Report On

INTERNSHIP AT UTTARA ADHUNIK MEDICAL COLLEGE & HOSPITAL

By

Mst. Armina Amjad Shila
20236008

Nowshin Jahan
20336012

A report submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of

Bachelor of Science in Biotechnology

Department of Mathematics and Natural Sciences
Brac University
November 2024

Declaration

It is hereby declared that

1. The internship report submitted is our own original work while completing a degree at BRAC University.

2. The report does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.

3. The report does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.

4. We have acknowledged all main sources of help.

Student's Full Name & Signature:

Mst. Armina Amjad Shila	**Nowshin Jahan**
20236008	20336012

Supervisor's Full Name & Signature:

Dr.M. Mahboob Hossain

Professor, Microbiology Program

Department of Mathematics and Natural Sciences

BRAC University

Letter of Transmittal

Dr.M. Mahboob Hossain

Professor

Mathematics and Natural Sciences

BRAC University

Merul Badda, Dhaka-1212

Subject: Submission of Internship Report

Dear Sir,

We are very pleased to present our internship report conducted at Uttara Adhunik Medical College & Hospital, which is the opportunity we obtained with your help.

We have attempted to complete the report with the required information and details about what we learned separated into different chapters.

We trust that the report will meet the requirements and your expectations.

Sincerely yours,

_____

Mst. Armina Amjad Shila (20236010)

Nowshin Jahan (20336012)

Mathematics and Natural Sciences

BRAC University

Date: 28th November, 2024

Non-Disclosure Agreement

This agreement is made and entered into by and between Uttara Adhunik Medical College & Hospital and the undersigned students, Mst. Armina Amjad Shila (20236008) and Nowshin Jahan (20336012), Biotechnology programme, Department of Mathematics and Natural Sciences at BRAC University, to ensure that the students will not disclose unauthorized information about the hospital.

UTTARA ADHUNIK MEDICAL COLLEGE HOSPITAL

House # 34, Sonargaon Janapath, Sector # 9, Uttara Model Town, Dhaka-1230.
Tele: Phone: 58951672, 55080711, 8932343, E-mail: uamch@yahoo.com

Date: 23-11-2024

TO WHOM IT MAY CONCERN

This is to certify that Mst. Armina Amjad Shila, Student ID 20236008, from BRAC University, Bangladesh has worked in Uttara Adhunik Medical College & Hospital (UAMCH) from 17-10-2024 to 02-11-2024, as an intern in the laboratory of Microbiology Department.

The Department of Microbiology of Uttara Adhunik Medical College & Hospital (UAMCH) is satisfied with her performance.

Brig Gen Dr. Mohammad Mizanur Rahman (Retd)
Director,
UAMCH

23.11.2024
Prof. Dr. Most. Fahmida Begum
MBBS, M. Phill
Head of Department, Microbiology,
UAMC&H

UTTARA ADHUNIK MEDICAL COLLEGE HOSPITAL

House # 34, Sonargaon Janapath, Sector # 9, Uttara Model Town, Dhaka-1230.
Tele: Phone: 58951672, 55080711, 8932343, E-mail: uamch@yahoo.com

Date: 23-11-2024

TO WHOM IT MAY CONCERN

This is to certify that Nowshin Jahan, Student ID 20336012, from BRAC University, Bangladesh has worked in Uttara Adhunik Medical College & Hospital (UAMCH) from 17-10-2024 to 02-11-2024, as an intern in the laboratory of Microbiology Department.

The Department of Microbiology of Uttara Adhunik Medical College & Hospital (UAMCH) is satisfied with her performance.

Brig Gen Dr. Mohammad Mizanur Rahman (Retd)
Director,
UAMCH

23.11.2024
Prof. Dr. Most. Fahmida Begum
MBBS, M. Phill
Head of Department, Microbiology,
UAMC&H

Acknowledgment

We would love to offer our sincere gratitude and appreciation to all whose guidance have helped us to complete our internship and prepare this report, We would love to convey our sincere gratitude to **Dr.M. Mahboob Hossain**, our advisor, and **Dr. Munima Haque**, course coordinator of Biotechnology Program, Department of Mathematics and Natural Sciences, whose advice, guidance and motivation have encouraged us to pursue our internship with determination.

We would like to sincerely thank **Uttara Adhunik College & Hospital** personnel for allowing us to complete our internship at their facility. I am thankful to the Director of Uttara Adhunik Medical College & Hospital, **Brig Gen Dr. Mohammad Mizanur Rahman (Retd)** for permitting us and providing us the opportunity to perform the internship.

We would also like to appreciate **Prof. Dr. Most. Fahmida Begum**, Head of the department of the **Microbiology lab** whose guidance has helped us to learn about the lab's facilities and functionalities, as well as, for explaining all laboratory experiments thoroughly, allowing us to perform certain experiments and learn from the experiments.

Executive Summary

This internship report consists of our practical experience in the Microbiology laboratory of Uttara Adhunik College & Hospital which we completed within 15 days. This internship started on October 17th, 2024, and was completed on November 2nd, 2024 as a requirement for our Bachelor of Biotechnology program under the Mathematics and Natural Science (MNS) Department of BRAC University.

Uttara Adhunik College & Hospital is a general hospital and diagnostic center where we gained theoretical and practical knowledge on blood sample collection, urinalysis, biochemical tests, various manual serological tests such as Widal, and Rapid diagnostic tests through kits to diagnose malaria, dengue, COVID-19 et cetera. Additionally, we saw demonstrations of certain biochemistry and hematology tests that were automated which suited the hospital's fast-paced environment by being less time-consuming and efficient. Altogether, it was a remarkable opportunity to be familiar with laboratory protocols and tests conducted in a hospital.

Keywords: Microbiology laboratory; Blood sample collection; Urinalysis; Biochemical tests; Serological tests; Widal test; Malaria diagnosis; Dengue diagnosis; HIV diagnosis; Biochemistry tests; Hematology tests; Laboratory protocols

Table of Contents

List of Tables

List of Figures

List of Acronyms

C/S	Culture and Sensitivity
CBC	Complete Blood Count
EDTA	Ethylenediaminetetraacetic Acid
EGFR	Estimated Glomerular Filtration Rate
Fig	Figure
HBsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus
NS1	Non-structural protein 1 (of Dengue Virus)
pH	Power of Hydrogen
RDT	Rapid Diagnostic Test
UTI	Urinary Tract Infection

Chapter 1

UTTARA ADHUNIK MEDICAL COLLEGE & HOSPITAL

1.1 Introduction to Uttara Adhunik Medical College & Hospital

Uttara Adhunik Medical College Hospital is a private hospital in Sector 9, Uttara, Dhaka. It has been providing hospitable 24/7 healthcare service in the Uttara area for over 21 years. In 1984, a visionary group of individuals founded the Bangladesh Medical Studies and Research Institute (BMSRI) to revolutionize medical education in Bangladesh. Amidst the dominance of government medical services, BMSRI introduced Bangladesh Medical College (BMC), the nation's first private medical college, paving the way for private-sector contributions to healthcare education. Building on this legacy, BMSRI established the Uttara Adhunik Medical College & Hospital (UAMC&H). The hospital began its journey with outdoor services on March 12, 2003, followed by indoor facilities on March 10, 2005. Now a 500-bed institution registered under the Ministry of Health and Family Welfare, it is a trusted name in healthcare. With experienced faculty and dedicated staff, UAMC&H has become a cornerstone of private medical education and healthcare in Bangladesh. It continues to uphold its mission of advancing medical knowledge and service, making significant contributions to the nation's healthcare landscape.

Uttara Adhunik Medical Hospital in Dhaka, Bangladesh, offers a wide range of medical services across various fields to cater to the diverse healthcare needs of patients. Some of the key fields and departments include:

1. **Internal Medicine**
    - Diagnosis and management of general medical conditions.

2. **Surgery**
    - General Surgery
    - Orthopedic Surgery
    - Neurosurgery
    - Pediatric Surgery
3. **Cardiology**
    - Heart disease diagnosis and treatment.
4. **Gynecology and Obstetrics**
    - Women's health, pregnancy, and childbirth.
5. **Pediatrics**
    - Child health and diseases.
6. **Neurology**
    - Disorders of the brain and nervous system.
7. **Oncology**
    - Cancer diagnosis and treatment.
8. **Urology**
    - Diseases of the urinary tract and male reproductive organs.
9. **Dermatology**
    - Skin, hair, and nail disorders.
10. **Ophthalmology**
    - Eye diseases and surgery.
11. **ENT (Ear, Nose, and Throat)**
    - Diagnosis and treatment of ENT-related conditions.

12. **Radiology and Imaging**
    - X-rays, CT scans, MRIs, and ultrasounds.
13. **Pathology and Laboratory Services**
    - Diagnostic testing and analysis.
14. **Emergency and Critical Care**
    - 24/7 emergency services and intensive care units (ICUs).
15. **Anesthesiology**
    - Pain management and perioperative care.

It also has standard cabins, men's and women's wards, ICUs, and more. Numerous tests are carried out in the hospital's Microbiology Lab. Some of these are:

1. Biochemical Tests
2. Antimicrobial Susceptibility Test
3. Microscopic examination
4. Widal test, NS1 test, Malaria, etc.
5. Hormone Tests
6. Urine tests, urine analysis, UA, etc.

1.2 Microbiology Laboratory of Uttara Adhunik Medical College Hospital

The Microbiology Lab is a well-equipped and well-staffed laboratory. It is located on the Ground floor, with a sample collection room on the same floor. The lab in itself is divided into 3 broad parts; the main area is equipped with automated and semi-automated machines to carry out serology, biochemistry, hematology etc tests. This area contains the following machines:

1. BD BACTEC^{TM} FX-40
2. ARCHITECT *i*1000SR immunoassay analyzer
3. Fully automated biochemistry analyzer Sysmex BX-3010
4. Fully automated Hematology Analyzer DYMind DF50
5. Semi-automated Biochemistry Analyzer Microlab 300
6. Immunofluorescence quantitative analyzer Getein 1100

There are two subsections on the side, one for manual blood tests and another for culture and urine sample storage, handling, and testing. The manual blood tests include:

1. Blood grouping
2. Widal test
3. COVID-19
4. Malaria
5. NS1 test
6. IgG/IgM and more.

The Microbiology lab is fully functional and operational 24/7. Samples are received at any time of the day and the tests are carried out in a timely manner. Reports are printed and then again cross-checked by the lab in-charge before being signed and delivered.

Chapter 2

Sample Collection and Culture

2.1 Blood, Urine, Stool, and other sample collection

The first step in the laboratory work is sample collection. Blood samples are collected in ready-for-use bottles called BACTEC™ FX40 (8-10 ml for adults & 1-3 ml for babies). The BD BACTEC™ FX40 system is a compact, automated blood culture instrument designed to detect microbial growth in blood samples. It uses advanced fluorescence technology for rapid and accurate results.

Figure 1: BACTEC™ FX40 Culture Vial (8-10 ml Adult)

2.2 Culture of Blood

1. Preparation for Blood Collection

- **Hand Hygiene**: Hands had been washed or sanitized with alcohol-based hand rub.
- **Equipment Gathered**:
    - Sterile blood culture bottles (aerobic and anaerobic) had been prepared.
    - Alcohol swabs, sterile gloves, a blood collection device (e.g., syringe or vacutainer), a tourniquet, and bandages had been assembled.
- **Bottles Labeled**: Bottles had been pre-labeled with the patient's details for identification.

2. Patient Prepared

- **Procedure Explained**: The patient had been informed about the purpose and steps of the procedure.
- **Tourniquet Applied**: A tourniquet had been placed above the venipuncture site to locate a vein.
- **Site Disinfected**: The venipuncture site had been cleaned with alcohol in a circular motion, and the area had been allowed to dry.

3. Blood Collected

- **Venipuncture Performed**: A sterile butterfly needle was inserted at a 45-degree angle into the chosen vein.
- **Bottles Filled**:
    - The required blood volume (usually 8–10 mL per bottle) had been drawn.
    - Then automatically blood had filled up to the mark.

4. Finalized Collection

- **Needle Removed**: The needle had been withdrawn carefully, and the site had been pressed with sterile gauze.
- **Site Covered**: A bandage had been placed over the puncture site.
- **Bottles Mixed**: Bottles were gently inverted to mix the blood with the culture medium.

5. Transport and Loading

- **Bottles Inspected**: Bottles had been checked for contamination, damage, or underfilling.
- **Samples Transported**: The bottles were transported promptly to the laboratory.
- **Bottles Loaded**: The bottles had been placed into the BD BACTEC FX40 system for incubation and monitoring.

Figure 2: BD BACTEC™ FX40 Machine

1. Bottle Preparation and Inoculation

- Blood or sterile fluid samples are collected and inoculated into BD BACTEC culture bottles containing a specific medium.
- The bottles are sealed and gently inverted to mix the sample with the medium.

2. Bottle Loading

- Inoculated bottles are placed into designated slots in the FX40 machine.
- Each slot is equipped with sensors to track (Scanner) individual bottles.

3. Automated Incubation

- The machine incubates the bottles at an optimal temperature (typically 35–37°C) to encourage microbial growth.
- Each bottle is monitored independently for changes in fluorescence.

4. Real-Time Detection

- If microorganisms grow in the sample, CO_2 is released, changing the fluorescence signal.
- The system detects this signal change in real time and marks the bottle as **positive** (incubation period 6 - 96 hours)

5. Alert System

- Positive bottles trigger a visual alert (Green color signal) on the FX40 system, notifying laboratory staff.
- Detailed data, including time-to-detection and bottle status, are displayed on the system interface or monitor.

6. Reporting and Further Analysis

- Positive bottles are removed for further analysis, such as Gram staining, subcultures, or molecular testing to identify the specific pathogen.
- Negative bottles are incubated for the full duration (e.g., 5–7 days) before being discarded.

Urine, Stool & other samples are collected in different types of containers manually from the patients. And the cultures are done in different cultural mediums.

Figure 3: Urine Container

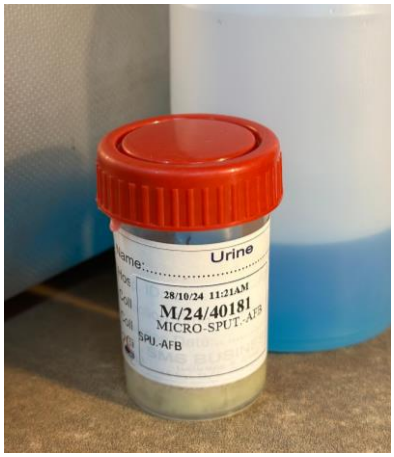

Figure 4: Sputum Container

Figure 5: Stool Container

2.3 Types of Tubes

Blood samples are collected in 4 different types of tubes, according to the prescribed tests. These tubes are identified by their color and are red, lavender, blue, gray, and black. Each tube has different reagents required for different tests hence blood is collected in specific tubes depending on the tests being conducted. The tubes and the tests they are used:

Fig 6: Grey Tubes (RBS/ Glucose test) *Fig 7: Black Tubes (ESR Test)* *Fig 8: Red tube (Serology & immunology tests)* *Fig 9: Lavender tube (Hematology, CBC tests)* *Fig 10: Blue tube (Coagulation)*

Red-top tubes had mainly been used for all types of Serology, Immunology & Hormone tests that had also been conducted in the microbiology lab at Uttara Adhunik Medical College & Hospital.

2.4 Sub-Culture Media Names & Preparation

1. Blood Agar Media:

1. The base media had been boiled to dissolve.
2. It had been autoclaved at **121°C for 15 minutes**.
3. The media had been cooled to **45°C**.
4. **5–10% defibrillated blood** had been added to the media.
5. The media had been gently mixed and poured into sterile Petri dishes.

Figure 11: Blood Agar Media

2. Chocolate Agar:

1. The media had been prepared by following the same steps as blood agar.
2. After the blood had been added, the media had been gently heated to lyse red blood cells, resulting in a chocolate-brown color.
3. The prepared media had been poured into sterile Petri dishes.

Figure 12: Chocolate Agar

3. CLED (Cystine Lactose Electrolyte Deficient Agar)

- **Composition**: 36.25 g/L.
    1. **36.25 g** of CLED media powder had been weighed.
    2. It had been dissolved in **1 liter of distilled water**.
    3. The solution had been boiled to dissolve completely.
    4. It had been autoclaved at **121°C for 15 minutes**.
    5. The media had been cooled to **45–50°C** and poured into sterile Petri dishes.

Figure 13: CLED Agar

4. MacConkey Agar

- **Composition**: As per standard protocols, typically 50.15 g/L.
- **Preparation Steps**:
    1. **50.15 g** of MacConkey agar powder had been weighed.
    2. It had been dissolved in **1 liter of distilled water**.
    3. The solution had been boiled to dissolve the medium completely.
    4. It had been autoclaved at **121°C for 15 minutes**.
    5. After autoclaving, the medium had been cooled to **45–50°C**.
    6. The cooled medium had been poured into sterile Petri dishes and allowed to solidify.

Figure 14: MacConkey Agar

5. Simmon Citrate Agar

- **Composition**: 24.2 g/L.
- **Preparation Steps**:

1. **24.2 g** of Simmon citrate agar powder had been weighed.
2. It had been dissolved in **1 liter of distilled water**.
3. The solution had been boiled to dissolve completely.
4. It had been autoclaved at **121°C for 15 minutes**.
5. The media had been cooled to **45–50°C** and poured into sterile tubes if slants were required.

6. MIU (Motility Indole Urea) Medium

- **Composition**: 18.9 g/950 mL.
- **Preparation Steps**:
    1. **18.9 g** of MIU medium base powder had been weighed.
    2. It had been dissolved in **950 mL of distilled water**.
    3. The solution had been boiled to dissolve the medium.
    4. It had been autoclaved at **121°C for 15 minutes**.
    5. The medium had been cooled before use.

7. Bile Media (e.g., Bile Esculin Agar or Bile Salt Media)

- **Composition**: As per the specific formulation, typically 64 g/L (for Bile Esculin Agar).
- **Preparation Steps**:
    1. The required amount of bile media powder (**e.g., 64 g**) had been weighed.
    2. It had been dissolved in **1 liter of distilled water**.
    3. The solution had been boiled to dissolve the medium completely.
    4. It had been autoclaved at **121°C for 15 minutes**.
    5. After autoclaving, the medium had been cooled to **45–50°C**.

6. The cooled medium had been poured into sterile Petri dishes (or tubes for slants) and allowed to solidify.

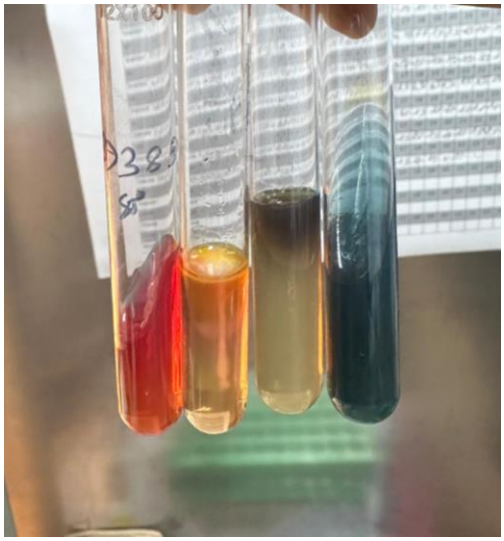

Figure 15: TSI, MIU, Bile, and Citrate test tubes

2.5 Sample Sub-Culture Process

2.5.1 Urine Sample Subculture Process (Plate Streaking)

1. The urine sample had been collected and prepared for subculturing.
2. A previously labeled CLED media plate with the sample's serial number had been taken.
3. A rod iron loop had been used for the process and the loop had been sterilized by burning it in a flame.
4. After sterilization, the loop was cooled to avoid damaging the sample or media.
5. The cooled loop had been used to take a small amount of the urine sample.

6. The plate had been oiled (spread with the sample) and streaking had been performed using the three-quadrant method at a 60° angle to spread the sample.
7. The plate had been closed properly and incubated at 37°C for 24 hours.
8. The loop had been burned again to sterilize it after use.
9. The next day, the results had been checked for bacterial growth.
10. Bacterial colonies, mostly E. coli, had been observed and identified as the common pathogen in urine samples.

Figure 16: E.coli growth after 24 hrs incubation in CLED media (slightly pink color)

The **stool** and **sputum** subculture process was the same as the urine subculture process, using the plate streaking method. However, the medias are different:

1. **MacConkey Agar**:
    a. It Has been used to isolate **Gram-negative enteric bacteria** and differentiate **lactose fermenters** (e.g., *E. coli*) from **non-lactose fermenters** (e.g., *Salmonella*, *Shigella*).
    b. Lactose fermenters had appeared **pink**, and non-lactose fermenters had appeared **colorless** on the medium.
2. **Blood Agar**:
    a. It Has been used to detect **hemolytic activity** of bacteria, particularly **Gram-positive cocci** like *Streptococcus* spp.

b. **Alpha-hemolysis** had appeared **green**, **beta-hemolysis** had appeared in clear **zones**, and **gamma-hemolysis** had shown **no change**.

3. **Chocolate Agar**:
    a. It Has been used to support the growth of **fastidious organisms** such as *Haemophilus influenzae* and **Neisseria spp.**
    b. The medium had turned **brown** due to the lysed red blood cells, providing nutrients for these pathogens.

Common Bacteria Found:

- **Stool**: *E. coli*, *Salmonella*, *Shigella*, *Enterococcus*.
- **Sputum**: *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Klebsiella pneumoniae*.

Growth Appearance:

Table 1: Observation of Different Types of Organisms

Result	Name of Organism	Observation	Sample
	*Eshcheria coli*	*E. coli* had been observed as **pink colonies** on MacConkey agar. Colonies had appeared smooth, moist, and slightly raised in texture.	Stool
	*Klebsiella pneumoniae*	*Klebsiella* spp. had been observed as **large, mucoid/saggy, pink colonies** on MacConkey agar.	Blood
	*Salmonella typhii*	*Salmonella typhi* colonies had been observed as **small, smooth, and colorless to slightly gray** on chocolate agar.	Blood

Chapter 3

Biochemical Tests

Biochemical tests are utilized to identify bacterial species by distinguishing them based on their biochemical activities. These tests represent one of the oldest methods for microorganism identification, complementing phenotypic analysis. Various biochemical tests are used to detect the presence of bacteria, which can be categorized into three main groups:

- **Universal**
- **Differential**
- **Specific**

3.1 Citrate Test

Simmons Citrate Agar is a selective and differential medium used to assess citrate utilization by bacteria. In this medium, citrate serves as the sole carbon source, while inorganic ammonium salts provide the sole nitrogen source, allowing the organism's metabolic abilities to be tested. Bacteria are inoculated into the medium, which contains sodium citrate and a pH indicator, bromothymol blue. This medium is an integral part of the IMViC tests. A positive result is indicated by bacterial growth and a blue coloration on the slant.

Certain bacteria, such as *Klebsiella*, *Enterobacter*, *Citrobacter*, *Providencia*, *Proteus*, *Serratia*, *Vibrio cholerae*, *Pseudomonas*, *Salmonella enteritidis*, and members of the subgenera *Salmonella* II, III, and IV, can produce positive results. The enzyme citrase breaks down citrate into oxaloacetate and acetate. Oxaloacetate is further metabolized into carbon dioxide and pyruvate. The metabolism of ammonium salts generates ammonia or sodium carbonate through the release of carbon dioxide, raising the alkalinity of the medium. This pH shift, above 7.6, changes the medium's color from green to blue, with bromothymol blue acting as the indicator.

To perform the test, bacterial colonies are collected using a straight wire and inoculated onto the slope of Simmons Citrate Agar. The medium is then incubated overnight at 37 °C.

Result Interpretation:

Citrate positive: The pH indicator (Bromothymol blue) turns blue.

Citrate negative: Remain Green color or no color change.

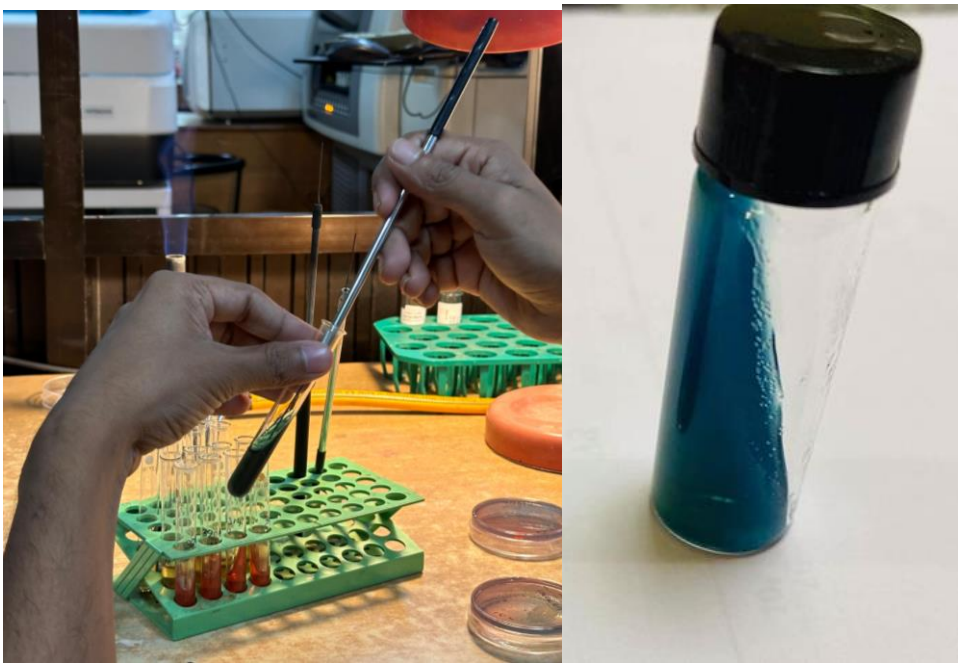

Figure 17: Result Interpretation of Citrate Test

3.2 Triple Sugar Test (TSI)

The TSI (Triple Sugar Iron) test is a microbiological assay designed to assess a microorganism's ability to ferment sugars and produce hydrogen sulfide (H_2S). The medium used for this test contains multiple sugars and is characterized by a pH-sensitive phenol red indicator. The formulation includes ingredients such as sodium thiosulfate, ferrous ammonium sulfate, and 1% each of lactose, sucrose, and glucose. These components allow the medium to solidify at a slanted angle, forming the TSI test tube, which consists of two sections: the butt and the slant.

For inoculation, a zigzag streak pattern is first made across the surface of the slant. Then, a straight needle is used to stab deeply into the agar butt. The inoculated tube is incubated for 18–24 hours to detect gas production, H_2S production, and sugar fermentation.

Possible Reactions in a TSI Tube

1. Yellow Butt and Red Slant:
    - Indicates glucose fermentation.
    - Lactose or sucrose is not fermented.
    - The slant remains red (alkaline) due to the limited glucose content and minimal acid formation.
2. Yellow Butt and Yellow Slant:
    - Indicates fermentation of lactose or sucrose.
    - A significant amount of acid is produced, turning the phenol red indicator yellow.

3. Red Butt and Red Slant:
    - Indicates no sugar fermentation.
4. Gas Formation (CO_2):
    - Splitting or cracking of the agar indicates CO_2 gas production.
5. H_2S Production:
    - Black precipitation in the butt indicates the presence of H_2S.

This test provides a comprehensive way to interpret a microorganism's metabolic activities based on the observed reactions in the TSI tube.

Figure 18: Alkaline slant/alkaline butt. Red/Red = glucose, lactose, and sucrose non-fermenter Acidic slant/acidic butt; Yellow/Yellow = glucose, lactose and/or sucrose fermenter gas (+ or -), H2S (+ or -).

3.3 Motility, Indole, Urease Test (MIU)

The MIU test, an abbreviation for Motility, Indole, and Urease, combines three assays in a single tube to identify and differentiate bacterial species, particularly Gram-negative bacilli such as members of the Enterobacteriaceae family. This test uses a semi-solid medium and requires precise inoculation with a straight needle.

1. **Motility Test**:
    - Assesses an organism's ability to move independently.
    - Motile organisms grow away from the stab line, while non-motile organisms grow along it.
    - Semi-solid media allow the observation of this movement.
2. **Indole Test**:
    - Detects the production of indole from tryptophan by the enzyme tryptophanase.
    - Indole reacts with **Kovac's reagent** (p-dimethylaminobenzaldehyde) to produce a red-violet compound, confirming indole production.
3. **Urease Test**:
    - Evaluates the ability of bacteria to hydrolyze urea into ammonia using the enzyme urease.
    - Ammonia raises the pH, turning the medium's **phenol red indicator** from yellow to pink, indicating an alkaline environment.

Procedure

1. **Preparation**:
    - A semi-solid agar medium is prepared in a test tube.
2. **Inoculation**:
    - Using a straight wire, make a single stab down the center of the medium to about halfway through the tube.
3. **Incubation**:
    - Incubate the tube at **37°C** for 24 hours under conditions favoring motility.
4. **Observation**:
    - After incubation, examine for:
        - **Motility**: Diffused growth indicates motile organisms, while growth restricted to the stab line indicates non-motile organisms.
        - **Indole production**: The addition of Kovac's reagent results in a red-violet layer if indole is present.
        - **Urease activity**: A color change from yellow to pink confirms urease activity.

This integrated test is a reliable method for identifying and differentiating bacteria based on their motility, indole production, and urease activity.

Result Interpretation :

Table 2: Result Interpretation of Motility

Motile	We will notice cloudy media and the organism will spread over the stab line.
Non-Motile	The media will not be cloudy, and the organism will grow along the stab line.

Figure 19: Motility positive (Left one) Motility negative (right one)

Table 3: Result Interpretation of Indole Test

Indole Positive	Indole-positive bacteria produce a red cherry ring upon the addition of Kovacs' reagent
Indole Negative	There did not form red cherry ring upon the addition of Kovacs' reagent.

Figure 20: Indole positive (Right one) Indole negative (Left one)

Table 4: Result Interpretation of Urease Test

Urease positive	pH indicator turns pink in the presence of ammonia.
Urease negative	No color change was noticed.

Figure 21: Urease negative (right one)Urease positive(left one)

3.4 Bile Test

The bile test had been performed to identify bacteria capable of growing in bile-rich environments and hydrolyzing esculin. This test had been particularly useful for differentiating Enterococcus spp. and Group D Streptococci from other Gram-positive cocci.

- Positive Result: Indicated by black precipitation in the medium, typical of Enterococcus faecalis and related species.
- Negative Result: No black coloration, suggesting the organism could not hydrolyze esculin or tolerate bile salts.

This test has been an important diagnostic tool for identifying bile-resistant, esculin-hydrolyzing bacteria in clinical microbiology.

Bile Test Procedure

1. The test was conducted to evaluate the ability of bacteria to grow in the presence of bile salts.
2. A bile esculin agar medium had been prepared, which contained bile salts, esculin, ferric citrate, and nutrients to support bacterial growth.
3. The bacterial sample had been streaked onto the surface of the medium or inoculated into the agar slant using a sterile loop.
4. The tube or plate had been incubated at 37°C for 24–48 hours.

5. A positive result had been indicated by the formation of a dark brown or black precipitate in the medium, caused by the hydrolysis of esculin to esculetin, which reacted with ferric citrate.

Result Interpretation of Bile Test

- **Positive Result**:
    - **Dark brown/black precipitate** forms, indicating the bacterium can **hydrolyze esculin** in the presence of bile.
    - Typically observed in **Enterococcus spp.** and **Group D Streptococci**.

- **Negative Result**:
    - No color change, indicating the bacterium cannot **hydrolyze esculin** or tolerate bile salts.
    - Seen in non-enterococcal Gram-positive cocci.

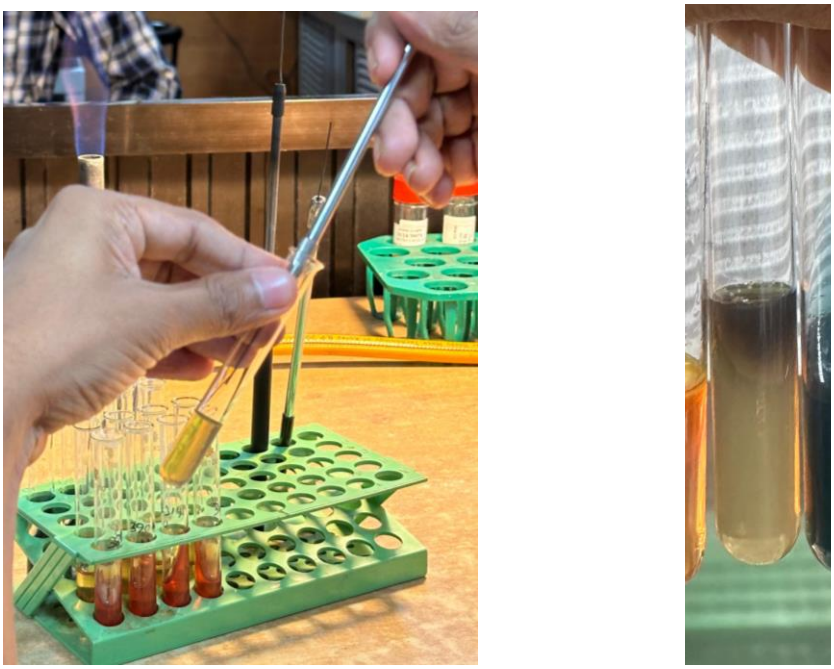

Figure 22: Left one negative, right one with black precipitation is a negative result

3.5 Oxidase Test

• Oxidase test:

The oxidase test is used to indicate if an organism has the cytochrome c oxidase enzyme. This test is used as an aid to distinguish between *Neisseria, Moraxella, Campylobacter, and Pasteurella spp.* which is oxidase positive. It is also used to distinguish the Pseudomonas from closely related species.

Again, for the electron transport chain (ETC), the cytochrome-c oxidase enzyme plays a vital role. It indicates the existence of the cytochrome-c oxidase enzyme when oxidizing TEMPEH (tetramethyl p-phenylenediamine dihydrochloride, the redox dye). The blue-purple color can be noticed after the oxidizing, which indicates an oxidase-positive result. The organism does not contain the cytochrome-c oxidase enzyme that remains colorless, which indicates an oxidase negative result. Oxidase-positive bacteria are considered aerobes, and oxidase-negative bacteria are considered anaerobe, aerobe, or facultative.

Figure 23: TEMPEH (tetramethyl p-phenylenediamine dihydrochloride, the redox dye)

Result interpretation for the oxidase test

Oxidase positive: A deep blue-purple color will indicate the presence of organisms.

like *Pseudomonas spp.*, etc., which successfully produce cytochrome c-oxidase within

5-10 seconds.

Oxidase negative: Colorless or no color change indicates the presence of organisms.

like *Escherichia coli*, etc., and the absence of cytochrome c oxidase.

Figure 24: Oxidase negative (white color one)

3.6 Catalase Test

This test illustrates the nearness of catalase, an enzyme that catalyses the discharge of oxygen from hydrogen peroxide (H2O2). Hydrogen peroxide (H2O2) could be a harmful by-product. It is utilized as a reagent, 6% hydrogen peroxide is utilized. Hydrogen peroxide (H2O2) is changed over into oxygen and water through the action of an enzyme named catalase. It is evident that the enzyme is displayed if a little inoculum of a bacterial isolate is included with hydrogen peroxide and the rapid elaboration of oxygen bubbles takes place. The non-appearance of catalase is evident from a lack of drowsy bubble generation. The culture shouldn't be more seasoned than twenty-four hours. By doing this, microbes guard themselves against hydrogen peroxide's dangerous impacts. Accumulation happens as a byproduct of aerobic carbohydrates in the digestive system.

Figure 25: Hydrogen peroxide solution

Result interpretation for the catalase test

• **Catalase positive:** Bubble formation noticed For example, *Staphylococcus aureus*

• **Catalase negative:** No bubble formation and absence of catalase enzyme to hydrolyze the hydrogen peroxide. For example, *Streptococcus spp.*

Figure 26: Catalase positive result (bubble formation noticed), no bubble one is negative.

3.7 Bacterial Identification

The process of identifying the kind of bacteria present in a sample by a variety of techniques, including molecular procedures, biochemical tests, and microscopy, is known as bacterial identification. For several reasons, including illness diagnostics, where it aids in identifying the precise pathogens causing infections, bacterial identification is essential. By determining which antibiotics work best, this knowledge helps choose the best course of therapy. Bacterial identification in food safety ensures consumer protection by identifying dangerous germs in food items. To determine the presence and effects of bacteria in water, soil, and air, environmental monitoring depends on their identification. Furthermore, identifying bacteria is essential for study since it helps us better understand how they interact and operate in various settings. It can be done in many ways. Mostly used two ways are discussed below:

3.7.1 Gram Staining

Gram staining is a differential staining technique used to classify bacteria into two groups based on their cell wall composition i.e gram positive and gram negative.

Steps:

- A smear was prepared. A drop of bacterial culture was placed on a clean slide, air dry, and heat-fix by passing the slide over a flame.
- Primary stain (Crystal Violet): The slide was flooded with crystal violet for 1 minute, then rinsed with distilled water.
- Mordant (Iodine solution): Gram's iodine was applied for 1 minute, then rinsed with water. This formed a crystal violet-iodine complex.
- Decolorization (Alcohol/Acetone): The slide was washed with 95% ethanol or acetone for 10-15 seconds until the runoff was clear, then rinsed immediately with water.
- Counterstain (Safranin): The slide was flooded with safranin for 1 minute, then rinsed with water.
- Dry and observe: The Blot was dried gently, and then examined under a microscope using oil immersion (100x objective).

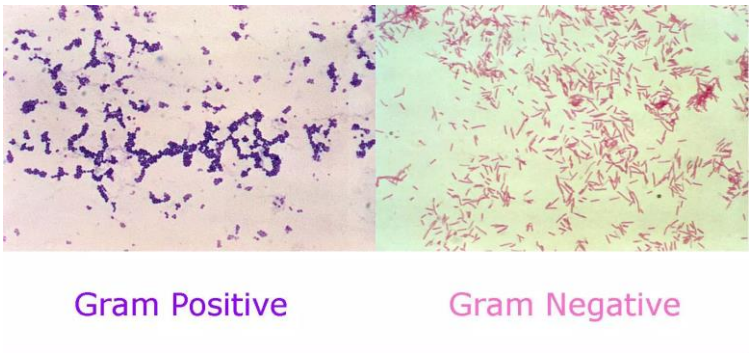

Figure 27: Gram positive and Gram negative

Result:

Gram-positive bacteria: It has a thick peptidoglycan layer, retains crystal violet, and appears purple/blue under a microscope.

Gram-negative bacteria: It has a thin peptidoglycan layer and an outer membrane, loses crystal violet during decolorization, and takes up the safranin counterstain, appearing red/pink.

3.7.2 Microscopic Observation

Steps:

The following are instructions for using an electron microscope:

1. If required, repair and stain the sample to get it ready.
2. The sample was inserted into the microscope chamber after mounting it on a specimen holder.
3. An atmosphere was established that is conducive to electron passage, vacuum the chamber.
4. The microscope was switched on and the magnification and voltage were adjusted.
5. The coarse was adjusted and fine adjustment knobs to adjust the focus.
6. Depending on the sample, the preferred imaging mode was chosen (transmission or scanning).
7. Using the camera or digital device attached to the microscope, the image was captured.

Figure 28: How to observe a sample under a Microscope

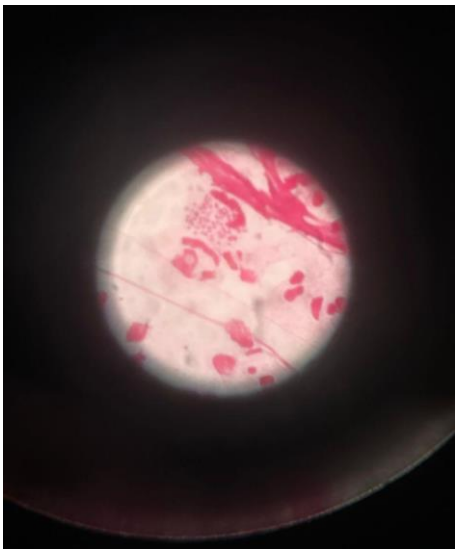

Figure 29: Neisseria gonorrhoeae under a microscope (Diplococci)

3.8 Antimicrobial Susceptibility Test (AST)

Under carefully monitored laboratory settings, antibiotic susceptibility testing (AST) assesses how well different antibiotics limit bacterial growth (Bayot & Bragg, 2024). We used the Kirby-Bauer disk-diffusion approach, a well-known AST methodology, in our investigation.

MHA (Mueller-Hinton Agar)

- **Composition**: 38 g/L.
- **Preparation Steps**:
    1. **38 g** of Mueller-Hinton agar powder had been weighed.
    2. It had been dissolved in **1 liter of distilled water**.
    3. The solution had been boiled to dissolve completely.
    4. It had been autoclaved at **121°C for 15 minutes**.
    5. The media had been cooled before being poured into sterile Petri dishes.

Bacterial Suspension Preparation

1. A bacterial suspension was made before the antibiotic susceptibility test.
2. 2 ml saline through syringe was used for this and was placed in an autoclaved test tube.
3. With this, using a sterile metallic loop, one loopful of bacterial colony from the 18-hour-old culture was collected and combined.
4. For the best bacterial growth, the saline and colony combination was vortexed and left for 15 minutes.
5. With a cotton swab, it was spread on Muller-Hinton agar.
6. The antibiotic discs were put according to the list of antibiotics.
7. It was inoculated for 18-24 hours at 37°C.

Figure 30: Antibiotic Susceptibility Testing (AST)

The selection of antibiotics for the susceptibility test was based on their recommended usage. The table below presents the list of antibiotics utilized in this research study, along with their corresponding zone of inhibition-

Table 5: Antibiotic List with their Diameter Zone of Inhibition

Antibiotic	**Antibiotic**	**Code**	**Disc**	**Diameter of zone inhibition**

group	**Name**		**Concentration (mcg)**	Susceptible (S) (mm)	Intermediate (I) (mm)	Resistant (R) (mm)
Penicillins	Ampicillin	AMP	2	≥17	14-16	≤13
Monobactams	Aztreonam	ATM	30	≥22	16-21	≤15
Cephalosporins (4th gen)	Cefepime	FEP/CPM	30	≥26	21-25	≤20
Amphenicols	Chloramphenicol	C	30	≥18	13-17	≤12
Macrolides	Azithromycin	AZM	25	≥15	10-14	≤9
Tetracyclines	Doxycycline	DO	30	≥16	13-15	≤12
Macrolides	Erythromycin	E	15	≥23	14-22	≤13
Aminoglycosides	Gentamicin	CN	10	≥15	13-14	≤12
Carbapenems	Imipenem	IPM	10	≥23	20-22	≤19
Aminoglycosides	Kanamycin	K	30	≥18	14-17	≤13
Fluroquinolones	Levofloxacin	LEV	5	≥17	14-16	≤13
Oxazolidinones	Linezolid	LZD	30	≥23	21-22	≤20
Lincosamides	Clindamycin	DA	2	≥21	15-20	≤14
Glycopeptides	Vancomycin	VA	30	≥17 (for gram positive like *S.aureus*)	N/A	N/A

Chapter 4

Manual Serological Tests

4.1 Widal Test (Test for Typhoid)

A serological diagnostic method, the Widal test is mainly used to identify typhoid fever, which is caused by the *Salmonella enterica* serotype Typhi, and, to a lesser degree, paratyphoid fever, which is caused by *S. Paratyphi*. This test, which was created by Georges-Fernand Widal in 1896, is still often used, particularly in areas with little access to sophisticated diagnostic equipment.

The test is done using four available (O and H) antigens of *Salmonella enterica* serotype Typhi. The four reagents are:

1. **TO:** O antigen of *Salmonella typhi*
2. **TH:** H antigen of *Salmonella typhi*
3. **AH:** H antigen of *Salmonella paratyphi A*
4. **BH:** H antigen of *Salmonella paratyphi B*

Figure 31: Four reagents for Widal test

Steps

1. 2 drops, each consisting of 10 microlitres of the patient's blood serum is taken on each glass plate using a micropipette.
2. Four reagents (TO, TH, AH, BH) are added serially using droppers in reagent-containing bottles.

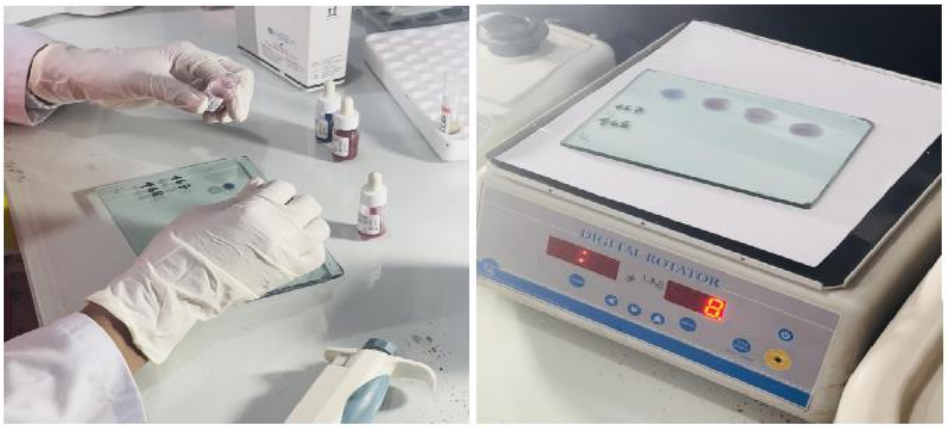

Figure 32: Mixing the reagents and the blood serum

3. Then the reagents and the serum drops were mixed.
4. For better mixing, the glass plate was put on a shaker rotator for 2 minutes.

Result

The antibody titers are used to interpret the results. Significant titers indicate a recent or ongoing infection; they are often 1:80 or 1:160 or above. However, because of possible cross-reactions with other bacterial illnesses and previous immunizations, care must be used when interpreting data (House et al., 2001). If any agglutination (on any of the mixed drops) is detected under white light, Typhoid's result will be positive. On the other hand, if no agglutination is detected under white light, the result will be negative for Typhoid.

➢ **Several important points for Typhoid-positive results:**

- **C/S test:** This test is done to determine appropriate antibiotic treatment and diagnosis in case of typhoid-positive result.
- **Blood culture:** A sample of the patient's blood is cultured to check for the presence of *Salmonella typhi* bacteria. If the bacteria are present, they will grow in the culture medium, confirming the disease.
- **Sensitivity Testing:** Once *Salmonella typhi* is identified, sensitivity testing is performed to determine which antibiotics are effective in treating the infection. This is crucial because *Salmonella typhi* has been found to develop resistance against several antibiotics.

4.2 Blood Grouping

Blood grouping is a method used to categorize blood based on the presence or absence of specific antigens on the surface of red blood cells (RBCs). The primary ABO system includes four major blood types: A, B, AB, and O. These groups are defined by whether A and B antigens are present on the RBC surface (Dean, 2005).

Additionally, the Rh system classifies blood based on the presence of the RhD antigen. If the RhD antigen is present, the blood type is Rh-positive; if absent, it is Rh-negative. This results in a total of eight possible blood types: A+, A-, B+, B-, O+, O-, AB+, and AB- (Daniels, 2013).

Blood group testing involves combining a blood sample with different antibody solutions. Observing the reaction between the blood cells and specific antibodies helps determine the blood type.

Steps:

1. A glass slide was divided into three sections labeled A, B, and D.
2. Three drops (approximately 20 microliters each) of blood were placed on the slide using a micropipette.
    - **Anti-A serum:** Detects the presence of A antigen.
    - **Anti-B serum:** Detects the presence of B antigen.
    - **Anti-D serum:** Identifies the Rh factor (RhD antigen).

Figure 33: Anti-A, Anti-B, Anti-D (Anti-Rh factor) antigens

3. The anti-seram with the blood samples was mixed.
4. The slide was placed on a shaker for one minute to observe agglutination reactions.

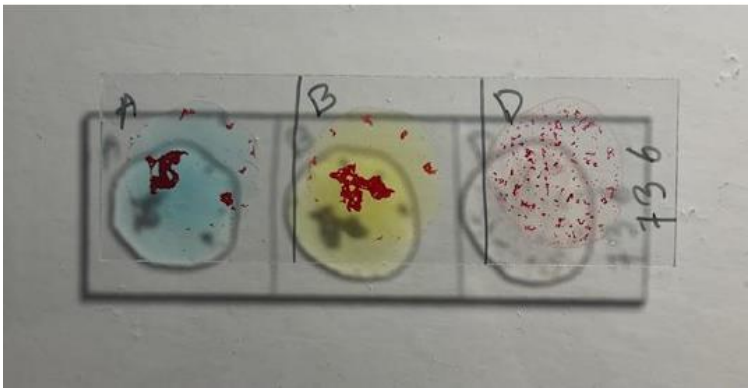

Figure 34: Agglutination is seen in all three portions; antigen-A, antigen-B, and Rh-positive antigens are present, therefore the blood group is AB+

Results Interpretation:

- Agglutination in the Anti-A serum indicates the presence of antigen A.
- Agglutination in the Anti-B serum indicates the presence of antigen B.
- Agglutination in the Anti-D serum indicates Rh-positive blood.

Chapter 5

Kit-based Serological Tests

The hospital's serology lab used rapid diagnostic tests (RDTs) to identify several illnesses, including COVID-19, dengue, and malaria. Usually, these tests are used to find out whether a blood sample contains antigens or antibodies. During the internship, the following examinations were administered:

Table 6: List of Kit-based Serological Tests for Rapid Detection

Name of Test	**Procedure**	**Result Interpretation**
COVID-19	The cotton swab was put in the nasal and sample was collected. Then the nasal sample was dipped in the buffer followed by 4 drops of buffer added to the kit.	Results were read after 15 minutes. 2 lines, one in C and one in T indicate positive while only one line in C means negative. No result interpretation after 20 minutes to avoid false results.
Malaria	10µl of whole blood followed by 2 drops of buffer added.	Results were read after 20 minutes. 2 lines or 3 lines mean positive i.e. C and Pf or Pv or C, Pf and Pv. Only one line in C means negative.
Dengue NS1	5µl serum was added followed by 2 drops of buffer.	Results were read after 15 minutes. Positive is indicated by 2 lines one in the C region and another in T. Only one line in C means negative. If negative then perform additional tests like IgG and IgM. When the infection has proceeded past the early stage (when NS1 antigen is evident) or when individuals exhibit symptoms past the acute phase, testing for IgM and IgG antibodies is especially helpful.

Dengue IgG-IgM	10µl of serum and immediately or within 20 seconds 4 drops of buffer added.	Results read after 15-20 minutes.Positive indicated by 2 lines and 3 lines i.e. one in C region and another in M or G or both. G indicates that the patient has been infected earlier and M indicates that the patient got infected recently. When both M and G are positive, it means the patient has been infected with dengue again.

The absence of lines in the control zone following incubation in any of the mentioned tests indicates an invalid result, necessitating a repeat test using a different kit. Since a kit's control line is meant to guarantee that the test runs correctly, the absence of a line in the C area indicates that the test did not run correctly. To get rapid answers and handle the large volume of samples in hospitals, kit-based testing is essential. Furthermore, gloves were used during the tests to reduce the possibility of infection and transmission. If the person conducting the tests has any cuts, the patient's blood may come into contact with them and cause an infection.

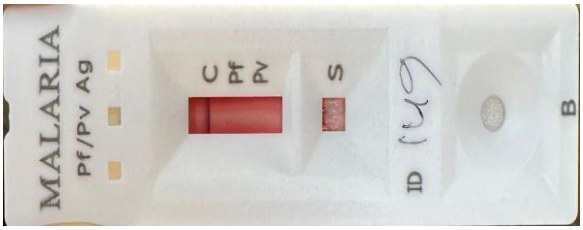

Figure 35: Tests for Malaria

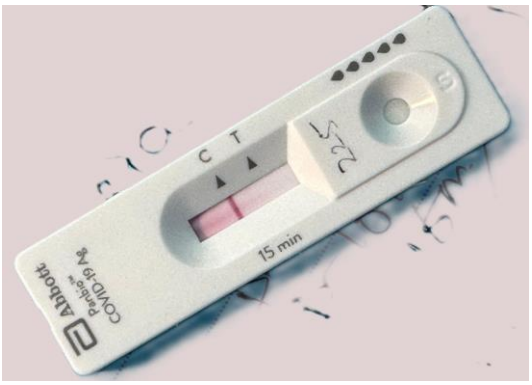

Figure 36: Tests for COVID-19

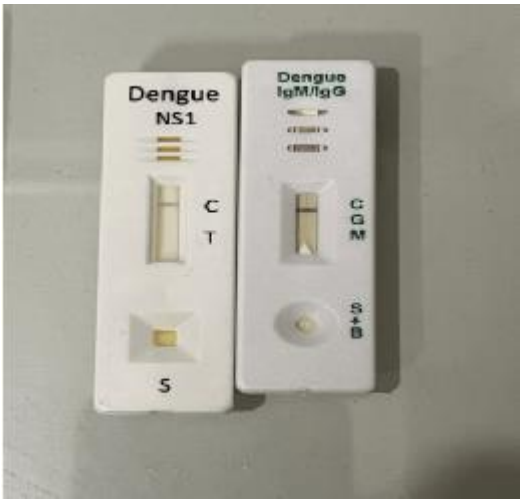

Figure 37: Tests for NS1, IgG, and IgM

Chapter 6

Machine-run Serological, and Hematology

6.1 The ARCHITECT i1000SR

Abbott Diagnostics created the automated immunoassay analyzer known as the ARCHITECT i1000SR. It is often used in clinical labs to carry out a range of diagnostic procedures, such as the identification of hormones, infectious illnesses, tumor markers, and cardiac markers. Chemiluminescent microparticle immunoassay (CMIA) technology is used by the analyzer to produce incredibly accurate and consistent findings.

- **How the ARCHITECT i1000SR Works:**

- Sample Preparation:
  - Blood or serum samples are collected and loaded into the analyzer.
  - The system automatically identifies each sample using barcodes, ensuring correct test association.
- Sample Dispensing:
  - The instrument aspirates a specified volume of the sample.
  - The sample is dispensed into a reaction vessel.
- Addition of Reagents:
  - The analyzer adds specific microparticle-based reagents and chemiluminescent labels to the sample.
  - These reagents contain antibodies or antigens that bind to the target analyte (e.g., hormone, virus).
- Incubation:

- ➢ The reaction mixture is incubated, allowing the antigen-antibody complexes to form.
- ➢ During incubation, magnetic microparticles assist in capturing the target molecules.

❖ Washing:

- ➢ Unbound substances are washed away to remove non-specific material.
- ➢ This step ensures that only bound complexes remain.

❖ Detection and Signal Measurement:

- ➢ A chemiluminescent substrate is added, triggering a light-emitting reaction.
- ➢ The amount of light emitted is proportional to the concentration of the target analyte in the sample.

❖ Data Analysis:

- ➢ The analyzer detects and measures the luminescent signal.
- ➢ Results are calculated using a calibration curve and displayed on the system's software.

❖ Result Reporting:

- ➢ Results are automatically recorded and can be transferred to laboratory information systems (LIS) for reporting.

Figure 38: The ARCHITECT i1000SR

Hormonal tests were commonly performed using the ARCHITECT i1000SR system including the detection and measurement of various hormones for diagnostic and monitoring purposes.

1. **Thyroid Function Tests**
    - Thyroid-Stimulating Hormone (TSH)
    - Free Triiodothyronine (FT3)
    - Free Thyroxine (FT4)
2. **Reproductive Hormones**
    - Luteinizing Hormone (LH)
    - Follicle-Stimulating Hormone (FSH)
    - Prolactin

- Estradiol (E2)
- Progesterone
- Testosterone

3. **Pregnancy and Fertility**
    - Human Chorionic Gonadotropin (hCG)
4. **Adrenal Function Tests**
    - Cortisol
    - Dehydroepiandrosterone Sulfate (DHEA-S)
5. **Parathyroid Hormones**
    - Parathyroid Hormone (PTH)
6. **Metabolic and Growth-Related Hormones**
    - Insulin
    - Growth Hormone (GH)

These tests utilize chemiluminescent microparticle immunoassay (CMIA) technology for high precision and accuracy.

Chapter 7

Antimicrobial resistance (AMR)

Antimicrobial resistance (AMR) refers to the ability of microorganisms, such as bacteria, fungi, viruses, and parasites, to resist the effects of medications that once effectively treated them. This resistance occurs when these pathogens evolve mechanisms to survive exposure to antimicrobials, including antibiotics, leading to treatment failures and increased health risks. The misuse and overuse of antibiotics in humans, animals, and agriculture are significant contributors to the rise of AMR. For instance, inappropriate prescribing practices and the availability of antibiotics without prescriptions have accelerated resistance development. Additionally, the agricultural sector's reliance on antibiotics for growth promotion in livestock further exacerbates this issue. The consequences of AMR are dire; it is estimated that AMR was directly responsible for 1.27 million deaths globally in 2019 and contributed to nearly 5 million deaths overall. Without concerted efforts to address this challenge, projections suggest that AMR could lead to 10 million deaths annually by 2050, highlighting an urgent need for effective strategies to combat this growing threat. The National AMR Surveillance Strategy aims to establish a robust, cross-sectoral AMR surveillance system to generate data that can inform policy decisions and the prudent production, distribution, and use of antimicrobials in Bangladesh. Uttara Adhunik Medical College and Hospital is included in this surveillance.

Conclusion

We learned about several facets of the healthcare sector during our internship at Uttara Adhunik College & Hospital, including sample collecting, diagnostic testing, and report production. Additionally, it has taught us to pay close attention to obtain correct findings by avoiding contamination and, if needed, repeating tests. In addition to the testing, we discovered how important it is to keep the equipment maintained so they are operating in optimal condition. To keep up with the fast-paced medical atmosphere, we also learned how important collaboration is in a lab. All things considered, it was a fantastic chance to learn new abilities that we can use later.

Reference

1. Daniels, G. (2013). *Human blood groups* (3rd ed.). Wiley-Blackwell. https://doi.org/10.1002/9781118493478
2. Dean, L. (2005). *Blood groups and red cell antigens.* National Center for Biotechnology Information. https://www.ncbi.nlm.nih.gov/books/NBK2261/
3. House, D., et al. (2001). Typhoid fever: Pathogenesis and disease. *Current Opinion in Infectious Diseases*, *14*(5), 573–578. https://doi.org/10.1097/00001432-200110000-00010
4. Bayot, M. L., & Bragg, B. N. (2024, May 27). *Antimicrobial susceptibility testing*. StatPearls - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK539714/
5. Aryal, S. (2022b, August 10). *Mueller Hinton Agar (MHA) – Composition, principle, Uses and preparation*. Microbiology Info.com. https://microbiologyinfo.com/mueller-hinton-agar-mha-composition-principle-uses-and-preparation/