Vidyasagar University

Internship Report

B. Sc. in Medical Laboratory Technology

Internship report submitted to Midnapore City College for

the Partial Fulfillment of the Degree of

BMLT/MLT

Submitted by

Name:

Registration No:

Internship Hospital and Address:

MIDNAPORE CITY COLLEGE

Dept. of Paramedical & Allied Health Sciences
Kuturiya, P.O. Bhadutala, Pin-721129
Paschim Medinipur
West Bengal, India

Preface

Internship for the six months in the Govt. / Govt. recognized hospital is an integral part of the BACHELOR OF MEDICAL LABORATORY TECHNOLOGY (BMLT) program and is designed to provide BMLT interns with an opportunity to integrate and apply previously acquired knowledge and technical skills in actual clinical settings.

This internship report is prepared with the intention to provide orientation to interns about various tasks that have been performed and/or observed in different laboratories during 6 months internship at the Govt. / Govt. recognized hospital. The main goal of the internship is to acquire the necessary practical skills in performing various laboratory tests in different laboratories in hospitals that will contribute directly to efficient laboratory diagnosis and improve health care services.

The main contents of this report are the tasks that interns are either performed or observed during the internship. The interns prepared this brief report after completion of the six months internship and submitted it to the College/University for the evaluation to complete their study to obtain their degree in BACHELOR OF MEDICAL LABORATORY TECHNOLOGY (BMLT).

Certificate of Internship

(Attach the photocopy of the certificate)

(Should be printed in College letter head)

Certificate

This is to certify that ……, a regular student of Midnapore City College, Midnapore, West Bengal, India has been studying Bachelor of Medical Laboratory Technology (BMLT), a 3 and ½ years regular degree course under the Vidyasagar University. His/her University Registration No. is…….. of the year …….

He/She has completed the course with the 6 months mandatory rotator internship at ……

…….form ……. to ……...

He/she is a very diligent and disciplined student.

I wish him success in life.

Signature Course coordinator/HOD/Principal/Director

Date:

Place: Midnapore City College, Paschim Medinipur

Acknowledgment

I would first like to acknowledge Dr. Pradip Ghosh, Hon'ble Founder Director, Midnapore City College, Paschim Medinipur for providing me the opportunity to study in this college and complete my Internship in Govt. Hospitals. I am gratefully indebted to him for his very valuable advice about our duty and responsibility as a Lab technologist during the Internship period and in future careers.

I would like to express my sincere thanks to the Principal, MCC for his kind suggestion, cooperation, and help during our time at MCC as well as during the internship.

I would like to express my deepest sense of gratitude to the ……(Head of the Hospital) for his valuable advice and unending guidance to complete my internship.

I would like to express my sincere thanks to HOD, Pathology; HOD, Biochemistry; HOD, Microbiology, HOD/In-charge Blood bank; HOD/In-charge Central Lab, ……(Internship institute) for their valuable advice, guidance, helping us to learn the modern techniques & applications in Biomedical aspects and co-operations.

I would like to convey my sincere thanks to the technical staff, …….. of the …….. (Internship institute) for their kind co- operation & help throughout the internship period.

I also convey my sincere thanks to the faculty members,……(Name and Designation), Dept. of Paramedical & Allied Health Sciences, MCC & all the honorary guest teachers for their inspiration & advice throughout the courses as well as for the internship training & report preparation. Without their passionate participation and input, the validation internship could not have been completed.

Finally, I must express my very profound gratitude to my parents and family members for providing me with unfailing support and continuous encouragement throughout my years of study and during the internship period, and this report preparation. This accomplishment would not have been possible without them.

Thank you.

(Name and Signature of the student)

Contents

1. Vision, Mission, Goals, and Objectives of the Internship

1.1. Vision: The BACHELOR OF MEDICAL LABORATORY TECHNOLOGY (BMLT) program is to be one of the leading programs at the national and regional levels recognizing our graduates for their professional competence, leadership quality, and competitive research in the field of MEDICAL LABORATORY TECHNOLOGY.

1.2. Mission: To provide innovative curricula responsive to the needs of the profession that result in medical laboratory technicians being able to join immediately the health care field with the theoretical knowledge and the technical skills necessary to provide quality laboratory services. Moreover, we will be prepared to be able to adapt to the future changes in the health care system and clinical laboratory science.

1.3. Goals: To prepare competent laboratory technicians who have acquired the necessary knowledge, skills, training, and proficiency in the laboratory diagnosis of various diseases.

1.4. Objectives:

Upon completion of the BACHELOR OF MEDICAL LABORATORY TECHNOLOGY program our graduates are expected to:

a. Have in-depth knowledge of the relationships between laboratory data and pathologic processes, and how laboratory data related to health and disease.
b. Have the talent to design, evaluate and implement new methods or protocols in different clinical laboratories.
c. Have experience with the performance and quality control of routine and specialized medical laboratory testing procedures and an understanding of the theoretical basis of these procedures.
d. Have experience trouble-shooting and resolving typical problems in the clinical laboratory and are familiar with laboratory quality assurance, laboratory safety, laboratory regulations, information systems, management, research design, and educational methodology.
e. Have the ability to work independently and as a team member to perform critical thinking and problem-solving skills in different diagnostic laboratory domains.
f. Have the capability to demonstrate an attitude of professionalism when working with colleagues with all other health professional staff working in the diagnostic laboratory.

2. INTERNSHIP SPECIFICS

2.1 Introduction:

The internship is an integral part of the program for Bachelor of Medical Laboratory Technology and is designed to provide interns with an opportunity to integrate and apply previously acquired knowledge and technical skills in actual clinical settings. Under the guidance of experienced Medical Laboratory Professionals and other qualified laboratory personnel and health professionals, interns learn more about diagnostic test procedures, quality control methods and programs, and instrumentation in the clinical laboratory. They also gain an understanding of the roles and functions of the Medical Laboratory technologist.

The internship provides applied learning experiences during which the intern should:

a. Acquire clinical laboratory skills
b. Perform quality control procedures
c. Learn and adapt new procedures
d. Operate and maintain various laboratory machines and instruments
e. Report accurate and precise results to supervisors
f. Understand the responsibilities, roles, and functions of the Medical Laboratory technologist.

The internship program is conducted in the laboratories of the Govt./ Govt. recognized hospital, where interns learn by participating in the workload of a supervising technologist/specialist/consultant. The emphasis in each internship discipline is given on: a) organization of work, b) use of automated instrumentation, c) the relation of laboratory results to patient diagnosis, and d) the establishment and use of programs for quality control and preventive maintenance of laboratory instruments.

2.2 Internship duration:

The training period for the internship is 6 months. It was offered after the 6th Semester.

2.3 Intern Information

1.	Name of the intern	
2.	College Address	
3.	Permanent Address	
4.	College ID card No	
5.	Registration no with Year	
6.	Roll no	
7.	Mobile:	
8.	E-mail:	
9.	Hospital name and Address where internship done	
10.	Duration (dd/mm/yyyy to dd/mm/yyyy)	

2.4 PROPOSED TRAINING SCHEDULE

Internship Discipline	Duration with date
Pathology	
Biochemistry	
Microbiology	
Blood Bank	
Central laboratory	

3. GENERAL LABORATORY SAFETY PROCEDURES AND RULES

3.1 Laboratory safety

We, the interns, read and understand the information regarding laboratory safety and emergency procedures before the first laboratory session. We adhere to written and verbal safety instructions throughout the internship period. Safety information was provided by the hospital safety officer before the beginning of the internship, following general safety guidelines that help us to work in a safe environment during the internship period. Discipline-specific safety precautions are provided by the specific sections.

3.1.1 General laboratory safety

a. Always wear a laboratory coat or apron while working. After work, leave the lab coat in an assigned cabinet or area.
b. Must wear personal protective equipment (gown, gloves, masks, face shield or glasses, etc.) when working with hazardous or toxic materials and change when contaminated.
c. Shoes should be fluid impermeable material and cover the entire foot.
d. The application of cosmetics within the laboratory is strictly prohibited.
e. Contact lenses should not be worn while working in the laboratory.
f. Always cover any cut, insect bite, or open wound with a water-proof adhesive dressing.
g. Gloves should be removed (unless stated to wear) before handling telephones, computer keyboards, laboratory equipment, doorknobs, etc
h. Eating, drinking, smoking, and chewing gum are prohibited in the laboratory.
i. Storage of food or drink is not allowed in laboratory refrigerators.
j. Mouth pipetting must not be done.
k. Laboratory working surfaces shall be decontaminated with a disinfecting solution after the spill of blood or body fluid.
l. Needles should not be recapped or removed from a disposable syringe.
m. Discard used syringes, needles, and other sharps (glass slides, glass pipettes, knives, etc.) in specified containers.
n. If equipment shows any problem while being used, report immediately to your supervisor. Never try to fix the problem yourself.
o. Follow the standard safety precautions when using a centrifuge.
p. Hands should be washed with soap and water after handling hazardous and infectious materials.
q. Biological safety cabinets (Class I or II) should be used to avoid aerosolization or droplets.
r. Equipment contaminated with blood or other body fluids should be decontaminated and cleaned before use.
s. All waste and contaminated materials (clinical specimens, bacterial cultures) should be disposed of in appropriate containers.
t. Inform your supervisor about any accidents, spills, or potential hazards.

3.1.2 Chemical safety

a. Know the color coding and numerical rating of chemicals or materials for health hazards, fire hazard, reactivity hazard, and specific hazards (reactivity with water) (NFPA label).
b. Use volatile and flammable compounds only in a fume hood.
c. Never return unused chemicals to their original container.
d. Dispose of chemical waste in proper containers according to manufacturer's instructions.

3.1.3 Emergency response

a. Read safety and fire alarm posters and follow the instructions during an emergency.
b. Know the location of fire extinguishers, fire exits, first aid kits, and eyewash solutions in your lab and know how to use them.
c. Know the building evacuation procedure in an emergency.

4. Internship for Laboratory: Pathology

4.1 Goal:

Interns need to acquire knowledge and skills of proper handling and documentation of clinical specimens for the pathology laboratory at reception during the internship period.

4.2 Objectives:

I. To know the guidelines and procedures for handling and documentation of clinical specimens.
II. To apply specimen acceptance/rejection criteria.
III. To familiarize with the computerized system of specimen entry and distribution in laboratories.
IV. To categorize specimens according to their turnaround time.

4.3 Tasks:

Sl. No.	Task	Observed/ Performed	Specific Learning
	Specimen collection		
	Laboratory test performed		
	Instruments handled		
	Analysis of results		
	Documentation Report preparation		
	Specific learning		

4.4 Methodology:

4.4.1 HISTOPATHOLOGY

Definition:

It is a branch of pathology in which cells deals with the study of disease in a tissue section. The tissue undergoes a series of steps before it reaches the examiner's desk to be thoroughly examined microscopically to arrive at a particular diagnosis.

To achieve this, the tissue must be prepared in such a manner that it is sufficiently thick or thin to be examined microscopically and all the structures in a tissue may be differentiated. The objective of the subsequent discussions will be to acquaint the staff with their responsibility, and the basic details of tissue handling, processing, and staining.

The term Histochemistry means the study of the chemical nature of the tissue components by histological methods.

The cell is the single structural unit of all tissues. The study of the cell is called cytology. A Tissue is a group of cells specialized and differentiated to perform a specialized function. The collection of different types of cells forms an organ.

Type of material obtained in the laboratory:

The human tissue comes from the surgery and in the autopsy room from surgery two types of tissue are obtained:

I. Biopsy-A small piece of lesions or tumor which is sent for diagnosis before final removal of the lesion or the tumor (Incision biopsy). If the tumor or lesion is sent for examination and diagnosis by the pathologist. It is called an excision biopsy.

II. Tissues from the autopsy are sent for the study of disease and its course, for the advancement of medicine.

Types of Histological preparation:

The Histological specimen can be prepared as

I. Whole-mount
II. Sections
III. Smears

Responsibility of a technician

The technician is responsible for

I. Specimen preservation.
II. Specimen labeling, logging, and identification.
III. Preparation of the specimen to facilitate their gross and microscopy.
IV. Record keeping.

To obtain these aims the following point needs consideration:

i. As soon as the specimen is received in the laboratory, Check if the specimen is properly labeled with the name, age, and Hospital registration No. And the nature of the tissue to be examined and their question form is also duly filled.
ii. Also, check if the specimen is in proper fixative. Fixative should be fifteen to twenty times the volume of the specimen and fixative matters have been taken care of.
iii. Check if the financial matters have been taken care of.
iv. Make the entries in the biopsy register and give the specimen a pathology number called an accession number. Note this number carefully on the requisition form as well as the container. This number will accompany the specimen everywhere.
v. If the specimen is large inform the pathologist who will cut the specimen so that proper fixation is done. The container should be appropriate to hold the specimen without distorting it.
vi. Blocks of tissues taken for processing should be left in 10 % formalin at 60°C till processing. These would be fixed in 2 hours.
vii. Slides should be released for recording after consultation with the pathologist.
viii. Specimens should be kept in their marked container and discarded after checking with the pathologist.
ix. Block must be stored at their proper number the same day. Note the blocks have to be kept preserved for life long. Slides should be stored in their proper number after 3 days. It gives time for the slides to be properly dried.

4.4.2. FIXATION:

Definition: It is a complex series of chemical events which brings about changes in the various chemical constituents of cell-like hardening, however, the cell morphology and structural detail are preserved. Unless a tissue is fixed soon after the removal from the body it will undergo degenerative changes due to autolysis and putrefaction so that the morphology of the individual cell will be lost.

Principle of fixatives

I. Coagulation and precipitation.
II. Penetration fixation is done by immersing the tissue in fluid containing the fixative.
III. Faster a fixative can penetrate the tissue better its penetration power depends upon the molecular weight e.g., formalin fixes faster than osmic acid.
IV. Solubility of fixatives- All fixatives should be soluble in a suitable solvent, preferably in water so that adequate concentrations can be prepared.
V. Concentration - The concentration of fixative must be isotonic or hypotonic.

Amount of fixative

The fixative should be at least 15-20 times the bulk of tissue. For museum specimens, the volume of fixative is > 50 times. If the specimen is large then see that the sections are made to make slices that have a thickness of 1.5 cm so that fixative can penetrate the tissue easily.

Reagents employed as fixatives (simple fixatives)

I. Formaldehyde- Formaldehyde is a gas but is soluble in water to the extent of 37- 40% w/v. This solution of formaldehyde in water is called formalin or full-strength formalin. Formalin is one of the commonly used fixatives in all laboratories since it is cheap and penetrates rapidly hours for small biopsies- 4-6 hours at 65°C fixative occurs in 2 hours.

II. Alcohol (Ethyl Alcohol)- Absolute alcohol alone has very little place in routine fixation for histopathology. It acts as a reducing agent, and becomes oxidized to acetaldehyde and then to acetic acid. It is slow to penetrate, hardens, and shrinks the tissue. Alcohol penetrates rapidly in presence of other fixatives hence in combination e.g. Carnoy"s fixative is used to increase the speed of tissue processing.

III. Acetone- Cold acetone is sometimes used as a fixative for the histochemical demonstration of some tissue enzymes like phosphatases and lipases. Its mode of action as fixative is similar to that of alcohol.

Preparation of the specimen for fixation

I. For achieving good fixation, the fixative must penetrate the tissue well hence the tissue section should be > 4mm thick so that fixation fluid penetrates from the periphery to the center of the tissue. For fixation of large organs, the perfusion method is used i.e fixative is injected through the blood vessels into the organ. For hollow viscera, the fixative is injected into the cavity e.g. urinary bladder, eyeball, etc.
II. The ratio of the volume of fixative to the specimen should be 1:20:3. The time necessary for fixative is important routinely 10% aqueous formalin at room temperature takes 12 hours to fix tissue. At higher temperatures i.e. 60-65°C the time for fixation is reduced to 2 hours.

Fixatives are divided into three main groups

I. Micro anatomical fixatives- Such fixatives preserve the anatomy of the tissue.
II. Cytological fixatives- Such fixation is used to preserve intracellular structures or inclusion.
III. Histochemical fixatives- Fixatives are used to preserve the chemical nature of the tissue for it to be demonstrated further. Freeze-drying techniques are best suited for this purpose.

Zenker's fluid

i. Mercuric chloride: 5 gm
ii. Potassium dichromate: 2.5 gm
iii. Sodium sulfate: 1.0 gm
iv. Distilled water: 100 ml
v. Add immediately before use: Glacial acetic acid: 5 ml

Specific features

- Good routine fixative
- Give fairly rapid and even penetration
- It is not stable after the addition of acetic acid hence acetic acid (or formalin) should be added just before use.
- Washing of tissue in running water is necessary to remove excess dichromate.

Bouin's fluid

i. Saturated aqueous picric acid:75 ml
ii. Formalin: 25 ml
iii. Glacial acetic acid: 5 ml

Specific features

- Penetrates rapidly and evenly and caused little shrinkage
- Excellent fixatives for testicular and intestinal biopsies because it gives very good nuclear details, in tests are used for oligospermia and infertility studies.
- Good fixatives for glycogen
- It is necessary to remove excess picric acid by alcohol treatment.

Carnoy"s fluid

i. Absolute alcohol 60 ml
ii. Chloroform 30 ml
iii. Glacial acetic acid 10 ml

Specific features

- It penetrates very rapidly and gives excellent nuclear fixation.
- Good fixative for carbohydrates.
- Nissil substance and glycogen are preserved.
- It causes considerable shrinkage.
- It dissolves most of the cytoplasmic elements. Fixation is usually complete in 1-2 hours.
- For small pieces 2-3 mm thick only 15 minutes in needed for fixation.

4.4.3. DECALCIFICATION

Definition:

Decalcification is a process of complete removal of calcium salt from the tissues like bone and teeth and other calcified tissues following fixation.

Decalcification is done to assure that the specimen is soft enough to allow cutting with the microtome knife. Unless the tissues in completely decalcified the sections will be torn and ragged and may damage the cutting edge of the microtome knife.

Steps of decalcification:

To ensure adequate fixation and complete removal of the calcium the slices must be 4-5 mm thick.

Calcified tissue needs 2-3 hours only, for complete decalcification to be achieved so it is necessary to check the decalcification after 2-3 hours.

Fixative of choice for bone or bone marrow is Zenker formal or Bouin"s fluid.

Unfixed tissue tends to be damaged 4 times greater during decalcification than a properly fixed tissue.

Decalcifying Agents:

Nitric acid- 5%-10% aqueous solution used. They decalcify vary rapidly but if used for longer than 24-48 hours.

Hydrochloric acid- 5%-10% aqueous solution decalcification is slower than nitric acid but still rapid. Fairly good nuclear staining.

Formic acid: Brings out fairly rapid decalcification. Nuclear staining is better. But requires neutralization and thorough washing before dehydration.

Aqueous nitric acid: Nitric acid: 5-10 ml. Distilled water up to 100 ml.

Procedure:

I. Place calcified specimen in large quantities of nitric acid solution until decalcification is complete (Change solution daily for best results).
II. Washing running water for 30 minutes.
III. Neutralize for at least 5 hours in 10% formalin to which excess calcium or magnesium carbonate has been added.
IV. Wash in running water overnight.
V. Dehydrate, clear and impregnate in paraffin or process as desired

4.4.4. TISSUE PROCESSING

Specific objective

The tissue processing is the heart of any tissue section which will be cut adequately only if the tissue is properly preserved and processed. The study of this topic is to understand the coarse and fine details of tissue processing so that excellent sections are obtained.

Definition

The term tissue processing refers to the treatment of the tissue necessary to impregnate it into a solid medium so that the tissue is rendered sufficiently firm yet elastic for the tissue sections of desirable thickness to be cut on a microtome.

Principle of tissue processing

The tissue is embedded in a solid medium by the help of first removing the tissue water which is then replaced by any solid medium such as paraffin wax so that the tissue is rendered firm enough to enable thin sections to be cut, at the same time, the tissue is a fort (not so hard) to enable microtome knife to cut the sections.

Before paraffin wax embedding and impregnation the tissue must be subjected to the following steps:

I. Fixation
II. Dehydration
III. Clearing- With a substance that is miscible with both the dehydrating agent which precedes it and the embedding agent which follows it.
IV. Embedding all these 4 processes depend upon complete impregnation of the tissue by the agent like paraffin wax being used. Before going into the details of these 4 stages it is important to understand the factors which influence the rate ad efficiency of tissue impregnation.

4.4.5. PARAFFIN WAX EMBEDDING:

Fixation-Usually tissue that is received at the laboratory is already fixed but before proceeding further check if the fixation is complete.

Dehydration-After fixation in an aqueous solvent the delicate tissue needs to be dehydrated slowly starting with 50% ethyl alcohol. The other routine tissue specimen may be put in 70% alcohol. A higher concentration of alcohol initially is inadvisable because this may cause very rapid removal of water and may produce cell shrinkage. An exception to this is in the case of Heineman's fixed tissue where it may be placed directly in 95% alcohol. Tissue transferred from alcoholic based fixative like Carnoy"s fixative may be placed in higher grades of alcohol or even in absolute alcohol36 for routine biopsy and post-mortem tissue of 4-7, thickness 70%, 90% and absolute alcohol (2-3 changes for 2-4 hours each) are sufficient to give a reasonably satisfactory result.

Clearing-It means the appearance of tissue after it has been treated by the fluid chosen to remove the dehydrating agent.

A clearing agent is required when the dehydrating agent is not miscible with the impregnating medium. A clearing agent needs to be miscible both in dehydrating agent as well as the embedding agent. Commonly used clearing agents are as follows:

Xylene- It has rapid action. Biopsy specimens of 3-4 mm thickness are cleared in 2-4 hours.

Toluene and Benzene are similar in properties to xylene but are less damaging to the tissues on prolonged exposure.

4.4.6. IMPREGNATION:

Definition- It is the complete removal of clearing reagents by substitution of paraffin or any such similar media.

Impregnation with wax-Impregnation with paraffin wax takes place in an oven heated to 56-60°C depending upon the melting point of the wax in use. A frequent check of the temperature of

paraffin baths is required since a temperature of 5°C above the melting point of the paraffin will cause tissue shrinkage and hardening.

4.4.7. EMBEDDING

It is the orientation of tissue in melted paraffin which when solidified provides a firm medium for keeping intact all parts of the tissue when sections are cut.

4.4.8. SECTION CUTTING

Objective:

I. To ensure good section cutting.
II. To overcome trouble shooters during section cutting.
III. To familiarize the staff with the equipment used for section cutting.

Microtome Knives: The knife is probably the greatest single factor in producing good sections.

Types of microtome knives: Microtome knives are classified by how they are ground and seen in their cross-section.

I. Plane wedge
II. Plano concave
III. Biconcave
IV. Tool edge

Sharpening of microtome knives:

Honig: Grinding of a knife on a hone to restore straight cutting edge and correct level.

Method of honing:

I. The hone is placed on a bench on a non-skid surface.
II. A small quantity of light lubricant oil is poured on the center of the hone and lightly smeared over the surface.
III. The knife is completed with a handle and the backing sheath is laid on the hone with the cutting edge facing away from the operator, and the heel in the center of the nearest end of the hone. Correct positioning of the fingers is achieved by holding the handle of the knife between the thumb and forefinger with the cutting edge facing away from the operator (so that the thumb is on the back). When the knife is on the hone the tips of the finger and thumb of the other hand rest on the other end of the knife ensuring even pressure along the whole edge of a knife during honing.
IV. The knife is pushed forward diagonally from heel to toe, turned over on its back, and moved across the hone until the heel is in the center 53 with the cutting edge leading, and then brought back diagonally. It is turned to its original position, thus completing the figure of 8 movements.
V. The process is continued until all jagged edges have been removed now the knife is ready for stropping.

Stropping:

It is the process of polishing an already fairly sharp edge. It removes burrs formed during honing. Fine quality leather is used leather strops may be either flexible/hanging or rigid. In stropping, usually, a firm surface is preferred. Action is reverse of honing to heel direction of stropping is also opposite.

Assessment of the sharpened knife-edge.

Examine the edge of the knife with reflected light and under the microscope to assess the honing and stropping.

Microtome: These are mechanical devices for cutting uniform sections of tissue of appropriate thickness. All microtomes other than those used for producing ultrathin sections for election microscopy depend upon the motion of the crew thread to advance the tissue block on the knife at a regulated.

Types of microtomes:

I. Hand microtome- Limited for use in botanical sections.
II. Rocking microtome.
III. Rotary microtome.
IV. Freezing microtome.
V. Base sled microtome.
VI. Vibrating knife microtome.

Section cutting of paraffin-embedded tissue.

Fixing of block:

I. Fix the block in the block holder on the microtome knife in such a position that it will be clear of the knife when it is in position, a block may be fixed directly or it may be fixed to a metal carrier which in turn is fixed to the microtome.

II. Insert the appropriate knife in the knife holder and screw it tightly in position. Adjust if required. The clearance angle should be set at 3-4 degrees and the angle of slope should be set permanently at 90°. It is important to tighten the knife clamp screw securely and block clamp screws must also be firm. The exposed ends of the knife must all the time be protected by magnetic or clip-on knife guards to avoid any accidents.

III. Trimming of tissue block: Move the block forward so that the wax block is almost touching the knife. To trim away any surplus wax and expose a suitable area of tissue for sectioning. The section thickness adjusters are at 15 microns.

IV. On exposing a suitable area of tissue the section thickness is set to the appropriate level for routine purposes to 4-6 microns.

V. Apply ice to the surface of the block for a few seconds and wipe the surface of the block free of water. This step is optional but makes sections cut easily.

VI. Note that the whole surface of the block will move parallel to the edge of the knife to ensure a straight ribbon of sections.

VII. The microtome is now moved in an easy rhythm with the right hand operating the microtome and the left hand holding the sections away from the knife. The ribbon is formed due to the slight heat generated during cutting, which causes the edges of the sections to adhere. If the difficulty is experienced in forming the ribbon it is sometimes overcome by rubbing one of the edges of the block with a finger.

VIII. During cutting the paraffin wax embedded sections become slightly compressed and creased. Before being attached to slides the creases must be removed and the section flattened. This is achieved by floating them on warm water. Thermostatically controlled water baths are now available with the inside coated black. These baths are controlled at a temperature of 4-6°C below the melting point of paraffin wax. It is easy to see creases if the inside of the water is black.

IX. The action in floating out must be smooth with the trailing end of the ribbon making contact with water first to obtain flat sections with the correct orientation, floating out with the shiny surface towards the water is essential. When the ribbon has come to rest on the water the remaining wrinkles and folds are removed by teasing apart by using forceps or seeker.

X. Picking up sections- The ribbon of sections floating on water is split into individual or groups of sections by use of forceps or seekers. Picking up a section on the slide is achieved by immersing the slide lightly smeared with adhesive vertically from the water, the section will flatten onto the slide. The sections are then blotted lightly with moistened blogging paper to remove excess water and to increase contact between section and slide. For delicate tissues or when several ribbons of sections are placed on the slide, omit the blotting instead keep the slide in the upright position for several minutes to drain.

XI. Drying of section- Sections are then kept in an incubator with a temperature 5-6°C above the melting point of wax i.e. at 60°C for 20-60 minutes. It is better to overheat than underheat. If the sections are not well dried they may come off during staining. The sections should not be allowed to dry without a good contact with the slide, such sections will come off during staining.

Faults in cutting:

I. Fault - Tear or scratch across the section or splitting of ribbon.
II. Fault - Tear or scratch across part of the section.
III. Fault - Holes in the sections.
IV. Fault – Cracks across the section parallel to the knife.
V. Fault – Section curl as they are cut.

4.4.9. STAINING:

The sections, as they are prepared are colorless and different components cannot be appreciated. Staining them with different colored dyes, having affinities of specific components of tissue, makes identification and study of their morphology possible.

Direct staining- Application of simple dye to stain the tissue in varying shades of colors.

Indirect staining- means the use of mordant to facilitate a particular staining method or the use of accentuate to improve either the selectivity or the intensity of the stain.

Progressive staining: Stain applied to the tissue in strict sequence and for specific times. The stain is not washed out or decolorized because there is no over-staining of tissue constituents.

Staining is controlled by frequent observation under the microscope.

Regressive staining: Tissue is first over stained and then the excess stain is removed from all but the structures to be demonstrated. This process is called differentiation and should always be controlled under the microscope.

Mordents: Substance that causes certain staining reactions to take place by forming a link between the tissue and the stain. The link is referred to as a lake. Without it, dye is not capable of binding to and staining the tissue. e.g. Ammonium and Potassium alum for hematoxylin. The most common stain applied for the histological study is Hematoxylin and Eosin.

Hematoxylin and Eosin staining: Hematoxylin is extracted from the wood of the logwood tree. When oxidized it forms haemitin, a compound that forms strongly colored complexes with a certain metal ion, notably Fe(III) and A(III) salts. Metal-hematin complexes are used to stain cell nuclei before examination under a microscope. Structures that stain with iron – or aluminum-hematein are often called basophilic, even though the mechanism of the staining is different from that of staining with basic dyes.

Ingredients:

Hematoxylin powder	- 1g
95% Ethyl alcohol	- 10ml
Ammonium or Potassium alum	- 20gm
Distilled water	-200ml
Mercuric Oxide	-0.5ml

Blueing: Alum Hematoxylin stains nuclei and red color, which is converted to bluish back color, when the section is washed in weak alkali. Tap water is usually alkaline enough to produce this color change.

Procedure:

I. Deparaffinize in a hot air oven.
II. Hydrate the section.
III. 3 dips in xylene (2 Min each)
IV. 3 dips in acetone/alcohol (2 Min. Each)
V. In running tap water for 5 minutes.
VI. Mayer's hematoxylin for 15 minutes.
VII. Wash in running tap water for 20 minutes.
VIII. Counter stain with eosin for 2 minutes.
IX. Dehydrate the section in 95% and absolute alcohol/acetone 2 changes (2 minutes each).
X. Clear in xylene 3 changes (2 minutes each)
XI. Mount in DPX.

Results: Nucleus –blue cytoplasm and background – pink

Causes of poor quality of staining

i. Poor or inadequate fixation of tissue.
ii. Over or under – ripened Hematoxylin.
iii. Overused or worked out Hematoxylin. Insufficient bluewing following differentiation.
iv. Failure to wash blueing agent out of section before counter staining with eosin (especially when

ammonia is used)

v. Insufficient differentiation of eosin during washing or dehydration.

vi. Insufficient dehydration and clearing of sections.

MCMANUS FOR GLYCOGEN (PAS)

Aim: Staining and identification of the various types of carbohydrates"s (Polysaccharides & mucopolysaccharides).

Principle: Tissue structures like liver & heart, and striated muscles are studied by the periodic acid stiff stain. Periodic acid reacts with the aldehyde group of the carbohydrates and after wards reaction with the Schiff"s reagent produces a red or purple-red color.

Reagents:

I. 0.5% w/v periodic acid solution.

II. Schiff's reagent –

(a) Dissolve 1.0 gm of basic fuchsine in ≈ 100 ml of boiling distilled water cool to about 60 degrees and filter.

(b) Add 20 ml of 0.1 N hydrochloric acid, cool further and add 1.0 gm of sodium metabisulphite and mix well.

(c) Keep in the dark for 24-48 hours. When the solution becomes straw-colored, add 300 mg of activated charcoal, shake vigorously, filter and store.

III. 1 N Hydrochloric acid.

IV. 0.1 gm of light green in 100 ml of 0.1% (v/v) acetic acid.

V. Harris hematoxylin stain.

Procedure

I. Deparaffinize and hydrate to distilled water.
II. Oxidize in the periodic acid solution for 5 minutes.
III. Rinse in distilled water.
IV. Schiff"s reagent solution for 15 minutes.
V. Wash in running water for10 minutes for the pink color to develop.
VI. Harris hematoxylin for 6 minutes or light green counter stain for a few seconds.
VII. Wash in running water.
VIII. Differentiate in 1% acid alcohol solution 3-10 quick dips.
IX. Wash in running water.
X. Dip in ammonia water to blue the sections.
XI. Wash in running water for 10 minutes
XII. Dehydrate in 95% alcohol, absolute alcohol, clear in xylene two changes each.
XIII. Mount in DPX.

Results:

With hematoxylin counter stain.

I. Nuclei – blue

II. Glycogen, mucin, hyaluronic acid, reticulin, colloid droplets, amyloid infiltration, thrombi. – purple-red.

III. Fungi – red Background – pale green (with light green counter staining).

4.4.2. CLINICAL PATHOLOGY

It comprises:

I. Urine test (Physical, Chemical & Microscopically).

II. Stool test (Physical, Chemical & Microscopically).

III. Occult blood test in urine and stool.

IV. Working in clinical pathology laboratory are required to examine various body fluids, like blood, urine, spinal fluid, Pleural fluid, serous fluid, and synovial fluid. Laboratory investigation in clinical pathology primarily focuses on the physical examination of fluid, clinical microscopy, and simple chemical screening.

4.4.3. EXAMINATION OF URINE

PHYSICAL EXAMINATION:

Color: Normal urine color is nearly colorless to dark yellow or straw color:

Specific gravity: The Specific Gravity at a constant temperature is the ratio of the weight of a volume of urine to the weight of the same volume of distilled water. The specific gravity determination is used to measure the concentration and diluting of the power of the kidney. The specific gravity of urine thus varies throughout the day and the normal range is 1.003 to 1.035. To measure the Specific gravity of urine, we normally use the Refractometer method**.**

Materials: Refractometer scale, Urine sample.

Refractometer method:

i. Clean the glass surface of the instrument with distilled water and a soft cloth.
ii. Close the cover over the glass surface and insert a drop of a urine sample with the help of a Pasteur pipette.
iii. Hold the instrument up to a light source.
iv. Read the Sp. Gravity from the scale at the point where the dividing line between the bright and dark fields meets.

CHEMICAL EXAMINATION OF URINE

pH Test: pH normal urine varies from 6 to 6.8 which is acidic.

A rough estimate of pH is made by indicator paper, blue litmus, or wide-range indicator paper.

Urine Albumin Test: To estimate the albumin of urine "**A heat and Acetic Acid test**" is required.

Materials:

i. Urine sample.
ii. 3% glacial acetic acid.

Heat and acetic acid test:

i. The urine should be acidic, a few drops of 3% acetic acid is added to alkaline urine to make it slightly acidic.
ii. Urine should be clear, and turbid.
iii. At first centrifuge the turbid urine in a test tube.
iv. Taken out the supernatant portion for examination.

v. Take urine in a clean test tube filling 2/3 portion.
vi. Boil the upper half-inch of the tube.
vii. A white cloud appears in a heated portion, due to the presence of protein or phosphate.
viii. Add 2 to 3 drops of 3% glacial acetic acid.
ix. If cloudiness disappears------- Phosphate.
If cloudiness persists ---------- Albumin.

Observation:

Negative	---	No cloudiness.
Trace	---	Barely visible cloudiness.
+	---	Definite cloud without granular flocculation.
++	---	Heavy and granular cloud without flocculation.
+++	---	Dense cloud without marked flocculation.
++++	---	Thick, cloudy precipitate and coagulation.

- **Urine glucose Test:**

To sugar test the urine "**Benedict's Test**" is essential.

Benedict's Test is used as a simple test for reducing sugars. Reducing sugar is a carbohydrate possessing either a free aldehyde or a free ketone functional group as part of its molecular structures. This includes all monosaccharides (i.e, Glucose, Fructose, Galactose) and many Disaccharides, including Lactose and Maltose. Benedict"s Test is most commonly used to test for the presence of glucose in urine. Glucose found to be present in urine is an indication of Diabetes Mellitus.

Principle: Reducing sugar under alkaline conditions tautomerase form enediols. Indios are powerful reducing agents. They can reduce cupric ions (Cu^{2+}) to cuprous ions (Cu^{+}), which is responsible for the change in color of the reaction mixture. This is the basis of "Benedict"s Test". When the conditions are carefully controlled, the coloration develops and the amount of precipitate formed (Cuprous Oxide) depends upon the amount of reducing sugars present.

Composition and Preparation of Benedict's Reagent

One liter of Benedict"s Solution can be prepared from 100 gm of anhydrous sodium carbonate, 173 gm of sodium citrate and 17.3 gm of copper (II) sulfate pentahydrate.

Component	Amount	Functions
Copper Sulphate	17.3 gm	Furnishes cupric ions (Cu^{++})
Sodium Carbonate	100 gm	Makes medium alkaline
Sodium Citrate	173 gm	Complexes with the copper (II) ions so that they do not deteriorate to copper (I) ions during storage
Distilled water	Up to 1000ml	Solvent

Material:

i. Benedict"s qualitative reagent.
ii. Urine sample.

Procedure:

i. 5ml Benedict"s qualitative reagent was taken in a test tube.

ii. Boil for 1 minute.
iii. Add 8 drops of urine drop by drop.
iv. Boil for 3 to 5 minutes.
v. Then cool the mixture.

Observation:

Negative	---	No change in color.
Trace	---	Pale green with slide cloudiness.
+	---	Green deposit.
++	---	Yellow deposit.
+++	---	Orange to red precipitate.
++++	---	Brick red precipitate.

➢ **Occult Blood Test in Urine:**

Method: Benzidine method.

Principle: The peroxides activity of hemoglobin decomposes hydrogen peroxide and liberates active oxygen oxidases the compound benzidine.

Reagents: Saturated solution of benzidine in glacial acetic acid; Hydrogen peroxide (3% v/v in distilled water).

Procedure:

i. Mix equal parts of two reagents.
ii. Add an equal volume of urine.
iii. The appearance of a green or blue color within 5 min indicates the presence of blood.

Report as follows.

Trace	---	Faint green
+	---	Green
++	---	Greenish blue
+++	---	Blue
++++	---	Deep blue

➢ **Determination of bile pigments:**

Requirements:

i. Centrifuge tubes or test tubes 10 X 75 mm and 15 X 125 mm.
ii. Pasteur pipette
iii. 10 gm / dl barium chloride
iv. Fouchet‟s reagents
v. Ehrlich reagents
vi. Whatman no. 1 filter paper (if necessary)

Procedure:

i. Place 3-4 ml of urine in a centrifuge tube by using a pasture pipette
ii. Add an equal amount of 10 gm/dl barium chloride, mix well
iii. Centrifuge at 1500 RPM for 10 minutes
iv. Place supernatant in another test tube for urobilinogen test
v. Add one to two drops of Fouchet‟s reagent to the sediments
vi. Add about 0.5ml of Ehrlich Reagents to the supernatant

Observation:

i) Sediment (in the test tube or the filter paper)
   a) No change in color; Bile pigments absent
   b) Colour change to green; Bile pigments present
ii) Supernatant
   a) Development of pale pink color; urobilinogen, present normal
   b) Development of cherry red color; urobilinogen, increased

MICROSCOPIC EXAMINATION OF URINE:

Materials:

i. Urine sample,
ii. Centrifuge tube,
iii. Glass slide,
iv. Cover slip,
v. Microscope.

Method:

i. Mix the urine very well and pour it into a 10 ml centrifuge tube.
ii. Centrifuge with another balanced test tube for 3-5 minutes at 2500 RPM.
iii. Pour off the supernatant quickly and completely into another test tube.
iv. Re-suspended the deposit by shaking the tube.
v. Place 1 drop of the deposit on a glass slide.
vi. Cover it with a cover slip.
vii. Observe it first under a low-power objective in subdued light.

Observation:

i. Red cells, pus cells, and renal epithelial cells are identified under high power objectives.
ii. Casts are identified under the low power objective, but finer structures are identified under HP [High power] objective.
iii. Crystals are examined under low power objectives.
iv. Bacteria, yeast cells, and trichomonas are examined under the HP objective.

4.4.4. HEMATOLOGY

I. Blood Sample Collection by Venipuncture:
II. Routine hematological test:
   - Hemoglobin estimation
   - Total count of RBC
   - Total count of WBC
   - Differential count of WBC in peripheral blood smear
   - Total platelet count
   - Erythrocyte sedimentation rate (ESR)
   - Packed cell volume
   - Red cell indices (MCV, MCH, & MCHC)
   - Reticulocyte count

III. Coagulation profile test:

- Clotting Time.
- Bleeding Time.
- Prothrombin Time.

IV. Special Heamatolgical Test:
- Absolute Eosinophil Count.
- HLA Typing (Human Lymphocyte Analysis)

I. Blood Sample Collection by Venipuncture:

The Volume of blood obtained by venipuncture is sufficient to carry out multiple tests. Venipuncture can be done either by the syringe method or the vacuum tube method. The latter is disposable and is not very popular in developing countries because of the high cost.

Procedure:

i. All the things are assembled and required during blood collection. The things are tourniquet, cotton, alcohol, syringe, container, anticoagulant, etc.
ii. The patient is identified and decided the total amount of blood needed for all the tests.
iii. The containers are selected and labeled with the patient's identification.
iv. Then the patient is asked to sit alongside the table used for blood collection, patient's arm is laid on the table and palm upward. For indoors patients are said to lay their arms in an outstretched position.
v. The puncture is selected carefully after toeing up the tourniquet.
vi. After feeling the vein in the left hand the skin is disinfected with a swab dipped in methanol or 70% alcohol.
vii. The syringe is then checked it the needle is fixed tightly.
viii. After holding the syringe in the right hand and bevel with the uppermost it is pushed firmly and steadily into the center of the vein at 30-40° angle.
ix. Then the blood has appeared in a barrel and the tourniquet is released without disbursing the needle.
x. A Swales of cotton over the hidden point of the needle are placed.
xi. The needle is removed from the vein and the patient is asked to press the cotton swabs for a few minutes.
xii. The needle is removed from the syringe and gently expelled the blood into an appropriate container.
xiii. The blood is mixed immediately and thoroughly but gently with anticoagulant (it is required).
xiv. Immediately the syringe and needle are rinsed without cold water to prevent clotting.
xv. If bleeding is topped, an adhesive tape is applied to the puncture site.

II. Routine Hematological Test:

Determination of Hemoglobin Concentration (HB%):

Hemoglobin is a conjugated protein present in RBCs. It carries oxygen from the lungs to the tissue cells and carbon dioxide- The gaseous waste from the cells to the lungs. Hemoglobin consists of two

components-haem (Iron+Proteoporhyrin) and globin (amino acid chains). There are three types of hemoglobin present in an adult person-

Structure		Normal %
a. HbA (adult HB)	---------	α2β2 97-98%
b. HbA2	---------	α2δ2 1.5-3%
c. HbF (fetal Hb)	---------	α2γ2 Absent

Clinical significance:

A decrease in hemoglobin concentration in blood below normal is a sign of anemia and this leads to reduced oxygen-carrying capacity leading to anoxemia ischemic changes and ultimately necrosis.

Elevated hemoglobin levels can be observed in Ploycythemiasandcongenital heart diseases.

Normal Values:

Male-15-18 gm% Female-11-16 gm%

Children (at1yr)-10-14 gm%

Infants-14-20 gm%

Cyanmethaemoglibin Method:

Principle: In the presence of potassium ferricyanide at alkaline pH, h emoglobin and its derivatives are oxidized to methemoglobin. Methemoglobin so formed reacts with potassium cyanide to form cyanmethemoglobin, a red-colored complex, which is measured colorimetrically at 540 nm (green filter). The color intensity is proportional to the Hb concentration of the blood sample.

Hemoglobin + Potassium ferricyanide-Methemoglobin. Methemoglobin + Potassium cyanide-Cyanmethemoglobin.

Reagents:

Reagent-1: Drabkin"s Solution (contain Potassium cyanide-50mg & Potassium ferricyanide 200mg & Distilled water-1000 ml)

Reagent-2: Cyanmethemoglobinstd.

Sample: Whole blood sample (0.02 ml) is used.

Procedure: Take test tubes for three test tubes for blank rendered and test. Level them and proceed in the following steps.

Pipette into test tubes	**Blank**	**Standard**	**Test**
Cyanmeth Reagent (Drabkin's solution)	1.0ml	---	5.0ml
Cyahmethemoglobin (std)	---	1ml	---
Sample	---	---	20µl

It is mixed thoroughly and after 5minutes the test sample is measured against blank at 540nm.

Standard: The O.D. of Reagent 2 (Cyanmethemoglobin standard) is directly measured either on the spectrophotometer on the colorimeter against Drabkin"s solution.

Calculations:

Blood Hb in gm % = OD of test/OD of std ×concentration of std. Mg% × Dilution factor
= OD of test/OD of std × 60 × 0.250
= OD of test/OD of std × 15

Precaution:

Store Drabkin"s reagent in the brown bottle, as it decomposes on light exposure. Once the hemoglobin cyanide solution has been prepared, Hbestimation must be carried out within 6 hours.

Drabkin"s solution should be clear and pale yellow. If it is turbid, it should be discarded. It can be stored at a cool temperature, 4-6°C in the refrigerator.

Acid haematin method (SahiMehod):

It is recommended for the place where a colorimeter is not available. It is not recommended because all forms of Hb are not measured by this method.

Principle: Hb is converted to acid hematin by reaction with HCL. The acid haematin solution is further diluted with HCL acid until its color matches exactly that of a permanent standard comparator box. His Hb concentration is read directly from the hemoglobinometer tube.

Reagent and Equipment:

0.1 N Hydro Choric Acid, SahliHaemoglobinometer, Haemoglobinometer pipette. Aemoglobinometer tube.

Procedure:

i. Fill the haemoglobinometer tube up to the 20 mark with 0.1 N Hydro Chloric Acid.
ii. Draw the blood onto the haemoglobinometer pipette (20µl) from the anticoagulated test blood sample.
iii. Wipe out the surface of the pipette with the wet cotton.
iv. Blow the blood into the acid solution of the haemoglobinometer tube by adding 0.1 N HCL, until the color match.
v. Read the Hb concentration directly from the mark reached. The reading may be in % or in gm/dl.

Determination of Total Count of RBC:

Introduction:- The Human red cell is normally a circular, non-nucleated, biconcave disc. The red blood cell contains hemoglobin. The surface area of the red cell is much greater than that of a sphere of the same size. There is the exchange of oxygen & carbon dioxide is maximal with the biconcave configuration.

Clinical significance:- The total RBC count is performed to assess the red cell mass in the blood. The change in erythrocyte number is frequently detected in clinical practice by ordering estimation of hemoglobin rather than total RBC count, as estimation of hemoglobin is easy and less expensive more over total RBC count is still performed in some conditions to detect the red cell population, especially if the count is expected to be a very high as in poly cythemia.

Principle:- The blood specimen is diluted with RBC diluting fluid which does not remove the WBC but allows the RBC to be counted under 400X magnification in a known volume of fluid. Finally, the number of cells in undiluted blood is calculated.

Specimen:- Well mixed anticoagulated (EDTA) venous blood is mostly used.

Requirements:

i. RBC diluting fluid
   - Trisodium citrate - 3.2 gm.
   - Formalin - 1.0 gm.
   - Distilled water - 100 ml

ii. Micro-pipette

iii. Graduated pipette

iv. Improved neubauer chamber with coverslip

Method: Heamocytometric method [include improved hemocytometer chamber]

Normal Range: Male: 4.5-6.0×10^6 Cell/µl of Blood

Female: 4.0-4.8×10^6 Cell/µl of Blood

Procedure:

i. Collect all the equipment which is needed for practical, & clean all the equipment properly.
ii. Take adequate RBC diluting fluid in a watch glass.
iii. Make a finger prick under aseptic conditions & such blood into the pipette & dilute the blood with RBC diluting fluid.
iv. After proper mixing takes some amount of mixed diluting fluid through the pipette.
v. Discard the first two drops of fluid from the pipette.
vi. Charge the Neubauer's chamber and allow two to five minutes for a proper charge by which the cells settle down easily.
vii. Place the counting chamber.
viii. Switch to low power objective, adjust light and locate the large square in the center.
ix. Now switch to high power objective.
x. The red blood cells are counted in the four squares including the center of Neubauer's chamber.

Calculation:

Total RBC Count=Total NO of RBC × Diluting factor/Total no of area count × area of each square × depth of fluid

If Total No of RBC=N Diluting factor=200

Total NO of area=5

Total NO of each area square=1/5

Depth of fluid=1/10-0.1

So, Total RBC Count=N × 200/5 × 1/5 × 1/5 × 1/10= N × 200 × 50=N × 10,000

Determination of Total Count of WBC (Leukocyte):

Introduction: Leukocytes [White Blood Cell] are nucleated cells that are involved in the defense mechanism of the body, unlike red cells, White cells use the bloodstream primarily for transportation to

their place of function in the body tissue. Leukocytes are classified as granulocytes, & agranulocytes. Granulocytes are neutrophils, eosinophils, basophils & agranulocytes are lymphocytes & monocytes.

Clinical Significance: Total leukocyte counter is a part of the routine hematologic investigation to assess the nature & severity of infection in some diseases, alteration in leukocyte count alone may be diagnostic, but frequently the leucocyte count is ordered with other investigations especially with the differential leukocyte count to aid in diagnostic. When the total leukocyte count increase above the normal the condition is called leucocytosis and when the count decrease below the normal range then the condition is called leukocytopenia.

Specimen: EDTA mixed venous blood or fresh capillary blood.

Principle: Blood is diluted with the acid solution, which removes the red cells by hemolysis & accentuates the nuclei of the white cells. The counting of the white cells then becomes easy counting is done using a microscope under a low power objective and with knowledge of the volume of fluid examined & the dilution of the blood obtained. The number of white cells/mm^2 of undiluted whole blood is calculated.

Method: Haemocytometric method [include improved hemocytometer chamber

Requirement:

i. Microscope
ii. Improved neubauer chamber
iii. WBC pipette
iv. WBC diluting fluid [Turk"s fluid] Composition of WBC diluting fluid
v. 1% glacial acetic acid solution.
vi. Creation violet stain or aqueous methylene blue [0.3%]
vii. Distilled water.

Normal Range:

Adults: 4,000-11,000/mm^3

Newborns: 10,000-25,000/mm^3

blood Infants: 6,000-18,000/mm^3

Children: 5,000-15,000/mm^3 of blood

Procedure:

i. Assemble all equipment needed for practical, clean it properly.
ii. Take adequate WBC diluting fluid in a watch glass.
iii. Make a finger prick under aseptic conditions & such blood into the pipette & dilute the blood with WBC diluting fluid.
iv. After proper mixing takes some amount of mixing diluting fluid through the pipette.
v. Discard the first two drops of fluid from the pipette.
vi. Charge the Neubauer chamber a n d allow two-five minutes for a proper charge by which the cells settle down easily.
vii. Place the counting chamber.
viii. Switch to low power objective, adjust light & located the large square in the center.
ix. Now switch to high power objective.
x. The white blood cells in the four corner squares are counted.

Calculation:

White cell total count = Total NO of WBC × diluting factor/Total number of area count × area of each square × depth of fluid

Where i) Dilution = 1:20,

ii) Area counted = 4

iii) Each square = 1mm
iv). Depth of fluid = 1/10 = (0.1)
v). No of white cells counted = N

Now Total white blood cells = N × 20/4 × 1 × 1 ×1/10 = N × 20 × 10/4 = N × 50

Determination of Differential Count of WBC:

Introduction: Differential count is the percent (%) distribution of various white cells in the peripheral blood as determined from a blood smear stained with a polychromatic stain.

Clinical Significance: Differential count is useful to identify a change in the distribution of white cells which may be related to the specific type of disorder. It also gives an idea regarding the severity of the diseases and the degree of response of the body.

Principle: The polychromic staining solution contains methylene glue and casing. The basic and acidic dyes induced multiple colors when applied to the cell. Methanol acts as a fixative and also a solvent the natural components of the cell are stained by both dyes.

Specimen: EDTA-Anti coagulated blood or fresh capillary blood.

Normal Range:

i). Neutrophils -40%-75% ii). Eosinophils -1%-6% iii). Basophils -0%-1% iv). Lymphocytes-20%-45% v). Monocytes-2%-10%

Requirements: Microscope slider & A glass spreader; Cedarwood oil

Reagent:

Leishman stain:

i.	Leishman powder	0.5gm
ii.	Acetone free methyl alcohol	100ml
iii.	Eosin	7gm

Buffer (pH 7)

Procedure: Thin smear is prepared by spreading a small drop of blood evenly o n a slide.

Blood film preparation:

i. Take a clean dry grease-free slide.
ii. Transfer a small drop of blood near the edge of the slide.
iii. Place the spreader slide at an angle of 30°degree. Pull back the spreader until it touches the drop of blood. Let the blood run among the edge of the spreader.
iv. Push the spreader forward to the end of the slide with a smooth movement.
v. Dry the blood smear at room temperature.

Staining the film:

i. Cover the smear with the staining solution by adding 10-15drops to the smear.
ii. Wait for 2 minutes.
iii. Add an equal number of drops of buffer solutions. Mix the reaction mixture adequately by blowing on it through a pipette. Wait for 10 minutes.
iv. Wash the smear by using tap water.
v. Stand the slide in a draining rack.

Examination of the film:

i. First, examine the stained smear under the low power (40 X) and adjust the film.

ii. Then examine the film under high power (60 X).
iii. Examine the film by moving from one field to the next field. Record the type of Leukocytes seen in each field.
iv. Count at least a total of 100 Leucocytes.

Determination of Platelet Count:

Introduction: Platelets are the smallest cells in blood circulation, they participate in the blood clotting process. They are membrane encapsulated fragments of megakaryocytes. Although they are enucleated and have a diameter of 1-4 (μ) microns. The life span of platelets is 9-10 days. Its determination is requested in the investigation of bleeding disorders.

Clinical Significance: A decrease in platelet count known as Thrombocytopenia is often associated with prolonged bleeding and poor clot retraction. It also occurs in-Aplastic anemia, Megaloblastic anemia, Hypersplenism, Acute leukemia, Cytotoxic chemotherapy, HIV infection, and Septicaemia.

An increase in platelet count is known as Thrombocytosis, Importantly a high platelet count may also place risk for bleeding. It occurs in conditions like Polycythemia vera, Chronic myelogenous leukemia, Splenectomy, and Renal failure.

Normal Range: 1.5-4.5 lakhs/mm^3

Specimen: EDTA anti=coagulated blood is the recommended specimen for platelet count. Capillary blood can be used but venous blood generally for satisfactory results as capillary blood gives lower values than venous blood.

Principle:- The diluents prevent coagulation as it fixes the platelets and prevents them from clumping. No attempt is made to lyses the RBCs. Platelets are identified by their size, shape, and darker. The dye provides the background during cell counting. This dye does not stain the platelet and it is essential for the counting procedure.

Reagents: Diluting fluid:

i. Tri-Sodium Citrate (.106 M) -3.8gm
ii. Neutral formaldehyde (40%) -0.2ml
iii. Brilliant cresol blue -0.1gm
iv. De-ionised water -100ml

Equipment:

i. Haemocytometer.
ii. Sahli's pipette.
iii. Microscope.
iv. Test tube.
v. Petri dish with filter paper.

Procedure:

i. Transferred 3.98ml. of diluents into a test tube.
ii. The blood specimen is gently mixed for about 2minute.
iii. 20/μl of fresh non-coagulated blood is added into the test tube and the contents of the pipette are rinsed with the diluting fluid for 3-4 minutes.
iv. Immediately the diluents are mixed with the specimen for at least 5 minutes.
v. The hemocytometer chamber is then placed on the stage of the microscope. By using Sahli"s pipette a drop of a specimen is transferred to each side of the counting chamber.
vi. Place the mounted hemocytometer on a moist surface and let it stay undisturbed for 15 minutes.

The coverslip is placed on the counting chamber.

vii. The red cell counting is focused under low magnification and then under high magnification of a microscope.

Observation:

Platelets are bluish and must be distinguished from debris. They are oval, round, or shaped in various sizes from 1-5µm.

Calculation:

Platelet count/µm or mm^3 = Number of platelets × dilution/ volume of fluid
= (N × 200)/.1
Where, volume of fluid = 1 × 0.1 = 0.1 µm
Dilution = 400/.02 = 200

Determination of Erythrocyte Sedimentation Rate (ESR) :

Introduction: The whole blood is allowed to settle, and sedimentation of the erythrocyte will occur the rate at which the red cells fall is known as the erythrocyte sedimentation rate.

Clinical Significance:

Erythrocyte sedimentation rate or ESR is a none specific test that reflects changes in plasma protein that accompany most of the acute & chronic infections of some of these pathologic conditions. Its means if there is high ESR, we can say that there is a disease but we cannot diagnose the disease ESR does not change in functional disease

Principle:

The erythrocyte sedimentation rate of ESR of blood may be determined by the height of mm of a column of clear plasma lying about a ventral column of blood at the end of 1hour where a sample of blood is treated with an anticoagulant is left standing in a long tube place vertically.

Specimen: Citrated ant coagulated blood determines ESR within 2 hours after blood collection.

Method: WESTERGREN Method, wintrobe method

Normal Range:

1). Westergern method - Male-5-15 mm/hr
- Female-5-20 mm/hr
2). Wintrobe method - Male-0-9mm/hr-
- Female-0-20mm/hr

Reagent:

1). Anticoagulant - 1mm of 3.8% Na citrate sol
2). Patient blood - 4ml

Westergren Method:

i. Westergren's pipette (open at both ends) is about 30 cm long with a bore diameter of about 2.5mm.
ii. The lower 20 cm are marked from 0 (top) to 200 (bottom).
iii. The anticoagulant used is a 3.8% trisodium citrate solution. One part of the anticoagulant is added to 4 parts of the blood.
iv. The pipette accepts about 1 ml of blood. Fill the pipette by sucking it till the 0 mark and clamp it vertically in the Westergre's rack.
v. Read the upper level of red cells exactly after 1 hour.

vi. This is a better method than windtrobe's since the reading obtained is magnified as the column is lengthier.

Calculation: Mean ESR = 1st hour reading + 2nd hour reading/2/2

Hematocrit Packed Cell Volume (PCV)

Introduction: Hematocrit means "Blood separation" the hematocrit measures the percentage of the volume of the packed red cells. Therefore, hematocrit is also known as packed cell volume [PCV].

Clinical Significance:

The value of hematocrit is used in the determination of blood indices, especially MCV & MCHC indices help in the diagnosis"s classification of various types of anemia.

Hematocrit is one of the important factors that determine the viscosity of blood.

Principle: Anticoagulated blood is taken in a wintrobe tube filled to the graduation mark & then centrifugal for the prescribed length of time the volume of packed cells is read directly from the graduation mark on the wintrobe.

Method: Wintrobe method

Normal Range: Adult Male - 46% [42%-52%] Adult Female - 42% [38%-48%]

Reagent: A sample of venous blood [fresh or EDTA blood]

Procedure:

i) Fill the wintrobe tube with blood with the help of the Pasteur pipette to the 10cm mark

ii) Place the wintrobe tube in one of the cups of the centrifuge.

iii) Centrifuge for 30 minutes at 3000 rpm [Rapid/minute]

iv) After 30 minutes, stop the centrifuge stake out the tube & read the packed cell volume.

Determination of Erythrocyte Indices

DETERMINATION OF MEAN CORPUSCULAR VOLUME (MCV)

MCV is derived from PCV and total erythrocyte count. This is the average volume of red cells. The volume is expressed in femtoliters (fl). MCV is calculated by the following formula.

MCV = PCV × 10/RBC count in million

Normal Values: MCV: 86±10 fl

DETERMINATION OF MEAN CORPUSCULAR HEMOGLOBIN (MCH):

The MCH is the average hemoglobin content of a red cell. The weight is expressed in a picogram (pg). It is calculated as follows:

MCH = Hb × 10/RBC Count in millions

Normal Values : MCH : 29.5±2.5 pg

MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (MCHC):

It is expressed as the average hemoglobin concentration per unit volume (100) of packed red cells. It is expressed in g/dl. MCHC is calculated by following two cannulas.

MCHC= (MCH/MCV) × 100

MCHC= Hb (g/dl) × 100/PCV (%)

Normal Values: MCHC : 32.5±2 g/dl or (%)

Determination of Reticulocyte count:

Introduction: Reticulocytes are Juvenile red cells that pass into the bloodstream from the bone marrow. Reticulocytes stay in circulation for about 24 hours and mature into erythrocytes.

Clinical Significance: The number of Reticulocytes in the blood circulations indicates the degree of activity of bone marrow, and when the marrow is very active their number increase this is known as Reticulocytosis, in the case of aplastic anemia the reticulocyte count is decreased.

Principle: Supra vital staining method is used for reticulocyte count. Blood is mixed with the stain and the stain enters the cells in living conditions. The RNA in the cell is precipitated by staining as a dark blue network or reticulum. The blood smear is made afterward, since a direct count is not possible, a relative count is taken against the number of red blood cells and expressed as a percentage of blood cells.

Specimen: EDTA-Anti coagulated blood is commonly used for fresh capillary blood.

Normal Range: 0.2-2% of RBC

Requirements:

i). Grease-free glass slides

ii). Test tube.

iii). Pasteur pipette with rubber teats.

iv). Capillary tube.

v). Test tube rack.

vi). Microscope.

vii). Reagent- Staining solution: It is prepared as follows:

- Brilliant cresyl blue 1.0gm or New methylene blue powder
- Sodium citrate 0.4gm
- Sodium chloride 0.85gm
- Distilled water 100 ml

Dissolve first sodium citrate in normal saline and then the dye. Filter it and store it in a plastic container. It is stable at 2-8°C.

Procedure:

i. Take 2-3 drops of blood and 1 drop of methylene blue powder and mix it properly.
ii. After gently mixing stay it for 20 minutes at room temperature.
iii. Now take this mixture through the Pasteur pipette
iv. Now drop one drop of this mixture on a slide.
v. Prepare a thin smear of the stained blood specimen with the help of a spreader slide and air dry it.
vi. Examine the smear first under the low power objective for scanning, and locate a thin portion of the smear when red cells are evenly distributed.
vii. Change to the oil immersion objective. Reticulocytes are identified by the fine deep violet filament and granules arranged in a network.

Calculation:

Reticulocyte percentage (%) = Number of reticulocyte count × 100/ Total number of RBC Count

III. COAGULATION PROFILE TEST:

Determination of Coagulation Time (CT)

Introduction:- This is also known as Lee & White clotting time, this method seems for coagulation factor.

Clinical Significance:- Only severe clotting factor deficiency is recognized by this method prolonged clotting time. This is indicting the missing coagulation factor.

Normal Range:- 4-9 minutes

Method:-

Lee & White method

i. Make a clean venipuncture with as little trauma as possible to the connective tissue between skin and vein.
ii. Withdraw 4-5 ml of blood from a dry glass syringe. Delivered 1ml of blood in each of the three 8 × 75 mm test tubes.
iii. The three tubes with blood are allowed to stand in the upright position at room temperature.
iv. Coagulation in the tube can be ascertained by tilting the tube or by gentle tipping. The first tube is gently tilted every minute and the remaining tubes are examined every half minute. The average clotting time in three tubes gives the result.
v. Since the speed of coagulation increased with temperature, the test should be done at a particular temperature (37°C).

DETERMINATION OF BLEEDING TIME (BT)

Introduction:- Determination of bleeding time recognizes vascular defect and platelet disorder.

Clinical Significance:- Prolonged bleeding time is generally found with thrombocytopenia (Platelet count < 50,000 cells/µl) and where there is a dysfunction, bleeding time is high with a normal platelet count.

Normal Range: Duke method: 2-5 minute

IVY method: 5-11 minute

Method: IVY"s method

i. A sphygmomanometer cuff is wrapped around the patient"s arm above the elbow and inflated to 40mm Hg and the same pressure is maintained throughout the test.
ii. The volar surface of the forearm is cleansed with rectified spirit and an area of skin devoid of superficial veins is selected. The skin is stretched laterally by thumb between thumb and forefinger of the left hand, and two separate punctures, 5-10 cm apart, are made in quick succession by freehand, using a disposable lancet or No.11 surgical blade. Any micro lancet with about one mm width and a cutting depth of 2.5 mm is suitable.
iii. Timing is begun as soon as the punctures are made and bleeding starts.
iv. Blood extruding from the cut is blotted off gently but completely with filter paper at 15/30 seconds intervals till the bleeding spots. The time is noted.
v. When the bleeding has ceased, a sterile adhesive strip is placed on the wounds.

Determination Of Prothrombin Time (PT)

Introduction: Prothrombin or factor II is synthesized in the liver under the influence of fat-soluble vit-E.

Clinical Significance:- Prothrombin (factor II) is synthesized in the liver in the presence of vitamin K. Factor VII is all so synthesized in the liver, which is related to prothrombin. In the clotting mechanism in stage 2, prothrombin is converted to thrombin, which transforms soluble fibrinogen into an insoluble fibrin clot. Abnormal prothrombin time suggests stage 2 defect. Prolonged prothrombin time is related to the deficiencies of factors II, V, VII, and X. Since excess of coumarin group drugs may lead to hemorrhagic conditions, prothrombin time determination is also used to monitor the drug therapy.

Normal Range: 14± 2 seconds

Specimen: Citrated plasma.

Name of the method: Quick"s method

Principle: Thronbokinase preparation (Thromboplastin preparation of human or rabbit brain) containing calcium ions is added to citrated plasma. IN the presence of factor VII, stage 2 of the coagulation mechanism is triggered and the clotting time is recorded after the addition of thrombokinase in the presence of calcium ions. Since factors XII, Xi, VIII, and platelets are bypassed, the test depends upon the activity of factors VII, V, X, II, and I. Deficiency of any of these factors may cause prolongation of clot formation in this test.

Requirements:

i. Water bath (37°C)
ii. Stopwatch.
iii. Test tubes (10 × 100 mm)
iv. Brain thromboplastin (or commercially available thrombokinase tablets)
v. 0.15 g/dl calcium chloride

Procedure:

i. Place a test tube containing about 2 ml of calcium chloride at 37°C
ii. Pipette 0.1 ml of plasma in a small test tube (10 × 100 mm).
iii. Add 0.1 ml of brain thromboplastin and mix (or use thrombokinase tablets according to manufacturer's directions).
iv. Wait for 2 minutes.
v. Add 0.1 ml of pre-warned calcium chloride solution, mix and start the stopwatch.

vi. Hold the tube in front of a source of light and keep tilting the tube gently. At the first appearance of a fibrin clot, stop the watch immediately.
vii. Record the time.
viii. Repeat steps 1 to 7 twice to check the reliability of the results.
ix. Repeat the procedure by using normal plasma.
x. Report prothrombin time in seconds.

IV. SPECIAL HEMATOLOGICAL TEST

Determination of Absolute Eosinophils Count

Clinical Significance: Increased eosinophils count is often associated with allergic reactions. Parasitic infections, brucellosis, and in certain leukemia. Increase in adrenal function. (Hyperadrenalism or Cushing's syndrome) is associated with a fall in eosinophils count.

Normal Range: 40-440/cu mm (µl)

Specimen: EDTA or heparinized blood

Principle: Blood is diluted with a special diluting fluid, which removes red cells and stains the eosinophils red. These cells are then counted under low power (10X) in a known volume of fluid by using a Neubauer counting chamber.

Requirements:

i. Microscope.
ii. Improved Neubauer chamber of Fuch Rosenthal counting chamber
iii. Diluting fluid: (Hingleman"s solution)

It is prepared as:

a) Yellow eosin
b) 95% phenol
c) Formalin
d) Distilled water.

Procedure:

i. Pipette 0.36 ml of diluting fluid in a test tube.
ii. Add 0.04 ml of blood (use Hb pipette, twice).
iii. Mix and keep for 10 minutes.
iv. Mix the diluents and charge the counting chamber.
v. Let it stand under a moist Petridis for about 2 to 3 minutes.
vi. Count the cells under low power objective with reduced light.

- if an improved Neubauer counting chamber is used, count cells in all nine squares.

Calculations:- Total number of eosinophils, cu mm (µl) = Number of cells counted X

10/0.9 X volume of fluid X area counted X depth.

4.4.5. CYTOLOGY

Specimen collection: In the collection and preparation of smears for cytological examination, the major objectives are:

1. Specimens should have a sufficient number of cells from the area in question.

2. Smears should contain well-preserved cells uniformly distributed so that each cell can be individually examined.

3. The staining procedure should clearly define the details of all structures. Scraping, obtained from the vagina, uterus, cervix, mouth, or ulcerated skin area is spread directly on a clean microscope slide. The smear is immediately fixed with a cytological spray fixative or in an alcohol-ether dip. Fixation or preservation is one of the most important steps in the procedure.

PAPANICOLAOU STAINING

The Papanicolaou staining procedure is used for examining exfoliated cells in secretions, exudates, transudates, or scrapings of various internal organs and tissue. Cells are fixed to a slide and stained first with hematoxylin, which stains the nuclei followed by OG_6 and EA-

50 or EA-65 as a counterstain.

Reagents:

EA-65 Multiple Polychrome stain

Light green S.F. Yellowish fast green FCF, Bismark brown eosin y, Phosphotungstic acid, Glacial acetic acid. Filter before use.

OG-6 Orange G stain

Orange G, Phosphotungstic acid. Filter before use.

Haematoxylin, Potassium alum, Glacial acetic acid, Sodium iodate. Filter before use.

Procedure:

Filter the Harris hematoxylin immediately before use.

1. Dip slide (s) gently 5-10 times in 95% ethanol.
2. Dip slide (s) gently 5-10 times in 70% ethanol.
3. Dip slide (s) gently 5-10 times in distilled water.
4. Stain 5 minutes in Harris Haematoxylin.
5. Place smears in distilled water. Rinse in successive changes of distilled water until the water remains colorless.
6. Dip slide (s) gently – times in 70% ethanol.
7. Dip slide (s) in a 1% solution of HCL in 70% ethanol until the smear shows a salmon color.
8. Rinse slide (s) well in 2 changes of 70% ethanol.
9. Dip slide (s) gently in a 3% solution of ammonium hydroxide in 70% ethanol until the smear takes on a blue color.

10. Rinse the slide (s) in two changes of 70% ethanol.

11. Dip slide (s) 5-10 times in 95% ethanol.

12. Stain slide (s) in OG-6 for 2 minutes.

13. Rinse slide (s) in two changes of 95% ethanol.

14. Stain slide (s) in EA-50 or EA-65 for 3-6 minutes.

15. Rinse slide (s) well in two changes of 100% methanol.

16. Rinse slide (s) in one part absolute methanol on part xylene.

17. Clean smear in xylene.

18. Mount with DPX or Canada Balsam.

Results: Nuclei are stained blue while cytoplasm displays varying shades of pink, orange, yellow and green.

PATIENT 'S REPORT

[Make a table and provide a report here]

5. Internship for Laboratory: Biochemistry

Goal: Interns need to acquire knowledge and skills of proper handling and documentation of clinical specimens for the biochemical analysis at reception during the internship period.

Objectives:

1. To know the guidelines and procedures for handling and documentation clinical specimens.
2. To apply specimen acceptance/rejection criteria.
3. To familiarize with a computerized system of specimen entry and distribution to respective laboratories.
4. To categorize specimens according to their turnaround time.

Tasks:

Sl. No.	Task	Observed/ Performed	Specific Learning
	Specimen collection		
	Laboratory test performed		
	Instruments handled		
	Analysis of results		
	Documentation Report preparation		
	Specific learning		

Methodology

5.1. CARDIAC PROFILE TEST

a. Total cholesterol
b. Triglyceride
c. HDL
d LDL VLDL
e. Serum LDH
g. Amylase

5.2. LIVER FUNCTION TEST (LFT)

a. Total bilirubin
b. Direct bilirubin
c. Indirect bilirubin
d. SGOT
e. SGPT
f. ALP
g. Total protein
h. Serum albumin
i. Serum globulin

3. KIDNEY FUNCTION TEST (KFT)

a. Urea
b. Creatinine
c. Creatinine clearance test
d. ACR

4. THYROID PROFILE TEST

a. TSH b. FT3 c. FT4

5. DIABETIC PROFILE TEST

a. Fasting plasma glucose
b. Post Prandial plasma glucose
c. Random glucose

Clinical biochemistry deals with the biochemistry laboratory to find out the cause of a disease. The clinical constituents of various body fluids such as blood (serum/plasma), urine, CSF, and other body fluids are analyzed in a clinical biochemistry laboratory. These determinations are useful in diagnosing various clinical conditions such as diabetes mellitus, jaundice, gout, hyperlipidemia, pancreatitis, rickets, etc. The biochemistry tests are very useful in determining the severity of disease of many organs such as the liver, stomach, heart, kidneys, and brain as well as endocrine disorders and related status of acid-base balance of the body.

Instruments, used in this department: (all steps must correlate with my project & literature)

CARDIAC PROFILE TEST

Clinical Significance:

Elevation of the total cholesterol values in plasma is considered to be a prime risk factor for coronary heart disease.

Increased triglycerides and VLDL values are taken as primary risk factors. A low serum triglyceride level is suggestive of intravascular lipolysis and enhanced formation of HDL. Hypertriglyceridemia, on the other hand, indicates less effective intravascular lipolysis and a reduced formation of HDL, which is associated with a higher atherogenic risk.

Elevated LDL is suggestive of atherogenic risk. Low levels of HDL-Cholesterol indicate a high risk of coronary heart disease.

DETERMINATION OF SERUM TOTAL CHOLESTEROL

Clinical significance:

Elevated levels of serum cholesterol are associated with atherosclerosis, nephrosis, diabetes mellitus, obstructive jaundice & myxedema. Decrease levels are observed in hyperthyroidism, malabsorption & anemia.

Method:

Enzymatic method (application on auto-analyzers). For photometric determination of cholesterol according to the “CHOD-PAP” method.

Normal range: 150-250 mg/dl

Sample material: Fasting serum or heparinized plasma

Test principle:

Cholesterol esters are hydrolyzed by cholesterol ester hydrolase to free cholesterol & fatty acids. The free cholesterol produced & pre-existing one is oxidized by cholesterol oxidase to cholesterol-4-en-3-one & hydrogen peroxide. Peroxidase acts on H_2O_2& liberated o2 reacts with the chromogen (4-aminophenazone/phenol) to form a red-colored compound which is read at 510nm (505-530nm).

Requirements:

1. Test tube
2. Micropipettes
3. Stopwatch
4. Semi autoanalyzer
5. Auto analyzer

Reagents:

1. Cholesterol working reagent
2. Chlosterol standard: 200mg/dl

Stability of the reagents:

All the reagents are stable at 2-8ºC

Procedure:

Dispense in the tubes labeled as follows-

	Test	Std.	Blank
Working reagent, ml	1.0	1.0	1.0
Serum, ml	0.01	-	-
Cholesterol Std, ml	-	0.01	-
Distilled water, ml	-	-	0.01

Mixed well & kept at 37ºC for 10 minutes. Read the absorbance of test & standard against blank.

Calculation: Serum total cholesterol, mg/dl= X 200

DETERMINATION OF SERUM HDL-CHOLESTEROL

When the serum is reacted with the polyethylene glycol contained in the precipitating reagent, all the VLDL and LDL are precipitated. The HDL remains in the supernatant and is then assayed as a sample for cholesterol using the cholesterol reagent.

Clinical significance of HDL-Cholesterol:

HDL-Cholesterol values below 40 mg% indicate the risk factor. The clinical risk factor is better

represented by the ratio of total cholesterol to HDL Cholesterol in case of heart ailments.

Risk Factor	Total Cholesterol/HDL Cholesterol	
	Female	Male
Average x 0.5	3.3	3.4
Average x 1.0	4.5	5.0
Average x 2.0	7.0	9.6
Average x 3.0	11.0	24.0

Normal reference values:

Prognostically	Standard	Risk Favourable	Risk level indicator
HDL Cholesterol	Male>55	35-55	<35
	Female>65	45-65	<45
LDL Cholesterol	Male<150	150-190	>190
Total Cholesterol	Male>3.8	3.8-5.9	<5.9
	Female>3.1	3.1-4.6	<4.6

Contents:

L1: Precipitating Reagent

S: HDL Cholesterol Standard

Specimen collection & preservation:

Blood should be collected in a clean dry container. Fasting blood is preferred for HDL-Cholesterol assay.

Plasma should be separated immediately from the cells. For plasma separation, any of the following anticoagulants may be used: EDTA 10 mg/ ml blood; HEPARIN 200 IU/ ml blood

HDL- Cholesterol value is stable in serum for 24 hours at 2-8ºC & 30 days when stored at

20ºC.

Procedure:

HDL separation:- Pre-warm at room temperature (25º-30ºC) the required amount of

Precipitating Reagent & Cholesterol working solution before use. Perform the assay as given below:

Pipetted into clean dry test tube: Precipitating Reagent(L1): 0.1 ml Sample : 0.1 ml

Mix well (cyclo- mixture machine may be used) & centrifuge at 2500-3000 r.p.m. for 10 minutes in a common laboratory centrifuge (1800 x g) to obtain a clear supernatant. Procedure for the Cholesterol assay (given from own performed literature).

Wavelength/filter: 505 nm (Hg 546 nm)/ Green

Temparature: 37ºC/r.t

Light path: 1 cm

Pipetted into clean dry test tubes labeled as Blank, Standard, & Test:

Addition Sequence	Blank(ml)	Standard(ml)	Test(ml)
Working reagent	1.0	1.0	1.0
Distilled water	0.05	-	-
HDL Standard(s)	-	0.05	-
Supernatant	-	-	0.05

Incubation:

Incubate the assay mixture for 10 minutes at 37ºC or 30 minutes at room temperature(25º-30ºC). After completion of the incubation, measure the absorbance of the assay mixture against blank at 510 nm. The final color is stable for two hours, if not exposed to direct light.

Calculation:

HDL-Cholesterol in mg/dl = (Abs. test/ Abs. standard) x 50 x 2 (Where 2 is the dilution factor due to the deprotinization step) Calculation of VLDL Cholesterol (mg/dl) = Triglycerides/5

Calculation of LDL Cholesterol (mg/dl) = Total cholesterol- (VLDL cholesterol+ HDL cholesterol)

DETERMINATION OF SERUM TRIGLYCERIDES

Determination of triglycerides involves enzymatic splitting with lipoprotein lipase. Indicator in quinone imine, which is generated from 4-amino antipyrine & 4-chlorophenol by hydrogen peroxide under the catalytic action of peroxidase.

Lipase

↓

Triglyceride (Glycerol+ Fatty acids)

↓

GK

↓

Glycerol + ATP Glycerol 3-phosphate +ADP

Glycerol 3- phosphate + o_2 →GPO Dihydroxyacetone phosphate + H_2O_2

$2H_2O_2$ + Aminoantipyrine + 4-Chlorophenol POD Quinonemine + HCL + $4H_2O$

Reagent:

1. Triglycerides working reagent
2. Triglycerides standard

Sample material:

Serum, heparin plasma, or EDTA plasma.

The stability in serum or plasma is 2 days between 20-25ºC. 7 days between 4-8ºC & min 1 year at -20ºC.

Assay procedure:

Wavelength 550 nm, Hg 546 nm

Lightpath 1 cm Temperature 20-25ºC/37ºC Measurement against blank

Pipette in the tubes as follows:

	Test	Standard	Blank
1. Working reagent(ml)	1.0	1.0	1.0
2. Serum (ml)	0.01	-	-
3. Standard(ml)	-	0.01	-
4. Distilled water(ml)	-	-	0.01

Mixed & incubated at 10 min at 20-25ºC or 5 min at 37ºC. Read the absorbance against the blank within 60 mins.

Calculation:

Serum triglycerides = OD Test/OD Standard x 100mg/dl

Clinical interpretation:

Epidemiological studies have observed that a combination of plasma triglycerides > 180 mg/dl (>2.0 mmol/l) & HDL cholesterol <40 mg/dl (1.0 mmol/l) predict a high risk of CHD.

Borderline levels (>200 mg/dl) should always be regarded in association with other risk factors for CHD.

DETERMINATION OF LDH

Method: UV-Kinetic(ref. no. 19)

Principle: LDH

L- lactate + NAD^+ ↔ Pyruvate + NADH + H

An increase in OD is measured after 45 sec by the interval of 1 min

Normal range: 70-240 IU

Sample: serum or heparinized plasma. Hemolysed serum should not be used for the test.

Reagent:

1. Buffer substrate
2. NAD^+ solution

Stability of the reagents: the reagents are stable at 2-8ºC

Procedure: Wavelength 340 nm Cuvette 1 cm light path

Temperature 37ºC

	1.0
NAD^+ solution, ml	0.2
Serum or plasma, ml	0.02

Mix, take after 45 sec & then by the interval of 1, 2, & 3 minutes. Determine the mean absorbencies change/ min (Δ A/ min)

Calculation: LDH, IU = 9807 x Δ A/ min. If the absorbance change Δ A/ min exceeds 0.100 at 340 nm , dilute serum 1:10 by using normal saline (result x 10). (i.e. 0.1 ml serum

+ 0.9 ml normal saline).

DETERMINATION OF SERUM AMYLASE

Amylase was originally referred to as diastase amylases a group of hydrolyses that split polysaccharides such as starch & glycogen. The enzymes split straight chains of polyglucans (amylase) at alternate α-1-4 links, forming maltose & some residual glucose. It also acts on branched starch amylopectine& glycogen forming, dextrins& residual glucose. Human serum amylase has an optimum pH of 6.9-7.0. full activity is displayed in the presence of various icons such as mono hydrogen phosphate, chloride, and bromide CTC.

Clinical significance:

Determination of serum & urine amylase is largely used in the diagnosis of disease of the pancreas & the investigation of the pancreatic function of acute pancreatitis, a transition rise in serum amylase activity occurs within 2-12 hours of the onset. Serum amylase levels return to normal by the 3^{rd} or 4^{th} day. Usually, high levels of amylase (4-6 food elevations above the reference limit) are observed during 12-72 hours.

The parallel increase in serum lipase confirms acute pancreatitis. elevation of serum amylase activities is reflected in the rise of urinary amylase activity. The urinary clearance of amylase is markedly increased in acute pancreatitis. Serum amylase also increases in traumatic lesions of the pancreas (including surgical trauma & radiological investigations) & carcinoma of the pancreas. Tumors of the lungs & serous tumors of the ovary can produce high levels of serum amylase with elevation as high as 50 times the upper reference limit. Both kinds of tumors can produce pleural effusion.

Salivary gland lesions caused by infection, obstruction, surgery, irradiation, & tumor have all been reported to elevate serum amylase (S. Type hyperamylasemia). Mumps & maxillofacial surgery can cause a twofold.

Biliary tract disease (such as cholecystitis) can cause up to a four-fold elevation of the serum amylase activity (due to primary or secondary pancreatic involvement). Macroamylase is complex between ordinary amylase (mainly S-type) & IgA, IgG, or other normal or abnormal high molecular weight proteins. Their presence may increase serum amylase activity(6-8 fold over the observed in health) since macroamylase can not be filtered through the glomeruli of the kidney due to their large size. In macroamylasemia, amylase activity in the urine is lower than normal because the kidney clears less amylase.

The amylase/creatinine clearance ratio (ACCR) has been defined & expressed as percentage as

follows:

Urine amylase (U/L) X Urine Creatinine (mg/l) ACCR % =

serum amylase (U/L) X Urine creatinine (mg/l)

Normal ACCR = 1-5%

In macroamylasemia: <1% In acute pancreatitis: >5% X 100

Elevations in ACCR are also observed in renal insufficiency, burns, ketoacidosis & light chain proteinuria.

Laboratory methods: Amylase was previously measured by iodometric&saccharogenic methods which required long incubation periods. Turbidimetric methods were relatively first but they required special instrumentation & it was difficult to prepare stable & reproducible starch solutions. The method based on a modification of wallenfels et al is a short & simple

rate of reaction (visible kinetic) method. A substrate used in this method is silyl-blocked-p- nitrophenyl-α-D-maltoheptoside, which undergoes spontaneous degradation by α-glucosidase

&glucoamylase. Resultant nitrophenol is the yellow-colored complex that can be measured at

405 nm.

Method: Colorimetric (amyloclastic, iodometric)

Principle:

Amylase in specimen acts on the substrate, such starch the products formed are dextrins& maltose. After the incubation, when the end product is treated with the color reagent (iodine reagent), decrease in the blue color is observed, compared to that of the product with the blank. The disappearance of the blue color is direct to the amylase concentration in the specimen & gives the measure of amylase concentration in the specimen.

Enzyme units: Caraways unite: Caraway defined the enzyme unit as the amount of enzyme that will hydrolyze 10 mg of starch in 30 mins to the colorless stage. (in the procedure, the substrate should be completely hydrolyzed by 800 units of amylase in 100 ml serum)

Sample material: Serum. The enzyme is quite stable. Activity loss is negligible even at room temperature it is stable for 3 months refrigerated.

Requirements:

1. Test tubes
2. Stopwatch
3. Water bath
4. Photometer

Procedure:

	Test	Blank
Buffer substrate, ml	2.5	2.5
Keep at 37ºC for 5 min.		
Serum, ml	0.1	-
Mix, 37ºC for 7 min.		
Working color reagent, ml	2.5	2.5
Serum, ml	-	0.1
Distilled water, ml	20	20

Mix & read test & blank against distilled water, at 660 nm.

Calculation:

$$\text{Serum amylase} = \frac{\text{OD of blank} - \text{OD of test}}{\text{OD of blank}} \times 400$$

D**ETERMINATION OF SERUM URIC ACID**

Uric acid is the end product of purine metabolism. Uric acid is excreted to a large degree by the kidneys & to a smaller degree in the intestinal tract by microbial degradation. Increased levels are found in gout, arthritis, impaired renal function, & starvation. Decreased levels are found in Wilson"s disease, Fanconi's syndrome & yellow atrophy of the liver.

Method: Uricase/ PAP method

Principle:

Uricase converts uric add to allantoin & hydrogen peroxide. The hydrogen peroxide formed further reacts with a phenolic compound & 4-amino antipyrine by the catalytic action of peroxidase to form a red-colored quinone imine dye complex. The intensity of the color formed is directly proportional to the amount of uric acid present in the sample.

Uricase→Uric acid allantoin + H_2O_2→Peroxidase →H_2O_2 + 4- amino antipyrine→ Red quinone imine + H_2O_2 + Phenolic Compound.

Normal reference values:

Serum/Plasma (male): 3.4 – 7.0 mg/dl

(Female): 2.5 – 6.0 mg/dl

It is recommended that each laboratory establish its own normal range patient population. Sample material:

Serum, plasma, and uric acid are stable in the sample for 3 – 5 days when stored at 2 – 8ºC.

Procedure:

Wavelength/filter: 520 nm (Hg 546 nm)/ Yellow green

Temperature : 37ºC/ r.t

Light path : 1 cm

Pipette into clean dry test tubes labeled as blank, standard, & test

Working reagent, ml	1.0	1.0	1.0
Distilled water, ml	0.02	-	-
Sample, ml			

Mix well & incubate at 37ºC for 5 min. or at r.t. (25ºC) for 15 min. Measure the absorbance of the standard, & test the sample against the blank, within 30 min.

Calculation:

Uric acid in mg/dl = (OD of test/ OD of standard) X 8

Linearity:

This procedure is linear up to 20 mg/dl. If values exceed this limit, dilute the serum with normal saline (NaCl-0.9%) & repeat the assay. Calculate the value using the proper dilution factor.

LIVER FUNCTION TEST (LFT):

Clinical significance of LFT

In the differential diagnosis of the different types of jaundice.

To assess the severity of liver damage in known liver disease.

To follow the trend of the disease.

To screen the suspected case during an outbreak of infective hepatitis.

DETERMINATION OF SERUM BILIRUBIN

Principle:

Bilirubin is estimated by reacting it with diazotized sulfanilic acid obtained from sodium nitrate &sulfanilic acid. It forms a pink-colored azo compound. Direct bilirubin (conjugated or soluble fraction) reacts very quickly & is read by measuring the color developed in 15 seconds of reaction. The unconjugated or free bilirubin takes a longer time to react & requires an accelerator. Hence total bilirubin is measured using caffeine as an accelerator allowing the reaction to proceed for 5 minutes.

Clinical significance:

Total & direct bilirubin estimation in serum or plasma is used for the diagnosis, differentiation & follows up of jaundice.

The following table gives the findings:

Specimen collection:

Fasting, fresh, clear, nonhemolytic serum is preferred. Plasma collected using heparin may be used.

Reagent:

1. Reagent 1 (sodium nitrate)
2. Reagent 2 (sulfanilic acid)
3. Reagent 3 (caffeine)
4. Bilirubin standard (5mg/dl) (ready to use)

Reagent storage & stability:

All reagents included in the kit are stable at room temperature until the expiry stated on the table. Reagent 1 is stable at 2 – 8ºC.

System parameters:

	TOTAL BILIRUBIN	DIRECT BILIRUBIN
Type of reaction	Endpoint	Endpoint
Wavelength	546 nm	546 nm
Factor	16.2	16.2
Sample volume	100µl (6.1 ml)	100µl (0.1 ml)
Reagent volume	1.10 ml	1.10 ml
Light path	1.0 cm	1.0 cm

Procedure:

	TOTAL		DIRECT	
	BLANK	TEST	BLANK	TEST
REAGENT 1	-	0.05	-	0.05
REAGENT 2	0.1	0.05	0.1	0.05
REAGENT 3	1.0	1.0	-	-
SAMPLE	0.1	0.1	0.1	0.1

	Incubate for 5 min. at r.t. (25º-30º C)	Incubate for 15 min. at r.t (25º-30ºC)
Read absorbance against distilled water at 546 nm (530 – 570 nm) or green filter		

Calculation:

Serum bilirubin (mg/dl) = (OD of test – OD of sample blank) X F F = factor 16.2

The use of a ready-to-use dye standard instead of pure bilirubin is a practical way & a compromise for convenience.

Procedure for factor determination:

Read the absorbance of standard (5 mg/dl) at 546 nm or at a green filter(530- 570 nm) against distilled water several times till you get a consistence reading. Record it, take the average absorbance of the standard & calculate the factor as follows.

Factor determination:

F = 5/ average absorbance of standard

Under standard is given parameters factors range from 14 – to 17. Once a factor is obtained this can be stored in the instrument, under normal conditions recalibration is not required for the same batch of reagent.

Normal values:

Total bilirubin : 0 – 1.0 mg/dl Direct bilirubin: 0 – 0.3 mg/dl **Linearity:**20 mg/dl for total & direct bilirubin.

DETERMINATION OF SERUM SGPT (ALT)

Summary & clinical significance:

Serum Glutamate Pyruvate Transaminase (SGPT), also called Alanine Aminotransferase (ALT), belongs to the transferase class of enzymes. It is found to be distributed mainly in the liver & to a lesser extent in the kidney & muscles. In hepatitis of different etiologies, SGPT is an important indicator not only in the diagnosis of the ailment but also in assessing the prognosis & process of the disease. An elevated SGPT level is characteristic in alcoholics.

Principle:

L-Alanine + 2- Oxoglutarate ----------→ Pyruvate + Glutamate

Pyruvate + 2, 4 DNPH ---------- →Brownish red-colored complex

SGPT (ALT) catalyzes the transfer of amino group from L- Alanine to 2- Oxoglutarate to form pyruvate & L-Glutamate. The pyruvate thus formed reacts with 2, 4 Dinitrophenyl Hydrazine (2,4 DNPH) to form a corresponding Hydrazone, a brownish red-colored complex in an alkaline medium. The color intensity is directly proportional to the SGPT concentration in the serum & is measured photometrically

at 505 nm (490- 546 nm) or with a green filter.

Reagent composition:

1. Reagent 1---------Substrate reagent --------L- Alanine 200 mmol/l

2- oxoglutarate 10 mmol/l

2. Reagent 2---------Color reagent ---------- 2, 4- DNPH 5 mmol/l
3. Reagent 3---------Sodium Pyruvate ---------170 U/L
4. Reagent 4---------Alkaline reagent --------- Sodium Hydroxide 4N

Reagent preparation & storage:

Dilute reagent 4 (Alkaline reagent) 1:10 with deionized water (1 part Alkaline reagent + 9 parts Deionised water). All the other reagents are ready to use. All the reagents are stable at 2

– 8ºC up to the expiry date mentioned on the label when properly stored.

Specimen:

The clear, hemolyzed serum is preferred (serum should be removed from the clot as soon as possible after collection because of the presence of approximately 10 folds greater concentration of SGPT in erythrocytes than in serum). For a similar reason hemolysis of the specimen must be avoided.

Normal range: Male: Up to 42 U/ L at 37ºC Female: Upto 38 U/L at 37ºC

Procedure:

Pipette into test tubes labeled as a blank, calibrator, control, test & proceed as per given below.

Reagent	Blank	Calibrator	Control	Test
Substrate reagent, ml	0.5	0.5	0.5	0.5
Deionized water, ml	0.1	-	-	-
Serum sample, ml	-	-	-	0.1
Calibrator	-	0.1	-	-

Mix & incubate at 37ºC for 30 minutes

Color reagent, ml	0.5	0.5	0.5	0.5

Mix & incubate at 37ºC for 30 minutes

Alkaline reagent, ml(diluted)	3.0	3.0	3.0	3.0

Read absorbancies of all the tubes against distilled water at 505 nm(490- 546 nm) or with a green filter.

Calculation:

SGPT (ALT) activity in U/L = OD of sample X Concentration of calibrator.

DETERMINATION OF SERUM SGOT (AST)

Clinical importance:

Serum Glutamate Oxaloacetate Transaminase (SGOT), also called Aspartate aminotransferase (AST), belongs to the transferase class of enzymes. This enzyme shows high levels of activity in the heart, liver, skeletal muscles & kidneys. Since its level seems to be increasing enormously following Myocardial Infection (MI). Elevated levels are also seen in viral/ toxic hepatitis, hepatic & cardiac necrosis, muscular dystrophy & pulmonary embolism.

Principle:

L- Aspartate + 2-Oxoglutarate ----------→Oxaloacetate + L- Glutamate

Oxaloacetate + 2,4 DNPH----------→Brownish red-colored complex

SGOT (AST) catalyzes the transfer of amino group from aspartic acid to 2-Oxoglutarate to form Oxaloacetate & L- Glutamate. The Oxaloacetate thus formed reacts with 2,4- Dinitrophenyl Hydrazine (2,4-DNPH) to form a corresponding Hydrazone, a brownish red-colored complex in an alkaline medium. The color intensity is directly proportional to the SGOT concentration in the serum & is measured photometrically at 505 nm (490- 546 nm) or with a green filter.

Specimen:

The clear, hemolyzed serum is preferred (serum should be removed from the clot as soon as possible after collection because of the approximately 10-fold greater concentrations of SGOT in erythrocytes than in serum). For a similar reason hemolysis of the specimen must be avoided.

Normal range:

Male: Upto 40 U/L at 37º C Female: Upto 37 U/L at 37º C

(The expected value should be used as a reference only. It is recommended that each

laboratory should establish its own normal range)

Procedure:

Pipette into test tubes labeled as a blank, calibrator, control, test & proceed as per given below

REAGENT	BLANK	CALIBRATOR	CONTROL	TEST
Substrate reagent, ml	0.5	0.5	0.5	0.5
Deionized water, ml	0.1	-	-	-
Serum sample, ml	-	-	-	0.1
Calibrator, ml(conc. 160U/L)	-	0.1	-	-

Mix & incubate at 37ºC for 20 minutes

Color reagent, ml	0.5	0.5	0.5	0.5
Serum sample, ml	-	-	0.1	0.1

Same serum sample which is used above

Mix & incubate at 37ºC for 20 minutes

Alkaline reagent, ml (diluted)	3.0	3.0	3.0	3.0

Read the absorbance of all the tubes against distilled water at 505 nm (490- 546) or with a green filter.

Calculation:

SGOT (AST) activity in U/L = ∆A/ min. X 1746

Linearity:

This method is linear up to 300 U/L. For samples above the linearity limit, dilute suitably with saline & assay. Multiply by the dilution factor to calculate the end result

DETERMINATION OF SERUM ALKALINE PHOSPHATASE

Summary:

Alkaline phosphatase (ALP) is an enzyme of the hydrolase class of enzymes & acts in an alkaline medium. It is found in high concentrations in the liver, biliary tract epithelium & the bones. Normal levels are age-dependent & increase during bone development. Increased levels are associated mainly with liver & bone disease. Moderate increases are seen in Hodgkin's disease & congestive heart failure.

Principle:

ALP at an alkaline pH hydrolyses p-Nitrophenylphosphate to form p-Nitrophenol& Phosphate. The rate of formation of p-Nitrophenol is measured as an increase in absorbance which is proportional to the ALP activity in the sample.

ALP

p-Nitrophenylphosphate ---------- →p-Nitrophenol + phosphate

Normal range:

Serum (Adult) ---------80-290 U/L at 37º C (children) ------245- 770 U/L at 37º C

(it is recommended that each laboratory establish its own normal range representing its patient population)

Reagent:

1. Buffer reagent (L1) ----------2X150 ml
2. Substrate reagent (T1)----- 2X 10 Nos.

Storage/ stability:

Content is stable at 2 – 8 ºC till the expiry mentioned on the labels.

Reagent preparation:

Working reagent: Dissolve 1 substrate tablet in 15 ml of buffer reagent. The working reagent is stable for at least 15 days when stored at 2 – 8 ºC.

The substrate is light & temperature sensitive. Take adequate care, especially after reconstitution.

Sample material:

Serum (free from hemolysis. ALP is reported to be stable in serum for 3 days at 2 -8 ºC.

Procedure:

Wavelength / filter : 405 nm

Temperature: 37ºC Light path: 1 cm

Pipette into a clean dry test tube labeled as a test (T)

Addition sequence	Test
Working reagent, ml	1.0
Incubate at the assay temperature for 1 minute & add	
Sample, ml	0.02

Mix well & read the initial absorbance A_o after 1 minute & repeat the absorbance reading after every 1, 2, & 3 minutes. Calculate the mean absorbance change per minute (ΔA/ min.)

Calculation:

ALP activity in U/L = ΔA/min. X 2754

Linearity:

The procedure is linear up to 700 U/L at 37ºC. If the absorbance change (ΔA/min.) exceeds

0.250, use only the value of the first two minutes to calculate the result, or dilute the sample

1+9 with normal saline (NaCl 0.9%) & repeat the assay (Result X 10)

TOTAL SERUM PROTEIN DETERMINATION

Clinical significance:

Total serum protein values decrease below the normal range in different clinical conditions associated with nephrotic syndrome, malnutrition, cirrhosis, liver & other liver diseases in which liver cells are severely damaged. Increased total protein values may be found in multiple myeloma & conditions associated with high globulin concentration.

Normal range:

Serum protein: 6 – 8 gm/dl

Method: Biuret method

Principle:

Proteins react with cupric ions in an alkaline medium to form a violet-colored complex. The intensity of the color produced is directly proportional to proteins present in the specimen and can be measured on a photometer at 530 nm(or by using a green filter).

Requirements:

1. Test tubes
2. Micropipette
3. Test tube stand
4. Semi autoanalyzer

Preparation of the reagent:

1. Protein reagent
2. Protein standard

Stability of the reagent: Depends on the kits expiry date

Procedure:

Mono step method

Pipette in three tubes labeled as follows

Addition sequence	TEST	STANDARD	BLANK
Protein reagent, ml	5.0	5.0	5.0
Serum, ml	0.05	-	-
Protein standard (6 gm/dl), ml	-	0.05	-
Distilled water, ml	-	-	0.05

Mix thoroughly & keep at room temperature (25º- 30ºC) for exactly 10 minutes. Measure the intensities of the test & standard by setting blank at 100% T, by using 530 nm(green filter)

Calculation:

Serum protein = (OD of test/ OD of standard) X 6 gm/dl

DETERMINATION OF SERUM ALBUMIN

Method:

Bromocresol green method

Principle:

Albumin present in serum binds especially with bromocresol green at pH 4.1 to form green colored complex, the intensity of which can be measured colorimetrically by using 640 nm or a red filter.

Normal range: 3.3 – 4.8 gm/dl

Requirements:

1. Test tubes ------15X125 mm

2. Serological & graduated pipettes, 10 ml, 5 ml
3. Test tube standard
4. Push button pipette of 0.05 ml or serological pipette of 0.1 ml
5. Semi-auto analyzer
6. Serum

Preparation of reagent:

1. Albumin reagent
2. Albumin standard
3. Distilled water as sample blank

Stability of the reagent: Depends on kits expiry

Procedure:

Mono step method

Pipette into three test tubes labeled as follows -----like protein estimation

Calculation:

Serum albumin = (OD of test/ OD of standard) X 4

DETERMINATION OF SERUM GLOBULIN

Normal range: 1.8 – 3.6 gm/dl

Calculation: Serum globulin gm/dl= (Total protein – Serum albumin)gm/dl

A/G ratio = (Serum albumin, gm/dl/ Serum globulin gm/dl)

KIDNEY FUNCTION TEST

The kidney function test contains with:

1. Determination of blood urea
2. Determination of blood creatinine
3. Creatinine clearance test

DETERMINATION OF BLOOD UREA

Clinical significance:

Elevated levels of urea are observed in pre- renal, renal, & post renal conditions. Pre-renal conditions such as diabetes mellitus, dehydration, cardiac failure, severe burn, high fever, etc,

& renal conditions such as disease of kidneys, post-renal conditions such as enlargements of the

prostate, stone in the urinary tract, tumor of the bladder. Decreased values have been reported in severe liver disease, protein malnutrition & pregnancy.

Principle:

Urea reacts with hot acidic Diacetylmonoxime in presence of thiosemicarbazide& produces a rose-purple colored complex, which is measured colorimetrically.

Method: DAM (Diacetylmonoxime method)

Sample: Serum/plasma(0.01 ml is required)

Reagent:

1. Reagent 1: Urea reagent
2. Reagent 2: DAM
3. Reagent 3: Working urea standard, 30 mg%

Normal range: 20 – 40 mg/dl

Procedure:

	BLANK	TEST	STANDARD
Reagent 1, ml	2.5	2.5	2.5
Sample, ml	-	0.01	-
Reagent 3, ml	-	-	0.01
Mix well			
Reagent 2, ml	0.25	0.25	0.25

Mixed well & kept the tubes in the boiling water exactly for 10 minutes & cooled immediately under running tap water for 5 minutes. Mixed by inversion & measured the color intensity within 10 minutes using a green filter against blank.

Calculation: Serum/Plasma Urea(mg/dl) = (OD of test/ OD of standard) X 30

DETERMINATION OF BLOOD CREATININE

Clinical significance:

Creatinine is a waste product formed in muscle from the high energy storage compound, creatine phosphate. The amount of creatinine produced is fairly constant & is primarily a function of muscle mass. Creatinine is removed from the plasma by glomerular filtration & then excreted in urine without any appreciable resumption by the tubules.

Creatinine is used to assess renal function; however, serum creatinine levels do not start to rise until renal function has decreased by at least 50%. Congestive heart failure, shocks & mechanical obstructions of the urinary tract may also contribute to an elevated level of serum creatinine. An elevated serum creatinine level due to obstructive may rapidly fall when the obstruction is removed by surgery.

Principle:

Acid reagent differentiates color developed using Jaffe‟s reagent by (1) Creatine + reactive

noncreatinine substances & (2) reactive noncreatinine substances.

Colored developed before & after the addition of acid reagent is measured photometrically at 510 nm (500 – 540 nm/ green filter) & the difference is proportional to creatinine concentration.

Creatinine + Alkaline Picrate-------- →Creatinine Picrate complex

Method:

Alkaline Picrate method

Normal range:

Men: 0.9 – 1.4 mg/dl Women: 0.8 – 1.2 mg/dl **Sample:**

Fresh, clear serum with no hemolysis is the specimen of choice. Plasma prepared using heparin as an anticoagulant may also be used.

Reagent:

1. Creatinine (Picrate reagent)
2. Creatinine (Alkali reagent)
3. Creatinine (Acid reagent)
4. Creatinine standard, 3 mg/dl

Storage: All the reagents are to be stored at room temperature.

Procedure:

Deproteinization of the sample is not required.

PIPETTE INTO TEST TUBES	**STANDARD**	**TEST**
Working reagent, ml	2.5	2.5
Sample, ml	-	0.2
Standard, ml	0.2	-

Mixed well & incubated for 15 minutes at room temperature.

Read absorbance of standard(1) & test(1) at 510 nm against distilled water.

3 creatinine, ml	0.1	0.1

Mixed well & kept for 5 minutes at room temperature.

Read the absorbance of standard(2) & test(2) at 510 nm against distilled water.

Calculation:

Creatinine concentration mg/dl = TS/STD X 3

Where , TS = corrected absorbance of sample = test(1) – test(2)

STD = corrected absorbance of standard = standard(1) – standard(2)

DETERMINATION OF CREATININE CLEARANCE

Introduction:

This test gives a relatively accurate & useful measure of the glomerular filtration rate & also the excretory capacity of the kidney. The reasons for the degree of accuracy of creatinine clearance are (1) creatinine is not absorbed by the tubules (2) the effect of fluid intake & excretion on creatinine clearance is much less than that of urea & (3) the blood creatinine values relatively stable. The creatinine clearance values may be greater than the actual glomerular filtration rate when plasma creatinine levels increase considerably above the normal range.

The clearance of any substance is defined as the number of ml of plasma that contains the amount of that substance, excreted in the urine in one minute.

$$\text{Clearance} = \frac{UV}{S(P)} \times \frac{1.73}{A}$$

In the case of creatinine clearance

U = mg/ml of urine creatinine

S (P) = mg/ml of serum (or Plasma) creatinine

V = ml of urine excreted per minute

1.73 = standard average surface area of the normal individual

A = surface area of the patient

Normal range: a) For male: 105 ± 20 ml/min b) For female: 95 ± 20 ml/min

Preparation of the patient:

Requirement

A polythene container to collect 24 hours of urine & should contain a few thymol crystals (preservative)

Urine collection

a) The patients should be instructed to empty the bladder at the beginning of the period (8 am) & discard the urine.

b) Collect all urine passed until 8 o clock the next morning, emptying the bladder at the time & adding this urine to the 24 hours specimen.

c) The urine should be kept in a cool place.

d) Note: The diuretic drinks & drugs should not be given to the patients during the 24 hours of urine collection

e) A blood sample is collected in a test tube for serum f) Patients height & age are noted

Laboratory requirements:

1. Patients serum
2. 24 hours urine specimen

3. All other requirements are the same as those used for the determination of serum & urinary creatinine

Procedure:

a) Measure the volume of the collected urine specimen. b) Determination of serum & urine creatinine

c) Calculate creatinine clearance from the following formula:

$$\text{Creatinine clearance, ml/min} = \frac{\text{Urine creatinine mg/dl} \times \text{V}}{\text{mg/dl} \times \text{min}} \times \frac{17.3}{\text{Serum creatinine A}}$$

DETERMINATION OF BLOOD GLUCOSE

Clinical significance:

Accurate measurement of glucose in body fluid is important in the diagnosis & management of diabetes, hypoglycemia, adrenal dysfunction & various other conditions.

High levels of serum glucose may be seen in the case of diabetes mellitus, in patients receiving glucose-containing fluids intravenously, during severe stress & in cerebrovascular accidents.

Decreased levels of glucose can be due to insulin administration, as a result of insulinoma, inborn errors of carbohydrate metabolism, or fasting.

Method: Trinder"s method (GOD – POD)

Principle:

α- D- glucose in the sample is rapidly converted to the β-isomer by the action of mutarotase which then is oxidized to yield gluconic acid & hydrogen peroxide in the presence of glucose oxidase. The enzyme peroxidase catalyzes the oxidative coupling of 4- amino antipyrine with phenol to yield a colored quinone imine complex, with absorbance proportional to the concentration of glucose in the sample.

Mutarotase

α- D- glucose ----------→ β- D- glucose

Glucose oxidase

β- D- glucose + H_2O + O_2 ---------- →Gluconic acid + H_2O_2

Peroxidase

H_2O_2 + phenol + 4AAP ---------- →Red dye + $2H_2O$

Reagent composition :

1. Tris-Phosphate buffer ---------- 50 mmol/l
2. Glucose oxidase ---------- ≥20000 U/L
3. Peroxidase ---------- ≥800 U/L

4. Mutarotase ---------- ≥1000 U/L
5. Phenol ---------- 10 mmol/l

Reagent preparation:

The reagents supplied are ready for use. Protect from bright light.

Stability & storage:

The unopened reagents are stable till the expiry date stated on the bottle & kit label when stored at 2 – 8ºC. The reagent develops a pink coloration on storage, this however does not affect the performance of the test.

Specimen collection & handling:

Use a fresh hemolyzed serum. The stability of glucose in the specimen is reduced by bacterial contamination & by glycolysis. Serum or plasma should be separated from the cells, as soon as possible, to prevent glycolysis. The addition of sodium fluoride is recommended to inhibit glycolysis. Serum/ plasma is stable for 3 days at 2 – 8ºC. It is recommended to perform the assay with freshly collected samples.

Requirements:

1. Semi-auto analyzer
2. cuvettes
3. pipettes
4. reagent
5. sample
6. Glucose standard (100 mg/dl)

Procedure:

Pipette in the tubes labeled as follows

	TEST	STANDARD	BLANK
Glucose reagent, ml	1.0	1.0	1.0
Serum/Plasma, ml	0.01	-	-
Glucose standard; 100 mg/dl , ml	-	0.01	-
Distilled water, ml	-	-	0.01

Mix & keep at 37ºC for 15 minutes or at room temperature for 30 minutes. Measure the intensity of the color at 505 nm.

Calculation: Serum/Plasma glucose, mg/dl = (OD of test/ OD of standard) X 100

Linearity: The linearity of the method is up to 500 mg/dl.

PATIENT 'S REPORT

[Make a table and provide a report here]

6. Internship for Laboratory: Microbiology

Goal: Interns need to acquire practical skills in standard microbiological examinations during the internship period.

Objectives:

1. To select appropriate media for various clinical specimens.
2. To process specimens for isolation of pathogenic microorganisms.
3. To identify microorganisms encountered in the clinical laboratory.
4. To exhibit knowledge of environmental influences on microbial growth.
5. To differentiate between normal flora and pathogens.
6. To interpret antimicrobial sensitivity patterns.
7. To apply methods of sterile techniques in the laboratory at all times.

Tasks:

Sl. No.	Task	Observed/ Performed	Specific Learning
	Specimen collection		
	Laboratory test performed A. Media Preparation B. Specimen inoculation and incubation C. Gram staining D. Special colony characteristic E. Important biochemical tests F. Bacterial identification: kits and automated systems G. Antibiotic susceptibility test: disk diffusion and automated system		
	Instruments handled		
	Analysis of results		
	Documentation Report preparation		
	Specific learning		

Methodology

A. Media :

Preparation:

1. Sterilization in the Autoclave and Hot air oven.
2. Preparation of cultural media.

B. Bacteriology:

1. Sample collection.
2. Staining of bacteria.
3. Methods of Inoculation.
4. Culture of Bacteria.
5. Biochemical test.
6. Anti-microbial Sensitivity test.
7. Diagnosis of leprosy.

C. Serology:

1. VDRL or RPR test.
2. ASO test.
3. CRP test.
4. RA test.
5. WIDAL test.
6. HBsAg test.

D. Mycology:

1. Diagnosis of Pathogenic fungi.

E. Protozoology:

1. Examination of blood for the malarial parasite.

INTRODUCTION TO DIAGNOSTIC MICROBIOLOGY

Microbiology involves the study of microscopic organisms. Although microorganisms are generally beneficial and essential for life, some are however pathogenic and cause infectious diseases. The diagnostic microbiology laboratory is engaged in the identification of infectious agents.
The infectious agents are broadly classified as viruses, bacteria, mycosis agents, and parasites. Identification of infectious agents is the principal function of the diagnostic microbiology laboratory.

Following are the steps which are involved in microbiology laboratory:

Collection of specimens for microbiological examinations: The collection of specimens for microbiological examinations needs great attention to avoid all risks of contamination. The specimen should be collected in a properly labeled sterile container obtained from the department of microbiology.

1. Urine- to avoid contamination of genital flora, collection of midstream urine is suggested. Urine should be collected in a sterile wide-mouthed container with a cotton plug. Before collecting urine the area is washed with soapy water and dried. If there is a delay in processing, the urine should be stored in a refrigerator at $4^{o}C$.

2. Blood: The blood is drawn by vein puncture and thorough disinfection of the skin. Three bottles of the broth should be incubated; one for aerobic growth, one for anaerobic growth, and the other is incubated in 5-10% carbon dioxide.

3. Stool: Care should be taken to avoid contamination during the collection of stool.

Cartons of waxed cardboard, which can be easily inserted, prove to be a good container to collect feces.

4. Sputum: For sputum collection, the patient is asked to rinse the mouth with clean water to avoid contamination of oral microflora. The first-morning sample is best for most purposes. It should be collected in a wide-mouthed, sterilized, and transparent bottle with a screw-capped lid.

5. Pus swab: In case of superficial lesions, such as skin abscess or urethral pus, the local lesion is first cleaned with spirit, and then a swab soaked in warmed normal saline is rubbed against the lesion.

6. Throat swab: Throat swab is suggested in case of throat infection. The patient"s mouth should be opened as widely as possible. The tongue is depressed against a spatula and the sterilized swab is rubbed against the lesion.

Types of Culture Media generally used in Microbiological Laboratory:

The most common media which are generally used in clinical microbiology laboratories are described here-

Pepton Water:- This is a common liquid medium that is used as a base in sugar fermentation and for the Indole test. It can be used for seed culture for antibiotic sensitivity testing by the Kirby-Bauer method.

Formula:-

Peptone-10gm

Sodium chloride – 5 gm

Distilled water-1000ml

Ph- 7.6

Preparation:

All the ingredients are mixed with gentle heating and the pH is adjusted.

The solution was distributed in small test tubes in 4 ml quantities.

Then autoclaved at 121^{O}c for 15 minutes.

Nutrient Agar:- It is a basic transport culture medium used in the preparation of blood agar and other media. it is used to maintain cultures of control organisms in semisolid form and in solid form.

Formula:

Peptic digest of animal tissue- 5gm.

NaCl– 5gm.

Beef extract -8gm.

Yeast extract – 1.5 gm

Agar -15 gm

Distilled water – 1000ml.

pH should be maintained between 7.2-7.6

Preparation:

At first solid ingredients except agar are kept in 1 litter conical flask.

Then 800 ml of D/W is added to that flask.

Then the ingredients are dissolved by using a glass rod, if necessary heat is provided to dissolve the ingredients.

The pH is adjusted in the range of 7.2-7.6

At last, agar is added and the solution is boiled for 1-2 mins.

The volume is made1000 ml by adding D/W.

The total volume is distributed in conical flasks, bottles, and tubes and sterilized by autoclaving at 121^{O}C for 15 mins.

Blood Agar:- It is a general-purpose enriched and solid medium, which supports the growth of most ordinary bacteria. Blood supplies a number of substances for the growth of fastidious

organisms.

Formula:

Nutrient agar – 200ml.

Sterile defibrinated sheep blood – 25 ml.

Preparation:

At first, sterilized nutrient agar is transferred to 50^{O}C in the water bath.

When it is collected to 50^{O}C, sterile defibrinated blood is added to it.

It is allowed to be mixed gently and dispensed aseptically in sterile Petri dishes.

the pH of the medium should be adjusted to 7.3

McConkey Agar:- It is a differential and the low selectivity medium used to distinguish lactose fermenting from non-lactose fermenting bacteria.

Formula:

peptic digest of animal tissue – 20 gm.

Lactose -5 gm.

Neutral red – 0.07 gm.

Agar - 20 gm.

D/W – 1000 ml.

Preparation:

At first 500 ml of D/W is taken in a conical flask and weight lactose, neutral red, and peptic digest of animal tissue are added.

It is then allowed to mix properly.

Then sodium taurocholate and agar are added and mixed.

Rest 500 ml of D/W is added to make the total preparation 1000 ml.

pH is maintained in the range of 7.2-7.6.

the suspended media is autoclaved and plating is done in sterile Petri plates.

After cooling it is kept in the refrigerator for maintaining its sterility.

MULLET- HINTON AGAR

This medium is used for the diffusion method of anti-microbial susceptibility testing of bacteria (Kirby-Bauer Method).

Formula:

Beef infusion – 300gm.

Casein acid hydrolysate – 17.5 gm

Starch – 1.5 gm

Agar – 17 gm

D/W – 1000 ml.

Preparation:

The solid ingredients are allowed to dissolve in 1000ml of hot D/W in a conical flask.

pH is maintained in the range of 7.2-7.6. It is then sterilized by autoclaving at $121^{O}C$ for 15 mins.

Then it is cooled to 50 – 55^{O}C and dispensed aseptically in the Petri dishes.

Sterilization in The Autoclave

An autoclave is the most commonly used equipment for sterilization. It works on the principle that when water is heated in a closed container, saturated steam is produced under pressure. Usually, the pressure used is 15 lb/square inch for 15- 20mins. At this point, the temperature in the autoclave will be 121^{O}C.

1. The boiler is fixed with water to a point just below the level of the bottom of the basket.

2. The plugs of the flask containing media are covered with wrapping paper tied with a string around the neck of the flasks. All the media to be sterilized are placed in the basket.

3. Then the lids are closed and screws are tightened.

4. Air outlet valve should be opened and safety valves are adjusted to the required pressure (15-20 lbs).

5. The source of the heat is turned on and all air should be expelled out through the vent cock as a steady flow of steam.

6. Then the vent-cock is closed and it is allowed to increase the pressure up to the required level.

7. The sterilization technique should be continued for about 20 minutes. The heaters are then put off and pressure gauze is allowed to turn to zero (O).

8. The vent-cock is opened slowly and the internal pressure is allowed to adjust itself with that of the outside atmosphere (it takes about 10-15 mins).

9. At last the autoclave is opened and all the satirized materials are taken out carefully.

Method of Inoculation a number of techniques have been employed for the isolation of microorganisms in natural environments. The most commonly used techniques for isolation are:

Streak plate technique:

Streak culture is the routine procedure for bacterial isolation in pure culture. The surface of a semisolid medium in Petri dishes may be inoculated with the specimen by several methods. The primary inoculation may be made by a loop, swab, or other suitable devices.

A platinum or nichrome wire loop of 2-4 mm diameter with 2-3 inch long wire is first sterilized in the Bunsen burner and cooled by touching an uninoculated part of the medium. A loop full of the specimen is gently smeared onto the surface of a well-dried plate of medium near the peripheral area. The inoculums are then thinly spread in parallel lines in different segments of the plate. The loop is sterilized between different sets of streaks. the plate is incubated at 37^{O}C overnight. Confluent growth occurs at the primary site of inoculation and well-separated colonies appear on the final series of streaks.

II. IDENTIFICATION OF BACTERIA AFTER CULTURING

The following characteristics have to be studied when a bacterium has been obtained in pure culture.

Motility testing:

The motility test of bacteria gives an idea of their shape, approximate size, and general structure.

This test is particularly important in differentiating certain groups of gram- negative rod-shaped bacteria.

Method:

Hanging drop method.

Requirements:

Coverslip.

Petroleum jelly.

Microscope.

Sterile saline.

Procedure:

1. Petroleum jelly is applied at the four corners of the coverslip.
2. A loopful of culture is placed in the middle of the coverslip (if the specimen is dry,

a drop of saline is put on the specimen and mixed it properly).

3. Then a cavity slide is inverted over the drop placing the concave portion downwards.
4. The whole preparation is inverted so that the coverslip is on the top.
5. The suspension is examined at first under a low power lens and then a high power lens.
6. The results of the observations are recorded by indicting “Motile” or “Nonmotile” organisms.
7. After finishing the observations, the cavity slide is placed in disinfectant solutions.

STAINING METHODS:

GRAM STAINING:-

This is a useful differential staining procedure used in bacteriology which in addition to determining gross morphology, serves to differentiate bacteria into two distinctly separate groups.

a. Gram-positive. b. Gram-negative.

Principle: When a bacterium is heated with Methyl violet/Zenson violet/ Crystal violet and later decolorized with an acetone alcohol mixture, it is seen that some organisms resist the decolorizing effects of acetone or alcohol or both and remain colored violet, they are called gram-positive. Those that become colorless are called gram-negative. For proper visualization of colorless, they are counterstained with safranin and become colored pink.

Reagents:

1. Crystal violet stain:-

a. Crystal violet- 10 gm.

b. Absolute methyl alcohol- 500 ml.

After well mixing the staining solution is kept for 24 hours and then filtered and stored in amber-colored dropping the bottle.

2. Grams iodine solution:-

a. Iodine crystal- 6 gm.

b. Potassium iodide – 12gm. c. Distilled water – 800 ml.

It is stored in an amber-colored dropping bottle after mixing.

3. Decolouriser:- 95% alcohol and acetone are mixed in equal proportion. If

alcohol is not available, a rectified spirit can be used. the mixture is stored in a white dropping bottle.

4. Safranin:-

a. Safranin- 10 gm.

b. Distilled water – 1000ml.

The mixture solution is mixed well, filtered, and stored in an amber-colored dropping bottle.

Stability of the reagents:

All the reagents are stable at room temperature.

Procedure:

Smear preparation:

A clean grease-free dry slide is taken and one drop of normal saline is placed in the center of the slide.

The inoculating loop (nichrome) is sterilized on the flame of a Bunsen burner.

A suspension is made by adding a specimen by the sterile nichrome loop.

The suspension is spread in an oval-shaped and a smear is made.

The smear is allowed to dry in the air.

The smear is fixed by passing the slide 3-4 times through the flame quickly with the smear side facing up.

STAINING:

The slide is placed on the staining glass rods.

The smear is covered with a crystal violet stain and waits for one minute.

After that, it is washed under running tap water.

Then smear is flooded with grams of iodine solution and waited for one minute.

The iodine is drained off.

The smear is decolorized with alcohol-acetone (or rectified spirit) for 20 to 30 seconds until the purple stain just stops coming on the slide.

Then the smear is counterstained with safranin for one minute.

The smear is washed out in running tap water and is dried carefully by using a blotting paper.

The dried smear is observed microscopically at first under low power objective afterward under high power objective and finally under oil-immersion objective.

Observation:

Gram-positive organism----------dark violet. Gram-negative organism---------- light pink.

1. Gram-positive organisms are:

Cocci in clusters: Staphylococcus species.

Cocci in chains: Streptococcus species.

Cocci in pairs: Pneumococcus sp.(streptococcus pneumonia)

Bacilli without spores*: Corynebacterium sp.* Bacilli with spores : *Anthraxsp*(aerobic) *Clostridium sp*(anaerobic)

2. Gram- negative organism are:

Cocci in pairs : neisseriasp

Bacilli : enterobacteriaceae(E. coli)

Pseudomonas sp.

Comma shaped: vibrio sp. Coccobacilli: Haemophilus sp.

ACID FAST STAIN

The acid- fast stain is another differential stain used mainly to detect Mycobacterium that causes tuberculosis (Mycobacterium tuberculosis) and leprosy (Mycobacterium lepra).

Ziehl-Neelsen method (hot stain) :

This is recommended for the preliminary diagnosis of tuberculosis infection by the direct method. It serves to differentiate bacteria into two distinctly separate groups –

a. Acid-fast

b. Non-acid-fast

Principle:

When some organisms are stained with color-dye derivative like basic fuchsine and later decolorized with dilute mineral acid like 20% H_2so_4 and remain color red, they are called acid-fast.

Those who become colorless due to decolorizing effect of H_2SO_4 are called non-acid fast organisms. For proper visualization of the colorless, they are counterstained with Methylene blue and becomes color blue.

Reagents:

1. Carbol-fuchsine solution (saturated solution of basic fuchsine)

a. Sol- A:

Basic fuchsine powder-0.3 gm

95% ethanol -10ml.

The fuchsine is dissolved in alcohol and gentle heat may necessary to dissolve.

b. Sol- B:

Phenol crystal – 5gm. Distilled water – 100ml.

The phenol crystal is mixed with water heated to 56^oC

c. Sol- C:

10 ml of sol-a is mixed with 90 ml of solution B. It is allowed to remain overnight at room temperature and then it is filtered.

2. Acid-alcohol solution: Conc. HCl : 3 ml.

95% ethanol: 97ml.

OR Conc.H_2SO_4: 25 ml. D/W 75 ml.

3. Methylene blue counter stain Methylene blue: 0.3 gm Distilled water: 100 ml.

It is allowed to dissolve thoroughly and filtered.

Procedure:

a. A film of the specimen is prepared and heat-fixed.

b. The heat-fixed slide is placed on the staining rack.

c. The carbol-fuchsin stain is heated in a test tube on a Bunsen burner and then

that heat-fixed slide is flooded with stain and waits for 8 to 10 minutes.

d. The slide is washed with water. It is continued until the water that runs off is colorless.

e. Then the slide is covered with 20%H_2SO_4 for 1 minute. Finally, it is washed well with running tap water.

f. The slide is then covered with methylene–blue and left for 30 to 1 min. g. Then it is washed with running tap water and allowed to dry or blot carefully.

h. The slide is observed microscopically first under low power objective and then under high power objective and finally under the oil-immersion objective.

Observation:

Acid-fast organism bright red. The non-acid-fast organism is dark blue.

Number of acid fact bacteria in 10 fields	Report
None	Absent
1 to 2	Positive,+
2 to 10	Positive,++
10 to 100	Positive,+++
Above 100	Positive,++++

DIFFERENT BIOCHEMICAL TESTS

Following biochemical methods are useful in the identification of the bacteria isolated from wound swab.

Coagulase Test:

This test is used to differentiate Staphylococcus aureus from S. epidermis and S.aprophyticus.

Principle:-

S.aureus produces the enzyme coagulase which causes the plasma to clot by converting soluble fibrinogen to insoluble fibrin.

Requirements:-

Oxalate or citrated plasma.

Glass slides.

Normal saline.

Procedure:

1. A drop of physiological saline is placed on each end of a slide.
2. Thick suspensions of the organisms are made in each drop.
3. A drop of plasma is added to one of the suspensions and it is mixed gently.

Observation:

Look for clumping of the organisms within 10 secs.

Results:

1. Clumping within 10 secs – S.aureus.

2. No clumping within 10 secs- No production of coagulase.

Tube test:

1. 1.0 ml of a saline suspension of the organism is prepared.

2. 1.0 ml of plasma is added.
3. It is then incubated at $37^{O}C$ for 24 hours.
4. Formation of clot indicates the presence of S.aureus organisms.

Catalase Test:

This test is used to differentiate catalase producing bacteria such as Staphylococci from non-catalase-producing bacteria such as streptococci.

Principle:-

Catalase produced by the organisms acts on hydrogen peroxide to produce water and oxygen (indicated by bubbles).

Requirement: 3% hydrogen peroxide, test tubes.

Procedure:

1. 2-3 ml of hydrogen peroxide sample is poured into a test tube.
2. The growth of organisms is immersed in the test tube solution by using a sterile glass rod.
3. Look for immediate bubbling.

Observation:-

1. Appearance of bubbles: the presence of catalase-producing organisms.
2. No formation of bubbles: the presence of non-catalase-producing organisms.

Oxidase test:

This test is used to help in the identification of organisms that produces the enzyme oxidase. For example Pseudomonas, Neisseria, vibrio, and Pasturella sp.

Principle:

A colony of the test organisms is smeared on a filter paper, and soaked with a few drops of oxidase reagent. If the organisms are oxidase- producing, the phenylenediamine in the reagent is oxidized to a deep purple color.

Requirements:-

Oxidase reagent: 1.0 gm/dl tetramethyl-p-phenylenediamine dichloride in D/W (it should be prepared freshly).

Filter paper strips.

Procedure:

1. A piece of filter paper is placed in a clean Petri dish.

2. Two to three drops of freshly prepared oxidase reagent are added.

3. A colony of organisms is smeared on the filter paper by using a glass rod.
4. The reaction is observed.

Observation:-

1. Blue purple color – positive test.
2. No blue-purple color – negative test.

INDOLE TEST

This test is important in the identification of enterobacteria such as E.coli, P. Vulgaris, etc and differentiates from Klebsiella and Salmonella.

Principle:-

This test organism is cultured in a media containing tryptophan. The organism breakdown tryptophan and indole are released, and it is detected by the Ehrlich reagent.

Requirements:-

Kovac's reagent:-

Motility indole urea (MIU) medium.

Kovac"s reagent.

Formula and preparation:-

a. P-dimethylaminobenzaldehyde – 2.0 gm.

b. Isoamyl alcohol:- 30 ml c. Conc. HCl: 10 ml.

These ingredients (a) and (b) are dissolved in 10 ml of conc. HCl is stored in a brown bottle.

Procedure:-

1. The MIU medium is inoculated with test organism colonies.
2. Kovac"s reagent (about 0.5 ml is added and it is inoculated at $37^{O}C$).

Observation:- Looked for forming the red color of the reaction mixture.

Results:-

1. Reddening of mixture: Positive test.
2. No red color:- Negative test

METHYL RED TEST

This test is performed to differentiate enterobacteria. This test is employed to detect the production of sufficient acid during fermentation of glucose by bacteria and sustained maintenance of pH below 4.5.

Principle:-

Some enterobacteria when cultured in a buffered glucose peptone water, it ferments glucose to produce sufficient acidity, which gives a red color with a methyl red indicator.

Procedure:-

1. A colony of test organisms is inoculated into 0.5 ml of sterile glucose phosphate broth.
2. It is then incubated at 35-37^{O}C.
3. A drop of methyl red indicator is added and the color is observed.

Observation:-

1. Bright red color – positive test.
2. Yellow/Orange color – Negative test.

Citrate utilization test:

This test is performed for the identification of Enterobacteria.

Principle:-

This test organism is cultured in a medium containing Na- citrate, an ammonium salt, and a bromothymol blue indicator. The organisms use citrate (the only source of carbon) and ammonia (the only source of nitrogen). The citrate utilization is followed by an alkaline reaction (change of the color from light green to blue) and growth in the medium is indicated by the appearance of turbidity.

Requirements:-

1. Simmon"s citrate medium:-

Formula and preparation:-

a. Potassium dihydrogen phosphate: 1.0 gm. b. Sodium ammonium phosphate: 1.5 gm.

c. Magnesium sulfate: 0.2 gm. d. Sodium citrate: 2.5 gm.

e. Bromothymol blue: 0.016 gm. f. Agar: 15 gm.

g. D/W : 1000ml.

These ingredients are dissolved in about 900ml of D/W and after diluting to 1 lit, pH of this medium is adjusted to 6.7- 6.9.

Test tube method:-

1. 3-4 ml of sterile Simmon citrate medium is inoculated with a broth culture of test organisms.
2. It is then incubated at $37^{O}C$ for up to 4 days.

Results:-

1. Turbidity and blue color: Positive.
2. No growth (no turbidity and persistence of original color: Negative.

UREASE TEST

This test helps in differentiating enterobacteria. Proteus strains are strong urease producers.

Principle:-

The test organisms are cultured in MIU medium (or in Christensen"s urea broth). If the the strain produces urease, it acts on urea ammonia carbonate is formed with the release of Urease production is not to be considered negative till a day-old culture is tested.

Requirements:-

Mobility Indole Urea Medium.

Test tubes.

Procedure:-

1. MIU medium is inoculated with a colony of the test organisms.
2. It is then incubated at $35^{O}C$ overnight.
3. The medium is examined by looking for a red-pink color.

Observations:-

1. Red pink medium:-Positive test.
2. No red-pink color: Negative test.

Biochemical reactions on triple sugar iron agar(TSI):

Principle:-

1. Alkaline reactions(red color) are shown by the organisms, who fail to ferment anyone of the sugars.
2. Fermentation of the sugars is indicated by yellow color since the pH range of phenol is 6.8- 8.4 and the color changes from yellow to red. Since the glucose present on the surface of the medium is used up and since the surface the is exposed to the atmosphere, under anaerobic

conditions, the acid reaction of the surface reverts to alkaline(red color) in 18-24 hrs(which is the critical duration for this observation). In the butt, since anaerobic conditions exist, the color of the butt remains yellow.

3. Gas production (CO_2) is indicated by the splitting of the agar.

4. Production of H_2S imparts a black shade to the slant by reacting with ferrous ions. It is an indication of H_2S-producing organisms.

Procedure:-

1. Streak the TSI slant with a loop and stab with a straight needle.

2. Incubate at 37^oC for 18- 24 hrs.

Observation:

The various reactions to the slants are as follows:-

Colour of the Slant/Butt	Gas	H_2S	Sugar fermented	Possible organisms
Yellow/Yellow Acid / Acid	+	-	Glucose fermented Lactose fermented Or both lactose and sucrose fermented	E.coli Klebsiella sp. Enterobacter sp.
Yellow / Yellow	+	+	Glucose and sucrose fermented	Proteus vulgaris
Red/yellow Alkaline / Acid	–	–	Glucose fermented Lactose or sucrose not fermented	Shigella sp. Proteus morganite Providencialrettgeri.
Red/yellow Alkaline/ acid	-	+	Glucose fermented Lactose or sucrose not fermented.	S.typhi
Red / Yellow Alkaline / Acid	+	+	Glucose fermented Lactose or sucrose not fermented.	Other salmonella sp.
Red / Yellow Alkaline / Acid	+	-	Glucose fermented Lactose or sucrose not fermented.	Providencia Alcalifaciens
Red / Yellow Alkaline / Acid	-	-	None of the sugar	Don't belong to Enterobacteriaceae.

Antimicrobial Susceptibility Test

In bacterial infections, the somististy of the causative organism to the different therapeutic antimicrobial agents may be determined. Because of the changes in the drug-resistant variants of many of the infecting microorganisms, antibiotic sensitivity tests are not conducted, an important part of the bacteriological investigation. The physician is dependent on the antibiotic susceptibility report from the pathological laboratory to find the most effective antibiotic for a particular infectious disease.

Importance of Antimicrobial Susceptibility Test:-

The antimicrobial sensitivity test is mainly necessary if usually effective agents fail to produce the desired effects. In the treatment and control of infectious diseases which are caused by pathogens that are drug-resistant, sensitivity testing is helpful in selecting effective antimicrobial drugs.

Determination of Antibiotic Susceptibility by Disc Diffusion Technique (Kirby –Bauer Method) :-

A sterile cotton swab is inoculated with the inoculums to the Muller-Hinton agar plate by streaking the swab three times over the entire agar surface. Then 3-5 mins are allowed to dry the surface of the agar before inoculating the antibiotic discs, using sterile forceps. O a plate of 100 mm diameter seven discs are applied, one in the center and six in the periphery. The plates are then incubated at 37^0C for 16-18 hrs.

The zones of complete growth inhibition around each of the discs are measured. The diameter of the discs is including this measurement. The interpretation of zone size into sensitive, intermediate, or resistant is based on the interpretation chart.

Observation	**Report**
Zone less than 4mm	Resistant
Zone 4-12 mm	Intermediate
Zone more than 12 mm	Sensitive

Specimen of the Diagnosis of Leprosy:-

Leprosy caused by Mycobacterium leprae is a disease of great antiquity and even today.

Mycobacterium leprae causes diseases of the skin, nerves, and often the nose, it is most easily found in smears made from skin or occasionally from the nose. Laboratory diagnosis is primarily based on direct examination of the smear, because unlike Mycobacterium tuberculosis, Mycobacterium leprae cannot be cultured in the laboratory on artificial media.

Procedure for skin scrapings:-

1. At first before taking the scrapings, the skin is disinfected with 80% alcohol using alcohol-dipped gauze which is firmly rubbed over the scraping area. A sharp clean scalpel is sterilized in a flame.

2. The skin is held tightly between the thumb and first finger to prevent bleeding at the time

of cutting.

3. A cut (5mm long and 2-3 mm deep) is made by a sterile scalpel blade into the dermis(papillary layer).

4. Skin scrapings are scraped without bleeding by holding the scalpel sideways.

5. A small circular smear of the scraped material is made on a clean grease-free slide.

6. The smear is gently heat fixed and stained by using the Ziehl-Neelsen method.

LABORATORY DIAGNOSIS OF PATHOGENIC FUNGI:

Fungi are saprophytic or parasitic eukaryotic microorganisms, which possess a complex cell structure. Very few fungi are pathogenic. Most of the fungi are beneficial to man as they are utilized in the making of bread, fermented drinks, cheese, antibiotics, etc.

Specimen collection:-

Skin scrapings, nails, and hairs:-

1. Specimens for dermatophyte infection are skin scrapings and hair. At first, the infected the area is cleansed with 70% alcohol and then with sterile water.

2. From skin lesions about 2- 3 mm scales should be scraped by using a blunt scale.

3. The nails are scrapped with a scalpel. The first superficial scrapings are discarded. The friable material from under the nail is preferred for a laboratory test.

4. The infected hairs are plucked by using sterile forceps and placed in a sterile Petri dish.

Microscopic examination:-

1. Specimens are placed on a glass slide in a drop of 10 g/dl, sodium hydroxide, or potassium hydroxide. It is then covered by a coverslip.

2. After 10-30 minutes it is observed under the microscope.

Observations:

Skin, nails – Branching hyphae

Hair -Spores.

Culture:-

Specimens are inoculated on a Saboraud"s agar slants and incubated for 2-3 weeks a room temperature.

III. SEROLOGY

The laboratory procedure in serology consists of the collection and preparation of serum specimens and immunologic test procedures for specific serodiagnosis. The most common routine test are:-

Determination of Rapid Plasma Reagin (RPR) (Carbogen Antigen for Syphilis Testing):-

Syphilis is a sexually transmitted (venereal) disease caused by the spirochete Treponema pallidum. After infection, the host forms treponemal antibodies to Treponema pallidum in addition the host also forms non-treponemalantilipoidal antibodies in response to the lipoidal material released from the damaged host cell. These antibodies are traditionally referred to as 'reagins'.

Reagents:-

1. A particulate carbon suspension coated with lipid complexes.

2. Positive control serum.

3. Negative control serum.

Principle:-

During the test procedure, the specimen, serum, or plasma is mixed with the reagent and allowed to react for four minutes. If anti-lipoidal antibodies are present in the specimen, they will react with the reagent forming visible black floccules.

Specimen: - Fresh serum or plasma is used for testing. Samples not tested immediately may be stored at 2-8oC up to 48 hours.

Test procedure:-

All the reagents and samples are brought to room temperature before testing.

Qualitative method

i) One drop of a test specimen, positive control, and negative control are pipette onto a separate reaction circle of the disposable slide using a sample dispensing pipette.

ii) One drop of reagent is added next to the test specimen.

iii) Using a mixing stick the serum and reagent are mixed uniformly over the entire circle.

The dropper tips should not be touched by the liquid on the slide.

iv) The slide is rocked gently back and forth for exactly eight minutes for observing agglutination macroscopically.

Interpretation:-

Agglutination visible within two minutes is to be interpreted as a positive test result.

VDRL TEST

VDRL test is also performed to diagnose Syphilis. It is the old procedure and the modem procedure is RPR. In this case, used Syphilis causing Ag (inactivated) and heat inactive serum or plasma (Ab). -

Reagents:-

1. Heat-inactivated VDRL Ag.

2. Positive control serum.

3. Negative control serum.

Principle:-

During the test procedure, the specimen, serum, or plasma is mixed with the reagent (Ag) and allowed to react for four minutes. If antilipoidal antibodies are present in the specimen, they will react with the reagent forming antigenic clumps which are visible under a low-power microscope.

Specimen:-

Fresh serum or plasma is used for testing. Samples were not tested immediately maybe

stored at 2-8°C for up to 48 hours.

Test procedures:-

All the reagents and samples are brought to room temperature before testing.

DETERMINATION ASO LATEX

Streptococcus belongs to the family of lactobacillacae and the majority are facultative anaerobes. The facultative anaerobic streptococci are divided into two categories

a) those which produce soluble hemolysin

b) those which do not produce soluble hemolysin.

The first group of streptococci is called β-hemolytic streptococci which can be

further

subdivided into a group(a), group(b), group(c), and group(d).most of the strains of streptococcus which are pathogenic for human beings belong to the group(a). the group(a)β hemolytic streptococci produce various exotoxins such as streptolysin O.

Clinical significance:-

The detection of ASO is very helpful for the diagnosis of streptococcal infections.

Increased ASOtitres be associated with rheumatic fever and glomerulonephritis. An elevated

ASO titer of more than 200 IU/ml indicates past or present acute streptococcal infection. The titer of ASO should be observed repeatedly over a time of 4-6 weeks to follow the course of an infection.

Principle:-

ASOslide test for detection of antibodies to streptolysin-O is based on the principle of

agglutination. The test specimen (serum) is mixed with polystyrene latex particles and allowed to react. If antibodies to streptolysin-O are present in concentrations more than 200

IU/ml but less than 4000 IU/ml, then a visible agglutination is observed. If antibodies to streptolysin-O are not present or are in concentrations less than 200 IU/ml then no agglutination will be observed.

Sample:-It is recommended that the test be performed only on serum. It is not necessary to

heat inactive the test or control serum. If the delay is anticipated before testing test sera should be stored in a refrigerator (2-8°C).

Reagents:-

Reagent 1: ASO latex antigen

Reagent 2: Positive control serum

Reagent 3: Negative control serum

Accessories:-

Glass slide with reaction circles

Disposable applicator sticks

Disposable plastic droppers

Rubber teats.

Storage and stability:-

The reagents and control sera are stable at 2-8°C till the expiry date mentioned on the

individual level.

Procedure:-

All the reagents and samples (Ire-brought to room temperature.

A. Qualitative slide test:-

i. With the help of a disposable -plastic dropper one drop of undiluted test serum is

placed within the circled area on me special slide provided in the kit.

ii. One drop of well-mixed ASO latex reagent is added to the drop of the test sample on the slide.

iii. Using a mixing stick the serum and reagent are mixed uniformly over the entire circle. The dropper tips should not be touched by the liquid on the slide.

iv. The slide is rocked gently back and forth for exactly two minutes for observing agglutination macroscopically.

Interpretation:-

Agglutination visible within two minutes is to be interpreted as a positive test result

corresponding to an ASO titer of more than 200 IU /ml. A test is considered negative when there is the absence of agglutination indicating an ASO titer of less than 200 IU/ml.

B. Semi-quantitative slide test:

i. Using isotonic saline serial dilutions of the serum sample positive in the qualitative method are prepared as for example 1:2, 1:4, 1:8, and 1:16, and so on.

ii. With the help of a disposable plastic dropper, one drop of diluted serum is dispensed into one of the circled areas.

iii. One drop of well-mixed ASO latex reagent is then added to it.

iv. The reagent and the drop of diluted serum are mixed together using an applicator stick.

v. It is observed for macroscopic agglutination for two minutes.

Interpretation:-

Agglutination in the highest serum dilutions corresponds to the amount of ASO in IU/ml

Present in the test specimen.

Serum dilution	Corresponding IU/ml
1:2	400
1:4	800
1:8	1600
1:16	3200

DETERMINATION OF CRP

C-reactive protein (CRP) is an acute-phase protein found in concentrations up to 5µg/and in the serum of healthy persons.

Clinical significance:

Elevated CRP levels are usually observed in a variety of infections and inflammatory

conditions where there is tissue destruction. This increase in CRP level may be detected as

early as 5-10 hours after tissue damage. The changes in concentration of CRP occur more rapidly than in the ESR. Elevated levels of CRP can usually be demonstrated in cases of acute myocardial infarction, rheumatoid arthritis, bacterial and viral infections, acute rheumatic fever with or without carditis, and in several types of malignancies.

Principle:

Uniform latex particles are coated with anti-human CRP. The specimen containing

CRP, on mixing with latex reagent agglutinates, showed a positive test result. If CRP is absent there will be no agglutination, indicating a negative test result.

Sample:

The fresh serum is preferable, in case of delay in testing, it should be stored at 2-8°C.

Plasma, haemolysed, or lipaemic serum should not be used.

Reagents:

Reagent 1: CRP latex reagent

Reagent 2: Positive control serum Reagent 3: Negative control serum

Accessories:

i) Glass slide with reaction circles

ii) Disposable applicator sticks

iii) Disposable plastic droppers iv) Rubber teats.

Storage and stability:

The reagents and control sera are stable at 2-80C till the expiry date mentioned on the individual level.

Procedure:

All the reagents and samples are brought to room temperature.

A. Qualitative slide test:

i. With the help of a disposable plastic dropper one drop of undiluted test serum is placed within the circled area on the special slide provided in the kit.

ii. One drop of well-mixed CRP latex reagent is added to the drop of the test sample on the slide.

iii. Using a mixing stick the serum and reagent are mixed uniformly over the entire circle. The dropper tips should not be touched by the liquid on the slide.

iv. The slide is rocked gently back and forth for exactly two minutes for observing agglutination macroscopically.

Interpretation:

Observation	Conclusion
Coarse agglutination	Strongly positive
Finer agglutination	Weakly positive
Smooth suspension without any noticeable change	Negative

B. Semiguantitative slide test:

i) Using isotonic saline serial dilutions of the serum sample positive in the qualitative method are prepared as for example 1:2, 1:4, 1:8, 1: 16, and so on.

ii) With the help of a disposable plastic dropper, one drop of diluted serum is dispensed into one of the circled areas.

iii) One drop of well-mixed CRP latex reagent is then added to it.

iv) The reagent and the drop of diluted serum are mixed together using an applicator stick.

v) It is observed for macroscopic agglutination for two minutes.

Interpretation:

CRP level can be calculated in terms of µg/ml by multiplying the highest dilution giving clear-cut agglutination with a factor of 6 (sensitivity of antigen 6 µg/ml).

Serum dilution	Corresponding µg/ml
1:2	12
1:4	24
1:8	48
1:16	96

DETERMINATION OF RA

Rheumatoid arthritis is an autoimmune disorder, where autoantibodies are produced against self-antigen (lgG). These antibodies are termed "Rheumatoid Factor"(lgM).

Clinical significance:

The RA test is done in order to detect the presence of rheumatoid factor in the serum of

patients with rheumatoid arthritis. The factor is non-specific and is found in other diseases as well as both related and none related to rheumatoid arthritis.

Principle:

RA test antigen consists of polystyrene latex particle coated with a specially modified preparation of human gammaglobulin (lgG) in order to avoid non-specific agglutination. The suspension of coated latex particles agglutinates visibly when mixed with serum containing rheumatoid factor.

Sample:

The fresh serum is preferable, in case of delay in testing; it should be stored at 2-80C. Plasma, haemolysed, or lipemic serum should not be used.

Reagents:

Reagent 1: latex gammaglobulin reagent

Reagent 2: Positive control serum

Reagent 3: Negative control serum

Accessories:

i). Glass slide with reaction circles

ii). Disposable applicator sticks iii). Disposable plastic droppers iv). Rubber teats.

Storage and-stability:

The reagents and control sera are stable at 2-8°C till the expiry date mentioned on the individual level.

Procedure:

All the reagents and samples are brought to room temperature.

A. Qualitative slide test:

i) With the help of a disposable plastic dropper one drop of undiluted test serum is placed within the circled area on the special slide provided in the kit.

ii) One drop of well-mixed RA latex reagent is added to the drop of the test sample on the slide.

iii) Using a mixing stick the serum and reagent are mixed uniformly over the entire circle. The dropper tips should not be touched by the liquid on the slide.

iv) The slide is rocked gently back and forth for exactly two minutes for observing agglutination macroscopically.

Interpretation:

Observation	Conclusion
Clearly visible agglutination	Positive result
The granularity of doubtful clumping	Negative result

B. Semi-quantitative slide test:

i) Using isotonic saline serial dilutions of the serum sample positive in the qualitative method are prepared as for example 1:2, 1:4, 1:8, and 1:16, and so on.

ii) With the help of a disposable plastic dropper, one drop of diluted serum is dispensed into one of the circled areas.

iii) One drop of well-mixed RA latex reagent is then added to it.

iv) The reagent and the drop of diluted serum are mixed together using an applicator

stick.

vi) It is observed for macroscopic agglutination for two minutes.

Interpretation:

RA level can be calculated in terms of IU/ml by multiplying the highest dilution giving clear cut agglutination with a factor of 10(sensitivity of antigen 10 IU/ml).

Serum dilution	Corresponding IU/ml
1:2	20
1:4	40
1:8	80
1:16	160

DETERMINATION OF WIDAL

Typhoid is an enteric fever caused by various species of Salmonella such as S.typhi, S.paratyphi A, and S.paratyphi_B. The disease is characterized by sustained high fever, severe headache, nausea, anorexia- and constipation initially and later diarrhea. Humans are the only reservoir of bacteria.

Clinical significance:

This test is applied for the diagnosis of the enteric fever including typhoid

(Salmonella typhi) and paratyphoid (Salmonella paratyphi) which are clinically similar and cannot be differentiated by laboratory cultures. The pathogen can be recovered from the blood during the first 7 to 10 days of illness but after two weeks the laboratory culture may not yield any useful information. Specific antibodies (agglutinins) are usually detectable in the patient's blood after six days of enteric fever.

Principle:

This test is based on the principle of a direct agglutination reaction. The smooth suspension of killed Salmonella bacilli carries homologous "0" and "H" antigens. When

the patient's serum (containing antibodies to S. Typhi and S. Paratyphi) is incubated with respective antigens, visible agglutination occurs. A rising titer of antibodies is indicative of

enteric fever.

Reagents:

1) S. Typhi "H"

2) S. Typhi "0"

3) S. Paratyphi "AH"

4) S. Paratyphi "BH"

5) Positive control

Materials:

1. Applicator sticks

2. Disposable plastic droppers

3. Rubber teats

4. Test tubes

5. Pipettes

6. Normal saline

Sample:

The fresh serum is preferable, in case of delay in testing, it should be stored at 2-8°C.

Plasma, haemolysed, or lipemic serum should not be used.

Procedure:

A. Rapid Slide (Screening) Test

i) Cleaned the glass slide supplied in the kit properly and wiped it free of moisture.

ii) Placed one drop of an undiluted test serum in each of the 1st four circles (1 to 4) and one drop of positive control serum in each of the last two circles (5 &6).

iii) Placed one drop of antigens 0, H, A(H),B(H) in circles 1,2,3 and 4 respectively

and O antigen in circle 5 and any one of the 'H' antigens {H, A(H) or B(H)} in circle 6.

iv) Mixed the contents of each circle with a separate applicator stick and spread it to the

the whole area of the individual circle.

v) Rocked the slide for 1minute and observed for agglutination. If agglutination is-

visible within 1minute the test is positive, proceed for quantitative slide test or tube test for the quantitative estimation of the titer of the appropriate antibody. If no agglutination is observed, the test is negative.

B. Semi-quantitative slide test

i) Cleaned the glass slide supplied in the kit well and wiped it free of moisture.

ii) Placed 5µl, 10 µl, 20µl, and 40µl, 80µl of undiluted serum in 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th}

circles respectively on the slide.

iii) Added 1 drop of the appropriate antigen suspension which showed agglutination

in the screening slide test, to each of the above circles.

iv) Mixed the contents of each circle with separate applicator sticks.

v) Rotated the slide slowly for 1 minute and observe for agglutination.

vi) The titer of the antibody is the highest dilution of serum up to which there is clear

agglutination.

vii) Repeated steps through with all the antigens, which showed agglutination in

the screening slide test.

The serum volumes in the quantitative slide test correspond approximately to the tube test titers as follows:

Serum Volume	Approximate Tube Test Titer
80µl	1:20
40µl	1:40
20µl	1:80
10µl	1:160
5µl	1:320

DETERMINATION OF HEPATITIS B SURFACE ANTIGEN (HBsAg)

The HBsAg test is a test to diagnose viral hepatitis/Australia antigen.

Principle –

The test is an immunoassay for the detection of hepatitis B surface antigen in serum. The membrane is pre-coated with the anti-HBs antibodies on the test line and anti-mouse antibodies on the control line. During testing, the serum or plasma reacts with the dye, which has been pre-coated in the test strip. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-HBs antibodies on the membrane and generate red lines. The presence of this red line indicates a positive test, while its absence indicates a negative test.

Requirements- Blood sample HBsAg test strip

Procedure:

i) The strip is removed from the pouch.

ii) The test strip is immersed in the serum sample within arrows pointing towards a sample.

iii) It has to wait for red lines to appear.

Observation-

The test is negative if only one line appears on the control window. The test is positive if lines appear on the test window as well as the on-control window.

IV. Mycology

Study of *Candida albicans*

Specimens:

Swabs and scrapings from surface lesions

Sputum

Exudates

Material removed from intravenous catheters

Vaginal discharge

Requirements:

10 g/dl, sodium hydroxide or potassium hydroxide

Glass slides and coverslips

Microscope

Direct examination

1) Mount skin and nail scrapping on a slide in a drop of 10%KOH (or NaOH). Place a coverslip and observe under the microscope after

15-30 minutes.

2) Make a thin film of the specimen. Place under a coverslip and observe

immediately, first under low power objective and afterward under high power objective.

3) Make stain for Gram staining.

Requirements:

10 g/dl, sodium hydroxide or potassium hydroxide

Glass slides and coverslips

Microscope

Observation:

1) 2 to 4 um size, thin-walled oval, yeast-like cells are observed. Occasionally, mycelia threads are seen with bidding cells.

2) Gram staining: budding Gram-positive cells.

V. Proto Zoology

Microscopic Identification of Malarial Parasite:

1. Sample collection.

2. Standard procedure for staining.

1. Sample collection:

Blood samples are collected with symptoms of fever for routine examination of

the malaria parasites. A thick and a thin film are made on the same slide.

• Items needed for making blood films

Cleaned, wrapped slides.

Sterilized lancets.

70% methanol.

Absorbent cotton wool.

Labels.

Register or record form.

The procedure of sample collection:

a. With the patient's left-hand palm upwards, select the ring finger.

With the pellet of cotton wool lightly socked in alcohol clean the finger. With the clean cotton wool dry the finger using firm strokes to stimulate blood circulation.

b. With a sterile lancet puncture the ball of the finger.

c. By Apply gentle pressure to express the first drop of blood and wipe it away with cotton wool.

d. Apply gentle pressure to the finger and collect a single small drop of blood onto the middle of the slide. This is for the thin smear.

e. Apply further pressure to express more blood and collect two to three large drops of blood onto the slide, about 1 km. from the drop intended for the thin smear.

f. Using another clean slide as a "spreader" with the slide with the I-st blood drop resting on a flat firm surface touch the small drop of blood with the spreader and allow the blood to run along its edge. Firmly push the spreader along the slide keeping the spreader at an angle of 45°. Make sure the spreader is in even contact with the surface of the slide all the time the blood is being spread.

g. For a thick smear using the corner of the spreader, quickly join the drops of blood and spread them to make an even thick smear. Blood should not be excessively stirred but can be spread in a circular or rectangular form with 3-6 movements.

h. Label the dry thin smear with the soft lead pencil by writing across the thicker portion of the smear the patient's name and date.

i. Allow the thick smear to dry in a flat, level position protected from flies, dust, and extreme heat.

J. The slide used for spreading the blood films may now be used for the next patient and another clean slide from the pack will be used as a spreader.

2. Standard procedure for staining: TECHNIQUE USED: GIEMSA STAINING

i. Preparation of stock solution of Giemsa

Staining formula

Giemsa powder: 0.75 gm

Methanol 65 ml

Glycerol: 35 ml

• Preparation:

a. A dark bottle or a chemically clean and dry, clear hard glass or polythene bottle is taken.

b. Put about 50 solid glass beads of methanol. c. Pour in the measured amount of methanol.

d. Add the stain powder.

e. Tightly stopper the bottle.

f. Allow the stain powder to sink slowly through the methanol until it settles down to

the bottom. Shake the bottle in a circular motion for 2-3 minutes.

g. Add a measured amount of glycerol and repeat the shaking process. Continue to

shake for 2-3 minutes at half-hourly intervals at least 6 times.

h. Leave the bottle unused for 2-3 days; shake it 3-4 times each day until the stain is thoroughly mixed.

i. Labe the bottle with the date of preparation. Keep the bottle tightly Stoppard in a cool, place, away from direct sunlight. This can be used for one month.

ii. Preparation of buffer water

Disodium hydrogen phosphate: 1.0 gm

Potassium Dihydrogen phosphate: 0.7 gm

Distilled water: 10000 cc pH: 7.2

STAINING PROCEDURE:

a. Dehaernoglobinize the thick smear with tap water with the help of a pipette; care should be taken not to touch the thin smear.

b. Allow drying.

c. Fix both thick and thin smears with methanol for one minute. d. Allow to dry & place the slide on the staining tray.

e. Add the freshly prepared working solution of Giemsa stain (1 :3) (1 part Giemsa stock solution & 3 part buffered water, pH 7.2) gently onto the slides, until the slide is

completely covered.

f Allow the stain to remain for 10minutes.

g. Wash the slides with clean water.

h. Dry the slides and observe under oil immersion object.

PATIENT 'S REPORT

[Make a table and provide a report here]

7. Internship for Laboratory: Microbiology

Blood Bank

Goal: Interns need to acquire practical skills in proper phlebotomy techniques and blood storage and acquire practical skills in standard blood bank techniques during the internship period.

Objectives:

i. To disinfect the blood collection site with appropriate disinfectant.

ii. To know how to apply a tourniquet and for a desirable time.

iii. To detect the preferred venous access sites.

iv. To insert the needle properly for blood withdrawal.

v. To take care of the patient to avoid complications during and after the blood collection process.

vi. To categorize specimens according to their turnaround time

vii. To develop technical accuracy and self-confidence by experiencing routine functions of the Blood Bank.

viii. To recognize and resolve discrepancies in blood grouping.

ix. To exhibit knowledge of standard techniques used for ABO and Rh typing, compatibility testing, antibody identification, antigen typing, and preparation of blood components.

x. To acquaint with the procedures of donor selection and issuing of blood and blood products for transfusion.

Tasks:

Sl. No.	Task	Observed/ Performed	Specific Learning
	Blood collection		
	Laboratory test performed		
	Instruments handled		
	Analysis of results		
	Blood storage		
	Documentation Report preparation		
	Specific learning		

Methodology

Blood bank & blood transfusion unit:

a. In-house blood collection

b. Urgent laboratory

1. Blood grouping & Rh factor
2. Compatibility testing or cross-matching
3. Direct & indirect Coombs test
4. D^u test

c. Component separation

1. R.B.C. or Packed cell separation
2. Platelet separation
3. F.P.P. or plasma separation
4. Wash R.B.C preparation
5. Cryoprecipitate

Clinical significance of blood transfusion:

Blood transfusion today is a major medical service that is rendered to patients needing replacement of whole blood or blood components. Since the beginning of the Second World War, transfusion of blood & blood products has become accepted as a routine & relatively safe procedure in the management of patients. The blood bank tries to select a donor"s blood that will be compatible with the recipient's blood. The recipient"s plasma should not have any reactive antibody towards the red cell or donor which may lead to haemagglutination or hemolysis of the donor's red cells.

ABO blood groups:

During a blood transfusion, an identical ABO blood group of the donor is ideal. Persons belonging to blood group "O" are considered to be "universal donors", i.e. their blood can be given to individuals of any other blood group. The red cells do not carry either A or B antigen & hence they do not react with their corresponding antibodies. On the other hand, those with blood group AB are "universal recipients". They will accept blood from any of the blood groups-A, B, AB, or O. This is because they neither have anti-A nor anti-B.

Transfusion of specific component blood, as needed by the patient, is preferred over transfusion of whole blood. Whole blood is needed in case of large- scale bloodless. The whole blood arrangements the recipient"s total blood volume which may lead to congestive heart failure. Anemic patients with adequate blood volume but who are deficient in the red blood cell population require packed red cells. Transfusion of plasma, plasma components & platelet concentrates is the therapeutic measure recommended for bleeding patients.

IN-HOUSE BLOOD COLLECTION

Blood collection procedure: Preparation:

Before starting to draw blood, sauce that the following materials are available & within

reachable distance properly labeled blood collection bottle with donors identification member; bleeding set & airway attached; pilot tubes; tourniquet/pacer cuff; forceps; stripper; adhesive tape; rubber bond; savlon; alcohol; iodine swab; local anesthetic & syringe & needle for injection.

Procedure:

1. The donor is advised to lie on the bleeding table & made sure he/she is relaxed & comfortable. The bottle is placed approximately 30 – 40 below the level of the table.
2. The tourniquet/pacer cuff is applied to the upper arm & selects a prominent antecubital vein.
3. The tourniquet is released & disinfected venipuncture site using savlon, iodine & alcohol swab in that order. The skin is allowed to dry before inserting the needle.
4. The tourniquet is re-applied & injected with local anesthetic subcutaneously at the site of venipuncture using an aseptic technique.
5. The venipuncture is performed with a 15 or 16-gauge needle. The bottle or bag is gently agitated to mix the blood with anticoagulant. For this purpose agitating machine may be used.
6. When the required amount of blood has been collected, a tube is clamped; the tourniquet is released & removed the object is from the donor"s hand.
7. When the required amount of blood is collected before the needle has been removed from the vein, the needle is drawn from the bottle & put at 5 – 10 ml. Sample into 2 sterile, dry pilot test tubes, each labeled with the same number as that of the main blood collection bottle.
8. An alcohol swab is placed over the needle & then removed. Pressure is applied over the puncture site with an alcohol swab. The donor is asked to press swales firmly over the area for 3 – 5 minutes & preferably with the arm held straight up in an extended position.
9. The airway needle from the blood collecting bottles is promptly removed & replaced with the cays after applying a 70% alcohol swab in open areas.
10. Finally, tally the members on the bottle with the donor slip, and record the book.
11. A rubber band is put around the pilot tubes & collecting bottles.
12. Did not let the donor"s stand up immediately after blood donation. Make sure that any bleeding from the venipuncture had stopped with a Band-Aid wound is covered.
13. The bottle is stored at 2 – 4ºC.

BLOOD BANK LABORATORY

DETERMINATION OF BLOOD GROUP BY CROSS-MATCH

Principle:

The house of blood transfusion has become steadily more frequent nowadays. To detect or to decide whether the donor"s blood matches the recipient"s blood, the blood group test is done.

Requirements:

1. Glass slide
2. Pasteur pipette
3. Applicator sticks

Reagents:

1. Anti-A sera
2. Anti-B sera
3. Anti-D sera
4. Normal saline

Procedure:

1. A suspension of RBCs in normal saline is prepared.
2. On 1 half of the glass slide is placed 1 drop of Anti-A blood grouping sera.
3. On the other half of the glass slide placed 1 drop of Anti-B blood grouping sera & Anti- D sera.
4. Using a Pasteur pipette added 1 drop of the cell suspension to each half slide.
5. With separator applicator sticks mixed each cell's sera mixture well.
6. Tilt the slide back & forth & observed for agglutination.

ANTI-A	ANTI-B	ANTI-D	PROBABLE BLOOD GROUP
+	-	+	A(+)
-	+	+	B(+)
-	-	+	O(+)
+	+	+	AB(+)
-	-	-	O(+)

Agglutination: +

No agglutination: -

Significance: The use of blood groups are:-

1. To ensure compatible blood transfusion,
2. To eliminate the hemolytic disease of the newborns due to Rh incompatibility,
3. To detect susceptibility to various diseases.

DIRECT & INDIRECT METHODS MUST GIVE COMPATIBILITY TESTING OR CROSS-MATCHING

Before the recipient receives a blood transfusion, a compatibility test must be run within the laboratory with the donor"s red cells & the recipient"s serum. This is called major cross-matching. The primary purpose of a major crossmatch is to find out any incompatibility of the donor"s cells with the patient"s

serum in order to avoid transfusion reactions. The minor crossmatch is rarely requested when the compatibility of the recipient"s red cells is tested against the donor's serum.

A compatibility test or cross-matching is performed subsequent to the ABO grouping & Rh typing of the recipient"s & donor"s blood. It is the final criterion as to the suitability of particular donor blood for a particular recipient.

The recipient's blood is obtained fresh while the donor"s blood is obtained from the pilot tube. ACD anti coagulated donor"s blood should not be more than 21 days & constantly be stored at 4ºC.

Principle:

The serum of the recipient is tested against the red cells of the donor under different conditions in order to establish their compatibility or non-agglutination. Agglutination in any of the conditions indicates the presence of incompatible antibodies inpatient, natural or immune.

The three phases of compatibility testing are listed below & illustrated in saline phases, where the immunologic reaction between red cells suspended in a saline medium & the antibody occurs at room temperature.

1. Thermophase with protein: Where the red cells are suspended in the antibody (serum)

with 22% albumin (protein) & incubated for 30 minutes at 37ºC.

2. Antihuman globulin(AHG) phase: Where the incubated cells are washed (to remove free globulin) & reacted with antihuman globulin serum (Coombs reagent or antihuman globulin).

CROSS-MATCHING BY SLIDE METHOD

The above slide method is commonly done in the blood bank for quick cross-matching.

Requirements:

1. Glass slide, marker
2. Disposable plastic sticks
3. Microscope
4. 4% red cell suspension of donor"s & recipient"s
5. Donor"s & recipient"s serum

Procedure:

1. Take a slide & draw a line centrally to divide into 2 parts.
2. Marked 1 part „P" for major cross-matching & part „D" for minor cross-matching.
3. On the „P" slide add 1 drop of patient"s serum & 1 drop of donor"s 4% cell suspension.
4. On the part of „D" add 1 drop of donor"s serum & 1 drop of patient"s cell suspension.
5. Mix the content of each slide by gently rotating the slide.

6. Incubate at room temperature (21 -25ºC) for 10 minutes.
7. Examine both macroscopically & microscopically for agglutination.

Result:

There is no agglutination on both sides of the side that indicates the donor"s blood is compatible with the recipient's blood.

COOMBS TEST

Antihuman globulin technique is very useful in recognizing weak immunologic reactions. It is widely used in the identification of Du compatibility testing, antibody screening & identification of sensitized red cells.

DIRECT COOMBS TEST

This recognizes sensitized red cells when the sensitizing occurs within the body, i.e, in hemolytic disease of the newborn (HDN) & autoimmune hemolytic anemia. This test is performed to detect anti-D antibodies or other antibodies attached to the red cell surface within the bloodstream.

Principle:

Antihuman globulin or Coombs test detects sensitized re cells where the red cell gets coated with IgG antibody or globulin but do not agglutinate when sensitized red cells come in contact with anti-human globulin reagent they agglutinate.

Reagents:

1. Anti-human globulin or Coombs reagent
2. Pre sensitized red cells or Coombs sensitive cells
3. Saline (0.85%)

Specimen:

The collected blood is preferred over whole blood (citrate) is required for a direct Coombs test. In case of an indirect Coombs test for antibody screening serum specimen will be needed.

Procedure:

1. Prepare 4% cell suspension in isotonic saline of the red blood cell to be tested.
2. With a clean pasture pipette add 1 drop of the prepared red cell suspension to a small test tube.
3. Wash 3 times with normal saline to remove all traces of serum or free globulin.
4. Decant completely after the last washing.
5. Add 2 drops of AHG serum to the sedimented cells.
6. Mix well & centrifuge for 1 minute at 1500 r.p.m.
7. Resuspend the cells by gentle agitation & examine macroscopically for agglutination.
8. Examine for agglutination by holding the tube against a lighted background & tapping the bottom of

the tube.

Observation:

If agglutination occurs----------Positive Coombs test

If no agglutination----------Negative Coombs test

INDIRECT COOMBS TEST

Principle:

Here the sensitization of red cells is done in the laboratory by incubating the red cells with the corresponding antibody at 37ºC for 30 minutes. The test is applied in detecting the presence of unexpected antibodies in the serum which will react with the corresponding antigen on the red cells.

Specimen:

In the case of the indirect Coombs test for antibody screening, is needed it need not be a fasting sample.

Procedure:

1. Label their test tubes as T (test serum), PC (positive control), or NC (negative control).
2. In the T marked tube add 1 drop of test serum.
3. In the PC marked tube add 1 drop of Anti-D serum.
4. In the NC marked tube add 1 drop of saline or bovine albumin (22%).
5. In each test tube add 1 drop of 4% saline suspension of the pooled Rh +ve („O" group) cells or Coombs sensitive cells.
6. Incubate all the three tubes at 37ºC for 30 minutes.
7. Wash the cells 3 – 4 times with normal saline to remove excess serum or free globulin.
8. Add 2 drops of Coombs serum (AHG serum) to each tube & mix well.
9. Keep for 5 minutes & centrifuge for 1 minute, at 1500 r.p.m.
10. Re-suspend the cells & examine macroscopically or microscopically for haemagglutination

Observation:

If agglutination occurs ---------- Positive Coombs test

If no agglutination ---------- Negative Coombs test

D^u TEST

Requirements:

1. Test tube
2. Micro-pipette
3. Pasteur pipette
4. Centrifuge machine
5. Water bath
6. Microscope

Reagents:

1. Anti-human serum (Coombs serum/ Anti-IgG)
2. Bovine albumin

Procedure:

1. Prepare 5% red blood cell suspension in isotonic saline.
2. Level one tube as „T" & add 1 drop of anti-D serum to it.
3. Level the second tube as „C" & add 22% Bovine albumin to it.
4. By a Pasteur pipette add 1 drop of the cell suspension to each & mix well.
5. Incubate at 37ºC for 15 minutes.
6. After incubation washes the cell with normal saline three times.
7. Decant the supernatant after washing.
8. Add 2 drops of anti-human serum to each tube.
9. Mix & centrifuge at 15,000 RPM for 1 minute.
10. Re-suspend the cells by gentle agitation & examine macroscopically & confirm the result microscopically.

Interpretation:

OBSERVATION NO.	ANTI-D (ANTI-Rho SERUM)	BOVINE ALBUMIN CONTROL	INTERPRETATION
1	0	0	D(Rho) – NEGATIVE
2	+	-	D(Rho) – POSITIVE
3	+	+	TEST INVALID

PATIENT 'S REPORT

[Make a table and provide a report here]

8. Internship for Laboratory: Central Laboratory

Goal: Interns need to acquire knowledge and skills of proper handling and documentation of clinical specimens that need to be analyzed in the central laboratory at reception during the internship period.

Objectives:

a. To know the guidelines and procedures for handling and documentation of clinical specimens in the central laboratory.

b. To apply specimen acceptance/rejection criteria.

c. To familiarize with the computerized system of specimen entry and distribution to respective laboratories.

d. To categorize specimens according to their turnaround time.

Tasks:

Sl. No.	Task	Observed/ Performed	Specific Learning
	Specimen collection		
	Laboratory test performed		
	Instruments handled		
	Analysis of results		
	Documentation Report preparation		
	Specific learning		

Methodology

PATIENT 'S REPORT

[Make a table and provide a report here]

9. INTERN FEEDBACK/COMMENTS ON INTERNSHIP

Year: **Lab Section:**

1. Intern Name:

2. Hospital Name:

3. Duration and Dates:

4. Name(s) of the supervisor under whom you were trained:

5. **Overview:** Check **(√) the** explanation that most closely represents your evaluation of this section.

I. Was the intern's responsibilities and privileges discussed with you?

- Clearly discussed
- Clear to some extent
- Not clear

II. **What is your opinion about training for interns in this section?**

- Excellent training
- Good training
- Adequately planned training
- Poorly planned training

III. **Do you feel that the responsibilities given to you in this section were according to your abilities to handle them?**

- The responsibilities given to me were suited to my ability to handle them.
- Some of the responsibilities were above my ability to handle them.
- The responsibilities given to me were too limited and too narrow.

IV. **Do you feel that you gained maximum benefits from the training in this section?**

- Yes
- To some extent
- No benefit

10. **Supervision and Instruction:** Please rate the section on each item below by **circling** the appropriate number on the rating scale.

The rating scale is: **0**=Not applicable **1**=Poor **2**=Adequate **3**=Above average **4**=Excellent

		Rating Scale				
A	Committed to the training program	0	1	2	3	4
B	Supervision of intern	0	1	2	3	4
C	Encouraging intern learning	0	1	2	3	4
D	Amount of feedback given to intern	0	1	2	3	4
E	Friendliness toward interns' questions	0	1	2	3	4

11. Summary and Conclusion:

Briefly describe your Clinical Laboratory Experience:

I. The list below the instruments/equipment you operated.

II. New techniques learned

III. List the types of tests you observed but did not perform.

IV. Academic/Clinical Correlation:

Note: Pls explain what correlation have you found between previously learned theories/concepts (at College) and their practical application during training in this section. Recommendations would you like to make to correlate your learning theories/concepts (at university) with the practical experience during training in this section]

Name and Signature of The Intern:

10. OUTPUT OF INTERNSHIP TRAINING

Through Internship training, I can able to gain knowledge about the application of ……(*instruments name and technique name such as ELISA Reader, Semi-automated Analyze, Auto analyzer, colorimeter, component separation machine, HPLC machine, Flame Photometer, Rotary Microtome, Autoclave, Hot Air oven, etc.)* in the biological science and they also familiar with different operative parts of the concern instruments. So, practical knowledge, as well as theoretical knowledge about some of the instruments, have been clear by this Internship training.

Different research programs of human health are going in the concerned laboratory have been focused and we can able to motivated to continue our higher education in the field and this visit is also a driving force to motivate as in research work.

The techniques of different hormone assay protocols, biochemical estimations, protein separation techniques, and issue section preparation have been learned by us, which is also helpful for us to clear our knowledge and confidence.

The role of discipline, the importance of devotion, and accountability for scientific work have been learned by us from this training.

11. CONCLUSION

The duties of medical laboratory technologists as undergone repaid change science the last decay. The advancement of knowledge, medical science, and technology have to keep place with the newest development in the fields of physiology, Biochemistry, Immunology, Endocrinology, Microbiology, etc.

The thrust of knowledge has increased the so much graduate coerces as even Doctorate course are now being may available to us. So that the knowledge accumulates.

In the words of a poet, regarding the advancement of knowledge we the graduate of Medical Laboratory Technology has miles to go, many more miles to go……..