Higher Secondary Schools. The development of the teachers' version of reference books has been a joint venture of the State Council of Educational Research and Training (SCERT) and the Directorate of Vocational Higher Secondary Education.

The SCERT welcomes constructive criticism and creative suggestions for the improvement of the book.

With regards,

**Dr. J. Prasad**  
Director  
SCERT, Kerala

# CONTENT

1. About the Course.....	
2. Job Roles .....	
3. Major Skills (with sub skills).....	
4. Learning Outcomes of the course.....	
5. Course Structure.....	
6. Syllabus &List of Practicals	
7. Learning Outcomes of the units (module-wise).....	
8. Scheme of Work.....	
9. Structure of Module 3.....	
10. Structure of Module 4 .....	
11. Class room activities (General) .....	
12. Practical activities (General) .....	
13. Overview of Module 3 .....	
(Laboratory Management, Clinical Biochemistry & Clinical Pathology)	
14. Unit 3.1Laboratory Management.....	
Over view	
Unit Grid	
Additional Information	
Assessment Activities	
List of items in Portfolio	
15. Unit 3.2 Clinical Biochemistry.....	
Over view	
Unit Grid	
Additional Information	
Assessment Activities	
List of items in Portfolio	
16. Unit 3.3 Clinical Pathology.....	
Over view	
Unit Grid	
Additional Information	
Assessment Activities	
List of items in Portfolio	
17. Extended Activities.....	
18. Overview of Module 4 .....	
(Diagnostic Microbiology, Histotechnology & Cyological techniques)	
19. Unit 4.1 Diagnostic Microbiology.....	
Over view	
Unit Grid	
Additional Information	

**Assessment Activities**

**List of items in Portfolio**

**20. Unit 4.2Histotechnology&Cyological techniques.....**

**Over view**

**Unit Grid**

**Additional Information**

**Assessment Activities**

**List of items in Portfolio**

**21. Extended Activities .....**

**22. On The Job Training .....**

**23. List of References .....**

## About the course

Medical Laboratory Technology is fast developing along with growing population and technological advancement. It is the most sought job titles in the global Health Care System.

Medical Laboratory Technology is a broad area comprising of different disciplines like Clinical Pathology, Hematology, Biochemistry, Bacteriology, Immunology, Virology, Mycology, Parasitology Histopathology, Cytology, Cytogenetics & Molecular biology etc.

In a country like ours, where fast and tremendous technological advancement and population growth happens, the demand and supply of trained man power is not at par. Introduction of a certificate course in medical laboratory technology at higher secondary level is the remedy to this major skill gap in the country.

Medical Laboratory Technology plays a crucial role in diagnosis of diseases, prognosis and treatment. Apart from the medical diagnostic scenario, application of Medical laboratory technology extends to detection of genetic disorders, epidemiology of infection diseases, detection of metabolic disorders and even to answer unraveled questions in forensic medicine.

The course is designed to provide multi skilled competent personal in the field of medical laboratory technology to meet the increasing demand. On completion of the course students acquire basic skills in branches of medical laboratory technology to cater entry level jobs. The course also provides inroads for students to undergo higher education including research in disciplines of laboratory medicine.

The structure of the course is designed in such a way that the first module of First Year Curriculum familiarizes the learners to the basics of Human Anatomy & Physiology and gives an idea about the important parts and features of a Diagnostic Laboratory. The topic also envisages the understanding of proper use and handling of common Laboratory Equipments and Glassware. A proper know - how about Blood, the commonest sample of any laboratory is given as part of the First module so the learner have a clear idea about the components, composition and collection of blood.

The second module deals with the common Hematological investigations done in a laboratory. The practical and theoretical exposure obtained during the period makes the learners competent in the field. The second module also covers the topic Blood Banking which has attained much relevance nowadays due to the regular need for blood transfusions.

The third module of the curriculum focuses on the effective management of Laboratory, various analytical methods and recent advances in clinical biochemistry and clinical pathology through different units. The unit familiarises the students with the different instruments used in Clinical biochemistry from the simplest micropipette to the most advanced fully automatic STAT Analyser.

Main aim of the fourth modules is introduce the learners into the fascinating world of microorganisms and familiarise both traditional and recent trends in microbiology to provide a basic knowledge and impart skill in diagnostic microbiology. Fourth module also covers

Histotechniques and cytological techniques, in order to get a basic idea about the various steps involved in the preparation of tissue for microscopy. This will help in their future studies or career.

The Curriculum also provides introduction to the automated machineries and techniques which can be experienced during the field visits or as part of OJT (On the Job Training). The laboratories as well as PTCs attached to schools provide ambient atmosphere for attaining perfection in performance for the students. The curriculum of VHSE which gives prime importance to practical is further skill enhanced with the scheduled 'On the Job Training Programmes' conducted in laboratories both on the government as well as private sector. The school curriculum is further enriched with introduction of ICT enabled teaching-learning methodologies as well as activity oriented tools like survey, camps, expo etc.

## Job roles

Govt./Semi Govt. Sector	Private sector	Self-employment
<ul style="list-style-type: none"> <li>• Laboratory Technician</li> <li>• Phlebotomist</li> <li>• Lab Technical Assistant</li> <li>• Laboratory Instructor</li> </ul>	<ul style="list-style-type: none"> <li>• Lab Technician</li> <li>• Phlebotomist</li> <li>• Lab Assistant</li> <li>• Laboratory Instructor</li> <li>• Hematology Technician</li> <li>• Blood Bank Technician</li> <li>• Biochemistry Technician</li> <li>• Clinical Pathology Technician</li> <li>• Microbiology Technician</li> <li>• Clinical Assistant</li> </ul>	<ul style="list-style-type: none"> <li>• Laboratory Owner</li> <li>• Laboratory Technician</li> <li>• Reagent Manufacturer</li> <li>• Diagnostic distributor</li> </ul>

## Major Skills

- ☞ Phlebotomy skill
- ☞ Skill in Haematological techniques
- ☞ Blood Banking Skill
- ☞ Laboratory Management Skill
- ☞ Skill in Biochemical techniques
- ☞ Skill in Clinical Pathological techniques.
- ☞ Skill in Microbiological techniques

## Sub Skills

- ☞ Measurement of BP and Pulse
- ☞ Skill in Handling and operation of common laboratory equipments
- ☞ Skill in safe handling of various chemicals

- ☞ Skill in First aid practice
- ☞ Skill in pipetting
- ☞ Skill in reagent preparation
- ☞ Tissue processing skill in histopathology

## Learning Outcomes of the course

### **Upon completion of the course, the learner will be able to:**

- Acquire basic knowledge of the structure and function of human body
- Collect and handle clinical specimens properly for performing investigations.
- Operate and take care of various laboratory equipment
- Perform Hematological, pathological, biochemical and microbiological diagnostic tests.
- Perform routine blood bank techniques
- Perform phlebotomy
- Act as an effective laboratory technician demonstrating good laboratory ethics & code of conduct
- Give awareness to the public about various health hazards
- Establish and run reagent manufacturing units
- Act as a distributor of diagnostic kits and reagents.
- Establish and run a diagnostic laboratory

## Course structure

Module No.	Name of Module	No. of Periods
Module 1	Anatomy, Physiology and Phlebotomy	340
Module 2	Haematology and Blood Banking	340
Module 3	Clinical Biochemistry, Clinical Pathology & Laboratory Management	340
Module 4	Diagnostic Microbiology & Histotechnology	340

# Syllabus

## Module 3

### LABORATORY MANAGEMENT, CLINICAL PATHOLOGY & CLINICAL BIOCHEMISTRY

#### Module 3      unit 1      laboratory management      40 periods

Unit No.	Unit	Period
3.1.1	<b>Lab safety</b> Introduction Signs and symbols used in a laboratory Handling and storage of chemicals in a laboratory. Laboratory Hazards-Physical, Chemical, Biological, Electrical, Fire, Radiation Laboratory Safety Precautions–Personal Hygiene Fire Extinguishers Biomedical Waste Management First Aid Practice in Laboratory	20
3.1.2.	<b>Laboratory Management</b> Introduction Code of Ethics of a laboratory Professional Role of communication in laboratory Organization of a Laboratory Components of a Laboratory Lay out plan of a multi-room laboratory Organizational pattern of a Laboratory Familiarization of Request forms and report forms. Ordering and Utilization of supplies Maintenance of Stock Registers- Consumables, Non-consumables Accreditation and Certification of Laboratories. Accrediting Agencies- NABL, ISO, CAP, CRISIL - Bar coding and Total Laboratory Automation(TLA) Familiarization of Common Laboratory Software	20

#### Module 3      UNIT 2      CLINICAL PATHOLOGY      100 Periods

Unit No.	Unit	Period
3.2.1.	<b>Introduction</b>	10



	Importance, Common specimens, General guidelines for sample collection	
3.2.2.	<b>Urine Analysis</b> - Importance, Types of urine samples Methods of collection, preservatives - Physical Examination - Chemical Examination-Sugar, Protein, Blood, Ketone bodies, Bile pigments, Bile salts, Urobilinogen - Microscopic Examination - hCG test in Urine	44
3.2.3.	<b>Sputum Examination</b> Importance, Specimen collection Physical examination Microscopic examination	5
3.2.4	<b>Stool Analysis</b> Importance, Specimen collection Physical examination Chemical examination- Occult blood, Reducing substances Microscopic examination- Saline & Iodine mount	15
3.3.4	<b>Semen Analysis</b> - Importance, Specimen Collection - Physical Examination, Liquefaction Time, Microscopy- Total Sperm Count, Motility, Morphology Chemical Examination-Fructose, Acid phosphatase	16
3.3.5.	<b>CSF and other body fluids</b> CSF- introduction Specimen collection Physical & Microscopic Examination Chemical Examination- protein, glucose, chloride (Name of method of estimation & clinical significance only) Other body fluids Recent advances in Clinical pathology	10

## UNIT 2 CLINICAL BIOCHEMISTRY

200

Periods

Unit No.	Name of Units	Periods
3.2.1	<b>Introduction to Biochemistry</b> -Types of chemicals and preparation of solutions. -Types of specimens in clinical Biochemistry -Collection and processing of specimens for biochemical analysis -Types of assays- Endpoint and Kinetic (definition and example only) -Cleaning of glass wares for biochemical analysis	12
3.2.2	<b>Instruments used in Biochemistry</b> Familiarise with Colorimeter, Spectrophotometer, Flame photometer, Centrifuge, Electronic balance, Distillation apparatus, Deionizer	8
3.2.3	<b>Blood Glucose Estimation</b> Introduction to Diabetes – features, types, complications, Types of samples- FBS, PPBS, RBS, Anticoagulant used Methods of estimation- GOD-POD in detail Normal value and Clinical Significance - Hyper and hypoglycaemia	28

	Mention Glucometer Technique GTT and GCT procedures, Mention relevance HbA1C	
3.2.4.	<b>Renal Function Tests</b> -Introduction, Common tests included <ul style="list-style-type: none"> <li>• Estimation of Blood Urea Mention common methods Urea-Berthelot method in detail, Normal value and Clinical significance Renal, Pre-renal, Post renal conditions of Uraemia</li> <li>• Estimation of S. Creatinine. Mention common methods. Jaffe's method in detail Normal value and Clinical significance</li> <li>• Estimation of Uric Acid. Mention common methods. Uricase method in detail. Normal value and Clinical Significance.</li> </ul> - Mention Clearance tests- Urea and Creatinine - Mention Importance of Micro-albumin and Cystatin- C	40
3.2.5.	<b>Liver Function Tests</b> <ul style="list-style-type: none"> <li>• Introduction, Common tests included Bilirubin-Formation of Bilirubin Types of Bilirubin- conjugated and unconjugated Estimation of Bilirubin. Malloy- Evelyn method in detail. Normal value and Clinical Significance</li> <li>• Estimation of Total protein- Biuret method in detail</li> <li>• Estimation of Albumin- BCG method in details Normal value and clinical significance of total protein and Albumin, A-G Ratio.</li> <li>• Other LFT Parameters- ALP, ALT, AST in brief.</li> </ul>	40
3.2.6	<b>Lipid Profile</b> <ul style="list-style-type: none"> <li>• Introduction - Relevance, tests included in the Profile</li> <li>• Estimation of Serum Cholesterol. Mention common methods, CHOD-PAP method in detail, Normal value and Clinical Significance Mention Triglycerides, HDL, LDL</li> </ul>	25
3.2.7	<b>Other parameters of Diagnostic importance</b> <ul style="list-style-type: none"> <li>• Serum Electrolytes- Serum Sodium and Potassium Normal value and Clinical significance</li> <li>• Clinically important Minerals- Calcium and Phosphorus ( normal value and significance only)</li> <li>• Name Diagnostically important Hormones T3, T4, TSH, FSH, LH, Prolactin, progesterone</li> <li>• Name Clinically important enzymes- Acid Phosphatase, S. Amylase, GGT,</li> <li>• Name Cardiac markers- Troponin-I, Troponin-T CPK, CK-MB, LDH, SGOT</li> <li>• Name Tumour Markers- CA-125, CEA, AFP,CA-19.9, PSA, Beta hCG</li> </ul>	21
3.2.8	<b>Quality control in Biochemistry</b> - Introduction,	10

	Common terms used in Quality control, Errors - random and systemic , L.J. Chart, External QC and Internal QC	
3.2.9	<b>Automation and Recent advances</b> Need for Automation, Advantages of Automation Types of Auto Analysers-Semi and Fully automated Electrolyte Analyser (ISE) in brief Advanced Diagnostic Methods in brief - C.L.I.A.,C.L.F.A, Turbidometry, Nephelometry, HPLC, Mention Point of care testing (POCT)	16

## Module 4 Unit 1 DIAGNOSTIC MICROBIOLOGY 290 Periods

Unit No.	Unit	Period
4.1.1	<b>Introduction to Microbiology</b> Classification of Microbes, pathogen, commensals, type of Infections, communicable diseases, Carriers Historical aspects in Microbiology	15
4.1.2	<b>Structure and classification of bacteria</b> <ul style="list-style-type: none"> <li>• Structure- Cell wall, flagella, fimbriae, capsule, spore, plasmid</li> <li>• Classification of bacteria based on morphology- Arrangement, Motility and oxygen requirement</li> </ul>	15
4.1.3	<b>Sterilization and disinfection</b> <ul style="list-style-type: none"> <li>• Importance of sterilization and Disinfection</li> <li>• Methods of sterilization</li> </ul> Physical methods- Dry heat, Moist Heat Chemical methods- alcohols, aldehydes, gases Mechanical methods- Filtration, Radiation <ul style="list-style-type: none"> <li>• Describe principle, parts, and use of               <ul style="list-style-type: none"> <li>- Hot air Oven, Autoclave</li> </ul> </li> <li>• Disinfectants and Antiseptics and their application</li> </ul>	45
4.1.4	<b>Growth &amp; Cultivation of Bacteria</b> <ul style="list-style-type: none"> <li>• Bacterial growth and replication</li> <li>- Mention essential growth requirements- Temperature, PH, Gaseous requirements</li> <li>• Culture media               <ul style="list-style-type: none"> <li>Classification of culture media with examples                   <ul style="list-style-type: none"> <li>• Preparation and use of common media</li> </ul> </li> </ul> </li> </ul> Peptone water, Nutrient Agar, Blood Agar, Chocolate agar, Mac Conkey Agar <ul style="list-style-type: none"> <li>• Bacteriological wire loop, Straight wire</li> <li>- Inoculation of Culture media- Liquid and Solid</li> <li>• Mention Streak, Stroke, Stab, Lawn culture</li> <li>- Mention Anaerobic techniques- Gaspak</li> </ul>	40
4.1.5.	<b>Basic identification Techniques</b> Introduction	50

	<p>Identification of bacteria</p> <ul style="list-style-type: none"> <li>• Different methods</li> <li>• Detection of motility <ul style="list-style-type: none"> <li>- Name different methods</li> <li>- Hanging drop method in detail</li> </ul> </li> <li>• Staining <ul style="list-style-type: none"> <li>- Principle, requirement, procedure and interpretation of Simple stain, Grams stain, AFB stain-Diagnostic significance</li> </ul> </li> </ul> <p>Biochemical tests- Coagulase, Catalase, IMViC</p>	
4.1.6.	<p><b>Immunology and its diagnostic applications •</b></p> <p>Introduction</p> <p>-Types of Immunity, Antigen ,Antibody</p> <p>-Structure of antibody</p> <p>Types of antibody- Ig G, IgM, IgA, IgD, Ig E</p> <ul style="list-style-type: none"> <li>• Antigen Antibody reactions- Specificity, Sensitivity, Avidity, Prozone ,post-zone, Titer</li> </ul> <p>Clinical applications of Agglutination, precipitation, flocculation, ELISA, Immuno-Fluorescence.</p>	35
4.1.7.	<p><b>Laboratory Diagnosis of Common Bacterial diseases</b></p> <ul style="list-style-type: none"> <li>• Collection, Processing and transportation of common specimens-Urine, Blood, Sputum, CSF, Stool, Pus, body fluids, swabs</li> </ul> <p>General considerations- Macroscopy , Microscopy, Culture</p> <ul style="list-style-type: none"> <li>• Mention common culture media and identification methods used.</li> </ul> <p>Antibiotic Sensitivity Testing (ABST)- Kirby Bauer Method</p> <ul style="list-style-type: none"> <li>• Common Disease and pathogens encountered -Typhoid, Tuberculosis, Cholera, Dysentery, Syphilis, Leptospirosis, Tetanus, Meningitis&amp; UTI</li> <li>• Common Serological Techniques for diagnosis of Bacterial diseases-</li> <li>• ELISA &amp; its commercial preparations - Immunochromatographic technique</li> <li>• WIDAL,RPR,-Procedure and interpretation</li> </ul>	30
4.1.8	<p><b>Laboratory Diagnosis of Common Viral diseases</b></p> <ul style="list-style-type: none"> <li>• Introduction to viruses</li> <li>• Common viral diseases and pathogens encountered - AIDS, Hepatitis, Dengue, Chickun Guinia, Rabies, Infuenza, Mumps and Measles.</li> <li>• Diagnostic techniques for viral infections <ul style="list-style-type: none"> <li>- Mention common Serological tests used, Latex agglutination, Card tests, ELISA, Tissue culture, PCR Technique</li> </ul> </li> </ul>	20
4.1.9.	<p><b>Laboratory Diagnosis of Common Parasitic diseases</b></p> <ul style="list-style-type: none"> <li>• Introduction to parasites</li> <li>- Parasite, Commensal, Symbiosis, Host (Intermediate &amp; Definitive host), Vector, Zoonosis <ul style="list-style-type: none"> <li>• Classification-Intestinal &amp; Blood Parasites</li> </ul> </li> </ul>	40

	<ul style="list-style-type: none"> <li>• Common blood parasites and their lab diagnosis <ul style="list-style-type: none"> <li>- Blood collection-</li> <li>-Time of collection</li> <li>-Preparation of smear-Thick and thin</li> <li>-Dehaemoglobinisation of thick smear</li> </ul> </li> <li>• <b>Lab Diagnosis of Malaria</b> <ul style="list-style-type: none"> <li>- Disease, mode of transmission, hosts causative agent, types of malaria.</li> <li>- Examination of thick and thin smear-Morphological identification of different stages of parasite</li> <li>- Other stains used- JSB</li> <li>- Other methods- Card method , QBC</li> </ul> </li> <li>• <b>Lab Diagnosis of Filariasis</b> <p>Disease, mode of transmission, host, and nocturnal habit</p> <ul style="list-style-type: none"> <li>- Lab diagnosis- wet smear examination, thick smear examination, Concentration technique.</li> </ul> </li> <li>• <b>Lab Diagnosis of Intestinal parasites</b> <ul style="list-style-type: none"> <li>- Introduction -Helminthic infections and parasites</li> <li>- Amoebiasis -Entamoeba histolytica- Disease, Mode of Transmission, Trophozoite &amp; Cyst</li> <li>Lab diagnosis -Macroscopic examination</li> <li>Microscopic examination -Stained &amp; Unstained preparation</li> </ul> </li> <li>• Common Helminths- Tape worm, Round worm, Hook worm, Whip worm, Pin worm, <ul style="list-style-type: none"> <li>- Lab diagnosis-Macroscopic &amp; Microscopic examination</li> <li>- Concentration Techniques of Stool sample- Mention Floatation &amp; Sedimentation methods</li> </ul> </li> </ul>	
--	---	--

**Module 4      Unit 2 HISTOTECHNOLOGY & CYTOLOGY      50 Periods**

Unit No.	Unit	Period
4.2.1	<b><u>HISTOTECHNOLOGY</u></b> <b>Introduction</b> -Methods of examination of Tissues and cells -Gross examination -Microscopic examination Examination of Unfixed Tissue Examination of Fixed Tissue -Collection of specimens-    -Biopsy    -Autopsy -Fixation -10% Formalin -Decalcification	20
4.2.2	<b>Tissue Processing</b> Steps in tissue processing -Dehydration -Clearing -Impregnation -Embedding	20

	Microtomes-Rotary Microtome,-Cryostat -Section cutting -Mention role of adhesives -Staining -H&E Staining -Mounting of Tissue sections -Filing and storage of tissue sections	
4.2.3	<b><u>DIAGNOSTIC CYTOLOGY</u></b> Introduction Types of specimens Processing Fixation Staining Advantages and applications in diagnostic cytology	10

## List of Practical

### MODULE 3

#### **3.1 Laboratory Management**

1. Demonstration of signs and symbols used in laboratory
2. Preparation of charts-signs and symbols.
3. Demonstration of Laboratory Safety Measures by mock drill
4. Demonstration of different types of Request forms- Pre-printed, written
5. Demonstration of different types of report forms.- Haematology, Biochemistry, Clinical Pathology, Serology, Mixed forms
6. Preparation of different models of report forms.
7. Model Lay out plan of a multi-room laboratory (Chart/ Model Preparation)
8. Draw a model lay out plan of a laboratory
9. Preparation of model of Order form
10. Demonstration of models of Stock Registers- Consumables, Non-consumables
11. Preparation of models of Stock Registers
12. Demonstration of Laboratory software.
13. Demonstration of colour coding for biomedical waste segregation
14. Chart preparation - colour coding of biomedical waste

#### **3.2 Clinical Pathology**

15. Physical examination of urine.-volume, colour, specific gravity, Reaction, pH
16. Chemical examination of urine- Test for sugar (Benedict's qualitative test), Test for protein (Heat and acetic acid test), Test for Ketone bodies (Rotheras test), Test for Bile pigments (Fouchets test) and test for Bile salt(Hays test)
17. Microscopic examination of urine- Wet mount preparation of sediments, examination and reporting of sediments.
18. HCG detection in urine
19. Physical examination stool- consistency ,colour, mucus and parasites
20. Chemical examination stool-occult blood, reducing substances

21. Microscopic examination stool-saline and iodine mount
22. Physical examination of semen-Volume, colour, reaction and liquefaction time
23. Microscopy of semen-Total sperm count, motility, morphology

### **3.3.Clinical Biochemistry**

24. Prepare chart showing different types of chemicals used in a laboratory.
25. Prepare chart showing specimens with important biochemical investigations
26. Estimation of Blood Glucose( GOD-POD Method)
27. Estimation of S.Cholesterol (CHOD-PAP Method)
28. Estimation of Blood Urea ( Berthlot method)
29. Estimation of S.Creatinine (Jaffe's method)
30. Estimation of S.Uric Acid (Uricase method)
31. Estimation of S.Bilirubin (Evelyn Malloy method)
32. Estimation of S.Total Protein(Biuret method)
33. Estimation of S.Albumin (BCG Method)

## **MODULE 4**

### **4.1Diagnostic Microbiology**

1. Prepare chart showing Infectious diseases and pathogens
2. Prepare chart of communicable diseases in Kerala and causative agents
3. Preparation of Album of pioneers in microbiology and their contributions
4. Structure of a bacterial 'cell ( Model/Chart Preparation)
5. Comparison chart preparation of sterilization methods in laboratory
6. List of antiseptics and disinfectants of common use- Chart Preparation
7. Demonstration of operation procedure of hot air oven
8. Demonstration of operation procedure of Autoclave
9. Preparation of liquid media - Peptone water
10. Preparation of solid media - NA, BA, CA, MA
11. Handling , sterilisation and use of bacterial wire loop
12. Demonstration of inoculation methods
13. Inoculation of Culture media- Liquid
14. Demonstration of hanging drop method of motility
15. Inoculation of Culture media- Solid
16. Demonstration of colony characters of commonest pathogens
17. Preparation of bacterial smear- from liquid media
18. Preparation of bacterial smear- from solid media
19. Perform simple staining
20. Perform Gram staining
21. Perform AFB staining
22. Demonstration of ABST
23. Model/Chart preparation of a typical immunoglobulin
24. Demonstrate and Perform RPR Test
25. Flow chart showing processing of clinical specimens in lab
26. Chart showing common viral diseases and pathogen
27. Staining and examination of thin smear for blood parasites
28. Dehaemoglobinisation, Staining and examination of thick smear for blood parasites
29. Demonstration of wet smear for filarial parasite
30. Perform direct smear of stool sample for parasites-saline and iodine
31. Demonstration of floatation/sedimentation Techniques for intestinal parasites

### **4.2Histotechnology and Cytology**

32. Demonstrations of Tissue block and stained H&E histology sections.

33. Chart preparation for different steps in tissue processing in histopathology lab.
34. Demonstration of Ayers spatula, Coplin jar and stained PAP smear.
35. Chart preparation of different steps in H&E Staining
36. Chart preparation of different steps in PAP staining procedure

### **Module 3 : Learning outcome of the Units**

- 3.1.1 To Identify Code of laboratory ethics and safe laboratory practice
- 3.1.2 To explain different laboratory safety precautions and first aid
- 3.1.3 To classify different methods of biomedical waste management
- 3.1.4 To explain organization of a laboratory, its organizational pattern and the role of communication in a laboratory
- 3.1.5 To identify and prepare lay out of a Medical Laboratory
- 3.1.6 To format various request forms, stock registers and order form
- 3.1.7 To identify the importance of accreditation , certification of laboratories and identify different accrediting agencies
- 3.1.8 To identify the importance of barcoding and Total laboratory Automation and use of Common Laboratory Software
- 3.2.1 To define Clinical biochemistry, grades of various chemicals and preparation of solutions
- 3.2.2 To differentiate different types of assays used in biochemistry.
- 3.2.3 To identify different types of specimens, their collection and processing for biochemical analysis.
- 3.2.4 To perform cleaning of glass wares
- 3.2.5 To explain the parts, working, use and to operate common instruments used in biochemistry
- 3.2.6 To explain Diabetes and to differentiate various blood samples used for blood sugar estimation
- 3.2.7 To identify different blood sugar estimation methods and to estimate blood glucose by GOD -POD method
- 3.2.8 To explain GTT, GCT procedures, Glucometer technique and importance of HBA1c
- 3.2.9 To explain the relevance of renal function test and to identify various tests included in the RFT panel
- 3.2.10 To identify common blood urea estimation methods and estimate blood Urea by Berthlot method
- 3.2.11 To identify common creatinine estimation methods and to estimate S.Creatinine by Jaffes method
- 3.2.12 To discuss the importance of uric acid and estimation of uric acid by uricase method
- 3.2.13 To identify the importance of microalbumin, Cystatin-C and clearance tests for the evaluation of renal function
- 3.2.14 To explain the relevance of Liver function test and to identify various tests included in the LFT panel
- 3.2.15 To explain jaundice and to perform Estimation of Bilirubin by Malloy- Evelyn method
- 3.2.15 To perform estimation of serum total protein by Biuret method
- 3.2.16 To perform estimation of serum albumin by BCG method
- 3.2.17 To identify the importance of ALT,AST& ALP parameters in the evaluation of Liver function
- 3.2.18 To explain the importance of Lipid profile and to identify various tests included in the Lipid profile
- 3.2.19 To perform estimation of S. Cholesterol by CHOD-PAP method



- 3.2.20 To identify the importance of Serum Electrolytes
- 3.2.21 To identify the clinical significance of S.Calcium & Phosphorous estimations
- 3.2.22 To identify the clinical significance of various special biochemical tests
- 3.2.23 To identify the importance of quality control in biochemistry and able to explain various terms used in quality control
- 3.2.24 To identify the need, advantages and recent advances of automation in a Clinical biochemistry laboratory
- 3.2.25 To mention advanced diagnostic methods in clinical biochemistry
- 3.3.1 To identify different specimens and describe the general guidelines for sample collection
- 3.3.2 To discuss the importance of urine analysis
- 3.3.3 To identify different type of urine samples and method of collection
- 3.3.4 To perform physical, chemical and microscopical examination of urine
- 3.3.5 To perform urine pregnancy test
- 3.3.6 To identify the importance sputum analysis
- 3.3.7 To identify the importance of stool examination
- 3.3.8 To discuss the importance of semen analysis and describe the method of semen analysis
- 3.3.9 To identify the importance and describe analysis of C.S.F and other body fluids.
- 3.3.10 To mention the recent advances in clinical pathology

## **Module 4 : Learning outcome of the Units**

- 4.1.1 To define Microbiology, to differentiate Pathogen, Commensals and type of infection
- 4.1.2 To summarize various historical aspects of microbiology
- 4.1.3 To explain the structure of bacteria
- 4.1.4 To differentiate bacteria based on morphology, Motility & Oxygen requirement
- 4.1.5 To identify the importance of sterilization
- 4.1.6 To categorize different methods of sterilization
- 4.1.7 To operate Hot air oven, Autoclave & Incubator
- 4.1.8 To distinguish Disinfectants and Antiseptics and their applications
- 4.1.9 To explain various growth requirements of bacteria
- 4.1.10 To classify different culture media and prepare Common culture media
- 4.1.11 To explain the different inoculation techniques.
- 4.1.12 To perform streak culture technique
- 4.1.13 To classify different methods for the identification of bacteria
- 4.1.14 To define Immunology and to explain different terms in immunology
- 4.1.15 To explain the structure of antibody and to classify different types of antibodies
- 4.1.16 To differentiate various Antigen- Antibody reactions and their clinical applications
- 4.1.17 To explain the Collection and transportation of different specimens
- 4.1.18 To summarize the different methods in the specimen processing
- 4.1.29 To differentiate common bacterial diseases and identify the pathogens encountered
- 4.1.21 To explain the method of Antibiotic sensitivity test
- 4.1.22 To differentiate and perform common serological tests
- 4.1.23 To define virology and classify viruses
- 4.1.24 To differentiate common viral diseases and the pathogen encountered
- 4.1.24 To explain common diagnostic serological tests for viral infections
- 4.1.25 To define parasitology and explain different terms in parasitology
- 4.1.26 To differentiate intestinal and blood parasites

- 4.1.27 To identify common blood parasites and explain their lab diagnosis
- 4.1.28 To differentiate the Causative agent, different species, host and mode of transmission of malaria
- 4.1.29 To name the different stages of Malarial parasite
- 4.1.30 To explain different methods for the diagnosis of malaria
- 4.1.31 To differentiate the Disease, mode of transmission, host and nocturnal habit of filarial parasite
- 4.1.32 To explain different methods for the lab diagnosis of filariasis
- 4.1.33 To identify common intestinal parasites and the method of examination of stool by concentration technique
- 4.1.34 To familiarize the disease, causative agent, mode of transmission and lab diagnosis of amoebiasis
- 4.1.35 To differentiate common helminthes and explain their lab diagnosis
- 4.2.1 To identify different types of specimens and the method of examination in histotechnology
- 4.2.2 To explain various steps in tissue processing
- 4.2.3 To identify various specimens and processing techniques employed in cytology

# Scheme of Work

## MODULE 3

Month	Name of Unit	Period
	<b>LABORATORY MANAGEMENT</b>	
June	Lab safety	20
June	Laboratory Management	20
	<b>CLINICAL PATHOLOGY</b>	
June	Introduction	10
June	Urine Analysis	20
June	Urine Analysis	24
July	Sputum Examination	5
July	Stool Analysis	15
July	Semen Analysis	16
August	CSF and other Fluids	10
	<b>CLINICAL BIOCHEMISTRY</b>	
August	Introduction	12
August	Instruments Used in Biochemistry	8
September	Blood Glucose Estimation	8
September	Blood Glucose Estimation	20
September	Renal Function Tests	40
September	Liver Function Tests	10
October	Liver Function Tests	30
October	Lipid Profile	25
October	Other Parameters of Diagnostic Importance	21
October	Quality Control in Biochemistry	10
October	Automation and Recent advances	16
	<b>TOTAL</b>	<b>340</b>

## MODULE 4

Month	Name of Unit	Period
	<b>DIAGNOSTIC MICROBIOLOGY</b>	
November	Introduction to Microbiology	15
November	Structural Classification of bacteria	15
November	Sterilization and disinfection	45
December	Growth and Cultivation of Bacteria	40
December	Diagnostic bacteriology	35
January	Diagnostic Bacteriology	15
January	Immunology and its Diagnostic Application	35
January	Laboratory Diagnosis of Common Bacterial Diseases	15
February	Laboratory Diagnosis of Common Bacterial Diseases	15
February	Laboratory Diagnosis of Common Viral Diseases	20
February	Laboratory Diagnosis of Common Parasitic Diseases	40
	<b>HISTOTECHNOLOGY AND CYTOLOGY</b>	
March	Histo Technology	20
March	Tissue Processing	20
March	Diagnostic Cytology	10
	<b>TOTAL</b>	<b>340</b>

## Structure of Module 3

### CLINICAL BIOCHEMISTRY, CLINICAL PATHOLOGY AND LABORATORY MANAGEMENT

Unit No.	Name of Units	Periods
Unit 1	LABORATORY MANAGEMENT	40 Periods
Unit 2	CLINICAL PATHOLOGY	100 Periods
Unit 3	CLINICAL BIOCHEMISTRY	200 Periods
	30% Theory 70% Practical	Total -340 Periods

# Structure of Module 4

## DIAGNOSTIC MICROBIOLOGY & HISTOTECHNOLOGY

Unit No.	Name of Units	Periods
Unit 1	DIAGNOSTIC MICROBIOLOGY	300Periods
Unit 2	HISTOTECHNOLOGY & CYTOLOGY	40 Periods
30% Theory 70% Practical		Total -340 Periods

## Class Room Activities

- Multi media presentation
- Discussion
- Debate
- Seminar
- Assignment
- Magazine preparation
- Album preparation
- Chart preparation
- Poster Preparation
- Role play
- Quiz
- Survey
- Collection
- Model Preparation

## Practical Activities

1. Demonstration
2. Case study
3. Hands on experience
4. Survey
5. Field study
6. Field visit
7. Exhibition
8. Camp

## Module3

# Clinical Biochemistry, Clinical Pathology & Laboratory Management

Second year programme for VHSE-MLT consist of two modules. The third module comprises mainly topics like Clinical Biochemistry, Laboratory management and Clinical pathology .Clinical Biochemistry and Clinical pathology are two vital areas of laboratory medicine-. More than 60% of specimens send for investigations to a diagnostic laboratory are reaching here. Laboratory Techniques are blunt with manual as well as mechanical methodologies. Clinical biochemistry has now a days become the hot spot area of laboratory diagnosis . Manual methods and principles are revolutionized and replaced by enzyme chemistry and the reagents are available in 'kit' format. The scope of investigations has almost changed the diagnostic scenario from a level of general health assessment to organ function tests. In order to strengthen the laboratory professional, inevitable aspects like laboratory management, Lab safety, code of conduct etc; are also discussed along with this.

Clinical Pathology laboratory pertains to analysis of body fluids which enables to reveal pathophysiological maladies. Techniques used here are of historical importance in the evolution of medicine and are the easiest and cheapest methods. Patients have little discomfort and stress for specimen collection and the tests provide information of immense value. But low sensitivity and less chance for automation makes clinical pathology results less popular. Yet with the supporting and supplementing information and investigations, now a days clinical pathology results are largely depended by the clinicians to resolve diagnostic dilemma.

### **Unit 3.1 Laboratory Management**

The effective operation of a medical laboratory and proper delivery of laboratory results to clinician and their patients are integral part of a well defined health care system. An effective laboratory management is essential for providing an accurate, reliable and timely laboratory results which forms the basis of almost all of the medical decisions and diagnosis made in the modern era. The task of laboratory management involves integration and co ordination of organizational resources like personal, equipment, money, time and space so that standardized planning organization and operation of a laboratory happens. It essentiate management skills in ensuring laboratory safety, handling of laboratory wastes and observing laboratory ethics, protocols accreditation and certification criteria. The unit familiarizes the learner with the Code of Ethics of Laboratory professional, safety measures to be taken in a laboratory, tips for personal hygiene and about the care and handling of chemicals in a laboratory. It also creates awareness about the different signs and symbols used in a laboratory, different types of hazards and about the segregation and management of laboratory waste.

Software based laboratory management systems have been evolved over years that support laboratory operations. Most of them utilize web enabled sample management, data analyzing and reporting facilities. Introduction of Barcoding and Total Laboratory Automation has caused tremendous improvement in the patient identification and time management mechanism.

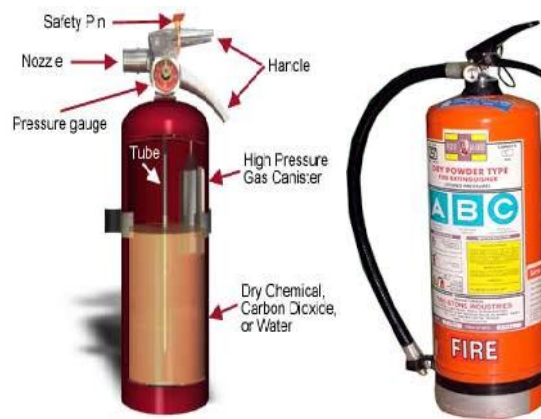
## Unit Grid:3.1 Laboratory Management

IDEAS/ CONCEPTS	LEARNING OUTCOME	SUGGESTED ACTIVITY	ASSESSMENT
<p><b>3.1.1Lab safety</b></p> <p>Code of Ethics of a laboratory Professional</p> <p>Laboratory Safety Precautions–Personal Hygeine</p> <p>Signs and symbols used in a laboratory</p> <p>Handling and storage of chemicals in a laboratory.</p> <p>Laboratory Hazards-Physical, Chemical, Biological, Electrical, Fire, Radiation</p> <p>First Aid Practice in Laboratory</p> <p>Laboratory Safety Precautions–Personal Hygiene- Fire Extinguishers</p> <p>Biomedical Waste Management in laboratory</p> <p>Skill</p> <p>-Observation -Participation -Classification -Charting</p>	<p>To Identify Code of laboratory ethics and safe laboratory practice</p> <p>To explain different laboratory safety precautions and first aid</p> <p>To classify different methods of biomedical waste management</p>	<p>Assignment Group Discussion</p> <p>Discussion Chart preparation Seminar Demonstration</p> <p>Chart preparation Assignment</p>	<p>Participation in the discussion</p> <p>Participation in the discussion</p> <p>Quality of chart</p> <p>Quality of chart</p>
<p><b>3.1.2 Laboratory Management</b></p> <p>Role of communication in laboratory</p> <p>Organization of a</p>	<p>To explain organization of a laboratory, its organizational pattern and the role of communication in a</p>	<p>Role Play Discussion</p>	<p>Involvement in role play Participation</p>

Laboratory	laboratory		
Components of a Laboratory	To identify and prepare lay out of a Medical Laboratory	Chart/Model Preparation	Quality of chart
Lay out plan of a multi-room laboratory			
Organisational pattern of a Laboratory			
Familiarisation of Request forms and report forms.	To format various request forms, stock registers and order form	Collection Chart Preparation	Quality of chart
Ordering and Utilisation of supplies			
Maintenance of Stock Registers- Consumables, Non-consumables	To identify the importance of accreditation, Certification of laboratories and identify different accrediting agencies	Reference Assignment	Portfolio assessment
Accreditation and Certification of Laboratories.			
Accrediting Agencies- NABL, ISO, CAP, CRISIL	To identify the importance of barcoding and Total laboratory Automation and use of Common Laboratory Software	Field Visit Assignment	
Familiarization of Common Laboratory Software			
Bar coding and Total Laboratory Automation(TLA)			
Skill			
-Observation			
-Communication			
-Sketching			
-Management			



## Additional Information



*Figure of Fire Extinguisher*

Extinguishing agents employed in the extinguisher may be CO<sub>2</sub>, Mono ammonium phosphate, Sodium bicarbonate, Potassium bicarbonate etc.

### How to use Fire extinguisher:

- It is important to know the locations and the types of extinguishers in your workplace prior to actually using one.
  - Fire Extinguishers can be heavy, so it's a good idea to practice picking up and holding an extinguisher to get an idea of the weight and feel.
  - Take time to read the operating instructions and warnings found on the fire extinguisher label. Not all fire extinguishers look alike.
  - Practice releasing the discharge hose or horn and aiming it at the base of an imagined fire. Do not pull the pin or squeeze the lever. This will break the extinguisher seal and cause it to lose pressure.
  - When it is time to use the extinguisher on a fire, Just remember to **P.A.S.S.!**
1. **Pull** : Pull the pin.
  2. **Aim**: Aim the nozzle or hose at the base of the fire from the recommended safe distance.
  3. **Squeeze**: Squeeze the operating lever to discharge the fire extinguisher agent.
  4. **Sweep**: Starting at the recommended distance, Sweep the nozzle or hose from side to side until the fire is out. Move forward or around the fire area as the fire diminishes.

Fires are classified into five (5) classes. They are described below:



**Class A**

A fire extinguisher labeled with **letter "A"** is for use on Class A fires. Class A fires are fires that involve ordinary combustible materials such as cloth, wood, paper, rubber, and many plastics.



**Class B**

A fire extinguisher labeled with **letter "B"** is for use on Class B fires. Class B fires are fires that involve flammable and combustible liquids such as gasoline, alcohol, diesel oil, oil-based paints, lacquers, etc., and flammable gases.



**Class C**

A fire extinguisher labeled with **letter "C"** is for use on Class C fires. Class C fires are fires that involve energized electrical equipment.



**Class D**

A fire extinguisher labeled with **letter "D"** is for use on Class D fires. Class D fires are fires that involve combustible metals such as magnesium, titanium and sodium.



**Class K**

A fire extinguisher labeled with **letter "K"** is for use on Class K fires. Class K fires are fires that involve vegetable oils, animal oils, or fats in cooking appliances. This is for commercial kitchens, including those found in restaurants, cafeterias, and caterers.



**SEGREGATION OF SOLID BIO-MEDICAL WASTE**

NON-INFECTED WASTE

CYTOTOXIC DRUG & CHEMICAL WASTE



INFECTED WASTE

SOILED WASTE

Infected Dressings, POP Casts



ANATOMICAL WASTE

Placenta, Pathological Waste & Body Parts



INFECTED PLASTICS

Syringes, Gloves & Plastic Waste.



SHARPS

Needles & Cut Glasses



## Assesment activities

- ❖ Assignment on Safe Laboratory Practice in Medical laboratory
- ❖ Seminar on Common Hazards in a Laboratory
- ❖ Prepare a chart on First Aid Practice
- ❖ Prepare a chart on different color labels for segregation of biomedical waste
- ❖ Prepare Lay out of an ideal laboratory
- ❖ Collect different types of report forms
- ❖ Prepare formats of different report forms, request forms & Stock registers

## List of items in portfolio

- Assignment report on Safe Laboratory Practice in Medical laboratory
- Seminar report on Common Hazards in a Laboratory
- chart prepared on First Aid Practice
- chart prepared on different color labels for segregation of biomedical waste
- Chart showing layout plan of Laboratory
- Different report, request forms collected and prepared by the student

## Unit 3.2 Clinical pathology

A change that takes place in the human body during the process of disease is always reflected in the chemical composition of body fluids. Clinical examination of these fluids reveals the presence of abnormal constituents, altered cellularity, microorganisms and other physical evidences. These evidences from a clinical pathology lab provide endless support to a physician in reaching an early and accurate diagnosis. Apart from the common importance like that of any other laboratory investigation, its importance is paramount in the sense that it includes most of common clinical investigations that are routinely done in a clinical laboratory. Hence an adequate and appropriate understanding of the accurate procedure of these investigations is very essential for a technician. Lack of automation, decreased sensitivity in microscopy and less specific chemical reactions in the absence of enzyme chemistry are some of the inherent limitations of clinical pathology analysis. Even though the advances in fibre-optic technique enables a pinpoint observation of Lower respiratory tract and gastro intestinal tract , the basic analysis of sputum and stool samples still remains in mainstay and so the case of other samples. Easy availability of samples, rapid results, and reasonable precision justifies the need of a clinical pathology lab in a hospital and in the curriculum too. Reporting of positive abnormal finding of the clinical pathology results are important equally to knowledge about the absence of abnormalities for correct diagnosis of a disease.

The analytical tests discussed here are mostly manual types which utilize principles of basic chemical reaction and primarily focuses on physical examination of fluids, microscopy, and simple chemical screening.

## Unit Grid: Unit 3.2 Clinical pathology

IDEAS/ CONCEPTS	LEARNING OUTCOME	SUGGESTED ACTIVITY	ASSESSMENT
<p><b>3.2.1 Introduction</b></p> <p>Importance, Common specimens,</p> <p>General guidelines for sample collection</p> <p>Skills</p> <ul style="list-style-type: none"> <li>-Discussing</li> <li>-Listing</li> <li>-Differentiating</li> </ul>	<p>To identify different specimens and describe the general guidelines for sample collection</p>	<p>Discussion</p>	<p>Participation in the discussion</p>
<p><b>3.2.2 Urine Analysis</b></p> <ul style="list-style-type: none"> <li>- Importance, Types of urine samples</li> </ul> <p>Methods of collection, preservatives</p> <ul style="list-style-type: none"> <li>- Physical Examination</li> <li>- Chemical Examination-Glucose, Protein, Blood, Ketone bodies, Bile pigments, Bile salts, Urobilinogen</li> <li>- Microscopic Examination</li> <li>- HCG test in Urine</li> </ul> <p>Skills</p> <ul style="list-style-type: none"> <li>-Observing</li> <li>-Experimenting</li> <li>-Identifying</li> <li>-Interpreting</li> </ul>	<p>To discuss the importance of urine analysis.</p> <p>To identify different types of urine samples and method of collection</p> <p>To perform physical, chemical and microscopical examination of urine</p> <p>To perform urine pregnancy test</p>	<p>Review of previous knowledge</p> <p>Discussion</p> <p>Discussion</p> <p>Assignment</p> <p>Demonstration</p> <p>Practical</p> <p>Chart preparation</p> <p>Demonstration</p> <p>Practical</p>	<p>Participation</p> <p>Quality of chart</p> <p>Performance</p> <p>Performance</p>
<p><b>3.2.3 Sputum Examination</b></p>			

<p>Importance, Specimen collection</p> <p>Physical examination</p> <p>Microscopic examination</p> <p>Skills</p> <p>-Observing</p> <p>-Describing</p>	<p>To identify the importance of sputum analysis</p>	<p>Discussion</p> <p>Assignment</p> <p>Multimedia</p> <p>Lab visit</p>	<p>Participation in the discussion</p>
<p><b>3.2.4 Stool Examination</b></p> <p>Importance, Specimen collection</p> <p>Physical examination</p> <p>Chemical Examination-</p> <p>Occult blood, reducing substance</p> <p>Microscopic examination-saline &amp; iodine prepn.</p> <p>Skills</p> <p>-Observing</p> <p>-Describing</p>	<p>To identify the importance of stool examination</p>	<p>Discussion</p> <p>Assignment</p> <p>Multimedia</p> <p>Lab visit</p>	<p>Participation in the discussion</p>
<p><b>3.2.5 Semen Analysis</b></p> <p>Importance, Specimen Collection</p> <p>Physical Examination, Liquefaction Time,</p> <p>Microscopy- Total Sperm Count, Motility, Morphology</p> <p>Chemical Examination- Fructose, Acid phosphatase</p> <p>Skills</p> <p>-Observing</p> <p>-Discussing</p> <p>-Analyzing</p> <p>-Explaining</p>	<p>To identify the importance of semen analysis and describe the method of semen analysis</p>	<p>Discussion</p> <p>Assignment</p> <p>Demonstration</p>	<p>Participation in the discussion</p>

<p><b>3.2.6 CSF and other body fluids</b></p> <p>CSF- Specimen collection Physical &amp; Microscopic Examination</p> <p>Chemical Examination</p> <p>Other body fluids</p> <p>Recent advances in Clinical pathology- Urine analyzer, Flow cytometry</p> <p>Skills</p> <p>-Observing - Explaining -Interpreting -Differentiating</p>	<p>To identify the importance of C.S.F and describe the analysis of CSF and other body fluids.</p> <p>To mention about the recent advances in clinical pathology</p>	<p>Discussion</p> <p>Multimedia presentation</p> <p>Reference</p> <p>Multimedia</p> <p>Lab visit</p>	<p>Participation in the discussion</p> <p>Participation</p>
--	--	--	---

### 3.2.2 Urine Analysis



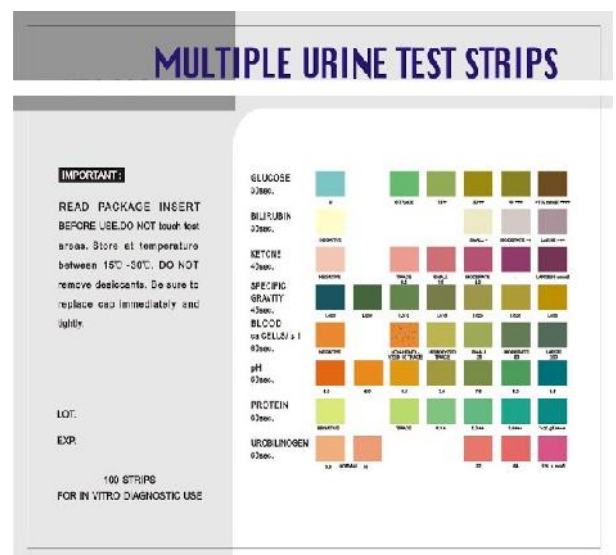
#### Additional Information

#### Reagent-strip Testing

A plastic strip is used, which contains pads that have incorporated within them the reagents for chemical reactions for the detection of a number of urine constituents. Urine is added to the pads for reaction by dipping the plastic strip into the urine and then slowly withdrawing it. The subsequent colorimetric reactions are timed to an endpoint; the extent of colors formation is directly related to the level of the urine constituent. The colors can be read manually by comparison with color charts or with the use of automated reflectance meters. Multi test strips and strips for individual detection are available.

The following are four general rules to be followed when performing urine reagent strip testing.

1. Test urine promptly; use properly timed test readings only.
2. Beware of interfering substances.



3. Understand the advantages and limitations of the test.

Manufacturers' information on sources of inhibitors and false-positive and false-negative results can be identified from the package inserts of the test strips. For example, ascorbic acid in urine can interfere with reagent-strip reactions for glucose, hemoglobin, bilirubin, and nitrite. Some reagent test strips have an additional test reaction that measures the levels of urinary ascorbic acid, and serve as a reminder of the possibility of interference from this source.

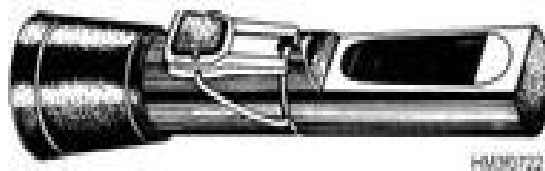
### Urine Analyser

A urine analyser is a device used in the clinical laboratory to perform urine analysis automatically. The unit can detect and quantify a number of analytes including bilirubin, protein, glucose, red blood cells etc. using urine strip readers. The instrument works on the principle of Reflectance photometry and can process several hundred strips per hour. Different types of urine analysers are available



Urine Analyser

### Refractometer



Refractometers work on the principle that light passing from a transparent medium of one density to a medium of another density, will change its velocity and therefore the direction in which the beam of light is moving. This change in direction, or the bending, of light is called refraction. The refractivity of a solution is dependent, in great part, on the total mass of solids dissolved in that solution. The refractive index scale can be calibrated to measure the specific gravity of urine sample

### Specific gravity corrections

**The correction of urine specific gravity for temperature is to add 0.001 for every raise in 3<sup>0</sup>c**

The *correction* of urine *specific gravity* for *protein* is to subtract 0.003 for each 1 g/dL of *protein* from the *temperature-corrected specific gravity*

**The correction of urine specific gravity for glucose is to subtract 0.004 for every raise in 1 gm/dl of glucose**

Hypersthenuria—The excretion of urine with an extremely high specific gravity. This can be due to severe restriction of water.

Hyposthenuria—Urine with a consistently low specific gravity (<1.010). This indicates a concentration problem.

Isosthenuria—A urine with a fixed low specific gravity that is approximately 1.010 and varies very little from specimen to specimen. This indicates poor tubular reabsorption.

**Bence Jones proteins** are light chain globulins seen in multiple myeloma condition, macroglobulinemias, lymphoma. These proteins have unusual property of precipitating at 40<sup>o</sup> -60<sup>o</sup>c & then dissolving when the urine is brought to boiling(100<sup>o</sup>c) & reappears when the urine is cooled.

**Microalbuminuria** is a term to describe a moderate increase in the level of urine albumin. It occurs when the kidney leaks small amounts of albumin into the urine, in other words, when there is an abnormally high permeability for albumin in the glomerulus of the kidney. Microalbuminuria is an important adverse predictor of glycemic outcomes in pre-diabetes.

Microalbuminuria is defined as levels of albumin ranging from 30 to 300 mg in a 24-hr urine

**Lactosuria:** Excretion of lactose (milk sugar) in the urine; a common finding during pregnancy and lactation, and in newborns, especially premature babies

### Assessment activity

The learner is instructed to perform the urine analysis with the given sample and record the results in practical log.

### 3.2.3 Sputum examination.



#### Additional information

Collection of sputum - Early morning deep cough sample is preferred. If unable to cough, induction of sputum can be done by a. 15% NaCl aerosol spray & propylene glycol for 20 min or b. Nebulized hypertonic saline and distilled water.

Microscopic examination of unstained sputum slide prepared by wet cover slip preparation or by concentration technique

Elastic fibres, Cruschmann's spiral, Charcot Leyden crystals, pigmented cells, bronchial casts, sulphur granules and pus cells are demonstrated

Microscopic examination of sputum for detection of parasites- Hydatid cyst of the lung, Entamoeba histolytica, Strngyloides stercoralis etc

For Concentration of sputum sample 10 % sodium hydroxide is commonly employed

### Assessment activity:

An assignment given on importance of sputum examination in Tuberculosis

### 3.2.4 Stool Analysis



### **Assessment activity:**

An assignment given on how microscopic examination of stool is performed

## **3.3.5 Semen Analysis**



### **Additional Information**

If semen is not liquefied, even after 60 minutes an additional treatment such as mechanical mixing or enzymatic digestion may be done for semen analysis.

### **Assessment Activity**

- Prepare a flow chart showing different procedures in semen analysis
- Prepare a chart showing precautions taken during collection of semen

## **3.2.6 CSF And Other Body Fluids**

### **CSF Examination**

#### **Assessment Activities**

Assignment on the importance CSF sample and its examination

#### **List of items in portfolio**

- Assignment report on importance of urine analysis
- Report in the practical log with regards to Physical, chemical and microscopic examination of urine
- Report in the practical log and pregnancy card with regards to Urine pregnancy test
- Assignment report on importance of sputum examination in tuberculosis.
- Assignment report on how Microscopic examination of stool is performed
- Chart showing precautions taken during collection of semen
- flow chart showing different procedures in semen analysis
- Assignment report on the importance CSF sample and its examination

## **Unit 3.3 Clinical Biochemistry**

Clinical Biochemistry is one of the most rapidly advancing areas of a clinical laboratory which deals with various biochemical parameters of the body. The marked increase in the number and availability of biochemical determinations has evolved the advancement of Laboratory medicine to a highly sophisticated molecular level. Advances in technique, practicing standards, and interpretation in this field have made the area, most multifaceted and complex. This unit of Clinical Biochemistry will make the learner familiar with

the basic biochemical analytical procedures as well as to get aware of the recent trends in clinical biochemistry.

This unit kept the importance emphasizing the application of clinical biochemistry to medicine. This unit gives the basic theoretical and practical information's in clinical biochemistry which are used for the diagnosis and treatment of diseases. In a clinical laboratory most of the investigations which the physicians rely are from clinical biochemistry. Every disease has a biochemical origin, which may alter the biochemical parameters. These parameters are estimated from the body fluids by processing different specimens. Initially learner should know the basic requirements for the biochemical investigations. Different biological specimens, their collection and processing for biochemical estimations. Brief knowledge of chemical, preparation of solutions and different types of assays. The knowledge of common instruments and the working procedures. The module suggests students activities a step by step guide to perform few of the biochemical estimation procedures to practice the procedures thought in the lessons. The module covers the routine biochemical investigations like blood sugar, renal function tests, Liver function tests, lipid profile and relevance of other clinical biochemistry estimations.

### Unit Grid: Unit 3.3 Clinical Biochemistry

IDEAS/ CONCEPTS	LEARNING OUTCOME	SUGGESTED ACTIVITY	ASSESMENT
<p><b>3.3.1 Introduction to Biochemistry</b></p> <p>-Introduction</p> <p>-Types of chemicals and preparation of solutions.</p> <p>-Types of Solutions-Percentage, Molar, Normal, Standard and Saturated solutions</p> <p>-Types of assays- Endpoint, Kinetic and fixed time assays.</p> <p>Collection and processing of specimens for biochemical analysis</p> <p>-Cleaning of glass wares for biochemical analysis</p> <p>Skills</p> <p>-Observing -Defining -Classifying</p>	<p>To define Clinical biochemistry, grade various chemicals and preparation of solutions</p> <p>To differentiate different types of assays used in biochemistry.</p> <p>To identify different types of specimens, their collection and processing for biochemical analysis.</p> <p>To perform cleaning of glass wares</p>	<p>Discussion</p> <p>Demonstration</p> <p>Discussion</p> <p>Reference</p> <p>Discussion</p> <p>Demonstration</p> <p>Practical work</p>	<p>Participation in the discussion</p> <p>Participation in the discussion</p> <p>Participation in the discussion</p> <p>Performance in practical</p>

<p><b>3.3.2 Instruments used in Biochemistry</b></p> <p>Familiarize with Colorimeter, Spectrophotometer, Flame photometer, Centrifuge, Electronic balance, Distillation apparatus, Deionizer</p> <p>Skills</p> <ul style="list-style-type: none"> <li>-Observing</li> <li>-Explaining</li> <li>-Performing</li> </ul>	<p>To explain the parts, working, use and to operate common instruments used in biochemistry</p>	<p>Review of previous knowledge</p> <p>Demonstration</p> <p>Practical work</p>	<p>Participation</p> <p>Performance</p>
<p><b>3.3.3 Blood Glucose Estimation</b></p> <p>-Introduction to Diabetes features, types, complications, Types of samples- FBS, PPBS,RBS</p> <p>anticoagulant used</p> <p>-Methods of estimation- GOD-POD in detail</p> <p>Normal value and Clinical Significance - Hyper and hypoglycaemia</p> <p>- Mention other common methods,</p> <p>- Glucometer Technique</p> <p>- GTT and GCT procedures, HBA1C</p> <p>Skills</p> <ul style="list-style-type: none"> <li>-Observation</li> <li>-Experimenting</li> <li>-Recording</li> <li>-Explaining</li> <li>-</li> </ul>	<p>To explain Diabetes and to differentiate various blood samples used for blood sugar estimation</p> <p>To identify different methods of blood sugar estimation and to estimate blood glucose by GOD POD method</p> <p>To explain GTT, GCT procedures, Glucometer technique and importance of HBA1c</p>	<p>Discussion</p> <p>Seminar</p> <p>Practical work</p> <p>Discussion</p> <p>Reference</p>	<p>Participation</p> <p>Evaluation of seminar report</p> <p>Performance</p> <p>Participation in the discussion</p>
<p><b>3.3.4 Renal Function Tests</b></p> <p>-Introduction, Common tests included</p> <ul style="list-style-type: none"> <li>• Estimation of Blood Urea</li> </ul> <p>Mention common methods Urea-Berthelot method in detail,</p>	<p>To explain the relevance of renal function test and to identify various tests included in the RFT panel</p> <p>To identify various blood</p>	<p>Recall previous knowledge</p> <p>Assignment</p>	

<p>Normal value and Clinical significance Renal, Pre-renal, Post renal conditions of Uraemia</p> <ul style="list-style-type: none"> <li>• Estimation of S. Creatinine.</li> </ul> <p>Mention common methods. Jaffe's method in details,</p> <p>Normal value and Clinical significance</p> <ul style="list-style-type: none"> <li>• Estimation of Uric Acid. Mention common methods.</li> </ul> <p>Uricase method in detail.</p> <p>Normal value and Clinical Significance.</p> <p>-Clearance tests- Urea and Creatinine</p> <p>-Importance of Micro-albumin and Cystatin- C</p> <p><b>Skills</b></p> <ul style="list-style-type: none"> <li>-Observation</li> <li>-Comparing</li> <li>-Experimenting</li> </ul>	<p>urea estimation methods and to estimate blood urea by Berthlot method</p> <p>To identify common creatinine estimation methods and to estimate s.creatinine by Jaffe method</p> <p>To discuss the importance of uric acid and estimation of uric acid by uricase method</p> <p>To identify the importance of microalbumin, Cystatin-C and clearance tests for the evaluation of renal function</p>	<p>Demonstration Practical</p> <p>Demonstration Practical</p> <p>Demonstration Practical</p> <p>Discussion Reference Lab visit</p>	<p>Performance</p> <p>Performance</p> <p>Participation in the discussion</p>
<p><b>3.3.5 Liver Function Tests</b></p> <ul style="list-style-type: none"> <li>• -Introduction, common tests included</li> <li>• Bilirubin- Formation of Bilirubin, Types of Bilirubin-conjugated &amp; unconjugated</li> </ul> <p>Jaundice, -Types of Jaundice</p> <p>Estimation of Bilirubin.</p> <p>Malloy- Evelyn method in detail.</p> <p>Normal value and Clinical Significance</p> <ul style="list-style-type: none"> <li>• Estimation of Total protein-</li> </ul>	<p>To explain the relevance of Liver function test and to identify various tests included in the LFT panel</p> <p>To explain jaundice and to perform Estimation of Bilirubin by Malloy-Evelyn method</p> <p>To perform estimation of</p>	<p>Review of Previous knowledge Assignment</p> <p>Demonstration Practical</p>	<p>Perfection in practical</p> <p>Perfection in</p>

<p>Biuret method in details</p> <ul style="list-style-type: none"> <li>• Estimation of Albumin- BCG method in details</li> </ul> <p>Normal value and clinical significance of total protein and Albumin, A-G Ratio.</p> <p>Other LFT Parameters- ALP, ALT, AST in brief.</p> <p>Skills -Comparing -Explaining -Experimenting -Judging</p>	<p>serum total protein by Biuret method</p> <p>To perform estimation of serum albumin by BCG method</p> <p>To identify the importance of ALT,AST &amp; ALP parameters in evaluation of Liver function</p>	<p>Demonstration</p> <p>Practical</p> <p>Discussion</p> <p>Practical</p> <p>Reference</p> <p>Lab visit</p>	<p>practical</p> <p>Participation in the discussion</p> <p>Perfection in practical</p>
<p><b>3.3.6 Lipid Profile</b></p> <ul style="list-style-type: none"> <li>• Introduction – Relevance, tests included in the Profile</li> <li>• Estimation of Cholesterol.</li> </ul> <p>Mention common methods,</p> <p>CHOD-PAP method in detail,</p> <p>Normal value and Clinical Significance</p> <p>Mention Triglycerides, HDL, LDL , Friedwald's equation</p> <p>Skills Observing Experimenting Interpreting Judging</p>	<p>To explain the importance of Lipid profile and to identify various tests included in the Lipid profile</p> <p>To perform estimation of S.Cholesterol by CHOD-PAP method</p>	<p>Discussion</p> <p>Assignment</p> <p>Demonstration</p> <p>Practical</p>	<p>Participation in the discussion</p> <p>Perfection in practical</p>
<p><b>3.3.7 Other parameters of Diagnostic importance</b></p> <ul style="list-style-type: none"> <li>➤ Serum Electrolytes- Serum Sodium and Potassium</li> </ul> <p>Normal value and Clinical significance</p> <ul style="list-style-type: none"> <li>➤ Calcium and Phosphorus normal value and significance</li> <li>➤ Diagnostically important</li> </ul>	<p>To identify the importance of Electrolytes</p> <p>To identify the Clinical significance of S.Calcium &amp; Phosphorous estimations</p>	<p>Discussion</p> <p>Lab visit</p> <p>Discussion</p> <p>Lab visit</p>	<p>Participation in the discussion &amp; Lab visit</p>

<p>Hormones</p> <p>TFT-Thyroid Hormones- T3, T4, TSH</p> <p>Infertility Hormones - FSH, LH, Prolactin, progesterone</p> <p>- Clinical enzymology- Acid Phosphatase, S. Amylase,</p> <p>- Cardiac markers- Troponin-I, Troponin-T</p> <p>- Cardiac enzymes- CPK, CK-MB, LDH, SGOT</p> <p>- Tumour Markers- CA-125, CEA, AFP,CA-19-9, PSA, Beta HCG</p> <p>Skills</p> <p>-Observing</p> <p>-Participating</p> <p>-Charting</p> <p>-Predicting</p>	<p>To identify the clinical significance of various special biochemical tests</p>	<p>Panel discussion</p> <p>Lab visit</p> <p>Chart preparation</p> <p>Reference</p>	<p>Quality of chart</p>
<p><b>3.3.8 Quality control in Biochemistry</b></p> <p>- Introduction,</p> <p>Common terms used in Quality control,</p> <p>Errors – random and systemic , L.J. Chart,</p> <p>External QC and Internal QC</p> <p>Skills</p> <p>-Observing</p> <p>-Analyzing</p> <p>-Tabulating</p> <p>-Judging</p>	<p>To identify the importance of quality control in biochemistry and able to explain various terms used in quality control</p>	<p>Discussion</p> <p>Assignment</p> <p>Reference</p>	<p>Participation in the discussion</p>
<p><b>3.3.9 Automation and Recent advances</b></p> <p>- Need for Automation,</p> <p>Advantages of Automation</p> <p>Types of Auto Analysers-Semi and Fully automated</p>	<p>To identify the need, advantages and recent advances of automation in clinical laboratory</p>	<p>Debate</p> <p>Multimedia presentation</p>	<p>Participation in the debate</p>

- Electrolyte Analyser (ISE) - Advanced Diagnostic Methods in brief Turbidometry, Nephelometry, C.L.I.A., H.P.L.C., C.L.F.A., Dry Chemistry-POCT  Skills -Observing -Differentiating -Participating -Listing	To mention advanced diagnostic methods	Field visit  Reference	
---	--	------------------------------	--

## Unit 3.3.1 Introduction to biochemistry

### Additional Information

#### Different methods of assay employed in clinical chemistry

##### 1. End Point Method:

This method is employed for the estimation of analytes, which would be completely consumed in the reaction. The end point for a particular analyte is normally achieved within 5 to 15 minutes at 37° C. The coloured complex/ non coloured complex thus formed at the end of reaction period is read for its absorbance using a spectro-photometer or Colorimeter.

In the end point methods the absorbance, at a specific wavelength, of the complex being formed is measured. This absorbance increases against the reagent blank absorbance, over a period of time. This increase in absorbance continues till it reaches a stable value, marking the end point of the reaction being reached. No further change in absorbance would occur, even if the conversion of the analyte being measured.

##### 2. Kinetic Method

The reagents employing this method are based on the principle of measurement of the difference in absorbance between two points over a period of specified time during the progress of the reaction. The assumption is that a constant amount of product is produced or Coenzyme utilized (NADH/NADPH) during the time interval being monitored. Usually, the reaction time is short to avoid any danger of enzyme degradation.

These tests are performed with a pre incubation, during which any substances in the sample, which might interfere with the test would have fully reacted with reagent system, so as not to interfere with the actual assay.

In kinetic test procedures, the difference in absorbance between two points, taken during the linear stage of progression of the test is taken into consideration, to yield the absorbance. This time interval could be typically like 1 minute or 20 seconds. The absorbance obtained would be multiplied by an appropriate factor for calculations, to give the quantity of the analyte being tested in the sample. The absorbance, that are consistent over a period of time are taken for calculation. These tests are standardized to give a linear absorbance over a period of time and up to the specific linearity mentioned for each analyte.

Kinetic Chemistries are classified as:

- Increasing Type:** In this type, the reaction proceeds in positive direction, where in, the initial absorbance is always lower than the latter absorbance.
- Decreasing Type:** This type of chemistry is also called as the negative direction type. In this method, the

difference between a latter point of absorbance and an initial point, over a specified period of time is always negative.

### Assessment activities

- Few used glass ware given to the learners and ask to demonstrate the cleaning procedure

## 3.3.2 Instruments used in Biochemistry

### 🔍 Additional Information

**Flame photometry** relies upon the fact that the compounds of the alkali and alkaline metals can be thermally dissociated in a flame and that some of the atoms produced will be further excited to a higher energy level. When these atoms return to the ground state they emit radiation which lies mainly in the visible region of the spectrum. Each element will emit radiation at a wavelength specific for that element.

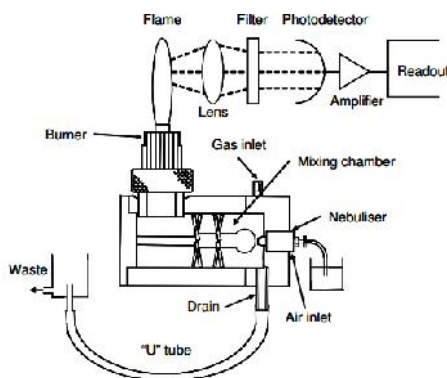
Sodium (Na) 589 Yellow

Potassium (K) 766 Violet

Over certain ranges of concentration the intensity of the emission is directly proportional to the number of atoms returning to the ground state. This is in turn proportional to the absolute quantity of the species volatilized in the flame, i.e. light emitted is proportional to sample concentration. It can be seen that if the light emitted by the element at the characteristic wavelength is isolated by an optical filter and the intensity of that light measured by a photo-detector, then an electrical signal can be obtained proportional to sample concentration. Such an electrical signal can be processed and the readout obtained in an analogue or digital form.

A simple flame photometer consists of the following basic components:

- The burner:** a flame that can be maintained in a constant form and at a constant temperature.
- Nebuliser and mixing chamber:** a means of transporting a homogeneous solution into the flame at a steady rate.
- Simple colour filters:** a means of isolating light of the wavelength to be measured from that of extraneous emissions.
- Photo-detector:** a means of measuring the intensity of radiation emitted by the flame.

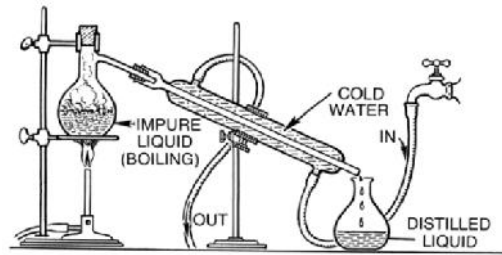


Schematic diagram showing the parts of a Flame photometer

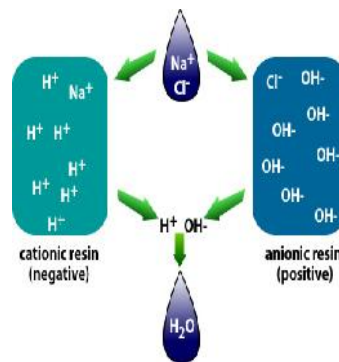
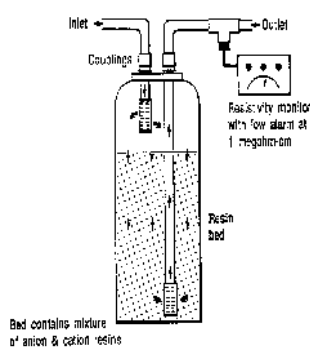
**Distillation** is a common operation in many laboratories for the purpose of obtaining purified water for the analysis. The apparatus used consists of three major parts distillation flask to heat the



mixture and volatilize the components, a condenser to cool the vapours back to liquid state, and a collection vessel.



**Deionized water** (DI water, DIW or de-ionized water), is water that has had almost all of its mineral ions removed, such as cations like sodium, calcium, iron, and copper and anions such as chloride and sulfate. Deionization is a chemical process that uses specially manufactured ion-exchange resins, which exchange hydrogen and hydroxide ions for dissolved minerals, and then recombine to form water. Because most non-particulate water impurities are dissolved salts, deionization produces a high purity water that is generally similar to distilled water, and this process is quick and without scale buildup. However, deionization does not significantly remove uncharged organic molecules, viruses or bacteria, except by incidental trapping in the resin.



### 3.3.3 Blood Glucose Estimation

#### Additional Information

##### ➤ Metabolism

Thousands of chemical reactions are taking place inside the cell, all these are collectively called metabolism. Metabolism provides adequate energy for life process and the synthesis of new materials. Metabolism allows the body to grow and function. There are two phases in metabolism- Catabolism and Anabolism

Catabolism - Energy rich complex macro molecule are degrade into smaller molecules and energy is released usually as: ATP.

Anabolism- The cells synthesize complex molecules from simple precursors by the expense of ATP



BLOOD SUGAR LEVEL	
Type of Specimen	Normal Range
Fasting Sample (FBS)	70-110 mg%
Post Prandial (PPBS)	80-140 mg%
Random sample (RBS)	80-120mg%

- Haemoglobin A1C Level(HbA1C) indicates increased risk of diabetes and levels of 6.5% or higher indicates Diabetes
- Estimation of **C-Peptide**, a new diagnostic method confirms the Type II Diabetes.

**Assessment activities:**

Three different serum samples provided to the students and direct them to do the blood sugar estimation by GOD POD Method, record the result in the practical log.

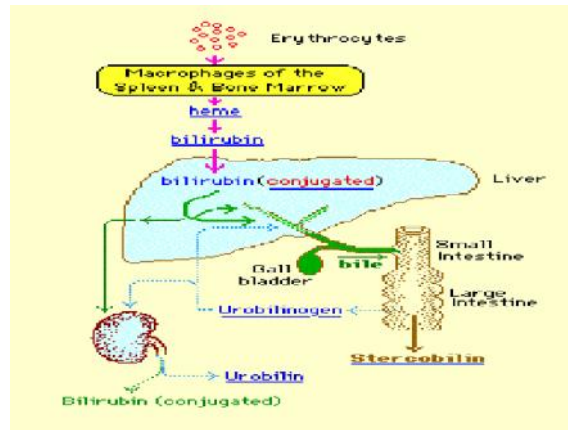
**3.3.4: Renal function tests**

**Assessment activities**

Determine S. Creatinine concentration in the provided serum sample using the Jaffe’s method.

**3.3.5 Liver function tests**

**Additional Information**



***Bilirubin Catabolism illustration***

**Differentiation of Three types of Jaundice**

	Pre Hepatic Jaundice	Hepatic Jaundice	Post Hepatic Jaundice
Causes	Due to Excessive haemolysis	Diseases of Liver cells viz. Viral hepatitis, Toxic hepatitis	Due to Obstruction of biliary passage
S.Bilirubin	Indirect- increased	Total & Direct Increased	Direct Increased
ALT	Usually normal	Marked Increase(+++)	Increased (++)
ALP	Normal	Slight Increase(+)	Marked Increase (++ or +++)

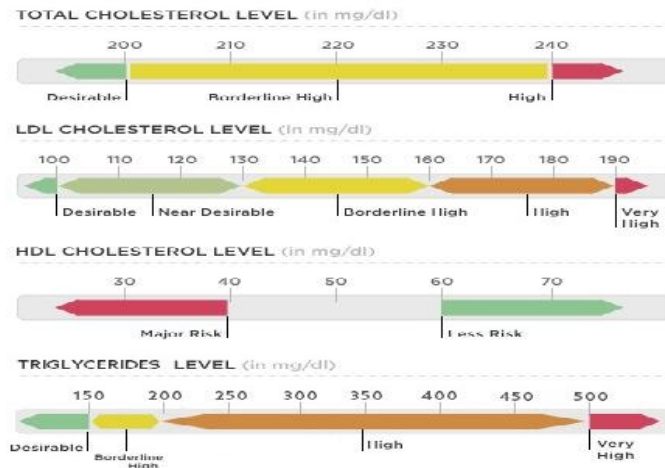
**Assessment activities:**

Ask the students to perform S. bilirubin concentration of serum sample given by Malloy Evelyn method and report.

### 3.3.6 Lipid Profile



#### Additional Information



**Limitation of Friedewald equation:** This method of indirect LDL-c estimation becomes unreliable when the triglyceride (TG) levels are high.

#### 'Bad' Cholesterol

Low-density lipoprotein (LDL) cholesterol, often referred to as "bad" cholesterol, is the type that tends to deposit on the walls of the arteries. White blood cells combine with the LDL cholesterol, forming artery-narrowing plaque, which restricts blood flow. The optimal level of LDL cholesterol for most people is 100 mg/dL or lower.

#### 'Good' Cholesterol

Not all cholesterol is bad. High-density lipoprotein (HDL) cholesterol is considered "good" cholesterol because it actually works to keep the LDL, or "bad" cholesterol from building up in arteries. HDL levels of 60 mg/dL and higher can help reduce risk for heart disease. Conversely, HDL levels of 40 mg/dL and lower are considered a high risk factor for developing heart disease.

**Atherosclerosis** is a disease in which plaque builds up inside the arteries. Plaque is made up of fat, cholesterol, calcium, and other substances found in the blood and hardens and narrows arteries. This limits the flow of oxygen-rich blood to organs such as heart, brain, arms, legs, pelvis, and kidneys and leads to serious problems, including heart attack, stroke, or even death. Coronary artery disease, occurs when plaque builds up in the coronary arteries. These arteries supply oxygen-rich blood to the heart. Plaque narrows the coronary arteries and reduces blood flow to the heart muscles leads to heart attack called myocardial infarction.

#### Assessment activities

Estimation of Serum Cholesterol by CHOD POD method.

### 3.3.7: Other parameters of Diagnostic importance

#### Additional Information

**Electrolyte panel** are usually ordered as part of a routine screening or as a diagnostic aid when a person has signs and symptoms, such as:

- Fluid accumulation (edema)
- Nausea or vomiting
- Weakness
- Confusion
- Irregular heart beat (cardiac arrhythmias)

It is frequently ordered as part of an evaluation when someone has a disease or condition or is taking a medication that can cause an electrolyte imbalance.

#### **ABG Analysis:**

Arterial blood gas (ABG) analysis test measures parameters such as pH,  $P_{O_2}$ ,  $P_{CO_2}$  &  $HCO_3^-$  in the blood for the Clinical management of acid-base imbalance, respiratory and metabolic disorders.

#### **Cardiac Markers and their Clinical Importance**

<b>Test</b>	<b>Approximate peak</b>	<b>Description</b>
Troponin test	12 hours	The most sensitive and specific test for myocardial damage Troponin is released during Myocardial infarction from cardiac muscles. Its subsequent release is prolonged with degradation of actin and myosin filaments. Troponins can also calculate infarct size but the peak must be measured in the 3rd day. After MI, troponin is released in 2–4 hours and persists for up to 7 days.
Creatine Kinase (CK-MB) test	10–24 hours	The CK-MB isoform of creatine kinase is expressed in heart muscle. Since it has a short duration, it cannot be used for late diagnosis of acute MI but can be used to suggest infarct extension if levels rise again. This is usually back to normal within 2–3 days
Lactate dehydrogenase(LDH)	72 hours	Lactate dehydrogenase catalyses the conversion of pyruvate to lactate. LDH-1 isozyme is normally found in the heart muscle and LDH-2 is found

		predominately in blood serum. A high LDH-1 level to LDH-2 suggest MI. LDH levels are also high in tissue breakdown or hemolysis. It can mean cancer, meningitis, encephalitis, or HIV. This is usually back to normal 10–14 days.
Aspartate transaminase(AST)	36 hours	This was one of the first used test. It is not specific for heart damage, and it is also one of the liver function tests
Myoglobin (Mb)	2 hours	Myoglobin is used less than the other markers. Myoglobin is the primary oxygen-carrying pigment of muscle tissue. It is high when muscle tissue is damaged but it lacks specificity. It has the advantage of responding very rapidly, rising and falling earlier than CK-MB or troponin.
Pro-brain natriuretic peptide		This is increased in patients with heart failure. It has been approved as a marker for acute congestive heart failure.

#### List of commonly used Tumour markers

<b>Tumour marker</b>	<b>Associated tumour types</b>
Alpha fetoprotein (AFP)	Hepatocellular carcinoma
CA19-9	Mainly pancreatic cancer, but also colorectal cancer and other types of gastrointestinal cancer
CA-125	Mainly ovarian cancer, but may also be elevated in endometrial cancer, fallopian tube cancer, lung cancer, breast cancer and gastrointestinal cancer.
Carcinoembryonic antigen(CEA)	gastrointestinal cancer, cervix cancer, lung cancer, ovarian cancer, breast cancer, urinary tract cancer
Human chorionic gonadotropin(hCG)	gestational trophoblastic disease, germ cell tumor, choriocarcinoma
prostate-specific antigen (PSA)	prostate Cancer

### Assessment activities:

Learners should prepare a chart showing various special biochemical tests and its clinical significance.

### 3.3.8 Quality control in biochemistry

#### Assessment activities

Make an assignment on importance of QC in Biochemistry and various terms used in quality control

### 3.3.9. Automation and Recent advances

#### Additional Information



Semiautoanalyser



Fully automatic analyser

#### Chromatography

Chromatography is a technique to separate mixtures of substances into their components on the basis of their molecular structure and molecular composition. This involves a stationary phase (a solid, or a liquid supported on a solid) and a mobile phase (a liquid or a gas). The mobile phase flows through the stationary phase and carries the components of the mixture with it. Sample components that display stronger interactions with the stationary phase will move more slowly through the column than components with weaker interactions. This difference in rates causes the separation of various components. Chromatographic separations can be carried out using a variety of stationary phases, including immobilized silica on glass plates (thin-layer chromatography), volatile gases (gas chromatography), paper (paper chromatography) and liquids (liquid chromatography)

Depending upon the phase system, HPLC is classified into

#### Normal Phase HPLC:

This method separates analytes on the basis of polarity. NP-HPLC uses polar stationary phase and non-polar mobile phase. Therefore, the stationary phase is usually silica and typical mobile phases are hexane, methylene chloride, chloroform, diethyl ether, and mixtures of these. Polar samples are thus retained on the polar surface of the column packing longer than less polar materials

### **Reverse Phase HPLC:**

The stationary phase is non polar (hydrophobic) in nature, while the mobile phase is a polar liquid, such as mixtures of water and methanol or acetonitrile. It works on the principle of hydrophobic interactions hence the more non polar the material is, the longer it will be retained

### **Size-exclusion HPLC:**

The column is filled with material having precisely controlled pore sizes, and the particles are separated according to their molecular size. Larger molecules are rapidly washed through the column; smaller molecules penetrate inside the porous of the packing particles and elute later

### **Ion-Exchange HPLC:**

The stationary phase has an ionically charged surface of opposite charge to the sample ions. This technique is used almost exclusively with ionic or ionizable samples. The stronger the charge on the sample, the stronger it will be attracted to the ionic surface and thus, the longer it will take to elute. The mobile phase is an aqueous buffer, where both pH and ionic strength are used to control elution time

### **List of Items in Portfolio**

- ❖ Practical log with regards to Glass ware cleaning method
- ❖ Seminar report on Diabetes.
- ❖ Practical log with regards to Blood Sugar estimation by GOD POD Method
- ❖ Practical log with regards to Blood Urea estimation by Berthlot Method
- ❖ Practical log with regards to S.Creatinine estimation by Jaffe's Method
- ❖ Practical log with regards to S.Uric acid estimation by Uricase Method
- ❖ Assignment on LFT
- ❖ Practical log with regards to S.bilirubin estimation by Malloy Evelyn Method
- ❖ Practical log with regards to S.Total Protein estimation by Biuret Method
- ❖ Practical log with regards to S.Albumin estimation by BCG Method
- ❖ Assignment on Lipid profile
- ❖ Practical log with regards to S.Cholesterol estimation by Oxidase Method
- ❖ Prepared Chart on special biochemical tests
- ❖ Assignment on QC in Biochemistry.

### **Extended activities**

- ✓ Organize a Visit to observe the design, layout, safety measures & Various procedures in a Clinical Laboratory
- ✓ To conduct Medical camp on diabetes detection
- ✓ Exhibition/Expo
- ✓ Prepare a Model of Ideal Laboratory
- ✓ Plan a visit to a Routine Medical Laboratory to observe various diagnostic tests in clinical pathology & Clinical biochemistry
- ✓ Organize a visit to speciality laboratory to observe various autoanalysers



# Module 4

## Diagnostic Microbiology & Histotechnology

### Overview

Medical Laboratory Curriculum for VHSE course has been designed in such a way that the programme concludes with diagnostic microbiology and histotechnology as the 4<sup>th</sup> module.

Laboratory diagnosis of infectious diseases with various conventional as well as modern technologies are the main stay of Diagnostic Microbiology procedures and protocols. Culture methods are still considered as the gold standard of diagnosis. Here an attempt has been made to introduce the novel and emerging immunological and molecular techniques and their applications.

Despite the advances in nanotechnology and nucleic acid level diagnosis, morphological characters of cells still remain as a preferred way of diagnosis in histological as well as cytological abnormalities. Information provided by a stained slide of tissue section have immense value in the present diagnosis of diseases especially that are of malignant origin. Different manual techniques that help in the preparation of specimens for histopathological and cytological studies are discussed here to introduce this special area of diagnostic laboratory to the students which may inspire them to pursue higher education in this field.

### Unit 4.1 Diagnostic Microbiology -Over view

Microbiology is an Oceanic subject having numerous branches which deals with all the microbial life in the universe. Medical microbiology is the part which touches the health and diseases of man. Diagnostic microbiology explains the various laboratory procedures involved in the process of diagnosis. It is quite cumbersome to discuss all these aspects within a short span of time .In spite of this all the fundamental scientific characters of clinically important microorganisms at least in the diagnostic angle have been discussed here. These include bacteria, protozoa and even viruses that encounter during the laboratory practice. Importance has been given for clinical and laboratory protocols that start from optimal specimen collection which eventually reaches up to an accurate diagnosis. In addition to the routine techniques employed to detect commonest pathogens, recent aspects that attribute an early diagnosis and immunological techniques with their application as well as interpretations, areas like sterilization and disinfection are also discussed in this unit without losing the necessary relevance

**Unit Grid:**

IDEAS/ CONCEPTS	LEARNING OUTCOME	SUGGESTED ACTIVITY	ASSESSMENT
<p><b>4.1.1 Introduction to Medical Microbiology</b>            Different branches            -Microbes, classification, pathogen, Commensals, type of Infections, Communicable diseases, Historical aspects</p> <p>Skills            -Observing            - Differentiating            - Charting</p>	<p>To Define Microbiology, to differentiate Pathogen, Commensals and type of infection            To summarize various historical aspects of microbiology</p>	<p>Discussion            Poster &amp; Album presentation</p>	<p>Participation in discussion            Quality evaluation of poster &amp; album</p>
<p><b>4.1.2 Structure and classification of bacteria</b></p> <ul style="list-style-type: none"> <li>• Structure</li> <li>• Classification- based on morphology- arrangement</li> <li>• motility, oxygen requirement</li> </ul> <p>Skill            -Explaining            -Observing            -Differentiating</p>	<p>Able to explain the structure of bacteria</p> <p>To differentiate bacteria based on morphology, Motility &amp; Oxygen requirement</p>	<p>Observation Chart            Multimedia presentation</p>	<p>Quality of Chart Notes</p>
<p><b>4.1.3 Sterilization and disinfection</b></p> <ul style="list-style-type: none"> <li>• Importance of sterilization and Disinfection</li> <li>• Methods of sterilization - mention with examples</li> <li>• Describe principle, parts, and use of               <ul style="list-style-type: none"> <li>- Hot air Oven,</li> <li>- Autoclave</li> </ul> </li> <li>• Disinfectants and Antiseptics and their applications</li> </ul> <p>Skills            -Observing            -Comparing            -Experimenting</p>	<p>To identify the importance of sterilization</p> <p>To categorize different methods of sterilization</p> <p>To operate Hot air oven, Autoclave</p> <p>To distinguish Disinfectants and Antiseptics and their applications</p>	<p>Discussion</p> <p>Flow chart preparation</p> <p>Demonstration Practical</p> <p>Discussion Preparation of Notes</p>	<p>Participation in discussion</p> <p>Perfection of Flow chart</p> <p>Perfection in practical</p> <p>Participation in discussion</p>
<p><b>4.1.4 Growth &amp; Cultivation of Bacteria</b></p> <ul style="list-style-type: none"> <li>• Bacterial growth &amp; Replication               <ul style="list-style-type: none"> <li>- Mention essential growth</li> </ul> </li> </ul>	<p>To explain various</p>	<p>Discussion</p>	<p>Participation</p>

<p>requirements- pH, Temperature. Gaseous requirements</p> <ul style="list-style-type: none"> <li>• Culture media <ul style="list-style-type: none"> <li>- General idea and Use,</li> <li>- Classification of culture media with examples</li> <li>- Preparation and use of common media</li> </ul> </li> </ul> <p>Peptone water, NA, BA, CA, MA</p> <ul style="list-style-type: none"> <li>• Culture methods <ul style="list-style-type: none"> <li>- Bacteriological wire loop, Straight wire</li> <li>- Inoculation techniques</li> <li>- Mention Streak,stroke,stab, carpet, liquid culture</li> </ul> </li> </ul> <p>-</p> <ul style="list-style-type: none"> <li>• Mention Anaerobic techniques- Gaspak</li> </ul> <p>Skills</p> <ul style="list-style-type: none"> <li>-Observing</li> <li>-Explaining</li> <li>-Experimenting</li> <li>-Classifying</li> </ul>	<p>growth requirements of bacteria</p> <p>To classify different culture media and prepare Common culture media</p> <p>Able to explain the different inoculation techniques.</p> <p>Able to perform streak culture technique</p>	<p>Flow chart preparation Demonstration Practical work</p> <p>Demonstration Multimedia presentation Practical work</p> <p>Multimedia presentation Field visit</p>	<p>in discussion</p> <p>Perfection of Flow chart</p> <p>Perfection in practical</p>
IDEAS/ CONCEPTS	LEARNING OUTCOME	SUGGESTED ACTIVITY	ASSESSMENT
<p><b>4.1.5 Basic identification techniques</b></p> <p>Identification of bacteria</p> <ul style="list-style-type: none"> <li>• Different methods</li> <li>• Detection of motility <ul style="list-style-type: none"> <li>- Name different methods</li> <li>- Hanging drop method in detail</li> </ul> </li> <li>• Staining <ul style="list-style-type: none"> <li>- Principle, requirement, procedure interpretation, diagnostic importance of Simple stain, Grams stain &amp;AFB stain-</li> </ul> </li> <li>• Mention Biochemical tests- Catalase, Oxidase, Coagulase, IMViC</li> </ul> <p>Skills</p> <ul style="list-style-type: none"> <li>-Observing</li> <li>-Explaining</li> <li>- Experimenting</li> </ul>	<p>Able to classify different methods for the identification of bacteria</p>	<p>Discussion Demonstration Multimedia presentation Practical work</p>	<p>Participation in discussion</p> <p>Perfection in practical</p>
<p><b>4.1.6 Immunology and its diagnostic applications</b></p> <ul style="list-style-type: none"> <li>• Introduction to Immunology &amp; Serology</li> </ul>	<p>Define Immunology and to explain different terms</p>	<p>Discussion Poster</p>	<p>Participation</p>

<p>-Types of Immunity, Antigen, Antibody</p> <p>-Structure of antibody, types of antibody</p> <ul style="list-style-type: none"> <li>• Antigen- Antibody reactions- -Mention terms-Specificity, Sensitivity, Avidity, Prozone, Post zone &amp; Titre - Mention types of Antigen- Antibody reactions and their clinical applications-Immunoassay -Agglutination, precipitation, flocculation, ELISA, Immuno fluorescence</li> </ul> <p>Skills</p> <ul style="list-style-type: none"> <li>-Observing</li> <li>-Analyzing</li> <li>-Explaining</li> <li>-Judging</li> </ul>	<p>in immunology</p> <p>Explain the structure of antibody and to classify different types of antibodies</p> <p>Differentiate various Antigen- Antibody reactions and their clinical applications</p>	<p>presentation</p> <p>Discussion Multimedia presentation</p> <p>Discussion Multimedia presentation Assignment</p>	<p>in discussion</p> <p>evaluation of poster</p> <p>Participation in discussion</p> <p>Participation in discussion Quality of assignment</p>
<p><b>4.1.7 Laboratory Diagnosis of Common Bacterial diseases</b></p> <ul style="list-style-type: none"> <li>• Collection and transportation of specimens- Urine, Blood, Sputum, CSF, Stool, Pus, body fluids and Swabs.</li> <li>• Specimen processing-General considerations-Macroscopy, Microscopy &amp; Culture - mention common culture media and identification methods used Antibiotic sensitivity test</li> <li>• Mention common bacterial diseases and pathogens encountered-- Typhoid, Tuberculosis, Cholera, Dysentery, Syphilis, Leptospirosis, Tetanus, Meningitis &amp; UTI</li> <li>• Common serological Diagnosis of Bacterial diseases Mention WIDAL, RPR, ASO, Leptospira card test, ELISA &amp; its commercial preparations- Immunochromatographic technique Use, procedure, interpretation of WIDAL &amp; RPR only</li> </ul> <p>Skills</p> <ul style="list-style-type: none"> <li>-Observing</li> <li>- presentation</li> <li>- Differentiating</li> <li>-Experimenting</li> </ul>	<p>To explain the Collection and transportation of different specimens</p> <p>To summarize the different methods in the specimen processing</p> <p>Able to explain the method of Antibiotic sensitivity test</p> <p>To differentiate common bacterial diseases and identify the pathogens encountered</p> <p>To differentiate and perform common serological tests</p>	<p>Discussion</p> <p>Discussion Multimedia presentation</p> <p>Demonstration</p> <p>Discussion Album preparation Seminar</p> <p>Multimedia presentation Demonstration Practical</p>	<p>Participation in discussion</p> <p>evaluation of album, Seminar report</p> <p>Perfection in Practical</p>

IDEAS/ CONCEPTS	LEARNING OUTCOME	SUGGESTED ACTIVITY	ASSESMENT
<p><b>4.1.8 Laboratory Diagnosis of Common viral diseases</b></p> <ul style="list-style-type: none"> <li>• Introduction to virology- Type of viruses ( RNA &amp; DNA)</li> <li>• Common viral diseases and pathogens encountered- AIDS, Hepatitis,Dengue, Chikungunia, Rabies, Influenza, Mumps and Measles</li> <li>• diagnostic tests for viral infections <ul style="list-style-type: none"> <li>- Mention Common serological tests (latex agglutination, Card tests and Elisa), Tissue culture, PCR technique.</li> </ul> </li> </ul> <p>Skills  -Observing  -Explaining  -Differentiating</p>	<p>To define virology, classify viruses</p> <p>To differentiate common viral diseases and the pathogen encountered</p> <p>To explain common diagnostic serological test for viral infections</p>	<p>Discussion</p> <p>Chart Preparation</p> <p>Demonstration Multimedia presentation Field visit</p>	<p>Quality of chart</p>
<p><b>4.1.9 Laboratory Diagnosis of Common Parasitic diseases</b></p> <ul style="list-style-type: none"> <li>• Introduction to parasitology -mention Parasite, host(Intermediate &amp; Definitive host),Vector, Zoonosis Classification-Intestinal &amp; Blood Parasites</li> <li>• <b>BloodParasites-</b> Common blood parasites and their lab diagnosis <ul style="list-style-type: none"> <li>-Blood Collection-Time of collection, preparation of smear (Thick &amp; Thin)</li> <li>Dehaemoglobinisation of thick smear</li> <li>Staining &amp; Examination.</li> </ul> </li> <li>• <b>Lab diagnosis of Malaria-</b> Disease, Mode of transmission, Host, Causative agent, Different species, Types of Malaria. <ul style="list-style-type: none"> <li>-Examination of Thick &amp; Thin smear</li> <li>Different stages of parasite</li> </ul> </li> </ul>	<p>To define parasitology and explain different terms in parasitology</p> <p>To differentiate intestinal and blood parasites</p> <p>To identify common blood parasites and explain their lab diagnosis</p> <p>To differentiate the Causative agent, different species, host and mode of transmission of malaria</p> <p>To name the different stages of parasite</p>	<p>Discussion Chart preparation</p> <p>Demonstration Multimedia presentation Practical work</p> <p>Demonstration Multimedia presentation Chart Preparation</p>	<p>Participation in discussion</p> <p>Perfection in practical</p>

<p>Other stains used-JSB Other methods-Card method, QBC</p> <ul style="list-style-type: none"> <li>• <b>Lab diagnosis of Filariasis-</b> - Disease, mode of transmission, host, nocturnal habit, -Lab diagnosis- wet smear examination, thick smear examination, Concentration technique.</li> <li>• <b>Lab diagnosis of Intestinal parasites</b> Introduction -Helminthic infections and parasites Amoebiasis -Entamoebahistolytica Disease, Mode of Transmission, Trophozoite&amp; Cyst Lab diagnosis -Macroscopic Microscopic - Stained &amp; Unstained preparation</li> <li>• Common Helminths- Tape worm, Round worm, Hook worm, Whip worm, Pin worm, - Lab diagnosis-Macroscopic Microscopic examination</li> <li>• Concentration Techniques of stool sample - Sedimentation &amp; Floatation methods</li> </ul> <p>Skills Observing Identifying Differentiating</p>	<p>To explain different methods for the diagnosis of malaria</p> <p>To differentiate the Disease, mode of transmission, host and nocturnal habit of filarial parasite</p> <p>To explain different methods for the lab diagnosis of filariasis</p> <p>To familiarize the disease, causative agent, mode of transmission and lab diagnosis of amoebiasis</p> <p>To differentiate common helminthes and explain their lab diagnosis</p> <p>To identify common intestinal parasites and the method of examination of stool by concentration technique</p>	<p>Demonstration Multimedia presentation</p> <p>Discussion Multimedia presentation</p> <p>Discussion Multimedia presentation</p> <p>Discussion Multimedia presentation Chart Preparation</p> <p>Discussion Multimedia presentation</p> <p>Demonstration</p>	<p>Participation in discussion</p> <p>Participation in discussion</p> <p>Quality of chart</p> <p>Participation in discussion</p>
--	---	---	--

### 4.1.1 Introduction to Medical Microbiology

#### Additional information

- Some unicellular protists are visible to the naked eye, and some multicellular species are microscopic.
- Microbes outnumber all other species and make up most of the living matter on the planet.
- In one single teaspoon of garden soil, there are over 100,000 microbes. In 1ltr of seawater, there are over 1billion microbes.

- Bacteria do many good things, such as decompose waste and give texture and flavour to food ,Cleaning up our waste, Killing pests,Making medicines,making of such things as leather, cheese and paper.
- **Factors of Virulence**
- Adhesion: The initial event in the pathogenesis of many infections is the attachment of the bacteria to body surfaces. This attachment is specific reaction between surface receptors and adhesive structures on the surface of bacteria (adhesins).
- Invasiveness is the ability of organism to spread in a host tissue after establishing infection. Less invasive organisms cause localized lesion. Highly invasive organisms cause generalized infection (septicemia).
- Toxigenicity. Bacteria produce two types of toxins – exotoxins & endotoxins
- A convalescent carrier is one who has recovered from disease but continues to harbor the pathogen in his body. Anthroponosis
- Zoonosis may be bacterial, (e.g. Plague from rat),rickettsial, (e.g. Murine typhus from rodent), viral, (e.g. Rabies from dog), protozoal, (e.g. Leishmaniasis from dogs), helminthic, (e.g. Hydatid cyst from dogs) and fungal (zoophilic dermatophytes from cats and dogs).

#### **4.1.2 STRUCTURE AND CLASSIFICATION OF BACTERIA**

##### **Additional information**

Classification of Bacteria is based on following characters

##### **Phenotypic classification**

- ❖ Morphological
- ❖ Anatomical
- ❖ Staining
- ❖ Cultural characteristics
- ❖ Nutrition
- ❖ Environmental factors
- ❖ Biochemical reactions
- ❖ Antigenic structure
- ❖ Phage typing
- ❖ Bacitracin typing

##### **Genotypic classification**

- ❖ DNA-DNA hybridization
- ❖ G+C content
- ❖ Actinomycetes are termed such because of their resemblance to sun rays when seen in tissue sections.
- ❖ Mycoplasma occur in round or oval bodies and in interlacing filaments. They are resulted from defective cell wall synthesis or by the action of a cell wall attacking antibiotic like penicillin
- ❖ Bacterial flagella is made up of protein called flagellin, possess H antigen, commonly used to detect immunologic response against which in enteric fever

- ❖ Motility of bacteria is an important criteria for differentiation and should be differentiated from Brownian movement
- ❖ Sporulation is not a method of reproduction and only one vegetative form arise from a single spore.
- ❖ Usually bacterial capsules are made up of polysaccharides while that of anthrax bacilli is of polypeptide (Protein) in composition

#### 4.1.3 .Sterilization and disinfection

##### Classification of disinfectants:

1. Based on consistency a. Liquid (E.g., Alcohols, Phenols) b. Gaseous (Formaldehyde vapor, Ethylene oxide)
2. Based on spectrum of activity a. High level b. Intermediate level c. Low level
3. Based on mechanism of action
  - a. Action on membrane (E.g., Alcohol, detergent)
  - b. Denaturation of cellular proteins (E.g., Alcohol, Phenol)
  - c. Oxidation of essential sulphhydryl groups of enzymes (E.g., H<sub>2</sub>O<sub>2</sub>, Halogens)
  - d. Alkylation of amino-, carboxyl- and hydroxyl group (E.g., Ethylene Oxide, Formaldehyde)
  - e. Damage to nucleic acids (Ethylene Oxide, Formaldehyde)

Methyl alcohol kills fungal spores, hence is useful in disinfecting inoculation hoods. Disadvantages: Skin irritant, volatile (evaporates rapidly), inflammable

##### *Assessment activities-1*

- ❖ Chart showing Endemic infections of our part of world can be prepared
- ❖ An infection cycle with various routes may be plotted
- ❖ An album showing pioneers in microbiology may be prepared
- ❖ A comparison chart showing the characters of different microorganisms can be prepared
- ❖ A role play of students may be planned with scientists as characters telling their observations from findings and difficulties faced
- ❖

##### *Assessment activities-2*

- ❖ Bacterial models can be prepared by using plaster of Paris or themocol to show the cell structure.
- ❖ Comparison chart may be prepared for prokaryotic and eukaryotic comparison
- ❖ Preparation of a model showing the arrangements of bacteria using Beads and plastic balls .
- ❖ Student groups may be assigned to collect the name of antibiotics that are commonly used
- ❖ A brainstorming session for motile bacteria without flagella

## Unit 4.1.4 BACTERIAL GROWTH

During bacterial growth The average time required for the population, or the biomass, to double is known as the generation time or doubling time.

During bacterial growth in liquid media, certain aerobic bacteria and those containing fimbriae (Vibrio & Bacillus) are known to grow as a thin film called 'surface pellicle' on the



surface of undisturbed broth. *Bacillus anthracis* is known to produce stalactite growth on ghee containing broth. Sometimes the initial turbidity may be followed by clearing due to autolysis, which is seen in pneumococci. Long chains of Streptococci when grown in liquid media tend to entangle and settle to the bottom forming granular deposits. Liquid media tend to be used when a large number of bacteria have to be grown. These are suitable to grow bacteria when the numbers in the inoculum is suspected to be low. Inoculating in the liquid medium also helps to dilute any inhibitors of bacterial growth. This is the practical approach in blood cultures. Culturing in liquid medium can be used to obtain viable count (dilution methods).

**Examples for growth factors are** Thiamine, nicotinic acid, folic acid and para-aminobenzoic acid etc:

## **Unit 4.1.8 Laboratory Diagnosis of Common viral diseases**

### **Additional informations**

#### **1. Direct Examination of Specimen**

1. Electron Microscopy morphology / immune electron microscopy
2. Light microscopy histological appearance - e.g. inclusion bodies
3. Antigen detection immunofluorescence, ELISA etc.
4. Molecular techniques for the direct detection of viral genomes (PCR)

#### **2. Indirect Examination**

1. Cell Culture - cytopathic effect, haemadsorption, confirmation by neutralization, interference, immunofluorescence etc.
2. Eggs pocks on CAM - haemagglutination, inclusion bodies
3. Animals disease or death confirmation by neutralization

#### **3. Serology**

The diagnosis of viral infections by detection of specific antiviral antibodies is a traditional method whose clinical utility is limited by the need for comparison of acute and convalescent antibody titers. However, detection of virus-specific IgM antibodies allows a diagnosis to be made from a single specimen. Viruses for which detection of virus-specific IgM antibodies are useful include EBV (IgM antibodies to the viral capsid antigen); CMV; hepatitis A virus; hepatitis B virus (IgM antibodies to the hepatitis B core antigen); parvovirus B19; measles, rubella, and mumps viruses; and the arboviruses such as St. Louis encephalitis virus.

Commonest serological methods including different types of ELISA, Immunochromatography, Heamagglutination, blot tests etc.

### **Methods of cultivation of viruses**

Viruses are obligate intracellular parasites. They do not grow on culture media used for bacteria. The methods used for cultivation of viruses are:

1. **Animal inoculation:** Monkeys were used for the isolation of the poliovirus earlier. Infant mice are used for the isolation of cox-sackie virus and arbo viruses (dengue, chikungunya). Mice may be inoculated by several routes – intra cerebral, subcutaneous, intra peritoneal or subcutaneous. Other animals like guinea pigs, rabbits and ferrets are used in some situations.

2. **Embryonated eggs:** Different parts of the egg are used for the cultivation of different viruses. Herpes simplex virus, when inoculated into the chorioallantoic membrane, produces visible lesions called pocks. Inoculation into the amniotic sac is done for the isolation of influenza virus. Yolk sac inoculation is done for the isolation of rabies virus.

3. **Cell culture:** This is routinely used for growing viruses.

#### Assessment activities

- ❖ Chart showing Names of common blood & Intestinal parasites
- ❖ A comparison chart showing the different morphological forms of malarial parasites in blood film.
- ❖ A chart may be prepared on identifying features of common parasitic eggs found in stool sample.
- ❖ An album preparation on images of ova/eggs of common parasites found in stool.

#### List of items in portfolio

- ❖ Chart Prepared on names of common blood & Intestinal parasites
- ❖ Comparison chart with regards to the different morphological forms of Malarial parasites in blood film.
- ❖ Chart prepared on identifying features of common parasitic eggs found in stool sample
- ❖ Album prepared on images of ova/eggs of common parasites found in stool.

## Unit 4.2 Histo technology& Cytology

### Overview

Specimens for histologic and cytology study usually consists of many different cells. Some are normal whereas others indicate pathologic conditions. Histological and Cytological techniques plays a crucial role in the diagnosis of diseased tissues or cells. Histopathological studies proved to be one of the most effective means in diagnosis of benign and malignant conditions of tissues. The unit describes various histological techniques adopted for the preparation of tissue sections or smears for examination under microscope.

Unit Grid:

IDEAS/ CONCEPTS	LEARNING OUTCOME	SUGGESTED ACTIVITY	ASSESSMENT
<p><b><u>4.2.1 HISTOTECHNOLOGY</u></b>  <b>Introduction</b>                      -Methods of examination of Tissues and cells                      -Gross examination                      Examination of Fresh specimens                      Examination of Fixed Tissue                      -Collection of specimens                      Labeling                          -Biopsy                          -Autopsy                      Fixation of the specimens                          -10% Formalin                          -Decalcification</p> <p>Skills                      Identifying                      Observing                      Differentiating                      Analyzing</p>	<p>To identify different types of specimens, collection , fixation and the method of examination in histotechnology</p>	<p>Discussion                      Multimedia presentation                      Field visit                      Assignment</p>	<p>Participation in discussion &amp; field visit</p> <p>Quality of Assignment</p>
<p><b>4.2.2 Processing of fixed tissue</b>                      Steps in tissue processing                      -Dehydration                      -Clearing                      -Impregnation                      -Embedding                      -Section cutting                      Microtomes-Rotary                      Microtome knives                      Frozen sections -Cryostat                      Technique of section cutting                      Adhesion of sections                      -Mention role of adhesives                      -Staining -H&amp;E Staining procedure                      -Mounting of Tissue sections                      -Filing and storage of tissue sections</p> <p>Skills                      -Observing                      -Differentiating</p>	<p>To explain various steps in tissue processing and H&amp; E staining techniques</p>	<p>Discussion                      Multimedia presentation                      Field visit                      Assignment</p>	<p>Participation in discussion &amp; field visit</p> <p>Quality of Assignment</p>

-Participating			
<b><u>4.2.3 DIAGNOSTIC CYTOLOGY</u></b> Introduction -Types of specimens Preparation of smears -Fixation -Staining technique- Papanicolaou method Advantages and applications in diagnostic cytology  Skills -Observing -Identifying - participating -Consolidating	To identify various specimens , preparation of smears and staining techniques employed in cytology	Discussion Multimedia presentation Field visit Reference	Participation in discussion & field visit

I  
ntro

## duction

### Additional Information

**Histotechnology** is the preparation of tissues for microscopic examination. It is an effective diagnostic tool in clinical path-ology. Histological preparations reveal normal tissue structure, tissue abnormalities and cancerous conditions. Branches of histotechnology are

- (1)Histology- the microscopic study of the normal tissues
- (2)Histopathology – the microscopic study of tissues affected by disease.
- (3) Histochemistry – the techniques provide information on the chemical composition of parts of tissues.
- (4) Cytochemistry – the techniques provide information on the chemical composition of parts of cells. And persons specializing in this technique are known as Histotechnologists

### Examination of fresh specimens

Fresh specimens can be examines as teased preparation, squash preparation, impression smears and frozen sections.

### Aims of Fixation :

- ✓ It should kill and preserve living tissues.
- ✓ It should prevent autolysis & putrefaction of the cell.
- ✓ It should penetrate evenly and rapidly.
- ✓ It should harden the tissues
- ✓ Increase the optical density
- ✓ Should not cause shrinkage or swelling of the cells

- ✓ To prepare tissue for staining and optical contrast. Must not react with the receptor sites & thus must not interfere with the staining procedure.
- ✓ To stabilize the tissue and cell structure for subsequent treatments (wax embedding, sectioning, mounting).
- ✓ To harden the tissue for section cutting

#### Types of fixation

There are different types such as

1. Immersion fixation
2. Perfusion fixation
3. Vapour fixation
4. Coating spray fixation
5. Freeze drying
6. Microwave fixation/ stabilization

#### Classification of fixatives

According to the component present fixatives are classified in to

1. Simple fixative - formaldehyde, glutaraldehyde
2. Compound fixative - Carnoy's fluid, Zenker's fluid, Bouin's fluid

**Based on the chemical action simple fixatives are further classified in to**

1. Aldehydes with examples its advantage and disadvantage
2. Oxidizing agent with examples its advantage and disadvantage
3. Protein denaturing agent with examples its advantage and disadvantage

**Fixatives are also Classified into three categories.**

1. **Tissue fixatives** Buffered formalin, Buffered glutaraldehyde, Zenker's formal saline, Bowen's fluid
2. **Cytological fixatives** -Ethanol, Methanol, Ether
3. **Histochemical fixatives**-Formal saline, cold acetone, Absolute alcohol

#### Additional Information

##### Technique of decalcification

1. Selection of tissues- Bone, teeth and calcified tissues
2. Fixation in formalin adequately

3. Decalcification by different methods
  - a) Acid decalcification
  - b) Ion exchange resins
  - c) Electrical ionization
  - d) Chelating methods
  - e) surface decalcification
4. Detection of end point
5. Neutralization of acid
6. Thorough washing

**Staining**-Staining is used to obtain contrast between the constituent parts of a tissue section. The depth of colouration is affected by chemical affinity, density, and permeability. Certain stains are metachromatic -i.e. they are capable of imparting one colour to certain constituents and another to others.

**Mordanting**- The salts of certain metals are capable of radically alter the behavior of particular stains. These salts are called 'mordants'. A mordant is capable entering into chemical combination with a stain. The resulting substance is called 'a lake'

**The freezing microtome**; The optimum cutting temperature is -20 degree Celsius. The freezing of the tissues is done by the carbon dioxide gas.

**The cryostat** Sectioning is done on unfixed tissue. The microtome is housed in a deep freezer cabinet. The temperature can be maintained between -15 to -30 degree Celsius.

### **Additional Information**

#### **Haematoxylin & Eosin staining Procedure :**

Deparaffinization with xylene.

Hydration with graded alcohols

Wash under running tap water

Stain with Haematoxylin for 15 min

Wash with running tap water ( Blueing)

Differentiate with 1 % acid alcohol

Wash with water for 10 min

Stain with 1% Eosin for 2 min

Wash with water.

Dehydration

Clearing with xylene

Dry

Mount in DPX

- **Result :**
  - The nucleus stains Blue
  - The cytoplasm stains pink.

# Diagnostic Cytology

## Additional information

### Specimens studied for exfoliative cytology

Urine  
Vagina & cervical secretions  
Pleural fluid  
Buccal smears  
Ascitic fluid  
Cerebrospinal fluid  
Aspirations from various lesions by fine needle

### Papanicolaou staining technique

1. Fix wet smears for 15- 30 minutes
2. Rinse in distilled water
3. Stain in haematoxylin for 4 minutes
4. Wash in tap water 1-2 minutes
5. Differentiate in acid alcohol for one minute
6. Blue in tap water
7. Rinse in distilled water
8. Transfer to 70 % and 95 % alcohol for few seconds
9. Stain OG 6 for 1-2 minutes
10. Rinse in 3 changes 95% alcohol
11. Stain in EA 36 for 1-3 minutes
12. Rinse 3 changes in 95% alcohol
13. Dehydrate in alcohol
14. Clear in xylene
15. Mount in DPX

Results    Nuclei – Blue  
                 Acidophilic cells- red  
                 Basophilic cells -blue green  
                 RBC's –Orange red

### Extended activities

- ✓ Organize a Visit to Microbiology & Histopathology depts. of a Laboratory/ Medical College to observe Microbial identification procedures and Histo & Cyto techniques.
- ✓ To conduct a Survey on various vector borne diseases.
- ✓ Participate in the various programs such as AIDS awareness, Filariasis eradication, health campaigns etc conducted by the health dept or NGOs.
- ✓ Exhibition/Expo

### On the job Training

On the Job Training OJT enables students to practice their acquired skills in real work situation and enhance their self esteem. It provides opportunity to familiarize with sophisticated equipment and recent methodologies in medical laboratory technology. A well conducted OJT

ensures competence of the students among the work force. Community involvement of the OJT contributes to the social and management skills of the student. The opportunity to interact with highly qualified professionals of the field inspires the students to higher academic achievements and motivates them to attain professional excellence.

OJT can be provided at • Govt. Medical College Laboratories, laboratories of District, Taluk Hospitals, Community Health Centers and in Public Health Laboratories • Laboratories attached to private hospitals, blood banks and independent clinical laboratories having standard specifications. • Laboratories of Co-operative societies, Kudumbasree units etc.

## List of References

### Recommended Text Books

#### Module 3

1. Text book of Medical Laboratory Technology - Ramnik Sood
2. Manual of Laboratory safety - Roshid Najat
3. Practical Clinical Biochemistry methods and interpretations- Ranjana Chawla
4. Laboratory procedures in haematology -Mehdi SR
5. Essentials of Blood banking -Mehdi SR
6. The short text book of Medical Laboratory for technicians- SatishGupte
7. A hand book of Clinical Pathology- Ganendrakumar Chakravarti
8. Medical Laboratory science: Theory and practice- J Ochei. A.Kolhatkar
9. Practical Haematology- Dacie and Lewis
10. Text book of Medical Laboratory Technology Vol I & II - Praful Godkar, Darsan Godkar
11. Medical Laboratory Technology , Procedure manual for Routine diagnostics Vol I, II & III Kanai Mukherjee
12. Lynch,s Medical Laboratory Technology- Stanley S Raphael, Mathew J Lynch.
13. Medical laboratory manual for Tropical countries vol I & II Monica Cheesbrough
14. Basic Medical Laboratory Techniques Barbara H.Estridge et al
15. Tietz-fundamentals of clinical chemistry

#### Reference Sites:

Web sites <https://en.wikipedia.org>,  
<https://internalmedicine.osu.edu>,  
<https://labtestsonline.org>  
<https://www.dcccd.edu>,  
<https://www.nlm.nih.gov>,  
[www.science.edu](http://www.science.edu),  
[www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov),  
[www.sciencedirect.com](http://www.sciencedirect.com),  
[www.encyclopedia.com](http://www.encyclopedia.com),  
[www.healthline.com](http://www.healthline.com), [www.webmed.com](http://www.webmed.com),  
[www.acb.org](http://www.acb.org)

#### Module 4

1. Prescott's Microbiology, 9th Edition  
Authors: Joanne Willey, Linda Sherwood and Christopher J. Woolverton
2. Jawetz Melnick & Adelbergs Medical Microbiology, 26th Edition  
Authors: Geo. Brooks, Karen C. Carroll, Janet Butel and Stephen Morse
3. Bailey & Scott's Diagnostic Microbiology, 13th Edition



Author: Patricia Tille

4. Greenwood Medical Microbiology, 18th Edition

Authors: David Greenwood, Richard C. B. Slack, Michael R. Barer and Will L Irving

5. District Laboratory Practice in Tropical Countries, Part 1&2, 2nd Edition

Author: Monica Cheesbrough

6. Medical Microbiology, 7th Edition

Authors: Patrick R. Murray, Ken S. Rosenthal and Michael A. Pfaller

7. Lippincott's Illustrated Reviews: Microbiology, 3rd Edition

Authors: Richard A. Harvey and Cynthia NauCornelissen

8. Microbiology: An Application Based Approach

Author: Michael J. Pelczar, ECS Chan and Noel R. Krieg

9. Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 6th Edition

Author: Elmer W. Koneman

10. Foundations in Microbiology, 8th Edition

Authors: Kathleen Park Talaro and Barry Chess

11. Textbook of Diagnostic Microbiology

Authors: Connie R. Mahon, Donald C. Lehman, George Manuselis

12. Medical Microbiology, 3rd Edition

Authors : Cedric Mims , Hazel Dockrell, Richard Goering

13. Ananthanarayan and Paniker's Textbook of Microbiology, 9th Edition

ArtiKapil

14. Medical microbiology Prep manual for undergraduates, 2nd Edition

Author: Asha P Pichare, B. S Nagoba

15. Textbook of Microbiology, 4th Edition

Author -Baveja

16. Basic laboratory procedures in clinical bacteriology 2nd Edition

World health organization

17. Parasitology 13th edition

K.D. Chatterjee.

18. Paniker's Textbook of Medical Parasitology

19. WHO- Basic Laboratory Methods in Parasitology

Reference sites

<http://www.asm.org/> American society for microbiology

<http://www.cdc.gov/> Centers for Disease control

<http://www.nih.gov/> National institute of health

<http://www.who.int/home-page/> WHO

END