

# Anti-d blood group essay sample



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The industrial attachment is an important part of the course structure of the university. This is the time students appreciate the theoretical work done in the lecture halls when seen practically. This attachment introduces the student to the outside world in terms of working experience and also prepares them for the future. The department of applied biology therefore introduced the industrial attachment program in its learning curriculum to give students a chance to combine theoretical knowledge with practical skills in the working environment. It requires a student to go for industrial attachment in his chosen field of profession for a period of eight weeks. At the end of the attachment program, the student is expected to write a report that incorporates work experiences gained linking it to the theoretical knowledge acquired in school.

#### OBJECTIVES OF SUPERVISED INDUSTRIAL ATTACHMENT

To provide the student an opportunity to apply his or her knowledge in real situation thereby bridging the gap between university work and actual practice.

To expose the student to work methods not taught in school and to provide access to equipment not available in the university environment.

To assess the interest of the student in the occupation he or she plans to take.

To expose students to the structures, operations and integration of different departments in laboratory such the microbiology, biochemistry, parasitology and hematology.

To expose the student to the behavior of organizations both in the internal

and external environment and encourage them to be productive and hardworking whilst developing chemists for the future.

## A BRIEF HISTORY OF THE HOSPITAL

### HISTORY OF THE MILITARY HOSPITAL

The Military Hospital is a general hospital situated about 4km from the Accra International Airport on the main Airport - Central road (Independence Avenue Road). It is a walking distance from the Golden Tulip Hotel, a popular and modern 4- Star Hotel and located directly opposite the Army Officers Mess. During World War II, it became operationally and logistically necessary for Britain to take over the defense and security of the West African sub - region. The responsibility was given to the war office with Lt Gen Giffard as the General Officer commanding the West Africa region. The General established his operational Headquarters in the Gold Coast. As a result of the war, there was an urgent need for Casualty Clearing Stations (CCS) and general Military Hospitals within the colonies to attend to the medical needs of the numerous service personnel deployed in combat in several theatres. General Giffard requested the war office in the UK for the necessary logistics and administrative support for setting up a Military Hospital in the Gold Coast.

The Public Work Department was commissioned by the colonial officers to construct the buildings and by 4th July, 1941 the Base Hospital in Accra was completed to begin operation as the 37th general Hospital within the British Empire. Soon after the war in 1946, the size of the hospital was re-designated Military Hospital to the Gold Coast to serve as one of the support service providers within the Armed Forces. Over the years, the hospital has

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grown to become the second largest medical facility in Ghana's capital, Accra and the third largest in the country after Korle - Bu (Accra) and Komfo-Anokye (Kumasi). As a Military institution and for operational flexibility, technical efficiency and administrative ease, the hospital has been well structured to carry out its roles and task. It is therefore organized into working units or subunits- Divisions and Departments on the basis of medical, paramedical and administrative lines, with their own heads.

The subunits operate with a Commanding Officer (CO) at the apex. The CO is responsible to higher Headquarters for the smooth running of the hospital. The pathology as one of the divisions is made up of the Chemical pathology and Immunology, Haematology, Microbiology, Parasitological, Histopathology, Blood Bank / Transfusion, Science / Serology and Morbid Anatomy Departments. It provides medical laboratory services to clinicians to help them monitor treatment and confirm the success or otherwise of such treatments. The 37 Military Hospital provides Medicare for troops, all civilian employees of the Ministry Of Defense (MOD) and their families and as well as the general public. It also serves as the United Nations Level IV medical facility in the West Africa sub regions. The hospital indeed lives to its motto " HEALTH FIRST" and all those who have passed through it can attest to it. CHAPTER TWO

#### THE DEPARTMENT AT THE LABORATORY OF 37 MILITARY HOSPITAL

1. PARASITOLOGY DEPARTMENT
2. HAEMATOLOGY DEPARTMENT
3. SEROLOGY
4. BLOOD BANK

5. CHEMISTRY DEPARTMENT

6. MICROBIOLOGY DEPARTMENT

7. HISTOPATHOLOGY

8. MORBID ANATOMY DEPARTMENT

SAFETY RULES AND REGULATIONS UNDERTAKEN IN THE LABORATORY

After taking a skin snip, disinfect the instruments. Thus needles and surgical blades.

Laboratory coats must be worn at all times in the laboratory. This will protect your clothing from contamination and discoloration.

Eating, drinking is forbidden at all times in the laboratory.

Gloves must be worn at all times when working on samples.

When a sample accidentally spill, disinfect the place immediately.

After using disposable equipment and samples, they should be disposed properly.

Clean the laboratory benches with disinfectant before and after work.

All samples should be properly labeled. This will prevent improper use of material.

After working in the laboratory, observe good hygiene by washing your hands with soap.

Collect samples (stool and urine) in clean specimen bottles. ACTIVITIES UNDERTAKEN IN THE PARASITOLOGY LABORATORY

## Routine Stool Examination (RST)

## Routine Urine Examination (RUE)

### ROUTINE STOOL EXAMINATION

#### Objective

The routine examination of stool is used to detect the presence of intestinal parasites including ova, larva, trophozoites and cyst of Helminthes and protozoa respectively.

#### Test Procedure

Emulsify about 1g of faecal material in physiological saline bringing it to suspension using an applicator stick

Label a clean glass slide using a grease pencil

Place a drop of stool suspension on each end of the viewing portion of the slide

Cover gently with a cover slip

Place the preparation under the microscope, lower the condenser to obtain a low light intensity

Observe for trophozoites of intestinal protozoa, ova, larva, Helminthes, red blood cells, crystals, white blood cells, Ascaris, Hook worms using the \*40

objective Reporting Test Results

Macroscopy: Describe stool specimen as to consistency (formed, semi

formed, loose, watery etc. and presence of blood and mucous) Microscopy:

Note the name of any parasite present or “ No ova, larva, or protozoa seen”.

### ROUTINE URINE EXAMINATION

#### Objective

The objective is to screen for the detection of diabetes, metabolic

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abnormalities, liver diseases, biliary and hepatic obstructions, haemolytic diseases, diseases in the region of the kidney and urinary tract as well as parasites and other formed elements.

### Test Procedure

Label a 5ml or 10ml centrifuge tube using a grease pencil

Mix urine and pour into the centrifuge tube until it is about half full of the specimen

Observe the appearance and colour of the urine specimen and record it

Dip the reagent strip into the urine for approximately 1 second

Remove 30 to 60 seconds later and record the biochemical reaction of the reagent strip to the colour chart

Ignore any colour change after 120 seconds (2 minutes). Spin the urine at 3000rpm for 3 to 5 minutes

Decant the supernatant and re-suspend the deposit in the residual fluid

Label a glass slide and place a drop of urine deposit onto it

Apply a cover slip gently on it

Place it under a microscope, lower the condenser to obtain a low light intensity

Examine the preparation preferably using the 40X objective

Observe for pus cells (WBC), RBC's, epithelial cells, cast (granular, blood and leucocytes); crystals, ova of *Schistosoma haematobium*, trophozoites of *Trichomonas vaginalis* and fungi (yeast-like cell) Reporting Test Results

### Macroscopic:

Colour: Normal freshly passed urine is amber (pale to dark yellow in colour).

The yellow is due the pigment urochrome, urobilin and porphyrins.

Appearance: Normal passed urine is clear. Abnormality may be due to:

Urinary tract infections in which the urine appears cloudy because it contains pus cells and bacteria

Urinary schistosomiasis in which the urine often appears red and cloudy because it contains blood (haematuria)

Black water fever and other conditions causing intravascular haemolysis in which the urine appears brown and cloudy because it contains free haemoglobin (haemoglobinuria)

Jaundice in the urine may appear yellow-brown or green-brown because it bile pigments or increased amount of urobilin (oxidized urobilinogen)

Bancroftian filariasis in which the urine may appear milky-white because it contain chyle  
PH: Normal reaction of freshly passed urine is slightly acidic, around 6.0  
Protein: Positive results indicate the urine might contain urinary schistosomiasis, urinary tract infections, and nephritic syndrome, renal disease such as pyelonephritis and glomerulonephritis and renal tuberculosis. It may also be found in pregnant women and sometimes healthy young individuals.  
Glucose: Found in the urine of diabetic patients and occasionally in healthy individuals.  
Ketone: Found in the urine of untreated diabetics or persons suffering from starvation  
Bilirubin: Found in the urine of persons with hepatocellular jaundice or cholestatic (obstructive) jaundice  
Urobilinogen: Found in the urine of persons with conditions of abnormal haemolysis  
Blood: Found in the urine in urinary schistosomiasis, bacterial infections, acute glomerulonephritis (inflammation of the glomeruli



of the kidney), sickle cell disease, leptospirosis, infective endocarditis, kidney stones and Specific gravity (S. G):

The normal specific gravity of urine varies between 1.002-1.025, depending on the state of hydration of the person and time of the day. High specific gravity is found in urine because of the following: glucose, protein, or other particles. Nitrite: Causes of nitrite in urine are because bacteria that produce the enzyme nitrate reductase are able to reduce nitrate to nitrite. Nitrate reducing bacteria are Escherichia coli, proteus species, Citrobacter species, Aerobacter species and Salmonella species A negative test cannot rule out a urinary tract infection because the causing organism may be one that does not reduce nitrate to nitrite, e. g. Enterococcus faecalis, Staphylococcus albus, Staphylococcus saprotyticus, Pseudomonas species or Candida species. NB: The following are some abnormal urine colours, straw, coke, light amber, deep amber etc. Haemorrhagic conditions

#### OCCULT BLOOD TEST (IN STOOL)

##### Objective

Occult blood test is done to detect the presence of occult (hidden) blood in stool samples. This test is not carried out during menstruation or diarrhea

##### Test Procedure

Open the front part of the hemoplus test card

Take a small sample of stool using the spatula, smear the red circles completely with the stool sample

Open the back part of the hemoplus card and drop two drops of hemoplus developing reagent on each of the circles

Close the hemoplus test card with its flap

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The result is read after some seconds according to the manufactures instructions Reporting Test Result

Positive results: A blue-black coloration of the sample occurs. Negative results: No change in coloration of the sample

#### ACTIVITIES UNDERTAKEN IN HAEMATOLOGY LABORATORY

The main activities include;

Blood Film (BF) for Malaria parasites

Sickling test

FBC (Full Blood Count)

ESR (Erythrocyte sedimentation Rate)

#### BLOOD FILM FOR MALARIA PARASITES

Objective

To detect the presence or the absence of malaria parasites

Principle

Stained thick films are used to diagnose malaria and other diseases caused by blood parasites Test Procedure

Place a large drop of well- mixed blood in the center of a clean glass slide

Using the corner of a second slide, carefully spread the drop of the blood over an area of 10-12mm

To determine the correct thickness of the film; place a piece of newspaper on the slide , the film must just be visible

Dry the film at room temperature

Prepare 1 in 10 dilution of the stock Giemsa stain

Cover the entire slide with diluted Giemsa and stain for at least 10 minutes

Wash gently in tap water and leave slide to dry in an upright position at

room temperature

Examine under \*100 objective for the presence or absence of malaria

parasites Reporting Test Result

+ = 1-10 parasites per 100 thick fields

++ = 11-100 parasites per 100 thick film fields

+++ = 1-10 parasites per single thick film fields

++++ = more than 10 parasites per single thick film field

To prepare Giemsa stock solution

Giemsa powder: 3. 8g

Absolute methanol: 250ml

Glycerol (pure): 250ml

Weigh and dissolve the powder in the alcohol in a dry conical flask. Stir using a glass rod until the powder has completely dissolved. Add the glycerol.

Filter and store in a reagent bottle and label. Working solution;

To prepare 5% Giemsa solution:

Giemsa stock 5ml Phosphate buffer 7. 2 or tap water 95ml Mix the solutions in a measuring cylinder.

## SICKLING TEST

Objective

Detection of Haemoglobin Sickling in human red blood cells

Principle

Under low oxygen tension, erythrocytes containing haemoglobin Sickling assume the characteristic sickle -shape (Crescent Shape). This is enhanced

by the addition of a reducing agent e. g. 2% Sodium Metabisulphite solution.

#### Test procedure

Combine an equal volume of blood and sickling fluid

Mix very well on the slide

Apply coverslip gently to avoid air bubbles

Allow to stand for at least 30 minutes

Examine under the microscope for sickled red blood cells Reporting Test

#### Result

Report as sickling Positive or Negative

To prepare 2% Sodium Metabisulphite Solution:

Sodium Metabisulphite 0.1 g Distilled water 5ml Weigh the salt and dissolve in 5ml distilled water in a test tube. Transfer into an appropriate bottle and label.

The blood samples are placed on a roller for 10-15 minutes to ensure well mixed anti-coagulated blood

They are then fixed under the SYSMEX BD HAEMATOLOGY ANALYSER (an automated machine) to be analyzed and the results are printed out automatically through a computerized printer. ESR (Erythrocyte sedimentation Rate)

Objective/Principle ESR measures the sedimentation of erythrocytes in their native. The numerical value in millimeters is obtained by measuring the distance between the lowest point of the surface meniscus to the upper limit of the erythrocyte sedimentation in a column of anti-coagulated and diluted blood that has stood in the selected tube for 60 minutes. Test Procedure

The ratio of blood to anticoagulant is 4 : 1

Fill a clean dry standard tube with the blood and adjust the level to the " O" mark

Place the tube in a strictly vertical position on the bench, not exposed to direct sunlight and free from vibration and draughts.

After exactly one hour , read the distance from the bottom of the surface meniscus to the top of the column of sedimentating red cells (where the full density is apparent) in millimeters and record as the ESR value Reporting Test Result

The test is reported mmfall/hr

Reference range:

Men up to 10mm/hr Women up to 14mm/hr

Erythrocyte Sedimentation Rate is a non-specific indicator of inflammation and tissue damage. To prepare sodium citrate anticoagulant: (100ml)

Tri-Sodium citrate 3. 8 g Distilled water 100ml

Weigh the sodium citrate and transfer it to a proof bottle. Add 100ml of water and mix to dissolve the chemical. Label the bottle and store preferably at 2-8 degrees Celsius. ACTIVITIES UNDERTAKEN IN THE CHEMISTRY

DEPARTMENT

The following are various tests undertake in the chemistry department

LFT [Liver Function Test]

BUE creatinine[Blood Urea Erythrocyte Creatinine]

FBS[Fasting Blood Sugar]

Lipid profile

Glycosylated hemoglobin

Glucose-6-phosphate dehydrogenase(G6PD)

Test procedure

Collect all blood samples and balance them in the centrifuge to separate the blood into their various components, thus plasma (serum) from the white (leucocytes) and red (erythrocytes) blood cells.

Pour the serum of the blood samples into the cups of the chemical analyser, these cups are fixed in cup adapters on the trays of the chemical analyser.

Fix the trays into the chemical analyser and allow to rotate

There are four trays in all, and each tray can contain only 10 cups, so as the trays rotate in the chemical analyser, the serum is collected by a pipette in the biochemical analyser which then undergoes several processes to produce specific results needed for diagnoses through a connected printer.

Objectives for carrying out these tests

BUE+CREATININE - Kidney function

FBS -Glucose metabolism

LFT (Liver function test) - Liver enzymes and protein

GLUCOSE 6 PHOSPHATE DEHYDROGENASE (G6PD) Principle

G6PD is involved in the generation of phosphorylated nicotinamide-adenine dinucleotide (NADPH) in the pentose phosphate pathway (hexose monophosphate shunt). NADPH is needed to provide glutathione (GSH) which maintains haemoglobin and other red cell proteins in a reduced active form. Exposure to an oxidant drug increases the need for NADPH and GSH. G6PD deficiencies prevent this need from being met, resulting in the oxidation of

haemoglobin into methaemoglobin. This precipitate to form Heinz bodies which attach to the red cell membrane. Cells containing Heinz bodies are easily damaged and lysed extravascularly. Test procedure

Set up 3 test tubes as follows:

Test Positive control	Negative control	EDTA Blood	1ml	1ml	1ml	Na Nitrite
Dextrose	0.05ml	0.05ml	-	Methylene Blue	0.05ml	- 0.05ml
Reporting result:	Defect or No defect					

To prepare Reagent:

Glucose 5.0g Dissolve in 100ml Sodium Nitrite 1.25g distilled water

Methylene Blue 0.15g Dissolve in 100ml  
**PROCEDURE TO DETERMINE BLOOD GLUCOSE LEVEL.**

#### URINE SUGAR TEST

The urine glucose test is used to monitor a person with diabetes. When the blood glucose level rises above 160mg/dl, the glucose will be detected in the urine; consequently glycosuria may be the first detector that diabetes or another hyperglycemia condition is present. **PROCEDURE**

Early morning urine is collected in a small container

The urine strip is dip in the urine for about 5 seconds

The rate of color change formed is compared with the color chart and the results are interpreted **REPORTING OF RESULTS**

If no color change occurs it is negative otherwise positive. Depending on the color changed of the strip; a positive results are recorded as +, ++, +++, +  
 +++ **PRINCIPLES**

This method employs a specific reaction of the enzyme glucose oxidase

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which catalyzes the conversion of glucose to gluconic acid and hydrogen peroxide second enzyme, peroxidase, promotes the reaction of hydrogen peroxide generated with chromogen to form a brown oxidized compound. The intensity of the color depends of the amount of sugar present in the urine

#### GLYCATED HEMOGLOBIN (HbA1C) TESTS

Summary and explanation:

Hemoglobin A1C is formed by the non-enzymatic glycation of the  $\beta$ -chain of hemoglobin A0. The level of hemoglobin A1C is proportional to the level of glucose in the blood over a period of glucose approximately two months. Thus, hemoglobin A1C is accepted as an indicator of the mean daily blood glucose concentration over the preceding two months. Studies have shown that the clinical values obtained through regular measurement of hemoglobin A1C leads to changes in diabetes treatment and improvement of metabolic control as indicated by a lowering of hemoglobin A1C values.

#### LIVER FUNCTION TESTS (LFTs)

The diagnosis of liver disease is often obvious from the clinical presentation and comprehensive diagnostic work-up might not be necessary. However, patients with mild jaundice, which may be hematological in origin, and anicteric patients who have disease of the liver (e. g. malignancy, alcoholism, etc.) will usually require further investigations. Evaluations of these patients should first focus on the routine LFTs and if necessary, followed up with specific LFTs. Routine LFTs done in the Military Hospitals pathology division (biochemistry laboratory) include: Total protein, Albumin, Bilirubin, Alanine, Aminotransferase, Alkaline phosphates, Gamma-



glutamyltransferase. The LFTs are done with the automated chemistry analyzer (ELLIPSE/BT 3000) manufactured by Assel company in Germany.

## TESTS

### TOTAL PROTEINS

### CLINICAL SIGNIFICANCE

In human plasma, albumin accounts for 50%-60% of total proteins; the remaining fraction usually contains globulins. Most plasmatic proteins are synthesized by the liver, except immunoglobulin. Increase of the plasmatic volume (salt retention syndrome, intoxication with water...), or its reduction (dehydration related to vomiting, diarrhea...), induce respectively relative hyperproteinaemia. Abnormal total protein rates only occur in the event of disorder affecting the concentration of albumin and immunoglobulin, thus, severe protein insufficiency (malabsorption, maldigestion, dietary insufficiency), renal and hepatic diseases result in hyperproteinaemia. If total protein concentration is lower than 40g/L, oedema can be observed.

Hyperproteinaemia can be seen in the case of hyperimmunoglobulinemia (multiple myeloma infection). **ACTIVITIES UNDERTAKEN IN THE BLOOD BANK**

They include

Blood grouping

Hepatitis B and C[HBsAg and HCV respectively]

Retro screening for HIV/AIDS

VDRL test

### BLOOD GROUPING

Objective/ principle

The ABO and rhesus blood grouping is done to ensure that blood donors and patients are grouped correctly since incompatible blood transfusion may result in the death of the recipient. An individual's ABO blood group depends on the A, B or O gene located on the chromosome inherited from each parent

### Reagents Needed

Anti-A reagent (Anti-A) Anti-B reagent (Anti-B) Anti-D reagent (Anti-D) Test procedure

Put three drops of patient's blood separate from each other on a white tile or slide

Put a drop of Anti-A by first drop of blood, Anti-B by the second drop of blood and Anti-D by the third drop of blood 25

Mix each of the content to a uniform mixture and place it on an orbital shaker to mix for about 3 minutes

Take the tile off the orbital shaker and observe for agglutination of red cells with the antiserum

### Reporting Test Results

#### Positives

Blood group A positive (A Rh " D" positive): Agglutination with Anti-A and Anti-D

Blood group B positive (B Rh " D" positive ): Agglutination with Anti-B and Anti-D

Blood group AB positive(AB Rh " D" positive): Agglutination with Anti-A, Anti-B and Anti-D

Blood group O positive (O Rh " D" positive ): Agglutination with only Anti-D

#### Negatives

Blood group A negative (A Rh " D" negative): Agglutination with Anti-A, but

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no agglutination with Anti-D

Blood group B negative ( B Rh “ D” negative): Agglutination with Anti-B, but no agglutination with Anti-D

Blood group AB negative (AB Rh “ D” negative): Agglutination with Anti-A and Anti-B, but no agglutination with Anti-D

Blood group O negative (O Rh “ D” negative): No agglutination with Anti-A, Anti-B and Anti-D HEPATITIS B SURFACE ANTIGEN (HBsAg)

#### AND HCV SCREEN

Objective/ principle

A chromatographic immunoassay for the qualitative determination of Hepatitis B surface antigen and Hepatitis C virus in serum or plasma Test procedure

Remove the dipstick from its wrapping pouch by tearing the pouch along the split

Holding the strip vertically, carefully dip it into the specimen (10-15 sec). Do not immerse the strip past the maximum line

Interpret the results within 15 minutes

Reporting Test Results

Positive Result: The presence of two purple colour bands within the result area regardless of which band appears first Negative Result: The presence of only one purple colour band within the result area RETROSCREENING/ RAPID

TEST FOR HIV-I/II ASSAY

Principle

The test involves the trapping of antibodies to HIV 1 and 2 by a capture reagent which is absorbed on a porous membrane. It is a sexually

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transmitted disease which is usually carried out to know the antigen-antibody reaction. A kit is usually used for this test. Test procedure

Remove cover from the test kit

Write the sample number on the device

Using the smaller pipette, discharge a drop of blood unto the kit

Add a drop of buffer solution unto the sample and allow to react

Read results within 10 minutes

Interpretation of results Negative: One small distinct red band (control band)

Positive for HIV 1 and or HIV2: Two small distinct red bands Invalid: No band shows

VDRL Objective / principle Venereal Disease Research Laboratory (VDRL) test is used to detect the presence of reagin (circulating antibodies directed against tissue components) in serum or plasma which is indicative of Syphilis. Syphilis is a sexually transmitted disease caused by spirochete, *Treponema pallidum*. Infection causes the production of antilipoidal antibodies. This type of antibody is produced as a result of the tissue damage that occurs in syphilis and is directed against tissue components.

Test procedure

Centrifuge blood sample at 3000rpm for 5 minutes

Remove cover from the VDRL test strip

Drop the strip 90 degrees into the serum of the blood sample to a required level

After some seconds remove strip and allow it to react for about 2-3 minutes

Reporting Test Results Positive:

Appearance of two bands, meaning the antigens coated on the strip reacted with the antibodies in the serum  
Negative: Appearance of one band, thus control band  
ACTIVITIES UNDERKEN IN THE MICROBIOLOGY DEPARTMENT

These include the following

Sputum for acid fast bacilli[AFB]

Culture media and sensitivity test

Semen analysis

High vagina swab[HVS]

Coagulase and catalase test

Skin snip

SKIN BIOPSY (SNIP) FOR MICROFILARIA

Objective/Principle

Physiological saline causes the microfilaria present in the skin tissue to come out and can be observed. The disease is also known as river blindness because invasion of the eye leads to loss of vision  
Test Procedure

Obtain a skin snip by cutting a piece of skin from the buttocks and back of the shoulder

Place the skin snips on the glass slide and add a drop or two of physiological saline

Apply a cover glass carefully and leave the wet mount to stand for at least 30 minutes

Examine the mount after the stipulated time period scanning with the \*10 and \*40 objectives for detailed morphology

. Look for the microfilaria of onchocerca volvulus and Mansonela streptococci

Onchocerca volvulus

Onchocercavolvuls causes river blindness because of microfilaria damage to the eye  
LEISHMANIA PARASITE

### Objective

To determine leishmania parasites

### Principle

Leishmania parasites occur as Amastigotes and are thus demonstrated microscopically in blood or other tissue smears

Sampling Conditions  
Anticoagulated blood, peripheral blood, bone marrow aspirates, lymph node fluid, spleen or liver aspirate, occasionally nasal secretion and skin are used

### Test Procedure

Prepare smear from aspirate or blood

Air dry the smear and fix in methanol for 2 minutes

Stain the smear in 1: 20 Giemsa for 30 minutes

Examine the smear microscopically using the oil immersion (\*100) objective

Look for Amastigotes which are small, round to oval shape with a nucleus and kinetoplast NB: The Leishmania parasite causes trypanosomiasis in human

### SPUTUM FOR ACID FAST BACILLI (AFB)

### Objective

To identify acid-fast bacilli in sputum in order to diagnose pulmonary tuberculosis (TB)

### Principle

The principle is based on the ability of AFB to hold the red dye of CarbolFuschin using the ZiehlNeelson technique

Test procedure  
Label the clean glass slides

Make a smear to cover 2/3 the length of the slide

Dry and heat fix by passing through flame 3 times

Flood the slide with strong CarbolFuschin

Heat gently with a flame until steam rises (avoid boiling) approximately 3 minutes

Repeat the steaming three or four times in the course of 5-10 minutes. The film must not be allowed to dry , add fresh stain if necessary

Wash thoroughly in running water

Decolorize with 20-25% H<sub>2</sub>SO<sub>4</sub> until no stain comes off

Wash well in running water

Counter stain with hydro soluble methylene blue for 15 to 30 seconds

Wash with water and air dry

Wipe the back of the slide clean, do not blot

Examine under the microscope using the oil immersion lens. Acid and alcohol fast bacilli should show up as bright red/ pink rods where as other organisms are blue. Reporting Test Result

No AFB / 100 fields

Scanty 1-9 AFB per 100 fields

1+ - 10-99 per 100 fields

2+ - 1-10 per 10 fields

3+- greater than 10 per field

Report as;

ZN AFB present (+, ++, +++)

ZN No AFB seen.

CULTURE MEDIA A culture media is a media containing nutrients such as

ions, moisture and correct pH for pathogens growth for microscopy and identification CULTURE PREPARATION

Blood agar: 8. 5g

Water: 15 ml

Test procedure

Prepare the agar medium as directed by the manufacturer

Sterilize by autoclaving at 121 degrees Celsius for 15 minutes and transfer to water broth of 50 degrees Celsius

When the medium cools to 50-55 degrees Celsius, mix well and dispense aseptically in sterile petri dishes

Give date and number to the medium in the petri dishes

Store at 2-8 degrees Celsius at room temperature

INOCULATION OF CULTURE MEDIA IN PETRI DISHES

The inoculation of culture media in petri dishes is referred to as looping out or plating out . before inoculating a plate of culture medium, the surface of the medium must be dried otherwise a single colony will not be formed Using a sterile loop, inoculate a small area of the plate and flame loop until sterile

SENSITIVITY TEST

Sensitivity test is an application of antibiotic drugs to see which of them will be sensitive to the micro organism. This can be done by using

Disc diffusion technique

A dilution technique

Disc diffusion technique

A disc of blotting paper is impregnated with known volume and appropriate concentration of antimicrobial, and this is placed on a plate of susceptibility.

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Testing agar uniformly inoculated with the test organism. The microbe diffuses from the disc the medium and the growth of the test organism is related to the susceptibility of the organism. Interpretation of zones

Resistant - Organism will not respond to treatment with the drugs

Intermediate-Infections respond to treatment in layer dose

Susceptible- Infection responds to treatment with drugs in normal dose HIGH

#### VAGINAL SWAB (HVS) ROUTINE EXAMINATION

##### Objective

To identify *Candida* species and *Trichomonas vaginalis*

##### Test procedure

##### Direct Saline Preparation

Prepare a smear or gram stain

Put a few drops of saline to cover the cotton bud of the swab

Mix thoroughly the saline with the sample on the cotton bud

Label the grease free glass slide using a marker

Make a wet mount from the contents in the tube i. e. put about 2 drops of the contents on the slide and apply a cover slip

Examine the wet mount under the microscope scanning with the 10\* objective and the 40\* objective for detailed morphology

Look for yeast-like cells (*Candida* sp) and motile trophozoites of *Trichomonas vaginalis*, pus cells and epithelial cells Reporting Test Result

Yeast-like cells (*Candida* sp.) and/ or *Trichomonas vaginalis* present Number of puss cells and epithelial cells per high field

Gram stain: report due cells and / or gram-negative intracellular diplococci (GNID) present or not seen.

## COAGULASE AND CATALASE TEST

### Objective /principle

To identify and isolate cultured microorganisms, most especially organisms in the genus *Staphylococcus* COAGULASE Test

### Test procedure

Pipette 9 drops of peptone water and add to 1 drop of blood plasma

Inoculate the cultured organism

Examine for the precipitation or clotting of the blood plasma Reporting Test

### Result

Coagulase positive: Precipitation / clotting of blood plasma e. g.

*Staphylococcus aureus* and *Staphylococcus epidermidis* Coagulase negative:

No precipitation / clotting of blood plasma. CATALASE Test

### Test procedure

Inoculate the organism in hydrogen peroxide oxide

Observe for oxygen bubbling

### Reporting Test results

Catalase positive: Presence of oxygen bubbling. E. g. *Staphylococcus epidermidis* Catalase negative: Absence of oxygen bubbling. E. g. *S.*

*epidermidis* NB: Coagulase and Catalase tests are used to identify and

isolate for the presence or the absence of *Staphylococcus aureus*,

*Staphylococcus epidermidis* etc. Other microbiological tests include Semen

Analysis, inoculation and culturing of various

## CHAPTER THREE

### EXPERIENCE/KNOWLEDGE ACQUIRED

Some experience/knowledge acquired is as follows

<https://assignbuster.com/anti-d-blood-group-essay-sample/>

I learnt how to culture urine samples, blood samples, stool samples and other samples. Every sample has a required medium on which to culture. Some samples may require more than one medium. For example fresh blood samples are first stored in a brain-heart infusion broth and later cultured on blood agar, MacConkey agar and chocolate agar. Also urine samples are cultured on Cystine Lactose Electrolyte-Deficient (CLED) agar. The chocolate agar is normally used for the growth anaerobic microorganisms such *Neisseria gonorrhoea*.

I also learnt that all stored media in the refrigerator must be dried first in the incubator at a temperature of 35°C - 37°C for about 15 minutes before using them for culture. The working area must be disinfected to prevent contamination. When culturing one must not talk. The wire loop must be flamed before and after use with a bunsen burner to keep it sterile.

Also, I learnt that the use of normal saline for the stool emulsification is mainly to keep the morphology of any parasite such as hookworm present in the stool. Slides prepared with urine and stool samples for the microscopy must not dry before viewing under the microscope. In routine urine examination the results for the biochemistry aspect must be read on time. Example, glucose is read 30 seconds after the test strip is dipped into the urine sample. The container containing the urine test strip must not be left open.

## CHAPTER FOUR

### CONCLUSION

In conclusion, my practical attachment at 37 Military Hospital, Accra has

been an immense success in my academic life as well as my social life; it has given me the experience in work ethics and has also broadened my knowledge on the application of microbiology and parasitology in the hospital and laboratory setting. It is therefore necessary to give proper attention to this compulsory industrial attachment to give students more knowledge and experience in and after school.

**RECOMMENDATION** I recommend that the school should have a link with various hospitals, research institutes, industrial sectors to alleviate the challenges of attachment location and students should be given more practical lessons on campus to enhance effective understanding whenever they go on industrial attachments. I also recommend that, the period of attachment should be extended to enable us acquire more experience in the work ethics and can as well be able to apply and learn more microbiologically related issues.

#### APPENDIX

EDTA - Ethylenediamine tetra-acetic acid

LFT - Liver Function Test

VDRL - Venereal Diseases Research Laboratory

R/E - Routine Examination

CLED - Cystine lactose electrolyte-deficient

SS - Salmonella Shigella

XLD - Xylose lysine deoxycholate

KIA - Kligler ion agar

TSI - Triple Sugar Ion

BA - Blood Agar

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CA - Chocolate Agar

MAC - MacConkey

Z-N - Zeihl-Neelsen

MRSA - Methicillin Resistant Staphylococcus aureus

LDC - Lysine decarboxylase

WBC - White Blood Cell

RBC - Red Blood Cell

#### BIBLIOGRAPHY

Monica Cheesbrough (2006). District Laboratory Practice in Tropical Countries, Second Edition, Part 2. New York: Cambridge University Press.