

INTERNSHIP REPORT

Submitted to

SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY

In partial Fulfillment for the Award of The Degree Of

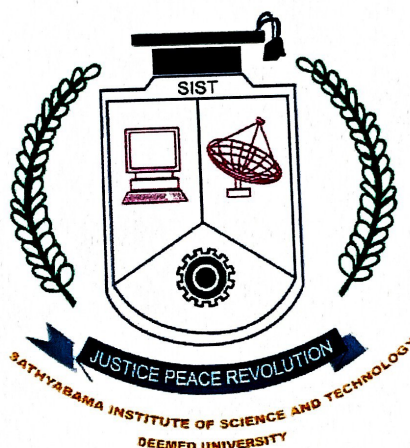
BACHELOR OF SCIENCE

In

MEDICAL LABORATORY TECHNOLOGY

By

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DEPARTMENT OF MEDICAL LABORATORY TECHNOLOGY

SCHOOL OF ALLIED HEALTH SCIENCES

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with Grade "A++" by NAAC

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APRIL 2024



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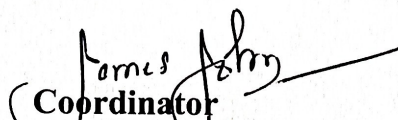
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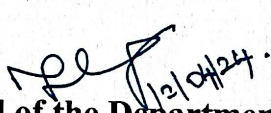
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BONAFIDE CERTIFICATE

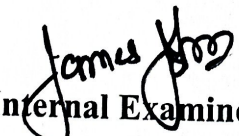
This is to certify that this internship report is the bonafide work of Arockiya Princy A who have done the project work as a team and carried out the training program at **Voluntary Health Services, Adyar and "EGMORE LIONS BLOOD BANK AND RESEARCH FOUNDATION- EGMORE"** under our supervision from November 2022 and November 2023.

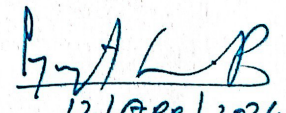

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DECLARATION

I, Ms. AROCKIYA PRINCY. A (41737002), hereby declare that the internship trainings done by me at **VOLUNTARY HEALTH SERVICES- ADYAR** under the guidance of **Dr.JAMES JOHN** and **"EGMORE LIONS BLOOD BANK AND RESEARCH FOUNDATION- EGMORE"** under the guidance of **Ms. D. JASMINE PRIYA** at Sathyabama Institute of Science and Technology is submitted in partial fulfilment of the requirements for the award of Bachelor of Science degree in **MEDICAL LABORATORY TECHNOLOGY**.

Date: 12/04/2024

Place: CHENNAI

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We would like to express our sincere and deep sense of gratitude to our Head of the Department Dr. **J. Premkumar** and Course Coordinator Dr. **James John** and **Ms. Jasmine Priya** and the for their valuable guidance, suggestions and constant encouragement paved way for the successful completion of our internship report.

We wish to express our thanks to all Teaching and Non-teaching staff members of the Department of Medical Laboratory Technology who were helpful in many ways for the completion of the internship.

We would like to express our thanks to our parents for their unwavering support to us during these years.

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1. INTRODUCTION

Place of visit

1. Lions blood bank, Egmore
2. Voluntary Health Service (VHS), Adyar.

I have got good experience of observing all kinds of tests done in Blood Bank and diagnostic laboratory. We have learnt the tests under the departments of Blood bank, Phlebotomy, Clinical pathology, Microbiology, Biochemistry, Hematology, Serology. Here I have prepared the report of my whole Internships.

2. PHLEBOTOMY

Phlebotomy is the process of drawing blood from patients for diagnostic testing, blood donations, or research purposes. I learnt about some of the common tasks performed in phlebotomy include choosing appropriate colour of tube to collect blood sample for testing and how to interact with patient during collection.

Procedure:

1. Identifying and verifying the patient's identity and medical records
2. Using Sterile equipment for blood collection mostly vacuum tube is used.
3. Selecting the appropriate vein for blood collection
4. Before blood collection recommended tube must be labelled with patient details for proper identification
5. Transporting blood samples to the laboratory for testing.
6. Properly disposing of used needles and biohazardous materials

Finally I learnt from this department is that primary responsibility of a phlebotomist is to ensure the safe and efficient collection of blood samples while providing a positive experience for the patient.

3. CLINICAL PATHOLOGY

In this session I gained knowledge about procedures for urine routine and physical examination of stool sample.

- Urine strip
- Urine deposit
- Wet mount for stool
- Physical examination of stool
- Occult blood

3.1 Urine routine

The purpose of a urine routine is to detect and diagnose various health conditions and diseases by examining the physical, chemical, and microscopic properties of a person's urine sample. It can help healthcare providers identify issues such as dehydration, urinary tract infections, kidney problems, diabetes, and liver disease. Urine routine tests are commonly performed during routine check-ups, when a person is experiencing symptoms such as frequent urination or pain while urinating.

Urine routine includes, urine strip and urine deposit.

Procedure for Sample collection:

Patient is given a sterile container and procedure to collect mid-stream urine.

1. Collect a clean, mid-stream urine sample in a sterile container.
2. Record the date and time of collection on the container.
3. Seal the container tightly to prevent leakage.
4. Send the sample to a laboratory for analysis within 2 hours.
5. The laboratory will perform a visual examination of the urine, measure its volume, and test for various substances such as pH, protein, glucose, ketones, blood, and nitrites.
7. The results will be interpreted to diagnose any underlying illnesses or monitor the progress of treatment.

I have thoroughly observed the procedure for urine strip and urine deposit.



Figure: 3.1 Urine strip

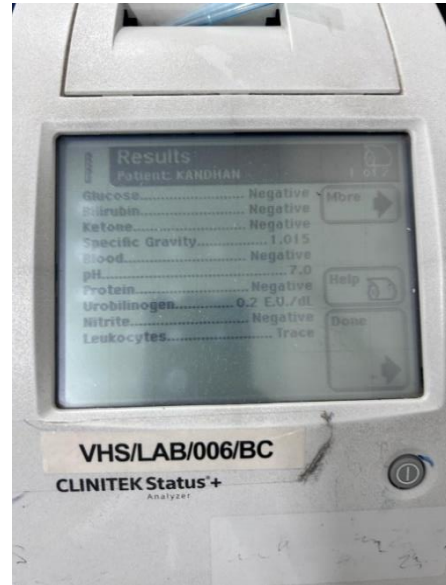


Figure: 3.2 Urine strip analyzer

Urine strip

Procedure:

1. Collect a fresh sample of urine in a clean container.
2. Dip the urine strip into the urine sample, making sure to immerse all of the reagent pads on the strip.
3. Remove the strip after the specified amount of time (usually around 1 minute) .
4. Gently shake off any excess urine from the strip. Using Kim wipes.
5. Compare the color changes on the reagent pads to the color chart provided by the manufacturer or by using urine strip analyzing machine. The color changes indicate the presence of various substances in the urine such as glucose, protein, blood, ketones, pH levels, etc.
6. Analyzer will record the result in the system.
7. Dispose of the urine strip and the urine sample properly according to waste disposal guidelines.

Urine deposit

The purpose of Urine deposit is to provide information about the presence of pus cell, RBC, Epithelial cells, any bacteria and any other substance.

Procedure:

- Transfer the urine from the container to centrifuge tube.
- Centrifuge the urine at 1500 rpm for 10 minutes.
- Discard the supernatant and take a deposit by using dropper.
- Place the coverslip on it, and examine under microscope.

Stool examination

Physical examination of stool is a common laboratory test used to evaluate the overall health of the gastrointestinal system. The procedure involves collecting a sample of stool and observing its color, consistency, odor, and presence of any abnormal substances.

Procedure:

1. Collection of the stool sample: The patient is asked to collect a small sample of stool in a clean container. It is important to avoid any contamination from urine or water during the collection process.
2. Inspection of color: The color of the stool can provide valuable information about the digestive system. Normal stool is usually brown in color. Abnormal colors such as red (indicating blood), black (indicating bleeding from the upper gastrointestinal tract), or pale (indicating liver or bile duct problems) should be noted.
3. Examination of consistency: The consistency of the stool can also provide important information. Stool that is watery may indicate diarrhea, while hard and lumpy stool may indicate constipation.
4. Smell: The odor of the stool can indicate certain gastrointestinal disorders, such as malabsorption or bacterial overgrowth.
5. Presence of abnormal substances: The stool sample is examined for the presence of any abnormal substances, such as blood, mucus, pus, parasites, or undigested food particles.

6. Testing for occult blood: A small amount of stool may be tested for occult blood using a chemical test called the guaiac test. This test can detect hidden blood in the stool, which may indicate gastrointestinal bleeding.

Occult blood

Purpose of occult blood:

The purpose of testing for occult blood in stool is to detect small amounts of blood that may be present but not visible to the naked eye. This can be a sign of various medical conditions such as gastrointestinal bleeding, ulcers. Detecting occult blood early can help in the early diagnosis and treatment of these conditions.

Procedure for occult blood:

1. Collect a stool sample: The first step in testing for occult blood is to collect a small sample of stool. This can be done at home using a special collection kit provided by your healthcare provider.
2. Perform a fecal occult blood test (FOBT): There are two types of FOBTs commonly used to test for occult blood in the stool - guaiac and immunochemical. The guaiac test involves applying a small amount of stool onto a test card containing guaiac, a substance that can detect blood. The immunochemical test uses antibodies to detect blood in the stool. Follow the instructions provided with the test kit to perform the test accurately.
3. Interpret the results: After performing the test, wait for the recommended amount of time for the results to develop. A positive result indicates the presence of blood in the stool, which may indicate a potential gastrointestinal issue. A negative result means that no blood was detected in the stool sample.

Procedure for wet mount:

1. Place a drop of physiological saline or tap water on a clean microscope slide.
2. Using a clean applicator stick, transfer a small amount of the stool sample onto the drop of water on the slide.
3. Gently mix the stool sample with the water on the slide using the applicator stick to form a homogenous mixture.

4. Place a coverslip over the stool sample on the slide, being careful to avoid trapping air bubbles.
5. Examine the wet mount preparation under a microscope using low power (10x) and high power (40x) objective lenses to observe any protozoa, helminths, or other parasites present in the stool sample.
6. Dispose of the slide and coverslip appropriately following standard biohazard disposal protocols.

4. HEMATOLOGY

Introduction

In this department of lab I learnt about all the tests related to blood like CBC, DC, ESR, and tests which comes under coagulation and also I observed the procedure for staining like PAP stain and Leishman stain.

Blood Sample:

- Ethelene Diamine Tetra Acetic Acid Tube
- Sodium citrate Tube

Tests:

1. Bleeding Time
2. Clotting Time
3. Complete Blood Count
4. Differential Count
5. Erythrocyte sedimentation Rate
6. HBA1C
7. Activated partial thromboplastin time
8. Prothrombin time

Procedures:

Bleeding Time

Bleeding time is a test used to measure the time it takes for a cut to stop bleeding

1. Gather all necessary materials and equipment, including a lancet, a stopwatch, blotting paper, a blood pressure cuff, and a ruler.
2. Select a suitable location for the incision, typically on the underside of the forearm.
3. Use the lancet to make a small, standardized incision, usually about 1-2 mm deep.
4. Immediately start the stopwatch as soon as the incision is made.
5. Blot the wound gently with sterile gauze every 15 seconds until the bleeding stops.

6. Record the time it takes for the bleeding to completely stop. This is the bleeding time.
7. Repeat the test two more times, making sure to use a different incision site each time.
8. Calculate the average bleeding time from the three trials.
9. Dispose of the used materials properly and clean up the work area.

Normal range: 2-7 minutes.

Clotting time:

Clotting time is a common test performed in the laboratory to assess the ability of blood to clot.

Method: Capillary tube method

1. A small sample of blood is obtained from the patient, usually through a finger prick or venipuncture.
2. The blood is then drawn into a capillary tube, which is a thin glass tube used to collect a small and specific volume of blood.
3. Wait for 5 minutes for a blood to produce fibrin clot.
4. After 5 minutes break a small portion of tube and observe the thread like fibrin clot.
5. The clot formation is noted as the time it takes for the blood to stop flowing within the capillary tube, and this is recorded as the clotting time.

Normal Range: 8-15 Minutes

Complete Blood Count and Differential Count

Machine: SYSMAX (3part)

Principle:

Blood is sampled and diluted, and moves through a thin tube which is enough that cells pass by one at a time. Characteristics about the cell are measured using lasers (fluorescence flow cytometry) or electrical impedance.

Sample preparation:

Blood is collected in EDTA (purple) tube. Mix thoroughly. Whole blood is used for testing.

Procedure:

- Whole blood sample is used to run this test.
- Sample is mixed gently before placing in to the reaction vial.
- 20samples can run at a time. Two sections are there to run.Each section can run 10 samples.
- After arranging the samples give a comment of run.
- Parameters of CBC
 - WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, RDW-SD, RDW-CV, PDW, MPV, P-LCR, PCT.
- Parameters of DC
 - Neutrophil, Lymphocytes, Monocytes, Eosinophil, Basophil and Immunoglobulins.

All the parameters are entered in both count and percentage.



Figure:4.1. Complete Blood Count analyzer

Erythrocyte Sedimentation Rate (ESR)

Machine: Transasia

Sample preparation:

Blood is collected in a flat tube for the convenience of ESR machine.

Procedure:

1. Turn on the ESR machine by pressing the power button.
2. Place the blood sample tube in the designated slot or holder on the machine.

3. Enter the necessary patient information and test parameters into the machine's software.
4. Select the appropriate test method and start the test run.
5. The machine will use a needle to puncture the cap of the blood sample tube and mix the blood with the anticoagulant reagent.
6. The machine will then measure the rate at which the red blood cells settle in the sample tube, which is known as the erythrocyte sedimentation rate (ESR).
7. The machine will display the ESR results on the screen once the test is complete.
8. Remove the blood sample tube from the machine and dispose of it properly.



Figure:4.2 Erythrocyte sedimentation rate analyzer

HbA1C

Machine: BIO RAD

Sample preparation:

Blood is collected in EDTA (purple) tube. Mix thoroughly. Whole blood is used for testing.

Procedure:

- Turn on the analyzer and allow it to initialize.
- Prepare the HbA1C test samples by collecting the blood in EDTA Tube and mix the sample by rotating the tube gently it allows the blood to mix with anticoagulant.

- Load the test samples into the analyzer. We need to make sure to correctly label each sample to avoid mix-ups during analysis.
- Start the analysis process on the analyzer. This may involve selecting the appropriate test protocol and running the analyzer according to the specified settings.
- Monitor the progress of the analysis on the analyzer's display screen
- Once the analysis is complete, review the results provided by the analyzer. Check for any errors or inconsistencies in the results and troubleshoot as necessary.
- Record the results of the HbA1C analysis in the patient's medical records or laboratory documentation.

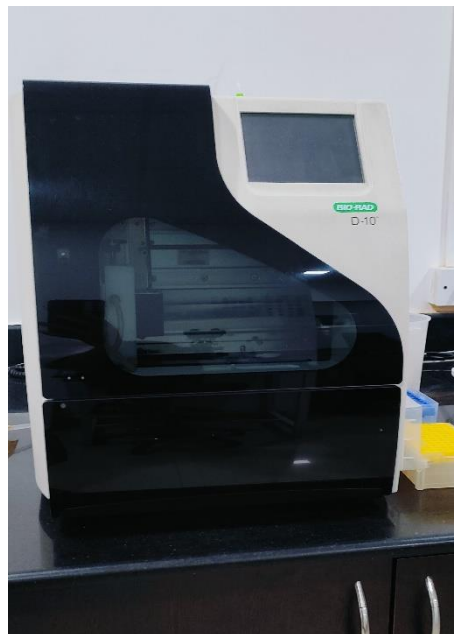


Figure: 4.3 HbA1C analyzer

Activated Partial Thromboplastin and Prothrombin

Machine: STart Max

Sample preparation:

Collect blood sample from the patient and deliver in to a Tube containing Sodium citrate (Blue) as an anticoagulant. Rotate the sample tube gently to mix the sample and sodium citrate. Centrifuge the sample for 10 minutes at 3000 rpm. After centrifuge we get Platelet poor plasma which is suitable for coagulation related tests.

Procedure for APTT

1. Prepare the reagents and equipment needed for the test, including APTT reagent, calcium chloride, and patient plasma sample.
2. Turn on the SStart Max coagulation analyzer and allow it to warm up for a few minutes.
3. Calibrate the analyzer according to the manufacturer's instructions.
4. Take a cuvette specifically manufactured for this machine. Add 50 μL of sample and add 50 μL of APTT reagent. Incubate for 170 seconds. Timer goes on.
5. 180th second add 50 μL of Calcium chloride reagent. Give a comment of run.
6. Once the test is complete, record the APTT value displayed on the analyzer.
7. Interpret the APTT value according to established reference ranges and clinical guidelines. Such as Normalized Ratio (NR)
8. Clean and maintain the analyzer according to the manufacturer's instructions.
9. Dispose of used reagents and any biohazardous waste properly.
10. Document the test results in the patient's medical record.

Procedure for PT

1. Prepare the reagent and equipment needed for the test, including PT reagent.
2. Turn on the SStart Max coagulation analyzer and allow it to warm up for a few minutes.
3. Calibrate the analyzer according to the manufacturer's instructions.
4. Take a cuvette specifically manufactured for this machine. Add 50 μL of sample and Incubate for 50 seconds. Timer goes on.
5. 60th second add 100 μL of PT reagent. Give a comment of run.
6. Once the test is complete, record the PT value displayed on the analyzer.
7. Interpret the PT value according to established reference ranges and clinical guidelines. Such as Normalized Ratio (NR)
8. Clean and maintain the analyzer according to the manufacturer's instructions.
9. Dispose of used reagents and any biohazardous waste properly.
10. Document the test results in the patient's medical record.



Figure:4.4 STart Max for coagulation studies

STAINING

Leishman's Stain

Leishman's stain is a type of stain used to distinguish between different cell structures, such as nuclei, vacuoles, and cytoplasm. It is commonly used in the detection of parasites, particularly the Leishmania parasite, which causes a disease called leishmaniasis. The stain works by binding to specific components within the cell, allowing researchers to visualize and study the cell's morphology and structure. Overall, Leishman's stain plays a crucial role in identifying and studying various microorganisms and can provide valuable insights into their biology and behavior.

PRINCIPLE:

Leishman's stain is based on a methanolic mixture of polychrome methylene blue and eosin. The methanolic stock solution is stable and also serves the purpose of directly fixing the smear thereby eliminating a prefixing step.

Materials required:

- Leishman stain,
- Phosphate buffer 0.66 M pH 6.8,
- KH_2PO_4 9.1 g/L in Solution A,
- Na_2HPO_4 9.5 g/L in Solution B,
- A Pasteur pipette,
- Kim wipes, a hair drier,
- Unstained peripheral blood smear slide

Procedure:

- ❖ Prepare a thin blood smear
- ❖ Keep for air dry
- ❖ Cover the slide with 8 drops of Leishman's stain
- ❖ Incubate for 3 minutes
- ❖ Wash the slide under tap water gently.
- ❖ Cover the slide with 16 drops of buffer
- ❖ Incubate for 12 minutes.
- ❖ Wash the slide under tap water gently.
- ❖ Air dry the stained smear and observe under microscope.



Figure:4.5 Microscope

PAP staining (Papanicolaou)

The purpose of Papanicolaou (PAP) staining is to detect abnormal cells in various body fluids, such as cervical smears or sputum samples. The stain highlights the cell's morphology and helps pathologists to identify precancerous or cancerous cells, as well as infectious agents or other abnormalities. PAP staining is routinely used as a Screening tool for cervical cancer and other conditions.

Materials required:

- Specimen slides
- Acid Alcohol
- Xylene 1&2
- Hematoxylin

- EA 50 solution
- OG 6 Solution
- Alcohol 1, 2&3(70%, 95%, 100%)
- Acetic acid
- Eosin
- Distilled water
- Cover slips
- Staining dish
- Timer

Procedure:

1. Prepare slides by fixing the specimen with 95% ethanol for 1-2 minutes.
2. Stain the slides with Hematoxylin for 2-3 minutes to stain the nuclei of cells blue.
3. Rinse the slides with water to remove excess Hematoxylin.
4. Stain the slides with Eosin for 30 seconds to stain the cytoplasm of cells pink.
5. Rinse the slides with water to remove excess Eosin.
6. Dehydrate the slides in graded ethanol solutions (70%, 95%, 100%) for 30 seconds each.
7. Clear the slides by immersing them in xylene for 1-2 minutes to remove any residual ethanol
8. Mount the slides with a mounting medium and cover with a coverslip.
9. Allow the slides to dry completely before examining under a microscope.
10. Analyze the stained cells for any abnormalities or changes in structure that may indicate disease or infection.

Conclusion:

I have got knowledge about instruments used in hematology lab and to operate them. Procedure for counting blood cells by staining method also I learnt.

5. MICROBIOLOGY

Introduction:

I have thoroughly learnt about all techniques which carried out in Microbiology laboratory. It is a place for working with a variety of microorganisms. Since several culture media are prepared and organic materials are present, chances exist for the presence of high spectrum of microbial community.

Samples used in microbiology LAB:

- Sputum
- Urine
- Stool
- Blood
- Pus
- Throat swab
- Wound swab
- Et tube
- Air sample
- Surface sample

Common media prepared in the microbiology lab

1. **MacConkey Agar:** Agar, peptone, lactose, sodium chloride, bile salt, and neutral red are among its constituents. The medium is selective. While bile salt stops the growth of many other bacteria, it does not stop the growth of Enterobacteriaceae.

Preparation: 12.88 grams of agar powder were combined with 250 ml of distilled water. After 15 minutes of autoclaving at 121°C and cooling, the media was put into the plates. Each dish will hold 25 ml.

2. **Chocolate agar:** It is prepared by heating blood agar. It is used for culture of Meningococcal, Pneumococcus, Gonococcus and Haemophilus.

Preparation: 250 ml of distilled water was added to 7 gm of nutrient agar powder. Autoclaving was done at 121°C for 15 minutes and cooled till 45°C then 5% of defibrinated blood was added. Heated slowly and evenly.

3. Blood Agar: Is used most commonly. For blood agar preparation 5-10% defibrinated blood is mixed with melted agar after cooling and the media is poured into the plates. 25 ml for each plate.

4. Muller Hinton Agar: 250 ml of distilled water was added to 9.5 gm of Agar powder. Autoclaving was done at 121C for 15 minutes and cooled after cooling the media is poured into the plates. 25 ml for each plate.

5. Xylose Lysin Deoxylate Agar: 50 ml of distilled water was added into 2.834 gm of XLD agar powder. Keep in boiling water. After becoming cool pour into plates.

6. Egg yolk agar: 60 ml of distilled water and 1.68 gm of nutrient agar was added. Autoclaving was done at 121C for 15 minutes. Add 3 ml of beaten egg and pour into the plates.

Preparation and sterilization of culture media:

Culture media are available commercially as powder; they require only the addition of water. Nutrient medium is a general purpose preparation for culturing microorganisms which are not nutritionally fastidious. The agar prepared has the same composition. The final pH of both media is 7.4.

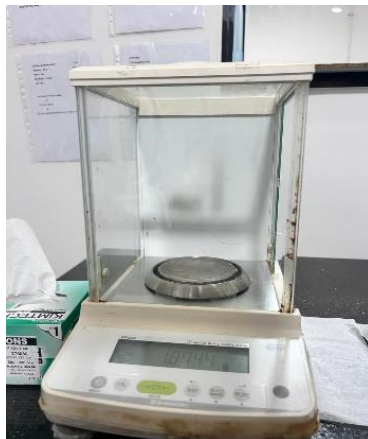


Figure:5.1 weighing machine



Figure:5.2 Media preparation

Autoclaving:

Autoclaving is a process that use moist heat and pressure so that all parts of the material to be sterilized reach 121 degree Celsius for 15 minutes.

An autoclave is a large pressure cooker; a chamber which sealed off against surrounding air.

Objective:

To prepare sterile condition for culturing microorganisms.

Materials required:

Commercial nutrient agar, Balance, Distilled water, Scott bottles, Measuring cylinder beaker, Forceps, Universal bottles.

Procedure:

1. Appropriate amount of agar powder is weighed and dissolved with distilled water into the measuring cylinder. They are mixed well.
2. The bottles are closed with non-absorbent cotton and wrapped with silver sheets. Set aside for sterilization.
3. All the media are sterilized at 121 degree Celsius for 15 minutes.
4. After autoclaving, the media is removed. The broth preparation is allowed to cool

STAINING**GRAM STAINING**

It is the most important and widely used microbiological differential staining technique. It was developed by Dr. Christian Gram in 1884, and differentiate bacteria according to their Gram character (Gram positive or Gram negative). In addition this stain also helps to determine of cell morphology, size, and arrangement.

Principle:

When the bacteria is stained with primary stain Crystal Violet and fixed by the mordant, some of the bacteria are able to retain the primary stain and some are decolorized by alcohol. The cell walls of gram positive bacteria have a thick layer of protein-sugar complexes called peptidoglycan and lipid content is low. Decolorizing the cell causes this thick cell wall to dehydrate and shrink, which closes the pores in the cell wall and prevents the stain from exiting the cell. So the ethanol cannot remove the Crystal Violet-Iodine complex that is bound to the thick layer of peptidoglycan of gram positive bacteria and appears blue or purple in colour.

In case of gram-negative bacteria, cell wall also takes up the CV-Iodine complex but due to the thin layer of peptidoglycan and thick outer layer which is formed of lipids, CV-

Iodine complex gets washed off. When they are exposed to alcohol, decolourizer dissolves the lipids in the cell walls, which allows the crystal violet-iodine complex to leach out of the cells. Then when again stained with safranin, they take the stain and appears red in colour.

Reagents Used in Gram Staining

- Crystal Violet, the primary stain
- Iodine, the mordant
- A decolourizer made of acetone and alcohol (95%)
- Safranin, the counterstain

Procedure of Gram Staining:

1. Take a clean, grease free slide.
2. Prepare the smear of suspension on the clean slide with a loopful of sample.
3. Air dry and heat fix
4. Crystal Violet was poured and kept for about 30 seconds to 1 minutes and rinse with water.
5. Flood the gram's iodine for 1 minute and wash with water.
6. Then, wash with 95% alcohol or acetone for about 10-20 seconds and rinse with water.
7. Add safranin for about 1 minute and wash with water.
8. Air dry, Blot dry and Observe under Microscope.

Interpretation

Gram Positive: Purple Colour

Gram Negative: Pink Colour

Sputum examination by Ziehl - Neelsen staining

Principle:

This procedure is used to stain mycobacterium tuberculosis and mycobacterium leprae. These bacteria are also called acid fast bacilli. They stain with carbol fuschin, which is a red dye. They retain dye when treated with acid, which is due to presence of mycolic acid in their cell wall.

Objectives of the Ziehl – Neelson staining

- To differentiate between acid-fast bacilli and non-acid-fast bacilli.

- To stain *Mycobacterium* species.

Reagents:

1. Carbol-Fuschin (Primary dye)
2. 20% sulphuric acid or acid-alcohol (Decolorizer)
3. Methylene Blue dye (counterstain) or malachite green

Procedure:

- On a clean sterile microscopic slide, make the smear of the sample culture and heat fix the smear over blue heat.
- Over the smear, pour and flood the smear with carbol fuschin and heat gently until it produces fumes.
- Allow it to stand for 5 minutes and wash it off with gently flowing tap water.
- Add 20% sulphuric acid and leave it for 1-2 minutes. Repeat this step until the smear appears pink in colour.
- Wash off the acid with water.
- Flood the smear with methylene blue dye and leave it for 2-3 minutes and wash with water.
- Air dry and examine the stain under the oil immersion lens.

Results and interpretation:

- Acid-fast bacteria retain the primary dye, carbol-fuschin, and stain pink.
- Non-acid fat bacteria take up the methylene blue dye and appear blue.

Fixing the smears.

- **Heat fix**

If the Smear is prepared from the culture, it should be fixed by heating it.

- **Methanol fix**

If the Smear is directly prepared from the sample, it should be fixed in methanol.

Cultures

Blood culture

Blood culture is a laboratory test used to identify the presence of bacteria or fungi in the bloodstream. It helps in diagnosing bloodstream infections (sepsis) and guides appropriate antibiotic treatment.

Principle:

The principle behind blood culture is to place a sample of blood into a culture medium, which provides the necessary nutrients for bacterial or fungal growth, and then monitor the sample for any signs of growth or presence of microorganisms.

Procedure:

1. The blood sample is collected under sterile conditions to minimize the risk of contamination. Usually, two sets of samples are collected from different sites to increase the chances of detecting any pathogens present. The most common sites for blood collection are the veins in the arm.
2. The collected blood samples are inoculated into culture bottles containing specific growth media. These media are designed to support the growth of bacteria or fungi and inhibit the growth of any contaminating microorganisms.
3. The inoculated culture bottles are placed in an incubator to provide optimal temperature and conditions for bacterial or fungal growth. The incubation period varies depending on the suspected pathogen and can range from a few hours to several days.
4. The culture bottles are examined multiple times during the incubation period to check for any signs of bacterial or fungal growth. Growth can be detected by visual inspection, changes in the colour or turbidity or appearance of the medium, or through the use of automated systems that detect microbial growth.
5. If growth is observed, the microorganisms are identified through various techniques such as Gram staining, biochemical tests, and molecular methods. This identification helps in determining the causative agent of the bloodstream infection.

Interpretation:

The interpretation of blood culture results depends on whether there is growth of microorganisms or not. The following interpretations can be made:

1. Positive blood culture: The presence of microorganisms in the blood culture indicates a bloodstream infection. The identified pathogens help guide appropriate antibiotic treatment, as susceptibility testing is performed to determine which antibiotics will be effective in treating the infection.
2. Negative blood culture: A negative blood culture indicates the absence of detectable microorganisms in the blood sample. However, it does not completely rule out the presence of infection as some microorganisms may be present in low numbers, missed due to inadequate culturing, or the infection may be caused by non-culturable microorganisms. Further clinical evaluation may be necessary to confirm the absence of infection.
3. Contaminated blood culture: Sometimes, blood culture samples may be contaminated with bacteria that are not causing the infection. This can occur due to improper collection or handling of the blood sample. Contaminated blood culture should be repeated to obtain accurate results.

Overall, blood culture is an important diagnostic tool for identifying bloodstream infections and guiding appropriate antibiotic therapy. It requires careful collection, handling, and interpretation to ensure accurate results.

Volume of Sample to be collected

Adult: 10ml of blood and 40ml of BHIB

Paediatric: 2ml of blood and 18ml of BHIB

Neonate: 1ml of blood and 9ml of BHIB

Brain Heart Infusion Broth (BHIB):

BHIB is commercially available in market. It is mixed with distilled water and autoclaved.

Urine culture

To identify and quantify the presence of microorganisms in urine specimens. This helps in the diagnosis of urinary tract infections (UTIs) and determining the appropriate treatment for patients. The culture allows for the growth of microorganisms, which are then identified and their susceptibility to antibiotics is determined.

Procedure:

1. A midstream clean-catch urine sample is usually collected, ensuring that the genital area is properly cleaned before collection to avoid contamination.
2. The urine specimen is inoculated onto different culture media, such as blood agar, MacConkey agar. These media provide optimal conditions for the growth of different bacteria.
3. The inoculated media are incubated at 37°C for 24 to 48 hours to allow for the growth of microorganisms.
4. After incubation, the colonies that have grown on the media are examined and identified based on their appearance, texture, and other characteristics. Different biochemical tests such as oxidase test, catalase test, and API strips may be used for identification.
5. Antibiotic susceptible the identified microorganisms are further tested for their susceptibility to various antibiotics. This helps in determining the appropriate antibiotic treatment for the infection.

Interpretation:

1. No growth: If no colonies are observed after incubation, it indicates that there is no significant bacterial growth and the urine sample is considered negative.
2. Significant growth: If there is significant bacterial growth, it suggests the presence of urinary tract infection. The colonies are identified and the antibiotic susceptibility testing is performed to guide the selection of appropriate antibiotic therapy.
3. Mixed growth: Sometimes, more than one type of microorganism can be present in the urine specimen. This is referred to as mixed growth. In such cases, identification and antibiotic susceptibility testing are performed for each type of microorganism present.

The interpretation of the urine culture results is done in correlation with the patient's symptoms, medical history, and other laboratory tests to make an accurate diagnosis.

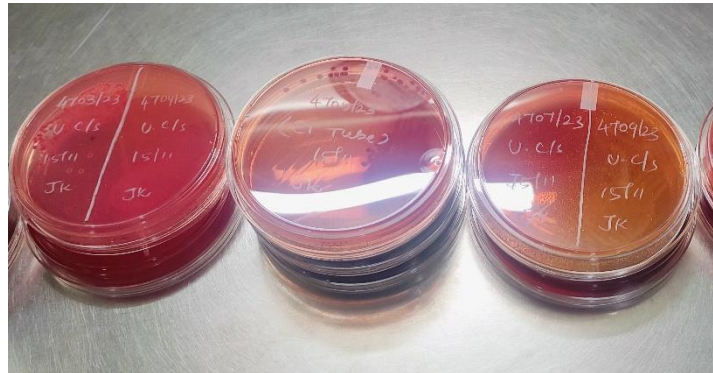


Figure:5.3 Urine culture plate

Stool culture

Stool culture is a laboratory test conducted on a stool sample to identify and determine the presence of disease-causing microorganisms, such as bacteria, viruses, parasites, or fungi, in the gastrointestinal tract. It helps in the diagnosis of gastrointestinal infections and identifies the causative agent for appropriate treatment.

Procedure:

1. Collection of Stool Sample: A clean, dry container is provided to collect a fresh stool sample, preferably obtained from different parts of the stool.
2. Streaking of Sample: The stool sample is streaked onto specific agar media plates, such as MacConkey agar, Salmonella-Shigella agar, or Campylobacter agar, using either a sterile swab or a loop.
3. The inoculated agar plates are then incubated at specific temperatures, usually between 35-37°C, and under specific atmospheric conditions, depending on the suspected pathogen.

Identification:

After an appropriate incubation period, the growth of microorganisms is observed on the agar plates. Colony morphology, such as size, shape, color, and texture, is examined to help identify different types of bacteria. Further tests, such as

Gram staining, biochemical tests, or antigen-antibody reactions, may be performed to identify specific pathogens.

Interpretation:

1. No Growth: If no growth is observed after incubation, it indicates the absence of significant bacterial, viral, or parasitic infection.
2. Normal Flora Growth: The growth of normal bacterial flora, such as *Escherichia coli* or *Lactobacillus*, is expected and doesn't necessarily indicate infection.
3. Pathogenic Growth: The presence of pathogenic microorganisms, such as *Salmonella*, *Campylobacter*, *Shigella*, or *Clostridium difficile*, indicates an infection or illness and requires appropriate treatment.
4. Mixed Growth: Sometimes, there can be a mixture of different microorganisms, both normal flora and pathogens, indicating a mixed infection. In such cases, further identification and testing are required to determine the significance of each organism.

It is important to note that the interpretation of stool culture results should be done in conjunction with the patient's clinical symptoms, medical history, and other diagnostic tests to establish an accurate diagnosis and guide appropriate treatment.



Figure:5.4 Stroglyoids

Wound and pus culture

Principle:

The principle of wound and pus culture is to isolate and identify the causative microorganisms present in a wound or pus sample. This is done to determine the type of infection and guide the appropriate treatment. The primary goal is to obtain a pure culture of the organisms present in the sample, which can then be subjected to various tests and identification methods.

Procedure:

1. The wound or pus sample is collected using a sterile swab or needle. Care should be taken to avoid contamination from the surrounding area.
2. The collected sample is streaked onto appropriate culture media. Commonly used media include blood agar and MacConkey agar. Each medium supports the growth of different types of microorganisms, allowing for the detection of various infections.
3. The inoculated media are placed in a suitable incubator and maintained at the optimal temperature for bacterial growth, which is typically around 37 degrees Celsius. Incubation time may vary depending on the suspected pathogens and the type of infection.
4. After incubation, the culture plates are observed for growth. Different colonies with variable characteristics (size, shape, color) may appear, indicating the presence of different microorganisms. These colonies are isolated onto separate plates for further study.
5. Once isolated, the microorganisms can be subjected to different identification tests, such as gram staining, biochemical tests, and molecular techniques like polymerase chain reaction (PCR). These tests help in determining the species and sometimes the strain or serotype of the bacteria.

Interpretation:

1. Identification: The microorganisms identified in the culture can provide crucial information about the infection. For example, the presence of *Staphylococcus aureus* might indicate a skin infection, while the presence of *Streptococcus pyogenes* might suggest a streptococcal infection.

2. Quantity: The number of microorganisms present in the culture can indicate the severity of the infection. High load of certain bacteria like *Pseudomonas aeruginosa* or *Enterococcus faecalis* may suggest a more significant infection.

Antibiotic sensitivity test

Principle:

The antibiotic sensitivity test, also known as the antibiotic susceptibility test or antibiotic disk diffusion test, is performed to determine the effectiveness of specific antibiotics against particular bacterial strains. The test helps guide the choice of antibiotics for treatment by providing information about which antibiotics will be most effective.

Procedure:

1. Isolate the bacterial strain: The first step is to isolate the bacteria to be tested. This is usually done by obtaining a clinical sample from the patient such as a urine, blood, or wound swab.
2. Prepare the agar plate: Prepare a sterile agar plate such as Mueller – Hinton agar (MHA) and spread the bacteria evenly on the surface using a sterile swab. Allow the liquid bacteria to dry.
3. Place antibiotic disks: Place sterile paper disks containing specific antibiotics onto the agar plate. These disks commonly contain predetermined amounts of different antibiotics.
4. Incubate the plate: Incubate the plate at the optimum temperature for bacterial growth for a specific time (usually overnight).
5. Measure the zone of inhibition: After incubation, examine the plate, and measure the zone of inhibition around each disk. The zone of inhibition is the area where the bacteria could not grow due to the antibiotic's effectiveness.

Interpretation:

The zone of inhibition is then compared to established criteria, such as the Clinical and Laboratory Standards Institute (CLSI) guidelines, to interpret the antibiotic sensitivity results. The measured zones are categorized as susceptible, intermediate, or resistant based on the diameter of the zone and the specific antibiotic.

- Susceptible: A larger zone of inhibition indicates that the bacteria are susceptible to the antibiotic, meaning the antibiotic is likely to be effective in treating the infection.
- Intermediate: An intermediate zone of inhibition suggests that the bacteria may be inhibited by the antibiotic in certain clinical situations or at higher doses.
- Resistant: A smaller or no zone of inhibition indicates that the bacteria are resistant to the antibiotic, and alternative antibiotics need to be considered.

Performing VITEK 2 machine in microbiology LAB for the identification of various Microorganism

1. Collect the bacterial or fungal sample to identify and test from a patient or a culture plate.
2. Inoculate the sample into a Vitek 2 ID card or relevant specific cards, depending on the type of microorganism. Ensure that the card matches the test organism and check the expiry date.
3. Carefully load the card into the Vitek 2 machine using the appropriate slots. Make sure the card is properly seated.
4. Input the necessary patient information and identification codes into the Vitek 2 software or interface.
5. Start the automated testing process by initiating the specific test protocol for the microorganism being analyzed.
6. The Vitek 2 machine will perform the necessary procedures for identification and susceptibility testing, including incubation, growth observation, and measurement analysis.
7. After the tests are completed, the Vitek 2 machine will generate a report with the identification of the microorganism and its susceptibility or resistance profile.
8. Review and interpret the results provided by the Vitek 2 system. This may involve comparing the obtained data with established reference standards or databases.
9. Report the final results and interpret them to assist in patient treatment.

It is important to follow the manufacturer's instructions and guidelines for each specific Vitek 2 machine and ID card used in the laboratory.



Figure: 5.5 Vitek 2 for microbiology



Figure:5.6 Vitek 2 analysing cards

Conclusion:

I have studied about Media Preparation, Blood, Wound, Pus, Sputum, cultures and all the staining done in Microbiology LAB

6. SEROLOGY LAB

Serology is the study of blood serum and other body fluids to detect the presence of antibodies, antigens, and other substances that can help diagnose and treat medical conditions. In this lab, I have explored various serological tests and techniques to help identify infectious diseases, autoimmune disorders, and other health conditions.

Samples

1. Blood
2. Urine
3. Saliva
4. Semen
5. Throat swab

Tests to be done in this LAB

- RA Factor
- CRP
- Widal
- VDRL
- HIV Screening Test
- HBsAg Screening Test
- HCV Screening Test
-

Rheumatoid Arthritis (RA) Factor

Principle: The Rheumatoid Factor (RF) test is used to detect the presence of antibodies against the Fc portion of IgG antibodies in the serum. RA is an autoantibody produced by the immune system in patients with autoimmune diseases such as rheumatoid arthritis. The presence of RA in the serum is indicative of an autoimmune disorder.

Procedure:

1. Blood sample is from the patient's vein, typically from the arm.
2. The serum sample is separated from the blood then the serum is placed on a special card that contains small wells or circles for mixing the serum with reagents that will react with the RA factor antibodies.
3. The card is rotated for a specific period of time to allow the reaction to occur.

4. After the rotation, the card is examined for any agglutination. During this process Positive and Negative controls are administered.

5. The test results will be interpreted by a healthcare provider and may be used in conjunction

Interpretation: A positive result on the RA factor test indicates the presence of RA antibodies in the patient's serum, which may suggest the presence of an autoimmune disorder such as rheumatoid arthritis.

However, it is important to note that a positive RA test result does not confirm a diagnosis of rheumatoid arthritis on its own. Additional tests and clinical evaluation are typically needed to confirm a diagnosis. Additionally, RA antibodies may also be present in healthy individuals or in patients with other inflammatory conditions, so the RA test results should be interpreted in conjunction with other clinical findings.

C-Reactive Protein (CRP)

Principle:

C - reactive protein (CRP) test is a quantitative measurement of the level of CRP in the blood. CRP is an acute phase reactant that increases in response to inflammation and infection in the body. The test helps in the diagnosis and monitoring of various inflammatory conditions, such as bacterial infections, autoimmune diseases, and cardiovascular diseases.

Procedure:

1. The patient's blood sample is collected from a vein in the arm using a needle and a syringe.
2. The blood sample is then centrifuged to separate the serum from the cells.
3. The serum is then tested using an automated analyzer that measures the concentration of CRP in the sample.
4. The results are reported in milligrams per liter (mg/L) of CRP in the blood.

Interpretation:

- ✓ Normal CRP levels are typically below 10 mg/L.
- ✓ Elevated CRP levels may indicate acute inflammation or infection.
- ✓ CRP levels can also be used to assess the effectiveness of treatment in inflammatory conditions.
- ✓ High CRP levels may also be associated with an increased risk of cardiovascular diseases.

It is important to note that CRP levels can be elevated for reasons other than inflammation, such as obesity, smoking, and aging. Therefore, the test results should be interpreted in conjunction with the patient's symptoms and other laboratory tests.



Figure:6.1 MISPA i2 for serology testing

WIDAL test

Principle:

The Widal test is a serological test used to diagnose typhoid fever caused by *Salmonella typhi* and paratyphi. It is based on the principle of agglutination, where antibodies in the patient's serum react with antigens on the surface of the *Salmonella* bacteria, causing them to clump together.

Procedure:

1. Collect a blood sample from the patient and allow it to clot.
2. Centrifuge the sample to separate the serum.
3. Prepare several test tubes containing varying dilutions of the patient's serum.
4. Add the *Salmonella* antigens (O and H) to each tube and mix well.
5. Incubate the tubes at 37°C for 2-4 hours.
6. Check for agglutination using a microscope or by visual inspection. Agglutination indicates the presence of antibodies in the serum that react with the *Salmonella* antigens.

Interpretation:

The Widal test results are interpreted based on the highest dilution of serum that shows agglutination for each antigen. The presence of agglutination at a certain dilution is indicative of a recent or current infection with *Salmonella typhi* or *paratyphi*. The interpretation is as follows:

- A single high titer of anti-O and anti-H antibodies (1:160 or higher) is suggestive of a current typhoid infection.
- Rising titers of antibodies between acute and convalescent samples also suggest active infection.
- A single high titer of anti-O antibodies without a corresponding rise in anti-H antibodies may indicate a past infection or vaccination.

It is important to note that the Widal test has limitations, including cross-reactivity with other bacterial infections and the need for careful interpretation of results in countries where typhoid is endemic. Confirmatory tests such as blood cultures are often recommended for accurate diagnosis.

VDRL(Venereal Disease Research Laboratory)

The Venereal Disease Research Laboratory (VDRL) test is a screening test for syphilis, a sexually transmitted infection caused by the bacterium *Treponema pallidum*. The VDRL test is a non-specific test and may also be used to monitor the effectiveness of treatment for syphilis.

Principle:

The VDRL test is a nontreponemal test that detects the presence of antibodies in the blood that are produced in response to the presence of syphilis. The test is based on the principle of flocculation, where the antibodies in the serum of the patient cause the antigen-antibody complex to flocculate or clump together. The clumping is visible to the naked eye, and the test is positive if clumping occurs.

Procedure:

1. A sample of blood is taken from the patient.
2. The serum is separated from the blood sample.
3. The sample and Positive and negative controls are placed in circles in the slide
4. The serum is mixed with a cardiolipin-cholesterol-lecithin antigen.

5. The mixture is observed for the presence of clumping or flocculation, which indicates a positive result.

6. The test results are interpreted based on the presence or absence of clumping.

Interpretation:

A positive VDRL test result indicates the patient with syphilis.

In conclusion, the VDRL test is a valuable screening tool for syphilis, but it should be used in conjunction with other tests to confirm the diagnosis and monitor the effectiveness of treatment.

HIV Screening test

The principle behind the HIV card method is to detect the presence of antibodies produced by the immune system in response to HIV infection. The test works by using a small sample of blood to detect these antibodies and provide a result within a short period of time.

Procedure

1. Collect all the things needed for HIV Screening test by card method. Which are provided in the kit.
2. Add 3 drops of buffer which provided in the kit, into the card.
3. After drying, add 1 drop of serum sample into it.
4. Add 5 drops of buffer and keep it for dry.
5. Add 2 drops of Enzyme conjugate after drying add 5 drops of buffer.

Interpretation

- ✓ If 3 dots present in the card it indicates that presence of type1 &2 HIV antibody
- ✓ If 2 dots in T1 and control it indicates that Type 1 HIV.
- ✓ C is always control.

For HCV also same procedure as HIV can be carried out. Kit only will be changed



Figure:6.2 HIV card

HBsAg

Principle: The HBsAg screening test by card method is based on the principle of immunoassay techniques, specifically lateral flow chromatographic immunoassay. The test uses specific antibodies that are capable of binding to the hepatitis B surface antigen (HBsAg) present in the patient's blood sample.

Procedure:

1. Collect a small amount of blood sample (usually from a finger prick) using a lancet.
2. Place the blood sample onto the sample pad of the test card.
3. Add a few drops of buffer solution onto the sample pad to allow the blood sample to flow through the test card.
4. The serum sample migrates along the test card by capillary action and encounters a specific antibody that binds to the HBsAg, if present.
5. The bound antibodies form a visible line on the test card to indicate a positive result for HBsAg.
6. The control line on the test card should also appear, indicating that the test was performed correctly.
7. Read and interpret the results according to the manufacturer's instructions provided with the test kit.

Interpretation:

- A visible line at the test line region and control line region indicates a positive result for HBsAg, indicating the presence of the hepatitis B virus in the blood sample.
- If only the control line appears and there is no line at the test line region, the result is negative for HBsAg, indicating the absence of the hepatitis B virus in the blood sample.

Other tests are also done in serology LAB.

Like, Cryptococci by strip method

Principle:

The strip method for identification of Cryptococci is based on the presence of specific antigens that react with antibodies on the test strip. The test strip contains multiple capture lines, each coated with a different monoclonal antibody specific to different Cryptococcus species. When the test sample is added to the strip, the antigens in the sample will bind to the corresponding antibodies on the test lines, resulting in the appearance of coloured bands on the strip.

Procedure:

1. Collect a sample from the suspected source of Cryptococci, such as blood.
2. Take a test tube and add 1 drop of diluent provided in the kit.
3. Add a 40µL of the sample to the test tube.
4. Insert the strip into the test tube.
5. Wait for the recommended incubation time, usually around 10 minutes.
6. Interpret the results based on the appearance of coloured bands on the test strip.

Interpretation:

- Presence of a coloured band on a specific line indicates the presence of Cryptococci species corresponding to that line.
- Absence of coloured bands on all lines indicates the absence of Cryptococci species in the sample.
- Faint lines or incomplete bands may indicate a weak positive result, and further confirmatory tests may be required.

7. BIOCHEMISTRY

Introduction:

In this lab, I was studying the chemical processes and reactions that occur within living organisms. By conducting various experiments and analyses, I gained a better understanding of the complex biochemical pathways that drive cellular function and metabolism. In this lab I was learning about Siemens Biochemical analyser.

Tests to be done in Siemens.

1. Liver function tests: These tests measure various enzymes and proteins in the blood to assess the health of the liver.

2. Kidney function tests: These tests measure levels of certain substances in the blood and urine to assess how well the kidneys are functioning.

3. Lipid profile: This test measures cholesterol and triglyceride levels in the blood to assess the risk of heart disease.

4. Glucose test: This test measures blood sugar levels to assess the risk of diabetes or to monitor blood sugar control in diabetic patients.

5. Electrolyte tests: These tests measure levels of important minerals in the blood, such as sodium, potassium, and calcium, to assess hydration status and overall health.

6. Protein tests: These tests measure levels of various proteins in the blood to assess nutritional status and overall health.

Individual tests under Liver Function Test

- Total Bilirubin
- Direct Bilirubin
- SGOT (Serum Glutamic Oxaloacetic Transaminase)
- SGPT (Serum Glutamic Pyruvic Transaminase)
- ALP (Alkaline Phosphatase)
- Total protein
- Albumin
- Globulin
- GGT (Gamma-Glutamyl Transferase)

Individual tests under Renal Function Test

- BUN (Blood urea nitrogen)
- Creatinine - blood.
- Creatinine clearance.
- Creatinine - urine.
- Urea
- Uric acid
- Bicarbonate
- Amylase
- Lipase
- Phosphorus
- Calcium

In this department of Laboratory, I have studied about Siemens Biochemistry analyser and Roche Electrolyte analyser.

Siemens Biochemistry analyser

Operating Procedure:

1. Turn on the Siemens biochemistry analyzer by pressing the power button.
2. Allow the instrument to warm up and run any necessary startup procedures.
3. Load the samples onto the sample tray according to the manufacturer's instructions.
4. Input the necessary information and parameters for the analysis, such as sample type, test type, and any other relevant data.
5. Begin the analysis by selecting the desired test on the analyzer's interface.
6. Wait for the analysis to be completed, during which the analyzer will measure and analyze the samples.
7. Once the analysis is finished, review the results on the screen or print out the results
8. Remove and dispose of any used samples according to biohazard guidelines.
9. Clean and maintain the analyzer according to the manufacturer's instructions to ensure accurate and reliable results for future use.
10. Power off the analyzer when finished using it.



Figure: 7.1 Siemens Biochemistry analyser

Roche Electrolyte analyser

Operating Procedure

1. Turn on the analyser by pressing the power button.
2. Follow the on-screen instructions to calibrate the analyser. This typically involves inserting calibration solutions and running the calibration process.
3. Check and ensure that the reagents are properly loaded in the analyser.
4. Prepare the samples for analysis according to the analyser's specifications. This may involve diluting the samples or adding them directly to the sample cups.
5. Load the samples into the analyser through probe which present in the analyser.
6. Start the analysis process by selecting the appropriate test menu on the analyser.
7. The analyser will automatically process the samples and present the results on the screen.
8. Record and interpret the results as needed for patient care or other purposes.



Figure:7.2Roche Electrolyte analyzer

8. BLOOD BANK

Introduction:

Blood banking is the process that takes place in the lab to make sure that donated blood, or blood products, are safe before they are used in blood transfusions and other medical procedures. Blood banking includes typing the blood for transfusion and testing for infectious diseases. A blood bank collects, separates, tests and stores blood until a patient needs it.

Blood Banking Saves Lives

Over the years, blood banking has helped save countless lives. Today, about 13.6 million units of blood are donated per year. About 36,000 units of blood are needed each day. Thanks to advances in medical technology, blood banks can safely store blood donations, and process and screen blood to ensure safety for all.

Blood donor:

Selection criteria

- Age between 18 and 60 years.
- Haemoglobin - not less than 12.5 g/DL.
- Pulse - between 50 and 100/minute with no irregularities.
- Blood Pressure -Systolic 100-180 mm Hg and Diastolic 50 - 100 mm Hg.
- Temperature - Normal (oral temperature not exceeding 37.50C)
- They should fulfil the following rules.
- Past one year - not been treated for Rabies or received Hepatitis B immune globulin.
- Past six months - not had a tattoo, ear or skin piercing or acupuncture, not received blood or blood products, no serious illness or major surgery, no contact with a person with hepatitis or yellow jaundice.
- Past three months - not donated blood or been treated for Malaria.
- Past one month - had any immunizations.
- Past 24 hours - taken alcoholic beverages
- Past 72 hours - had dental work or taken Aspirin
- Present - not suffering from cough, influenza or sore throat, common cold
- Women should not be pregnant or breast feeding her child
- Women donor should not donate during her menstrual cycles

- Free from Diabetes, not suffering from chest pain, heart disease or high BP, cancer, blood clotting problem or blood disease, unexplained fever, weight loss, fatigue, night sweats, enlarged lymph nodes in armpits, neck or groin, white patches in the mouth etc.

Individuals under certain conditions are deemed ineligible to donate blood:

- Those who have certain medical conditions or illnesses such as HIV, hepatitis, or certain types of cancers
- Individuals who have recently travelled to areas with high risk of infectious diseases
- Those who have recently undergone surgery or received a blood transfusion
- People who have a history of injecting drugs or engaging in high-risk sexual behaviours
- Those who have a history of heart disease or high blood pressure
- Pregnant women or individuals who have recently given birth
- Individuals who have received certain vaccinations within a specific time period
- Those who are currently taking certain medications that may affect blood or clotting factors.
- Have asthma with active symptoms, and severe asthma patients.
- Consumed alcohol in the past 24 hours.
- Ever had TB, bronchial asthma or allergic disorder, liver disease, kidney disease, fits or fainting, blue or purple spots on the skin or mucous membranes, received human pituitary - growth hormones etc.

Types of blood bag

Single Blood Bag

- For whole blood collection.
- The bag contains CPDA solution.
- Available in capacity of 350ml and 450ml.

Double Blood Bag

- For whole blood collection.
- Separation of 2 different blood components (red blood cells and plasma) obtain through the process of centrifugation and extraction.

Triple Blood Bag

- Triple Blood Bag with SAGM, for whole blood collection.
- Separation of 3 different blood components (red blood cells, plasma and platelets).
- The primary bag contains CPD and one satellite bag contains SAGM.



Figure:8.1 Triple Blood Bag

Blood donor request form

Blood donor request form tells about the donor's health details. Before donating blood the donor should consult who is present in the blood bank itself. The doctor investigates the donor's name, age, Are you taking any continuous tablet? Are you a diabetic or thyroid patient? Then finally doctor checks donor's BP. If everything is alright doctor permits the donor to donate blood. Otherwise the donor will be rejected.

The image shows a detailed blood donor request form from Madras Egmote Lions Blood Bank Research Foundation. The form is titled 'BLOOD DONOR REQUEST FORM' and contains various sections for donor information, medical history, and donor consent. It includes fields for name, age, sex, blood group, and date of birth. There are also sections for medical history, including whether the donor is a regular smoker, has any chronic diseases, or is taking any medication. The form also includes a section for donor consent, where the donor signs and provides their contact information. The form is printed on a white background with black text and red lines.

Figure:8.2 Blood donor request form

Blood donation

- **Registration** (10 Minutes)
 - Donor fills up the registration form and gives his consent for donation.
- **Medical Check-up** (10 Minutes)
 - Donors Medical History & life style is asked, check-up of temperature, blood pressure, pulse and haemoglobin.
- **Donation** (8 Minutes)
 - Phlebotomist draws unit (350ml/450ml) of blood. A new sterile needle is used to draw each unit and the needle is destroyed after it has been used. It takes less than 10 minutes.
- **Refreshment** (10 Minutes)
 - Enjoy Snacks and drinks juice to replenish fluids.



Figure:8.3 Blood donation

Donor counselling:

One unit of donated blood can help save the lives of more than one person.

- Trauma victims, cancer patients and those with inherited blood disorders require most of the donated blood.
- There is no substitute for blood and only source is human blood.

- People who are anaemic cannot donate blood. However, they should undergo treatment for anaemia and can donate blood once the haemoglobin is within the normal range.
- Whole blood donation can be made safely at an interval of 3 months if the donor has no risk of anaemia, particularly iron deficiency anaemia. Repeated blood donation at this interval does not cause any sort of weakness. As premenopausal women are more prone to iron deficiency, their donation interval is usually longer.
- There is absolutely no risk of acquiring AIDS or any other disease such as hepatitis B and C) from donating blood.
- An individual can safely donate blood from the age of 18- 60 years without any risk to their health.
- The donor is examined before donation for his/her suitability to give blood.
- The donor must know that he/she needs to be healthy and screened for blood donation.
- Donation blood does not take a long time. The actual blood collection procedure takes about 8-10 minutes.
- Individuals who have suffered or suffer from hepatitis B, hepatitis C or AIDS should not donate blood.

Blood grouping:

The test to determine your blood group is called ABO typing. Your blood sample is mixed with antibodies against type A and B blood. Then, the sample is checked to see whether or not the blood cells stick together. If blood cells stick together, it means the blood reacted with one of the antibodies.

- Gather the necessary supplies: A blood grouping kit, alcohol swabs, lancets, cotton balls, and bandages.
- Wash your hands thoroughly with soap and water.
- Prepare the patient by making them comfortable and choosing a suitable location for the procedure.
- Clean the patient's fingertip or earlobe with an alcohol swab to disinfect it.
- Use a lancet to prick the patient's fingertip or earlobe and allow a small drop of blood to form.

- Place the blood drop in the appropriate wells on the blood grouping kit according to the manufacturer's instructions.
- Add the appropriate reagents to the blood drop and mix gently according to the instructions provided in the blood grouping kit.
- Observe the reaction of the blood drop with the reagents to determine the blood group. This is typically done by observing the presence or absence of agglutination (clumping of blood cells).
- Record the blood group result and inform the patient of their blood type if necessary.
- Dispose of the lancet and any other used supplies properly according to biohazard waste guidelines.
- Clean the patient's finger or earlobe with a cotton ball and apply a bandage if necessary.
- Wash your hands again with soap and water to ensure proper hygiene.
- Document the blood grouping procedure in the patient's medical record along with the results.

Blood Typing

Blood typing helps your doctor determine what type of donor blood is compatible with your own. Some blood types contain antibodies that trigger immune reactions against other blood types. In general:

- If you have type A blood, you should only receive types A or O blood.
- If you have type B blood, you should only receive types B or O blood.
- If you have type AB blood, you can receive types A, B, AB, or O blood.
- If you have type O blood, you should only receive type O blood.

If you have type AB blood, you're known as a "universal recipient," and can receive any ABO category of donor blood. If you have type O blood, you're known as a "universal donor," as anyone can receive type O blood. Type O blood is often used in emergencies when there isn't enough time to perform blood typing tests.

Anti-sera



Figure:8.4 Anti A



Firgure:8.5 Anti B



Figure:8.6 Anti D

Blood screening test:

There are five screening tests for donated blood.

- ✓ HIV (Human Immuno Deficiency Virus)
- ✓ HCV (Hepatitis C Virus)
- ✓ HBV (Hepatitis B Virus)
- ✓ VDRL (Venereal Disease Research Laboratory)
- ✓ Malaria

These are test done in blood bank to ensure the donated blood is healthy. Without screening even, a drop of blood will not move away from the blood bank.

HIV:(Human Immuno Deficiency Virus)

For the ELISA test, the blood sample will be sent to a laboratory for analysis. A lab technician will add the sample to a device that contains HIV antigen and anti-HIV antibodies.

An automated process will add an enzyme to the device. The enzyme helps speed up chemical reactions. Afterward, the reaction of the blood and the antigen will be monitored. If the blood contains antibodies to HIV or antigens of HIV, it will bind with the antigen or antibody in the device. If this binding is detected, the person may have HIV.

HCV:(Hepatitis C Virus)

- Holding the pipette vertically drop 1 drop (approximately 25µl) of the plasma or serum sample into the sample well.
- Immediately add 2 drops of buffer (approximately 80 µl) to the sample well and start the timer.
- Wait for the coloured line(s) to appear. Results should be read at 10 minutes.

HBsAg:(Hepatitis B Virus)

- The method uses monoclonal antibody conjugated to colloidal gold and polyclonal antibodies immobilized on nitrocellulose strip in thin line.
- As the test sample flows through the membrane assembly of the test device, the coloured monoclonal anti-HBsAg-colloidal gold conjugate complexes with the HBsAg in the sample.

VDRL:(Venereal Disease Research Laboratory)

- ❖ Patients' serum is inactivated by heating at 56°C for 30 minutes in a water bath to remove non-specific inhibitors (such as complement).
- ❖ VDRL antigen suspension (colloidal suspension of tissue cardiolipid or chemically synthesized cardiolipin), controls and samples are brought to room temperature.
- ❖ One drop (50 µl) of the test specimen, positive and negative controls is pipetted onto separate reaction circles of the disposable slide.
- ❖ A drop of diluted antigen suspension is added to the measured volume of specimen, positive and negative controls.

- ❖ Using a mixing stick the test specimen and the VDRL reagent is mixed such that it thoroughly spreads uniformly over the entire reaction circle.
- ❖ The slide gently rotated and continuously either manually or on a mechanical rotor at 180 r.p.m.
- ❖ Flocculation is checked microscopically using 10X objective and eye piece at about 8 minutes.

Malaria:

Malaria parasites can be identified by examining under the microscope a drop of the patient's blood, spread out as a “blood smear” on a microscope slide. Prior to examination, the specimen is stained (most often with the Giemsa stain) to give the parasites a distinctive appearance.

Component separation:

Blood component separation is the process of separating whole blood into its individual components such as red blood cells, white blood cells, platelets, and plasma. This separation is typically done through a process called centrifugation, which involves spinning the blood at high speeds to separate the different components based on their density.

Procedure

1. **Collection:** Blood is typically collected from a donor or patient using a sterile needle and collected in a blood bag containing an anticoagulant to prevent clotting.
2. **Centrifugation:** The blood bag is placed in a centrifuge machine and spun at high speeds. This causes the blood to separate into layers based on the density of the components.
3. **Plasma separation:** The first layer that separates out is the plasma, which is the liquid portion of the blood. Plasma is collected and can be used for various purposes such as transfusions or plasma-derived products.
4. **Red blood cell separation:** The next layer that separates out is the red blood cells. These can be collected and used for transfusions in patients with anaemia or blood loss.

5. **Platelet separation:** Platelets are typically found in a thin layer between the red blood cells and plasma. These can be collected and used for patients with low platelet counts or clotting disorders.

6. **White blood cell separation:** White blood cells are less commonly separated from whole blood but can be done using specific techniques. They can be used for certain medical procedures or research purposes.

Overall, blood component separation allows for the different parts of blood to be used for specific medical purposes, helping to treat a wide range of conditions and save lives.

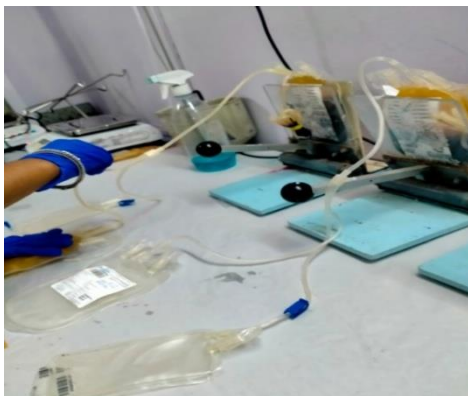


Figure:8.7 Blood pressure



Figure:8.8 Refrigerated centrifuge

Storage

1. **Whole Blood** contains red cells, white cells, and platelets (~45% of volume) suspended in blood plasma (~55% of volume).

Colour: Red

Shelf Life: 21/35 days

Storage Conditions: Refrigerated

Key Uses: Trauma, Surgery

2. **Red blood cells** (RBCs), or erythrocytes, give blood its distinctive colour. Produced in our bone marrow, they carry oxygen from our lungs to the rest of our bodies and take carbon dioxide back to our lungs to be exhaled. There are about one billion red blood cells in two to three drops of blood.

Colour: Red

Shelf Life: Up to 42 days

Storage Conditions: Refrigerated

Key Uses: Trauma, Surgery, Anaemia, Any blood loss, Blood disorders, such as sickle cell.

- 3. Platelets** or thrombocytes, are small, colourless cell fragments in our blood whose main function is to stick to the lining of blood vessels and help stop or prevent bleeding. Platelets are made in our bone marrow.

Colour: Colourless

Shelf Life: 5 days

Storage Conditions: Room temperature with constant agitation to prevent clumping

Key Uses: Cancer treatments, Organ transplants, Surgery

- 4. Plasma** is the liquid portion of blood; our red and white blood cells and platelets are suspended in plasma as they move throughout our bodies.

Colour: Yellowish

Shelf Life: 1 year

Storage Conditions: Frozen

Key Uses: Burn patients, Shock, Bleeding disorders

- 5. Cryoprecipitate (Cryo)** is a portion of plasma rich in clotting factors, including Factor VIII and fibrinogen. These clotting factors reduce blood loss by helping to slow or stop bleeding due to illness or injury.

Colour: White

Shelf Life: 1 year

Storage Conditions: Frozen

Key Uses: Haemophilia, Von Willebrand disease (most common hereditary coagulation abnormality), Rich source of Fibrinogen



Figure:8.9 Bags with plasma



Figure:8.10 Refrigerator for storing blood

Blood issuing

If you need a blood transfusion or transplant, your doctor can use blood typing and cross matching to learn if your blood is compatible with donor blood or organs.

Blood typing reveals what type of blood you have. This depends on the presence of certain antigens on your red blood cells (RBCs). Antigens are proteins that trigger your immune system to produce antibodies.

There are four main types of blood:

- type A, which contains type-A antigens
- type B, which contains type-B antigens
- type AB, which contains type-A and type-B antigens
- type O, which contains neither type-A nor type-B antigens

Your blood will also be classified as Rh positive (+) or Rh negative (-), based on the presence or absence of a particular protein on your RBCs, known as Rhesus factor. Crossmatching is a test used to check for harmful interactions between your blood and specific donor blood or organs. It can help your doctor predict how your body will react to those donor materials.

Blood typing:

Blood typing helps your doctor determine what type of donor blood is compatible with your own. Some blood types contain antibodies that trigger immune reactions against other blood types. In general:

- If you have type A blood, you should only receive types A or O blood.
- If you have type B blood, you should only receive types B or O blood.
- If you have type AB blood, you can receive types A, B, AB, or O blood.
- If you have type O blood, you should only receive type O blood.

If you have type AB blood, you're known as a "universal recipient," and can receive any ABO category of donor blood. If you have type O blood, you're known as a "universal donor," as anyone can receive type O blood. Type O blood is often used in emergencies when there isn't enough time to perform blood typing tests.

Crossmatching:

Cross matching is a procedure performed prior to transfusion of blood or blood products to detect any serological incompatibilities in the blood of donor and recipient. Before a donor's blood is transfused into a recipient, there should be no antigens or antibodies in both, that would react with each other resulting in transfusion reaction. Cross matching is designed to prevent such transfusion reactions which may occur after transfusion.

Cross matching plays important role to detect:

- Most recipient antibodies directed against donor's red blood cell antigens.
- Most donor antibodies directed against recipient's red blood cell antigens.
- Major errors in ABO grouping labelling and identification of donor and recipients.

1. **Major Cross Match:** It involves testing the donor's red cells with recipient's serum to determine the presence of any antibody which may cause hemolysis or agglutination of donor red cells. This is more important than minor cross match.
2. **Minor Cross Match:** It involves testing of donor's plasma with recipient's red cells to determine the presence of any antibody which may cause hemolysis or agglutination of recipient's red cells.

Procedure for Cross matching

There are different methods for cross matching. Among them most commonly used technique is Anti-human globulin (AHG) cross match.

Major Cross Match

1. Prepare donor and recipient's blood sample: Donor's red cells and recipient's serum/plasma.
2. Prepare 3-5% saline cell suspension of red cells.
3. Label a test tube.
4. Add two drops of recipient's serum and one drop of donor cell suspension.
5. Mix and incubate the tubes at 37 degree Celsius for about 60 minutes.
6. Decant the serum completely and wash the cells three times in saline.
7. Add two drops of Anti-human Globulin (AHG) and mix. Allow to stand at room temperature for 5 minutes.
8. Centrifuge at 1500 rpm for 1 minute.
9. Observe macroscopically and microscopically for agglutination.
10. If macroscopic agglutination is not observed, transfer a small amount onto a glass slide and examine for microscopic agglutination. Rouleaux is not an indication of incompatibility.

Minor Cross Match

1. Prepare donor and recipient's blood sample: **Recipient's red cells and donor's serum/plasma.**
2. Label a test tube.
3. Add two drops of donor's serum and one drop of recipient's cell suspension.
4. Mix and incubate the tubes at 37 degree Celsius for about 60 minutes.
5. Decant the serum completely and wash the cells three times in saline.
6. Add two drops of Anti-human Globulin (AHG) and mix. Allow to stand at room temperature for 5 minutes.
7. Centrifuge at 1500 rpm for 1 minute.
8. Observe macroscopically and microscopically for agglutination.
9. If macroscopic agglutination is not observed, transfer a small amount onto a glass slide and examine for microscopic agglutination. Rouleaux is not an indication of incompatibility.

Compatible donor and recipient blood should show no agglutination in both major and minor cross match. Blood which shows incompatibility in major cross match should never be transfused, because the large plasma volume of the recipient blood containing antibodies can destroy the donor's red cells easily. The minor incompatibility is less important because the donor's serum which contains the antibodies is diluted in the recipient's own plasma, making the antibodies very dilute and ineffective.

Cross matching



Figure:8.11 Centrifuge



Figure:8.12 Coomb's card



Figure:8.13 Coomb's Card centrifuge

Blood banking records:

It records and monitors all processes from blood donation to post-transfusion follow-up in adherence to international standards. It also employs warning, guidance and decision support tools which ensure error prevention and fast processing

Blood Bank documents / Records like all medical information must not be released or made available to unauthorised persons. The system should ensure confidentiality of donor and patients records.

Records to be maintained:

1. Blood donor register:

Indicating serial number, date of bleeding, name donor with particulars, age, weight, haemoglobin, blood pressure, medical examination, signature of the medical officer bleeding the donor, bottle bag number and patient's detail for whom donated in case of recipient donation, remarks on donation (voluntary/ replacement/ professional)

2. Blood stock register:

Indicating bottle bag number, date of collection, date of expiry, result of ABO/Rh group, result for testing HIV antibodies, malarial, VDRL, hepatitis B surface antigen (if any), name of donor with particulars, utilisation issue number, components prepared or discarded, certified by medical officer in charge.

3. Issue register:

Indicating serial number, date and time of issue, bottle bag number, ABO/Rh group, total quantity in ml. name of recipient, group of recipients, unit/institution, details of cross matching report, and indication for transfusion.

Register for: Bottles / packs giving details of firm, batch number, date of supply, result of testing.

- Register for diagnostic reagent used.
- Transfusion adverse reaction record.

Records of purchase, use and stock in hand of disposable needles, syringes, plastic bags, sets shall be maintained

REGISTER			SCREENING REGISTER														
Sl. No.	Age	Sex	Barcode	Ref. No.	Ref. Name	Ref. Address	Ref. Phone	Ref. Email	Ref. Date	Ref. Time	Ref. Location	Ref. Status	Ref. Remarks	Ref. Signature	Ref. Stamp	Ref. Date	Ref. Time
1	25	M	YLD1280548	01	✓	N	N	N	N	N	N	N	N	N	N	N	N
2	25	M	YLD1280517	01	✓	N	N	N	N	N	N	N	N	N	N	N	N
3	25	M	YLD1280516	01	✓	N	N	N	N	N	N	N	N	N	N	N	N
4	25	M	YLD1280514	01	✓	N	N	N	N	N	N	N	N	N	N	N	N
5	25	M	YLD1280504	01	✓	N	N	N	N	N	N	N	N	N	N	N	N
6	25	M	YLD1280533	01	✓	N	N	N	N	N	N	N	N	N	N	N	N
7	25	M	YLD1280532	01	✓	N	N	N	N	N	N	N	N	N	N	N	N
8	25	M	YLD1280531	01	✓	N	N	N	N	N	N	N	N	N	N	N	N
9	25	M	YLD1280520	01	✓	N	N	N	N	N	N	N	N	N	N	N	N
10	25	M	YLD1280519	01	✓	N	N	N	N	N	N	N	N	N	N	N	N
11	25	M	YLD1280518	01	✓	N	N	N	N	N	N	N	N	N	N	N	N
12	25	M	YLD1280534	01	✓	N	N	N	N	N	N	N	N	N	N	N	N

Figure:8.14 Screening records

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9. CONCLUSION

Day 1

First day I saw the departments in the blood bank. First it was reception. Lab, component separation room, screening room, storage room etc. We went lab first. There I saw centrifuge machine. After that I saw the refrigerators in the lab for each blood components to store them in a suitable temperature.

Day 2

I watched thoroughly blood grouping and typing for the patient those who are in need of blood. Then I did too. And also cross matching. I saw the separate centrifuge machine for the cross matching and incubator.

Day 3

I saw the collection of blood from the voluntary donor and the storing procedure of that. Antibody screening for the collected blood in the gel card. Then I saw the pooled cell preparation. From three different donor's blood of same group by mixing with Liss and centrifuge it.

Day 4

I saw component separation for the donated blood then I did. Then I watched Screening for HIV, VDRL, HBsAg, HCV and Malaria. In ELISA and card method.

Day 5

I saw all the records in the blood bank and discarding the expired and screening positive blood bags. And different stickers for different use.

Day 6

Last day I went for blood donation camp in Clive factory. There I saw many donors and I thoroughly watched the blood collection. And I checked Haemoglobin levels for all donors by copper sulfate method. Then I saw how to label the blood bag. For every donor they give a certificate. There I got new experience.

I have learnt all these techniques and I gained the knowledge about tests done in Clinical laboratory.



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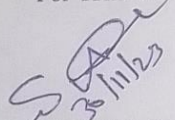
30.11.2023

TO WHOMSOEVER IT MAY CONCERN

This is to certify that **Ms.AROCKIYA PRINCY.A**
(Reg.No.41737002), B.Sc (MLT) student of Sathyabama Institute of
Science and Technology had done her internship in the Department
of Laboratory for the period from 01.11.2023 to 30.11.2023 at our
Institution.

We wish her all the best for her future endeavors.

For THE VOLUNTARY HEALTH SERVICES


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Date: 12th Dec 2022

TO WHOMSOEVER IT MAY CONCERN

This is to certify that Ms. Arockiya Princy, A. BSc in MLT, 2nd year of Sathyabama Institute of Science and Technology, Chennai - 119, undergone Internship training in Madras Egmore Lions Blood Bank & Research Foundation, Egmore, Chennai - 08. She has been observed blood bank procedures including Bleeding the Donors, Bloodgrouping, Cross-matching, Component separation, TTI screening and attend Blood donation camp for the period from 28th Nov 2022 to 3rd Dec 2022.

During her internship, performance was good and she was able to complete the project on time.

We wish him all the best for her upcoming career.

L. J. Madanagopal Rao
Chairman



This project made possible through the co-operation
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