ABIA STATE UNIVERSITY UTURU

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CODE: MLS 332

TITLE:

BASIC MEDICAL LABORTARY PROCEDURES

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PREPARATION OF CULTURE MEDIA AND PLATING TECHNIQUE

To study and identify m/orgs, they have to be cultivated and isolated in pure culture. Most bacteria can be cultured in artificial media. If the media meets the nutritional and physical growth requirement of the bacteria.

The media used for growing bacteria are either liquid or solid media .They must contain water and sources of mineral salts, nitrogen, carbon and essential vitamin. Some bacteria may require additional specific substances which may be added to the medium for their growth.

COMPOSITION OF CULTURE MEDIA MY MAC WP

WATER- Water is the main solvent for most of the ingredient used in preparation of culture media. It must not contain substances that may inhibit the growth of bacteria. Preferably deionized water or distilled water should be used to prepare media.

PEPTONE- Peptone is the water soluble product of protein hydrolysis. Protein in meat, milk, and soya bean are hydrolyzed by acid or by the enzymatic actions of enzymes such as pepsin, trypsin, papain. All peptones are heat stable Peptone from animals provides nitrogen for growing micro organism and soya beans (plants) protein is a source of carbohydrate.

A good brand of peptone must have the following qualities;

1) Must have neutral PH. CHUKWU ANTHONY WP MY MCA MY MAC WP
2) Must be light in color.
3) Must be hydroscopic.
4) Must contain large amount of tryptophan for indole production.

MEAT EXTRACTS-Beef extracts such as lab lemco provides organism with further supply of amino acids and also with essential growth vitamin and mineral salts and elements. Infused or enzyme digested meat extracts are also used for the same purpose.

YEAST EXTRACT-This is contained in many culture media as a bacteria growth stimulant, for example; in xylose lysine deoxycholate medium (XLD), Modified

New York City (MNYC) medium and Thiosulphate citrate bile salt (TSBS) medium.

- MINERAL SALT-Many trace elements are required by bacteria for their growth and activity. These elements are derived from salts, sulphate salts are a source of sulphur, and phosphates as a source of phosphorous. Many culture media also contain traces of magnesium, potassium, iron, calcium and other elements. Sodium Chloride is also an essential ingredient of most culture media.

- CARBOHYDRATES-It serves as a source of carbon and energy in culture media. They may also be added to perform some specific function, for example; lactose is added to CLED and MacConkey agar to differentiate the lactose fermenting organisms from the non lactose fermenting organisms.

- AGAR-This is an inert polysaccharide extract obtained from a variety of red-purple seaweeds (Rhodophyceae) which form the agarophyte group of marine algae. It consist of 2 main Polysaccharides agarose (70-75) and agaropectin (20-25). For use in culture media, Agar must be clarified and free from pigments and substances toxic to bacteria. Agar is resistant to actions of m/orgs. Agar is used to solidify culture media because of its high jelly strength and its setting temperature of 32-39 degree Celsius and melting temperature of 90-95 degree Celsius. Agar also provides m/orgs with calcium and other organic ions.

TYPES OF CULTURE MEDIA

1) Basic
2) Enriched or enrichment
3) Selective
4) Differential
5) Transport

BASIC MEDIA- These are simple media such as nutrient agar and nutrient broth that will support the growth of m/orgs that do not have special nutritional requirements.

They are often used in the preparation of enriched media, to maintain stock cultures of control strains of bacteria and sub-culture pathogen from differential or selective media prior to performing biochemical or serological identification tests.

ENRICHED MEDIA-These are media that are enriched with whole blood, lysed blood, serum, extra peptones, or vitamin to support the growth of pathogen that require growth stimulants. The term enrichment is used to describe a fluid medium that increase the numbers of a pathogen by containing enrichments, or substances that discourages the multiplication of unwanted bacteria e.g. selenite F broth is used as an enrichment medium for salmonellae in faeces or urine prior to subculturing on Xylose Lysine Deoxycholate agar.(XLD).

SELECTIVE MEDIA-These are media which contain substances that prevents or slow down the growth of m/org other than the pathogen for which the media are intended. eg X LD selects salmonellae and shigellae by containing bile salts that inhibits growth of other faecal bacteria. Antimicrobials can also be used as a selective agent in culture media eg Modified New York City (MNYC) medium for isolating Neisseria gonorrhoeae from Urogenital specimens and Butzters medium for campylobacter species in faeces. Lowenstein jensen agar slope for M.tuberculosis

DIFFERENTIAL (INDICATOR) MEDIA CHUKWU • ANTHONY

There are media to which indicators, dyes or other substances are added to differentiate M/orgs eg TCBS agar contains indicator Bromothymol blue which differentiate sucrose fermenting from non sucrose fermenting vibrio species. MacConkey agar differentiates lactose and non lactose fermenting m/orgs. Blood agar differentiates haemolysis. Many media are both differential and selective e.g TCBS agar, MacConkey agar and DCA. Enriched media can also be made selective and or differential e.g crystal violet blood agar is an enriched selective and differential medium for streptococcus pyogenes (Group A streptococcus)

TRANSPORT MEDIA- These are mostly semisolid media that contain ingredient to prevent the over growth of commensal and ensure survival of aerobic and anaerobic pathogens, when specimen cannot be culture soon after collection. They are useful when transporting microbiological specimens from health centers to district or reference microbiology laboratory. E.g includes Cary- Blairs medium for presenting enteric pathogens and Amies transport medium for ensuring the viability of gonococci and other pathogens in specimens collected in swabs.

FORMS OF CULTURE MEDIA

Culture media can be used in 3form

Solid

Semisolid

Fluid

→ Solid culture media – They are mainly used in petri dishes as plate cultures, it can also be used in bottles or tubes as stab (deep culture) or slope culture.

→ Fluid culture media- The growth and multiplication of bacteria in fluid medium is usually described in four stages or phases as follows.

- Lag phase, during which the organism adjust to their new surrounding.
- Logarithmic phase, during which the bacteria reproduce rapidly using up the food substances in the medium and introduce into it toxic products of metabolism.
- Stationary phase, during which there is no further increase in the concentrations of living bacteria in the medium. There is a balance number of bacteria dying and those being produced.
- Decline phase, during which the concentrations of living organism is reduced as the number of dying bacteria increases in the medium.

→ Semisolid culture media, they are mainly transport media and for motility testing.

PREPARATION OF CULTURE MEDIA

Almost all laboratories prepare their culture media from either dried or dehydrated products or use ready to use media which are available commercially. The preparation of media from raw materials is no longer practiced in routine laboratories. There are many advantages of using dehydrated products or ready to use culture media.

1) They are easy to prepare.
2) They give good performance.
3) They are highly standardized
4) They ensure reproducibility.
5) They are cheaper.

The successful preparation of culture media requires training and experience. A media preparation manual should be prepared by the head of the laboratory, giving details of preparation, sterilization, sterility test, quality control, shelf life and storage of all media used in the laboratory.

Media section- A section of the laboratory should be designated the media department and manned by an experienced person. The media section usually composes the media "kitchen" where all the weighing, sterilizing and steaming are carried out. The media pouring room should be the most sterile section in the laboratory. It is where media both solid and liquid are dispensed. It should have a flat smooth slab and cabinet. Only person immediately engaged in dispensing media are allowed in the pouring room.

Stages in the preparation of media

1) Weighing and dissolving.
2) The adjustment of PH.
3) Sterilization- Autoclaving. Heat sensistive ingredients should be added when the medium has cooled to about 50^{oc}.
4) Dispensing culture media. This should be done in the media pouring room using clean sterile containers and aseptic precautions.

Sterility testing- The simplest way to test for contamination of prepared media is to incubate the entire batch overnight at $35\text{-}37^{oc}$. Each plate is checked for visible growth, liquid media are examined for evidence of turbidity. All media, even those that have been sterility tested at the time of preparation, should always be checked visually for contamination immediately before being inoculated.

Performance Test- (Quality Control)

The performance test of culture media is another name for Quality control. The objectives are to test the ability of,

1) Nutrient Agar to support the growth from inocula.
2) Differential media to ensure that organism growing on them show characteristic features.
3) Selective and enrichment media inhibit bacteria other than those they are designed for,
4) Biochemical test media for their ability to show expected reactions.

The choice of the organism dispends on the nature of the media. The medium is best tested with the organism that is known to grow very well on it and another that does not grow on it. The sample should also be incubated at the correct temperature and proper atmosphere.

E.g of quality control organism for various media

Culture media	Positive control	Negative control
Alkaline peptone water	Vibrio sp	Eschenchia coli
Mannitol salt agar	Staph aureus	E. coli
Sabouraud agar	Candida albicans	
MacConkey agar	E. coli LF	Shigella NLF
Butzlers medium	campylobacter jejuni	E. coli

CAUSES OF FAULTY CULTURE MEDIA

The main cause of error and fault in culture media can be traced to the preparatory stages in the preparation of media. Some of them and the causes are

1) Poor growth promoting capacity.(Causes)
   i) overheating/ over sterilization
   ii) Repeated remelting of solid agar
   iii) Incorrect ph adjustment
   iv) Contamination with inhibitory metallic salts
2) Reduced gelling consistency.(Causes)
   i) Too much water used to dissolve ingredients
   ii) Inadequate amount of agar in the medium
   iii) Over sterilization
   iv) Repeated remelting
3) Darkened color of media.(causes)
   i) Caramalization of sugar
   ii) Over sterilization
4) PH change.(causes)
   i) Repeated remelting
   ii) Incomplete mixing

iii) Over sterilization
iv) Hydrolysis of ingredients

Storage of culture media

Dehydrated media and other ingredient such as agar, peptone should be kept in cool dry place at an even temperature away from sunlight.

Blood, serum, urea, antimicrobial disc should be kept at 2-6^{oC}. Plate and tubes of culture media should be stored at 2-6^{oC}, most plate culture media if properly stored have a shelf life of about 7-10 days.

PLATE CULTURE METHOD

The petridish (plate) is universally accepted container for solid culture medium and when colonial characteristics of an organism are to be studied. The technique of inoculating media in petridishes is called "plating out. In order to isolate single colonies, the surface of the plate must be "dried" usually 10min at 45°c is enough with the flat surface of the lid into the incubator shelf angling the media container dish on the edge of the lid, the media down wards.

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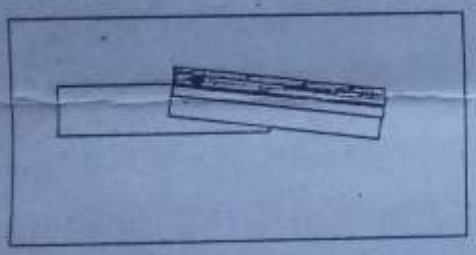
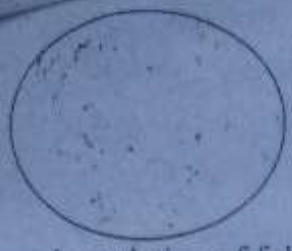


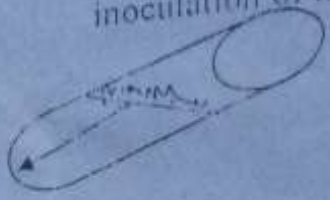
With a sterile wire loop or using the swab of specimen, apply the inoculums to a small area (1)of the plate. Sterilize the loop in the bunsen flame, cool and streak over area (2). Repeat the same procedure over area 3, 4 and 5. Then incubate the medium at appropriate temperature.



Inoculation of full plate

inoculation of half plate

Inoculation of slope culture



inoculation of butt and slope

stab culture e.g MIU medium.

Inoculation of nutrient broth and other liquid culture media are carried out with the aid of wire loop, straight wire or pastuer pipette. Blood culture media are inoculated with needle and syringe

PREPARATION OF STOOL SAMPLES FOR MICROSCOPIC EXAMINATION

The easiest and the simplest technique for the direct microscopic examination of faeces is WET MOUNTS.

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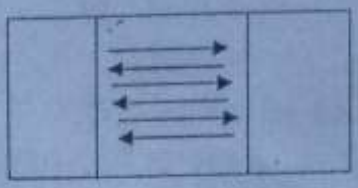
WET MOUNTS can be prepared directly from the faecal material in saline and iodine. Other useful mounts are buffered methylene blue (BMB) and eosin. The saline wet mount is used for preliminary microscopic examination of faeces to detect protozoan trophozoites and cysts,and helminths larvae and eggs.

In the iodine wet mount, most cysts can usually be specifically identified because iodine stains their nuclei and glycogen, if present; however the parasite are not motile in iodine. BMB wet mount is useful only when amoebic trophozoites are seen. It stain the amoebic trophozoites but not cysts. Eosin wet mount is very good for the detection of cysts and trophozoites. Eosin does not stain them but they can be much more easily seen against a pink- red background of eosin.

TECHNIQUE

- Place a drop of saline in one end of a slide and place a drop of iodine or eosin on the other end.
- With an applicator stick, pick up a small portion of the faeces about (2) and mix in the saline, do same on the eosin or iodine.
- Cover the emulsified smear with a cover slip
- Press on the cover slip with a tissue to make a thin preparation.
- Examine systematically the entire saline preparation using the X10 objective with the condenser iris closed sufficiently to give a good contrast, then use X40 objective to identify small parasite.



Preparation and examination of wet mounts.

947 CONCENTRATION TECHNIQUES FOR FAECAL PARASITES

It may be necessary to use concentration methods for the detection of faecal parasites for the following reasons. CHUKWU ANTHONY

I. To detect parasites when they are not found in a direct examination but the symptoms of intestinal parasitic infection still persist.
II. To detect the eggs of parasites whtch are often few in number such as those of schistosoma species and taenia species.
III. To check whether treatment has been successful.
IV. To investigate the prevalence and incidence of parasitic infection for epidemiological purposes.

There are two types of concentration methods,

1) FLOATATION TECHNIQUES
2) SEDIMENTATION TECHNIQUES

1) FLOATATION TECHNIQUES

A floatation techniques uses a liquid with high specific gravity for the separation of protozoan cysts and certain helminthic eggs and larva from faecal debris. The

parasites floats in the surface film while the debris remains at the bottom. However some helminth egg e.g operculated egg may not concentrate because they are ruptured by the zinc sulphate solution used for the test. The saturated sodium chloride is useful for concentrating hookworm and Ascaris egg in field survey. There are two methods viz 33% zinc sulphate solution and saturated sodium chloride solution (Brine).

Zinc sulphate floatation technique

Reagent – 33% zinc sulphate solution. Dissolve 330g of dry zinc sulphate crystals in one litre of distilled water. The specific gravity should be 1.18.

METHOD

1) About one quarter fill the tube with zinc sulphate solution.
2) Add an estimated 0-5gm of faeces, emulsify the specimen in solution.
3) Fill the tube with zinc sulphate solution, mix well.
4) Stand the tube in a vertical position in a flat surface.
5) Add more zinc sulphate solution until the tube is filled to the brim..
6) Carefully place a clean grease free cover glass on top of the tube.
7) Leave undisturbed for 30-45mins to give time for the parasites (cyst and eggs) to float through the solution
8) Carefully lift the cover glass from the tube by straight pull upwards, place the cover glass face down wards on a slide.
9) Examine microscopically the entire preparation using X10 objective and also X40 to identify smaller parasites.

Preparation of Brine solution (saturated sodium chloride solution)- Prepare a saturated solution of sodium chloride (Nacl) till some salt remain undissolved at the bottom of the container. The solution should have a specific gravity of 1.2.

The technique is the same as the zinc sulphate floatation method.

2) SEDIMENTATION TECHNIQUES

There are two types

- Formalin ether technique in which the parasites are sedimentated by centrifugal force. Ether is used to dissolve faecal fats and to separate the faecal matter from the sedimented parasites

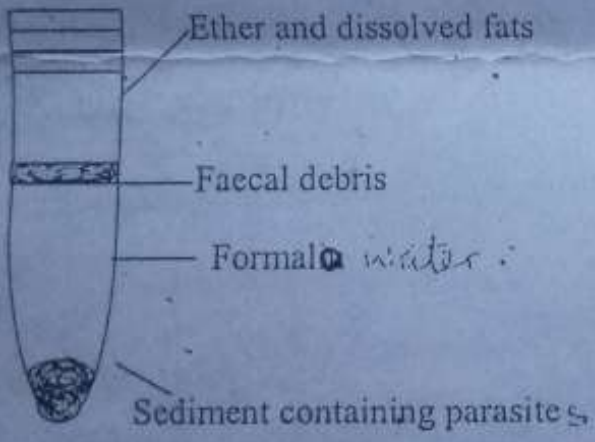
- Formalin detergent technique in which the parasites are sedimented by gravity using a solution of low specific gravity with detergent added to "clear" the faecal matter.

The recommended technique for Hospital laboratories is the formalin ether technique. It is rapid and gives good concentration of the parasitic cysts, larva, eggs and larva in fresh or preserved faeces.

Formalin – ether sedimentation technique method.

1) Transfer about 1g of faeces to 10ml of 10% formal water in a tube
2) Emulsify for about 30 seconds.
3) Sieve the emulsified faeces, collecting the sieved suspension in a beaker.
4) Transfer the suspension to a centrifuge tube, add equal volume of ether (Diethylether) 3-4 ml, close the tube with a glass stopper, and shake well for 30secs. (Do not use rubber stopper as it may get dissolved in the ether).
5) Centrifuge immediately at 3000 rpm for 1 minutes.

After centrifuging, the parasites will have sedimented to the bottom of the tube and the faecal debris will have collected in a layer between the ether and formalin water.



6) Loosen the faecal debris from the side of the tube.
7) Rapidly invert the tube to pour of the ether debris and formalin, the sediment will remain.
8) Transfer the sediment on a slide, and cover, with a cover slip.
9) Examine microscopically the entire preparation using X10 object and X40 to identify smaller parasites

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PREPARATION OF STAINS AND BUFFERS USED IN PARASITOLOGY LABORATORY.

The parasites which can be detected in blood are

(a)-Plasmodia (b) Trypaposmes (c) Leishmania (d) Filaral worms.

Some parasite such as microfilariae and Trypanosomes can be detected in the direct wet mount of fresh blood by the characteristic shape and motility.

Blood films should be stained as soon as possible as delay may result in stain retention. Rowmanowsky stains such as Giemsa, Leishman or Field can be used be used for staining.

GIEMSA STAIN

Giemsa stain is a Romanowsky stain that requires dilution in buffered water or buffered saline PH 7.1-7.2 before use. It gives the best staining of malaria parasite in thin film and also in thick film provided the thick film is dried thoroughly i.e (left to dry overnight or dry for a very long time). Care must be taken to ensure that water does not enter the stock solution of the stain, if this happens the solution will not stain well .

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Giemsa stain (stock solution)

Giemsa stain powder	0.6g
Methanol absolute (Acetone free)-	50ml
Glycerol	50ml

Dissolve the Giemsa stain in methanol in a brown bottle containing a few glas beads. Add glycerol, mix and place the bottle n a water bath at 50 – 60°c for tw hours to dissolve the stain shake gently a half hour interval, the stain should stan at room temperature for three week and should be filtered before use. If kept ai tight the stain is stable for several months.

METHODS

1) Immediately before use, dilute the Giemsa stains as required 3% solution f 30 minutes staining measure 50ml of buffered water or saline PH 7.1-7. add 1.5ml of Giemsa stain and mix gently. 10% solution for 10minut

staining measure 45ml of buffered water, PH 7.1-72 in 50ml cylinder. Add 5ml of Giemsa stain to the 50ml mark and mix gently.

2) Place the slides face downward in a shallow tray supported on two rods, in a coplin jar or a staining rack.
3) Pour the diluted stain into the staining rack, stain as follows

30minutes if using a 3% stain solution

10minutes if using a 10% stain solution.

4) Wash the stain using clean water.
5) Wipe the back of each slide and place in a draining rack for the preparation to dry.

BUFFER SOLUTION

A buffer solution is that which tends to resist changes in the PH on the addition acid or alkali. Buffer solutions are made up of weak acid and a salt of a strong base, or a weak base and salt of a strong acid.

Buffers are used in parasitology to help in the proper staining of the component of the parasite.

Example of buffer used in parasitology is PHOSPHATE BUFFER

Buffered water PH 7.1 -7.2.

This is best prepared from stock phosphate buffer solutions A and B as follows

Stock phosphate solution A

Sodium dihydro phosphate -27.6g

1 – hydrate phosphate ($Na\ H_2Po4\ H_20$)

Distilled water - liter

Weight accurately and mix

Label the stock phosphates solution 'A' store in cool dry place, the solution in stable for several months

Stock phosphate solution B

Disodium hydrogen phosphate -28.39g

Anhydrous (Na_2HPO_4)

Distilled water -1liter

Weigh accurately and mix, like solution A.

To make 1litre of buffered water stock phosphate solution A - 140ml

360ml

Stock phosphate solution B

500ml

Distilled water

Measure accurately and transfer to a clean bottle and mix well.

Check the PH using PH strips or meter label and store at room temperature and the buffer is stable for several months.

FIELDS STAIN

Fields stain is a water based Romanowsky stain. It is composed of two solutions A and B. Field solution A contain methylene blue, azure and phosphate salt while solution B contain eosin and phosphate salt. Buffer solutions are included in the staining solution. Neither solutions need to be diluted for thick films, but solution B is diluted 1:5 before use for thin films.

Advantages of the fields stain are that it is quick, stable, isotonic with malarial parasites and Trypanosomes. It penetrates unfixed parasites rapidly and gives particularly good result with fresh thick blood films. It is not suitable for staining microfilariae in thick films.

In the thick film staining the red blood cells are lyzed during the staining.

Because thick blood films are not fixed, pathogens are not killed and therefore even after staining preparation must be handled with care.

Fields stain A

Fields stain powder: 6g

Distilled water (Hot) 500ml

1) Weigh the powder and transfer to a pyrex beaker.
2) Measure the water and heat to boiling point.
3) Add the hot water to the stain and mix to dissolve the powder.
4) When cool, filter the stain into a clean bottle

5) Label the bottle and store at room temperature, the stain is stable indefinitely

Field stain B

Fields stain B -5g
Distilled water (Hot) - 500ml
Same method as A

FIELD THICK FILM STAINING TECHNIQUE METHOD

1) Holding the slide of the dried thick film facing downwards, dip the slide into field's stain 'A' for 5 secs.
2) Wash gently for about 5 sec in clean water
3) Dip the slide into field's stain B for 3 secs
4) Wash gently in clean water, wipe the back of the slide and place upright in a draining rack to dry
5) Examine the film microscopically with X100 object

For the thin film staining technique, you have to fix the thin film with absolute methanol for 1-2mins and fields stain B must be diluted 1:5 with buffered water i.e 1ml of stain B and 4ml buffered water.

Preparation of thick and thin films for examination of malaria parasites

Blood films for malaria parasite can be prepared directly from capillary blood or from EDTA (sequentrene) anticoagulated capillary or venous blood providing the films are made within 1hr of collecting blood into EDTA. A thick film made from anticoagulated blood, however needs to be handled with special care to avoid the blood being washed from the slide during staining

Need for thick and thin blood films

About twenty times more blood can be examined in a thick film than in a thin film. A thick film is more suitable for the rapid detection of malaria parasite.

More blood can be examined in a thick film because the film is not fixed and therefore the red blood cells are lyzed during staining. The parasites can be detected among the white blood cells.

A thin film is fixed and therefore the parasites can be seen in the red blood cells. Depending on the species, the parasitized red cells may become enlarged, oval in shape and show stripling. These features, together with the parasitic form present can greatly assist in confirming a mixed infection which are more difficult to differentiate in thick film.

Thick film allows a large volume of blood to be examined, thus making it easier to detect light infections with few parasites while species identification is difficult. Thin films are necessary to see the morphorlogical characteristic of the parasites and identify them.

Making Of Thick and Thin Blood Film

THICK FILM

TO MAKE A THICK FILM

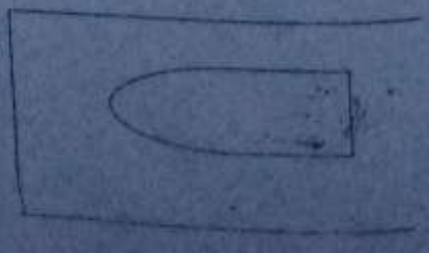
1) Place two or three small drop of fresh blood without anticoagulant on a clean slide
2) With a corner of another slide, mix the drop in a circular motion over an area of about 2cm in diameter. CHUKWU ANTHONY
3) Continue mixing to prevent formation of fibrin strands that may obscure the parasite after staining.
4) Allow the film to dry in air at room temperature

THIN BLOOD FILMS

1) Place a drop of blood on a slide
2) Place the slide on a flat surface.
3) Place a spreader just in front of the drop of blood at angle 45°c
4) Draw it back slightly to touch the drop of blood and allow the blood to spread along the contact, line.
5) Push the spreader forward, smoothly and rapidly, maintaining the contact between the slide and the spreader. The smear formed should be 3-4cm long slightly thicker at the base and thin at the tail end without ragged tails
6) Allow the smear to dry in air.

Thick Film

Thin Film



Results

Giemsa stain

Malarial parasites

Chromatin of parasite : Dark red

Cytoplasm of parasite : Blue

Schuffner's dots : Red

Maurer's dot : Red – mauve

Trypanosomes

Nucleus Mauve Red

Kinetoplast Dark Red

Cytoplasm Pale Mauve

Flagellum pale Mauve

Leishmania

Nucleus Mauve Red

Kinetoplast Dark Mauve Red

microfilaria

Nuclei Dark Purple

sheath of W. bancrofti Dark Pink

Sheath of Loaloa Pale grey or Unstained

Blood cells

Red cells – Grey to pale mauve pink

Reticulocytes – Grey Blue

Nuclei of Neutrophils –Dark purple

Granules of Eosinophils - Red

Granules of neutrophils -mauve purple

Granules of mononuclear lencocytes - Blue or blue grey.

Fields stain Results is similar to Giemsa result.

EXAMINATION OF STAINED BLOOD FILM FOR PARASITES

THICK BLOOD FILMS

In a thick blood films, the greatest concentration of blood cells occurs at the center of the film. An area which appears mauve in colour usually near the edges indicates an area of good colour balance between the blue and red stains and will show correct staining of parasites. In the thick film most of the red blood cells are lysed and therefore no longer visible. The blood layer is thicker therefore one should focus up and down to see the parasite located at different levels, the parasites in thick film appear smaller than those in thin films. Scanning for microfilaria is carried out at low magnification (X10) and identification confirmed with the oil immersion lens. The search for malarial parasites, Trypanosomes and leishmania should be carried out under oil immersion lens.

When properly stained, the thick film under oil immersion lens should show a clean background, free from debris, with a mottled grey colour due to lysed red blood cells.

THIN BLOOD FILMS.

Initial screening of a thin film should be carried with low power (X10) obj lens, when microfilaria are searched, they are commonly found near the edges of the thin film, once detected the microfilaria should be identified under oil immersion lens. Near the end of a thin film, the red blood cells spread out evenly into a single layer. This area should be examined under the oil immersion for malarial parasites, trypanosomes and leishmanial etc. Also the morphology of the red cells and that of parasite is most clearly seen in the thin films.

WET PREPARATION FOR DETECTION OF BLOOD MICROFILARIAE

Several methods can be used for the detection of microfilariae in blood

1. WET SLIDE PREPARATION (WET MOUNT)

2,LYSED CAPILLARY BLOOD TECHNIQUE.

In this, 100ul of Blood (0.lm) of capillary blood is haemolyzed in 1ml of lyzing fluid. The microfilariae are concentrated by centrifugation or overnight sedimentation, and the sediment examined microscopically for the microfilariae. The addition of methlylene Blue helps to identify the specie. The number of microfilariae counted multiplied by 10 gives the number per ml of blood (mf/ml)

METHOD

1) Collect 0.lml of capillary blood from the ear lobe and dispense into a centrifuge tube containing 1ml of saponin- saline (lyzing slolution)
2) Mix the blood gently and leave about 2mins for red cells to lyze.
3) Centrifuge for 5mins at 2000rpm (low speed). Do not centrifuge at high speed as it may cause the pathogenic microfilariae to loose its sheath. If a centrifuge is not avalliable add a drop of formalin into the tube to kill the microfilariae and allow the tube stand overnight.
4) Using a Pasteur pipette, discard the supernant fluid and transfer the sediment to a slide, add a drop of methylene blue and cover with a cover slip.
5) Examine the entire field microscopically using X10 obj or preferably examine by dark field microscopy.
6) Count the number of microfilariae in the entire preparation and multiply the number counted bly 10 to give an appropriate number of microfilariae per ml of blood (mf/ml)
7) If unable to identify the microfilariae, you then fix the smear with absolute methanol or ethanol for 2-3 mins and stain using Giemsa or modified fields stain and delafields haematoxylin.

KNOTT CONCENTRATION METHOD

When a light infection is suspected, the microfilariae in peripheral blood can also be detected by haemolyzing the red cell with formalin and using the centrifuged deposit for direct examination and to prepare thick and thin film.

Delafield's Haematoxylin stain for microfilariae

This is a rapid staining method for staining nuclei and sheaths of microfilariae which helps in the identification of species

METHOD

1) Prepare a thin smear from fresh blood or from the blood sample concentrated before.
2) Fix the smear in methanol for 1minute and wash with water
3) Cover the smear with diluted 1ml of field's stain B
4) Add equal volume of field's stain A and mix well, allow to stain for1 minute, wash with water
5) Cover with dilute 1:10 delafield's haematoxylin and stain for 5mins, wash off with buffered water PH 7.1 -7.2
6) Dry and examine microscopically using X10 obj ,X40 and oil immersion obj for identification of species.

Results	
Nuclei of microfilariae	blue
Sheath of W. bancrofti	Grey
Sheath of Brugia malayi	Dark Grey
Sheath of Loa loa	Pale grey