A REPORT ON:

STUDENT INDUSTRIAL WORK EXPERIENCE SCHEME (SIWES)

FROM AUGUST TO OCTOBER 2024

UNDERGONE AT

HILLSTAR HOSPITAL

MICROBIOLOGY LABORATORY

BY

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SUBMITTED TO

DEPARTMENT OF BIOLOGICAL SCIENCES

APPRECIATION

I give thanks to God Almighty the one who gives life and sustains it, for his protection, mercy and kindness for the successful completion of my industrial training.

I also thank God for my family members for their unwavering support during the period of my training.

ACKNOWLEDGEMENT

I am grateful to the hospital I interned at, Hillstar hospital and to all my fellow IT student, it was a great pleasure working with you at HILLSTAR HOSPITAL. I appreciate my supervisors; Mrs. Olaitan and Mr Bamidele and the head of department, Dr Farohunbi, I appreciate you all for your unwavering support.

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CHAPTER ONE

1.1 GENERAL LABORATORY EQUIPMENTS

1. THE LIGHT MICROSCOPE: The microscope employs a hollow, extremely intense cone of light concentrated on the specimen. The field of view of the objective lens lies in the hollow, dark portion of the cone and picks up only scattered light from the object. The clear portions of the specimen appear as a dark background, and the minute objects under study glow brightly against the dark field. This form of illumination is useful for transparent, unstained biological material and for minute objects that cannot be seen in normal illumination under the microscope.
2. AUTOCLAVE: The autoclave is effective equipment used for steam sterilization at pressures above the atmospheric pressure. Thus, it is possible to steam at higher temperature then the boiling point, which a lot of microorganisms cannot withstand
3. REFRIGERATOR: This is used to preserve samples, reagents etc, which are used for daily analysis and cannot be exhausted at once.
4. INCUBATOR: The incubator is mainly used to incubate culture media as microbes have different optimum temperatures for growth and reproduction.
5. WATER BATH: This is required to incubate bottle of culture media, liquids in flasks or other large Containers, and when incubating samples in the test tube racks.
6. WEIGHING BALANCE: This is a delicate instrument used for weighing essential, reagent, stains and culture.
7. WIRE LOOP: Made up of a thick metallic lower part and a straight thin upper metallic part curved into a small circle usually made up of platinum which is used generally for inoculating samples and picking colonies sterilized by flaming red hot before streaking.
8. GLASS SLIDES: Used for preparation of slides for microscopy. Sterilization is by flooding with alcohol and flaming off excess alcohol.
9. COVER SLIPS: This is use for covering wet smears of preparations. It is sterilized by flooding with alcohol and flaming off excess alcohol.
10. PETRI DISH: Used for the preparation of culture media. It is usually bought sterilized. The disposable type cannot used a second time while the glass ware type can be reused be usually sterilized by autoclaving

11. FORCEPS: A pair of forceps is a metallic object used for handing hot object to sterilize contaminated materials.

2.0 BACTERIOLOGY SECTION

This section deals with the study of bacteria. Bacteria are prokaryotic organisms with different shapes such as cocci (spherical), bacilli (rod shaped, microscopy), vibro (comma shaped)

Others include:

Other Laboratory equipments include includes sterilized slide, Giemsa Stain, needle, syringe, ethanol, sterilized bottle, agar (MacConkey or Chocolate), Gram positive, Gram negative sensitivity kit , cotton wool, EDTA, microscope, oil immersion, ethanol, sterilized slides, swab sticks, cotton wool, spirit, giemsa stain, lancets, surgical blades, oil immersion, pipette, light microscope, hot plate (dryer), centrifuge, hand gloves, microhaematocrit centrifuge, capillary tubes for measuring PCV, sealant, microhaematocrit reader, anaerobic jar, test tubes, bottles, water bath, weighing balance, microscope, pipette, beakers, bio safety cabinet, cotton

2.2 SAFE WORKING PRACTICES IN A MEDICAL LABORATORY

The following are some of the important points which apply when working with infectious materials:

1. Always use safe measuring and dispensing devices.
2. Do not eat, drink, smoke, store food, or apply cosmetics in the working area of the laboratory.
3. Use an aseptic technique when handling specimens and cultures.
4. Always wash your hands after handling an infectious material in the laboratory, when leaving the laboratory and before attending to patients.
5. Wear appropriate protective clothing when working in the laboratory. Ensure it is decontaminated and laundered correctly.
6. Wear protective gloves and when indicated a face mask, for all procedures involving direct contact with infectious materials. When wearing gloves, the

hands should be washed with the gloves on, particularly before doing ant clerical work.

7. Centrifuge safely to avoid creating aerosols. Know what to do should a breakage occur when centrifuging.
8. Avoid practices which could result in needle stick injury.
9. Do not use chipped or cracked glassware and always deal with a breakage immediately and safely.
10. Avoid spillages by using racks to hold containers, work neatly and keep the bench surface free of any unnecessary materials.
11. Decontaminate working surfaces at the end of each day's work and following any spillage of any infectious fluid.
12. Report to the laboratory officer in charge, any spillage or other accident involving exposure to infectious material.
13. Know how to decontaminate specimens and other infectious materials.
14. Use and control an autoclave correctly.
15. Dispose laboratory waste safely

1.3 AT THE RECEPTION

The receptionist on seat, collects samples from patients waiting to be transferred to the laboratory, put bills on the patients cards depending on the kind of tests to be done, register the patients cards and then also register results before they are given out to patient, they also give out universal, anticoagulant bottles to patient and give them necessary instructions on how to collect into the bottles that is being given to them. Some of the laboratory materials are stored in the reception. Listed below are a few steps to follow when dispatching microbiological specimens:

1. Keep a register of all specimens dispatched. Record the name, number, and ward or health center of the patient, type of specimen, investigation required, date of dispatch, and the method of sending the specimen. When the report is

received back from the microbiology laboratory, record the date of the receipt in the register.

2. Check the specimen container is free from cracks, and the cap is leak-proof.
3. Use sufficient packaging material to protect a specimen especially when the container is a glass tube. When the specimen is fluid use sufficient absorbent material to absorb it should a leakage or breakage occur.
4. Mark all specimens that may contain highly infectious organisms.

1.4 PHLEBOTOMY

Phlebotomy is the practice of drawing blood from patients for various purposes, including medical testing, donation, or transfusion.

Methods of phlebotomy;

Venipuncture:

- The most common method, involving inserting a needle into a vein, usually in the arm, to collect blood for tests or donations.

Capillary Puncture:

- Also known as a finger stick or heel stick (for infants), this method involves pricking the skin to collect a small amount of blood. It's often used for rapid tests or in pediatric cases.

  Material used; Needle, Syringe, Alcohol swab, Torniquete, EDTA bottle.

  Procedure;

- After identifying the site of collection, it was disinfected with the alcohol swab
- The torniqute was tied above the site of collection

- A needle was inserted into the vein at a slight angle, and blood was drawn into the syringe
- Once the required amount is collected, the tourniquet is released.
- The tourniquet was released and a cotton wool was placed on the area

CHAPTER THREE

BACTERIOLOGY SECTION

This section deals with the study of bacteria. Bacteria are prokaryotic organisms with different shapes such as cocci (spherical), bacilli(rod shaped, microscopy), vibro(comma shaped)

This diagnostic method is used in staining, and culture and sensitivity test. The specimen used in detecting bacterial pathogens include urine, sputum, swabs.

3.1 CULTURE OF MICROORGANISMS

Materials; Sample (urine), wire loop, burner flame, prepared medium (blood agar), incubator.

Procedure: The wired loop was flamed until it was red hot then allowed to cool, then it was dipped into the sample (urine) to pick an inoculant and transferred into the prepared medium (blood agar), then streaked properly and stored at 37 c for 24 hours

Observation: After 24 hours of incubation, the culture plates were observed and colonies of Escherichia coli was observed.

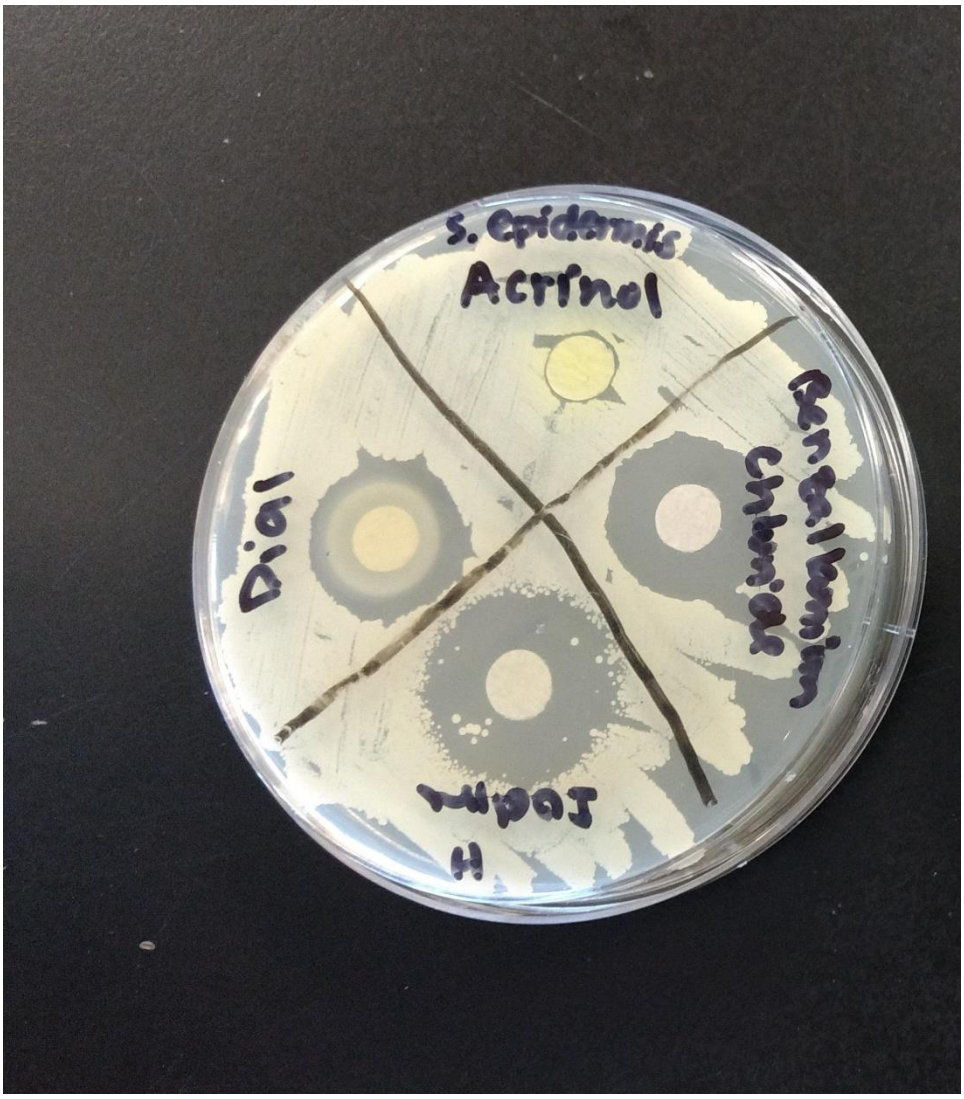
3.2 SENSITIVITY TEST

This is a test carried out to know the actual antibiotics that is susceptible or resistant

Materials; sensitivity disks, forceps, inoculating loop by the bacteria isolated from the patient sample

Procedure; It is done by subculturing the identified microorganism into a fresh non inhibitory medium, An inoculating loop is used to pick the identified microorganism and streaked on the fresh medium then the antibiotics disks are inserted on the plate in an incubator for 24hr at 37C

Observation; The zone of inhibition around each antibiotics was observed which determines the susceptibility of the microorganisms those that create no zone of inhibition are resistant to antibiotics.



3.3 High Vaginal Swab

High Vaginal Swab is a test done to diagnose infections in the vaginal area, Infections like yeast infections, sexually transmitted infection.

The swab sample is cultured to identify the presence of specific bacteria, Fungi or parasites.

Materials; Sterile swab, Culture media (blood agar)

Procedure;

- A sterile swab is inserted into the patient's vagina

- It is gently inserted high into the vagina, avoiding contact with the lower vagina walls to prevent contamination
- The swab is rotated in the vaginal to collect cells and secretion
- After collection, the swab is streaked on the blood agar and placed in the incubator for 24 hours at 37o c

  Observation*; Staphylococus aureus, Escherichia coli, yeast cells, Neisseria gonorrhoeae* can be found on the plate

3.4 Throat Swab

Throat Swab is a test done to diagnose bacterial infections in the throat, detect other pathogens causing sore throat.

Materials; Sterile Swab, Culture media

Procedure;

- The patient is asked to open their mouth wide
- A sterile swab is gently rubbed over the tonsils and the back throat avoiding contact with the tongue, teeth or cheeks.
- After collection, the swab is streaked on the blood agar and placed in the incubator at 37oC for 24 hours.

Observation; *Streptococcus pneumonia, Streptococcus pyogens* can be found on the plate

3.5 PARASITOLOGY SECTION

This is a section that deals with the study of parasites e.g *Ascaris lumbricodes (round worms caused by hand to mouth contact), Giardia lamblia(intestinal infection caused by swallowing contaminated water), Schistosoma haematobium(blood flukes caused by fresh water snails)* . Investigations of parasitic infection in the laboratory includes; Urine microscopy, Stool microscopy, Malaria parasites diagnosis.

Microscopy; This is an observation, investigation or experiments that involves the use of microscope. There are two types of microscopy;

i. Direct microscopy/ Wet preparation
ii. Non direct microscopy

Direct microscopy; The specimen is dropped on a slide then a coverslip is placed on it. It is mostly done with urine specimen, stool specimen, semen specimen.

Non direct microscopy; The specimen are usually stained and dried before viewing under the microscope. Eg Gram staining.

3.6 URINE ANALYSIS, MCS

Collection of urine

Urine is collected in clean universal bottles. The mid part of the first early morning sample is preferred.

MACROSCOPIC EXAMINATION

Appearance: the normal urine color should be either amber or yellow. Other colors could be red brown black or white.

Turbidity: it could be slightly turbid, turbid or clear.

MICROSCOPIC EXAMINATION

Note: Urine is cultured before spinning the urine samples in the centrifuge to avoid contamination the samples.

The urine samples are poured inside test tubes and labeled with the laboratory number of the patient. It is then arranged inside the centrifuge and allowed to spin for 10minutes, so as to separate the urine into layers.

The supernatant part of the spinned urine is then disposed off into a container containing a disinfectant and then the sediment is placed on the glass slide. The sediment of urine sample on the slide is covered with a cover slip and then examined under the microscope. The following could be seen under the microscope: bacteria cells, epithelial cells, cellular casts, red blood cells and white blood cells.

HOW TO CULTURE URINE

Materials; Blood Agar, Petri dish, inoculating loop, Bunsen burner,

PROCEDURE-

- A wired loop is dipped into the universal tube containing the urine (open the cork of the tube with the side of your palm and keep holding the cover while you dip the wire loop into the urine) and then inoculated (streaked) on the plate
- It is placed in the incubator for 24 hours at 37^{o} C

  Observation; *Escherichia Coli* was observed on the plate

3.7 STOOL MICROSCOPY

EXAMININATION OF FAECES

First it was viewed macroscopically for the following: Form, color, Smell, consistency, presence of blood, and mucus, nematode, tapeworm, and segments.

When viewed under the light microscope at x10 and x40

It is viewed under microscope in normal saline

- One drop of normal saline was placed on a thin slide with the pipette in the normal saline bottle.
- A tiny bit of the stool sample was taken and make a smear in the normal saline with it.

- It was viewed under the light microscope for cellular exudates such as helminthes egg, protozoa cyst, and actual larva of nematode worms.
- When viewed under the microscope in iodine it is the same process as listed in the first two steps above, just use iodine in this case and not normal saline. When viewed under the light microscope, stained protozoa cysts are more visible. Other things that could be seen under the microscope are: fat globules, undigested starch, vegetable cells, and air bubbles. Cysts can be concentrated by the formal ether technique or by a simple floatation in concentrated zinc sulphate

3.8 MALARIA PARASITE TEST

Malaria is a disease caused by protozoan parasite of the genus plasmodium. It has five causative agent; P. falciparum, P. ovale, P. malariae, P. vivax, P.Knowlesi. Malaria disease in human is transmitted through the bite of female anopheles mosquito. This parasite can be detected in the laboratory through;

- Microscopy of stained blood film
- Use of rapid diagnostic test kit

Microscopy of stained slide; There are two methods; **Thick blood film and thin blood film**.

THICK BLOOD FILM

Procedure

- A drop of blood is placed on a slide
- A spreader is placed on the slide and used to make a circular movement of 1cm
- Allow the blood to air dry
- The slide is stained for geimsa stain for 45 minutes
- The film is rinsed with distilled water and air dried
- A drop of immersion oil is placed on the stained film
- View under x100 lens

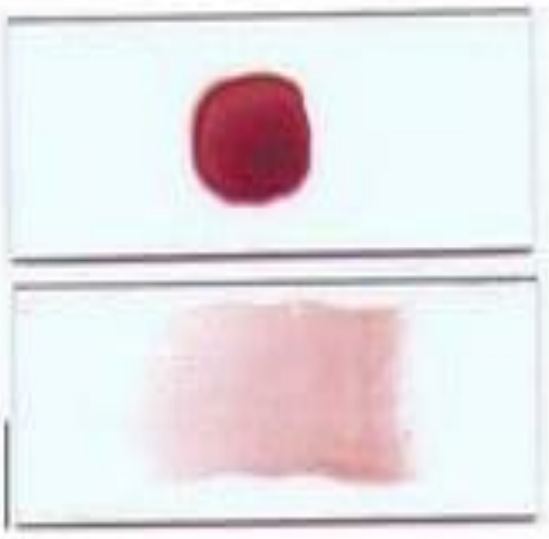
THIN BLOOD FILM

Procedure

- A drop of blood is placed on a slide

 Using another slide at a 30-45 degree angle, the drop of blood was touched and spread gently across the slide by moving the second slide in a straight line across the first slide.

- Aim for a thin, even layer without breaks. The ideal film should be roughly the size of a standard microscope cover



3.9 SEMEN ANALYSIS

Semen Analysis with Microscopy

This involves the analysis of semen by culturing and performing sensitivity test.

Physical examination

- Volume: 1ml, 2ml and above
- Viscosity: Watery or Normal
- Appearance: creamy, whitish or Creamy – whitish

Microscopy

- Motility
- High power
- Normal
- Abnormal

NOTE The best sperm count is about $90x10^6$ total counts but normal count is 45×10^6, but when the total count is 25×10^6 the diagnosis could be infertility.

SEMEN CULTURE

After the examination in the first process, the semen was cultured

Procedure;

- After sterilizing the inoculating loop
- The semen sample was streaked on blood agar It was incubated for 24 hours

Examine the colony if there

(i) Examine the colony if there).

HOW TO CARRY OUT SENSITIVITY TEST:

Procedure;

- Nutrient agar was prepared and poured into a petri dish
- The microorganism on the chocolate agar was picked and it was streaked on the nutrient agar
- The antibiotic disc was placed on the streaked plate and incubate for 24 hours
- At the end of the stipulated time any antibiotic surrounded by a region where no microorganism grew can proof useful against the microorganism discovered present

CHAPTER FOUR

SEROLOGY

4.1 WIDAL AGGLUTINATION REACTION

Materials; Widal kit, white tile, pipette, test tube, centrifuge, stop watch, blood sample

Procedure • Venous blood was collected into sample bottle and spinned for 5 minutes

• The serum was taken with the aid of a pipette and put on white tile in different spots of 4 per row making two rows

• First row is labeled O, OA, OB, OC and the second row H, HA, HB, HC respectively.

• Antiserum from the widal kit for each spot are released on top of the pipette blood.

• The tile is then rocked for 3 minutes

Observation

Expected results ratio:

• Highly reactive…….1:320(positive)

• Very reactive……1:160(positive)

• Weak reaction……..1:80(positive)

• Non Significant……1:40(negative)

• Non Significant……1:20(negative)


Salmonella O A
Group D

4.2 BLOOD GROUP TEST

Blood grouping is done on the basis of agglutination. Agglutination means the collection of separate particles like RBCs into clumps and it occurs if an antigen is mixed with its corresponding antibody.

This blood test is done to determine an individuals blood type for several reasons like blood transfusions etc.

Materials; EDTA bottle, Antiserum A, B, D, White tile

Procedures

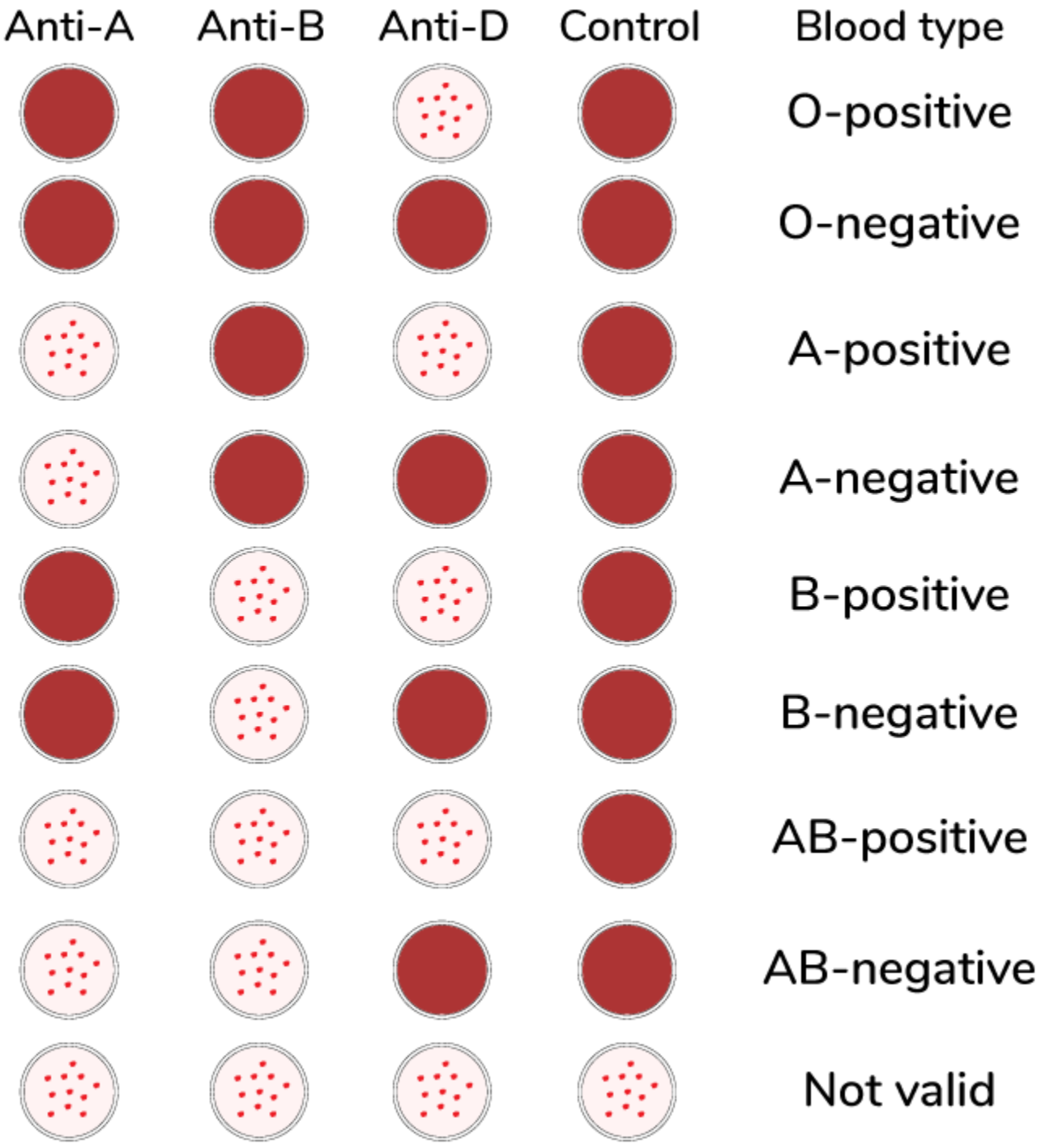
• Venous blood was collected into EDTA sample bottle

• Antiserum A, B, and D were placed on the white tile separately in three spots

• Three separate drops of blood were dropped unto each of the spots

• Each spot was then mixed together with the tip of a clean glass slide or an inverted rubber pipette

• The tile was rocked for three minutes to view agglutination


Anti-A
Anti-B
Anti-D
Control
Blood type
O-positive
O-negative
A-positive
A-negative
B-positive
B-negative
AB-positive
AB-negative
Not valid

4.3 PREGNANCY TEST

Materials used; Pregnancy test strip, Blood sample, centrifuge, test tube

- When sufficient blood has been collected, it is Centrifuge for 3-5 minutes
- Immediately after centrifuging, a pregnancy test strip was inserted into the bottle containing the blood sample
- It was allowed to read for about 3-5 minutes

Observation;

The strip shows whether the patient is pregnant or not if

• Positive (double line): the patient is pregnant

• Negative (single line): the patient is not pregnant

• Invalid: No visible band at all. The test is repeated

Precautions

• Test kit must not be beyond expiry date

• The test device must not be reused

• The test kit is for in vitro diagonostic

4.4 PACKED CELL VOLUME

The packed cell volume also called haematocrit, is used to calculate the mean cell haemaglobin concentration of the blood. These red cell indices are used in the investigation of anaemia.

Materials: Microhaematocrit reader, centrifuge, needle, syringe, capillary tube.

Procedure;

- A plain capillary was filled with well mixed EDTA anticoagulated blood
- Then both ends of the capillary tube are sealed using a cotton wool
- The capillary tube was placed in a centrifuge and spinnned for 5 minutes
- Immediately after centrifuging, read the PCV. Firstly check that there has been no leakage of blood from the capillary or breakage.

- Read off the PCV from scale. The reading point is the top of the red cell column, just below the buffy coat layer (consisting of WBCs and platelets). Results Above the packed red cells is a white layer of platelets. Plasma is usually straw colored, but if bright yellow; it is jaundiced.
- The normal PCV range for male is 39% - 53%. The normal PCV range for female is 35% -49%.

Factors that affect PCV result;

- Quality of capillary tube

• Time and speed of centrifugation

• Spectrum collection: quantity of anticoagulant

4.5 HUMAN IMMUNE DEFICIENCY SCREENING

HIV tests are used to detect the presence of the human immunodeficiency virus in serum, saliva, or urine. Such tests detect HIV antibodies.

Materials; Blood serum, Abort determine HIV-1 and HIV-2 test kit, and centrifuge

Procedure

• The blood was spinned in the centrifuge for 5 minutes

• The HIV test strip is then immersed into blood serum with the narrow end pointing towards the blood

• It must be immersed past the mark line. The strip is taken out after 3 seconds and laid on a flat clean dry non-absorbent surface.

• The test result is read after 10 minutes.

Result

- Positive: Distinct color band appear on the control and test regions. This indicates the presence of HIV-1 and HIV-2
- Negative: Only one color band appears on the control region. No apparent band on the test region. This indicates that the patient is HIV negative
- Invalid: No visible band at all. The test is repeated

4.6 ERYTHROCYTE SEDIMENTATION RATE

This is a blood test that measures how quickly red blood cells settle at the bottom of an ESR tube over a specific period of time.

ESR is done to detect Inflammation caused by infections, diagnose disease like arthritis.

Materials; ESR tube, Blood Sample, Rack/ Stand

Procedure;

- The blood sample is poured into an ESR tube

- The tube is left undisturbed for one hour in a rack
- After one hour, the rate at which the red blood cell settle is measured in millimeter.
- A higher than normal ESR suggests inflammation.

CHEMICAL PATHOLOGY

The following tests are carried out in Chemical pathology laboratory

• Fasting Blood Sugar
• Random Blood Sugar

4.7 FASTING BLOOD SUGAR

The blood sugar concentration or blood glucose level is the amount of glucose (sugar) present in the blood of a human or animal. Normally, in mammals the body maintains the blood glucose level at a reference range between about 3.6 and 5.8 mM (mmol/L). It is tightly regulated as a part of metabolic homeostasis.

Materials; Blood sample, glucomter

Procedure;

• Blood is collected from the thumb of the patient

• The blood is made to drop at the tip end of the glucometer and then left for few minutes (about 3-5minutes)

• The reading is then taken and written down Results The normal range is 70-100mg/dL. If the result from the reading is very much less than 70mg/dL, the patient is said to be hypoglycemic and needs sugar transmission, if the result is far higher than 100mg/dL the patient is said to be hyperglycemic and needs insulin transfusion.

4.8 RANDOM BLOOD SUGAR

This test is similar to fasting blood sugar, the difference being that the test can be carried out anytime on a patient (that is, whether the patient has or has not eaten is irrelevant) and it is useful in the case of emergency.

Materials Blood sample, glucometer

Procedure

• Blood is collected from the thumb of the patient

• The blood is made to drop at the tip end of the glucometer and then left for few minutes (about 3-5minutes)

• The reading is then taken and written down.

Results The normal range is 100-180mg/dL. If the result from the reading is very much less than 70mg/dL, the patient is said to be hypoglycemic and needs sugar transmission, if the result is far higher than 100mg/dL the patient is said to be hyperglycemic and needs insulin transfusion.

CHAPTER FIVE

5.1 PROBLEMS ENCOUNTERED

In most cases, safety rules are not taken into consideration and the necessary safety gadgets and equipment are not usually in place.

I also would want to say that more time should be given to students for their SIWES program.

5.2 RECOMMENDATIONS

I recommend that government should provide placements for microbiology students undergoing SIWES.

I recommend that more preference should be given to the power sector so as to provide adequate light to various Medical laboratories in the country