GOA UNIVERSITY

INTERNSHIP REPORT

SUBMITTED BY

NAME: - KANHAIYA GHANSHYAM DUBE

ROLE NUMBER: - 21P050008

INTERNSHIP INSTITUTE: - GOA MEDICAL COLLEGE, BAMBOLIM

AIM: - TO LEARN MICROBIOLOGICAL AND PATHOLOGICAL TECHQNIQUES

DURATION: - 1 MONTH (1ST DECEMBER 2022 TO 31ST DECEMBER 2022)

Department of Pathology,
Goa Medical College,
Bambolim-Goa.

Date: 02/01/2023

CERTIFICATE

This is to certify that Kanhaiya Dube student of M.Sc. in Marine Biotechnology curriculum Part II, Goa University has completed internship training in Pathology Department from 16/12/2022 to 31/12/2022.

This certificate issued at his own request.

(Dr. R.G.W. Pinto)
Professor. & Head
Department of Pathology

R G WISEMAN PINTO
Prof & Head
Department of Pathology
Goa Medical College
Bambolim Goa
Reg No 232

DEPARTMENT OF MICROBIOLOGY
GOA MEDICAL COLLEGE
BAMBOLIM- GOA

Date: 03/01/2023

TO WHOMSOEVER IT MAY CONCERN

This is to certify that Mr. Kanhaiya Dube, student of Goa University has undergone Internship training in various sections of the Department of Microbiology, Goa Medical College from 01/12/2022 to 15/12/2022.

This certificate is issued at his request.

Dr. Maria J.W Pinto
(Professor & Head)

DR. MARIA JOSE W. PINTO
PROFESSOR AND HEAD
DEPARTMENT OF MICROBIOLOGY
GOA MEDICAL COLLEGE
BAMBOLIM - GOA

Acknowledgment

First of all, I would like to express my gratitude to the Department of Biotechnology (DBT) India, Dean of the School of biological sciences and Biotechnology ‘Dr. Savita Kerkar’ and marine biotechnology program director Dr. Sanjeev Ghadi for allowing me to intern at Goa medical college, Bambolim. For me, it was a unique experience to learn about microbiology and pathology techniques. This internship was a great chance for learning and professional development.

Next, I express my deepest gratitude to Dr. Maria (Department of Microbiology, Goa medical college) and Dr. Pinto (Department of Pathology, Goa medical college) for allowing me to work in the respective laboratories. It helped in the understanding of the various techniques which are used in the diagnosis of the diseases. Working in the bacteriology, serology, TB, HIV, hematology and histopathology labs was a great experience for me.

I would also like to say thanks to the Goa medical college staff for the necessary guidance and advice. I would express my sincere gratitude to them for providing me with the most valuable guidance and affable treatment at every stage, which helped me learn microbiology and pathology-related procedures which helped me to add a feather to my cap.

Last but not least my sincere gratitude to all people who knowingly or unknowingly supported me, in my moral to make this internship a reality.

Bacteriology lab

Urine sample: -

- Most commonly found pathogen E.coli
- UTI infections include klebsiella species, pseudomonas sp., enterococcus sp., staphylococcus sp., saprophyticus sp.
- Directly streaked in the MacConkey agar (selective and differential for Gram-negative rods) are probably the most commonly recommended and used media for routine urine cultures also on the blood agar (non-selective medium)
- Incubated for the 24 hours
- Growth was seen on the plates for the infected people
- Further tests were done like oxidase test, catalase test, triple sugar test
- Urine sample is directly spread on the MH agar commonly used for the routine susceptibility testing of non-fastidious microorganism by the Kirby-Bauer disk diffusion technique.

Stool sample: -

- Sample is directly streakedon the DCA, XLD, MacConkey agar then on slide (saline suspension) and alkaline peptone water
- After the 24 hours of the incubation subcultured on TCBS and MacConkey agar
- Antibiotic sensitivity done on the recommendation

Miscellaneous: -

- Pus sample, tissue sample, sputum, cough
- Streaked directly on the blood agar and MacConkey agar and inoculated in GB

Oxidase test: -

The oxidase test detects the presence of a cytochrome oxidase system that will catalyse the transport of electrons between electron donors in the bacteria and a redox dye- tetramethyl-*p*-phenylene-diamine. The dye is reduced to deep purple color. This test is used to assist in the identification of *Pseudomonas, Neisseria, Alcaligens, Aeromonas, Campylobacter, Vibrio, Brucella* and *Pasteurella,* all of which produce the enzyme cytochrome oxidase.

Principle of Oxidase Test

Cytochrome containing organisms produce an intracellular oxidase enzyme. This oxidase enzyme catalyzes the oxidation of cytochrome c. Organisms which contain cytochrome c as part of their respiratory chain are oxidase-positive and turn the reagent blue/purple. Organisms lacking cytochrome c as part of their respiratory chain do not oxidize the reagent, leaving it colorless within the limits of the test, and are oxidase-negative.

Oxidase positive bacteria possess cytochrome oxidase or indophenol oxidase (an iron containing haemoprotein). Both of these catalyse the transport of electrons from donor compounds (NADH) to electron acceptors (usually oxygen). The test reagent, N, N, N', N'-tetramethyl-p-phenylenediamine dihydrochloride acts as an artificial electron acceptor for the enzyme oxidase. The oxidised reagent forms the colored compound indophenol blue.

The cytochrome system is usually only present in aerobic organisms which are capable of utilising oxygen as the final hydrogen receptor. The end product of this metabolism is either water or hydrogen peroxide (broken down by catalase)

1. A strip of filter paper is soaked with a little freshly made 1% solution of the reagent tertramethyl-p-phenylene-diamine dihydrochloride.
2. A speck of culture is rubbed on it with a platinum loop.
3. A positive reaction is indicated by an intense deep-purple hue, appearing within 5-10 seconds, a "delayed positive" reaction by coloration in 10-60 seconds, and a negative reaction by absence of coloration or by coloration later than 60 seconds.

Catalase Test

This test demonstrate the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide (H_2O_2). It is used to differentiate those bacteria that produces an enzyme catalase, such as *staphylococci*, from non-catalase producing bacteria such as *streptococci*. Normally 3% H_2O_2 is used for the routine culture while 15% H_2O_2 is used for detection of catalase in anaerobes.

Principle of Catalase Test

The enzyme catalase mediates the breakdown of hydrogen peroxide into oxygen and water. The presence of the enzyme in a bacterial isolate is evident when a small inoculum is introduced into hydrogen peroxide, and the rapid elaboration of oxygen bubbles occurs. The lack of catalase is evident by a lack of or weak bubble production. The culture should not be more than 24 hours old.

Bacteria thereby protect themselves from the lethal effect of Hydrogen peroxide which is accumulated as an end product of aerobic carbohydrate metabolism.

- The morphologically similar *Enterococcus*or *Streptococcus* (catalase negative) and *Staphylococcus* (catalase positive) can be differentiated using the catalase test.
- Also valuable in differentiating aerobic and obligate anaerobic bacteria.
- Semiquantitative catalase test is used for the identification of Mycobacterium tuberculosis.
- It is used to differentiate aerotolerant strains of *Clostridium*, which are catalase negative, from *Bacillus* species, which are positive.
- Catalase test can be used as an aid to the identification of Enterobacteriaceae.

Slide Method

1. Use a loop or sterile wooden stick to transfer a small amount of colony growth in the surface of a clean, dry glass slide.
2. Place a drop of 3% H_2O_2 in the glass slide.
3. Observe for the evolution of oxygen bubbles.

Positive: Copious bubbles produced, active bubbling

Examples: *Staphylococci, Micrococci, Listeria, Corynebacterium diphtheriae, Burkholderia cepacia, Nocardia*, the family Enterobacteriaceae (*Citrobacter, E. coli, Enterobacter, Klebsiella, Shigella, Yersinia, Proteus, Salmonella, Serratia*), *Pseudomonas, Mycobacterium tuberculosis, Aspergillus, Cryptococcus, and Rhodococcus equi.*
Negative: No or very few bubbles produced.
Examples: *Streptococcus and Enterococcus* spp

The Triple Sugar Iron (TSI) Test

Most bacteria have the ability to ferment carbohydrates, particularly sugars. Among them, each bacteria can ferment only some of the sugars, while it cannot ferment the others. Thus, the sugars, which a bacteria can ferment and the sugars, which it cannot is the characteristic of the bacteria and thus an important criterion for its identification.

The Triple Sugar Iron (TSI) test is a microbiological test named for its ability to test a microorganism's ability to ferment sugars and to produce hydrogen sulfide.

An agar slant of a special medium with multiple sugars constituting a pH-sensitive dye (phenol red), 1% lactose, 1% sucrose, 0.1% glucose, as well as sodium thiosulfate and ferrous sulfate or ferrous ammonium sulfate is used for carrying out the test.

All of these ingredients when mixed together and allowed solidification at an angle result in a agar test tube at a slanted angle. The slanted shape of this medium provides an array of surfaces that are either exposed to oxygen-containing air in varying degrees (an aerobic environment) or not exposed to air (an anaerobic environment) under which fermentation patterns of organisms are determined

Principle: -The triple sugar- iron agar test employing Triple Sugar Iron Agar is designed to differentiate among organisms based on the differences in carbohydrate fermentation patterns and hydrogen sulfide production. Carbohydrate fermentation is indicated by the production of gas and a change in the color of the pH indicator from red to yellow.

To facilitate the observation of carbohydrate utilization patterns, TSI Agar contains three fermentative sugars, lactose and sucrose in 1% concentrations and glucose in 0.1% concentration. Due to the building of acid during fermentation, the pH falls. The acid base indicator Phenol red is incorporated for detecting carbohydrate fermentation that is indicated by the change in color of the carbohydrate medium from orange red to yellow in the presence of acids. In case of oxidative decarboxylation of peptone, alkaline products are built and the pH rises. This is indicated by the change in color of the medium from orange red to deep red. Sodium thiosulfate and ferrous ammonium sulfate present in the medium detects the production of hydrogen sulfide and is indicated by the black color in the butt of the tube.

To facilitate the detection of organisms that only ferment glucose, the glucose concentration is one-tenth the concentration of lactose or sucrose. The meagre amount of acid production in the slant of the tube during glucose fermentation oxidizes rapidly, causing the medium to remain orange red or revert to an alkaline pH. In contrast, the acid reaction (yellow) is maintained in the butt of the tube since it is under lower oxygen tension.

After depletion of the limited glucose, organisms able to do so will begin to utilize the lactose or sucrose. To enhance the alkaline condition of the slant, free exchange of air must be permitted by closing the tube cap loosely.

Composition: -
Ph sensitive phenol red, 1% lactose, 1%sucrose, 0.1% glucose, sodium thiosulfate and ferrous sulfate or ferrous ammonium sulfate
Slant: - surface either exposed to the o2 containing air in varying degree (an aerobic environment) or not exposed to air (anaerobic environment)

Types of the agar used:-

MacConkey Agar

MacConkey agar (MAC) was the first solid differential media to be formulated which was developed at 20th century by Alfred Theodore MacConkey. **MacConkey agar** is a selective and differential media used for the isolation and differentiation of non-fastidious gram-negative rods, particularly members of the family Enterobacteriaceae and the genus *Pseudomonas*.
Final pH 7.1 +/- 0.2 at 25 degrees C.
Principle of MacConkey Agar

MacConkey agar is used for the isolation of gram-negative enteric bacteria and the differentiation of lactose fermenting from lactose non-fermenting gram-negative bacteria. **Pancreatic digest of gelatin** and **peptones (meat and casein)** provide the essential nutrients, vitamins and nitrogenous factors required for growth of microorganisms. **Lactose monohydrate** is the fermentable source of carbohydrate. The selective action of this medium is attributed to **crystal violet** and **bile salts,** which are inhibitory to most species of gram-positive bacteria. **Sodium chloride** maintains the osmotic balance in the medium. **Neutral red** is a pH indicator that turns red at a pH below 6.8 and is colorless at any pH greater than 6.8. **Agar** is the solidifying agent.
Uses of MacConkey Agar

1. MacConkey agar is used for the isolation of gram-negative enteric bacteria.
2. It is used in the differentiation of lactose fermenting from lactose non-fermenting gram-negative bacteria.
3. It is used for the isolation of coliforms and intestinal pathogens in water, dairy products and biological specimens.

Result Interpretation on MacConkey Agar

Lactose fermenting strains grow as **red or pink** and may be surrounded by a zone of acid precipitated bile. The red color is due to production of acid from lactose, absorption of neutral red and a subsequent color change of the dye when the pH of medium falls below 6.8.
Lactose non-fermenting strains, such as *Shigella* and *Salmonella* are **colorless** and **transparent** and typically do not alter appearance of the medium. *Yersinia enterocolitica* may appear as small, non-lactose fermenting colonies after incubation at room temperature.

Deoxycholate Citrate Agar (DCA)

- Deoxycholate Citrate Agar is a modification of Leifson formula and is recommended for the isolation of *Salmonella* and *Shigella* spp.
- This medium is similar to deoxycholate agar in comparison but is moderately more selective for enteric pathogens owing to increased concentrations of both citrate and deoxycholate salts.
- Sodium deoxycholate at pH 7.3 to 7.5 is inhibitory for gram-positive bacteria. Citrate salts, in the concentration included in the formulation, are inhibitory to gram-positive bacteria and most other normal intestinal organisms.
- It is thus a selective and differential medium, used commonly for the isolation of enteric pathogens.
- HI solids is a source of carbon and nitrogen and result in the inhibition of coliforms.
- Proteose peptone provides carbon, nitrogen, vitamins and minerals.
- Coliform bacteria and gram-positive bacteria are inhibited or greatly suppressed due to sodium deoxycholate, sodium citrate, and ferric ammonium citrate.
- Dipotassium phosphate buffers the medium.
- Lactose helps in differentiating enteric bacilli, as lactose fermenters produce red colonies while lactose non-fermenters produce colourless colonies.
- Coliform bacteria, if present form pink colonies on this medium.
- The degradation of lactose causes acidification of the medium surrounding the relevant colonies and the pH indicator neutral red changes its color to red. These colonies usually are also surrounded by a turbid zone of precipitated deoxycholic acid due to acidification of the medium.
- Sodium deoxycholate combines with neutral red in an acidic environment, causing the dye to go out of the solution with the subsequent precipitation of deoxycholate.
- The reduction of ferric ammonium citrate to iron sulfide is indicated by the formation of black iron sulfide.
- Salmonella and Shigella species do not ferment lactose but Salmonella may produce H2S, forming colorless colonies with or without black centers.

Organisms	**Growth**
Escherichia coli	Poor growth; pink with bile precipitate negative reaction for H_2S
Salmonella Enteritidis	Good-luxuriant growth; colorless; positive reaction for H_2S, black centered colonies
Salmonella Typhimurium	Good-luxuriant growth; colorless; positive reaction for H_2S, black centered colonies
Shigella flexneri	Good growth; colorless
Salmonella Abony	Good-luxuriant growth; colorless; positive reaction for H_2S, black centered colonies
Shigella sonnei	Colonies are smooth and initially colorless, becoming pale pink on further incubation due to late lactose fermentation

Enterobacter/Klebsiella spp.	Large, pale mucoid colonies with the pink center

- Citrate and iron (Fe) combination has a strong hydrolyzing effect on agar when the medium is heated, producing a soft and unelastic agar.

esult Interpretation on Deoxycholate Citrate Agar (DCA)

Lactose non-fermenters produce transparent, colorless to light pink or tan-colored colonies with or without black centers.
Lactose fermenters produce a red colony with or without a bile precipitate.

Mueller Hinton Agar (MHA)

Mueller and Hinton developed Mueller Hinton Agar (MHA) in 1941 for the isolation of pathogenic *Neisseria* species. Nowadays, it is more commonly used for the routine susceptibility testing of non-fastidious microorganism by the Kirby-Bauer disk diffusion technique.
Five percent sheep blood and nicotinamide adenine dinucleotide may also be added when susceptibility testing is done on *Streptococcus species*. This type is also commonly used for susceptibility testing of *Campylobacter*.

Principle of MHA

Mueller Hinton Media contains Beef Extract, Acid Hydrolysate of Casein, Starch and Agar. **Beef Extract** and **Acid Hydrolysate of Casein** provide nitrogen, vitamins, carbon, amino acids, sulphur and other essential nutrients. **Starch** is added to absorb any toxic metabolites produced. **Starch** hydrolysis yields dextrose, which serves as a source of energy. **Agar** is the solidifying agent.
The use of a suitable medium for testing the susceptibility of microorganisms to **sulfonamides and trimethoprim** is essential. Antagonism to sulfonamide activity is demonstrated by **para-aminobenzoic acid (PABA)** and its analogs. Reduced activity of **trimethoprim**, resulting in smaller inhibition zones and innerzonal colonies, is demonstrated on unsuitable Mueller Hinton medium possessing high levels of **thymidine**. Both the **PABA and thymine/thymidine** content in Mueller Hinton Agar are reduced to a minimum, thus markedly reducing the inactivation of **sulfonamides and trimethoprim** when the media is used for testing the susceptibility of bacterial isolates to these antimicrobics.

1. It is a non-selective, non-differential medium. This means that almost all organisms plated on here will grow.
2. It contains starch. Starch is known to absorb toxins released from bacteria, so that they cannot interfere with the antibiotics. It also mediates the rate of diffusion of the antibiotics through the agar.
3. It is a loose agar. This allows for better diffusion of the antibiotics than most other plates. A better diffusion leads to a truer zone of inhibition.
4. MHA shows acceptable batch-to-batch reproducibility for susceptibility testing.
5. MHA is low in sulfonamide, trimethoprim, and tetracycline inhibitors (i.e. concentration of inhibitors thymidine and thymine is low in MHA).
6. Both the para-aminobenzoic acid (PABA) and thymine/thymidine content in Mueller Hinton Agar are reduced to a minimum, thus markedly reducing the inactivation of

sulfonamides and trimethoprim when the media is used for testing the susceptibility of bacterial isolates to these antimicrobics.

Blood Agar

Blood Agar (BA) are **enriched medium** used to culture those bacteria or microbes that do not grow easily. Such bacteria are called "fastidious
" as they demand a special, enriched nutritional environment as compared to the routine bacteria.
Blood Agar is used to grow a wide range of pathogens particularly those that are more difficult to grow such as *Haemophilus influenzae, Streptococcus pneumoniae* and *Neisseria* species. It is also required to detect and differentiate haemolytic bacteria, especially *Streptococcus* species. It is also a **differential** media in allowing the detection of hemolysis (destroying the RBC) by cytolytic toxins secreted by some bacteria, such as certain strains of *Bacillus, Streptococcus, Enterococcus, Staphylococcus, and Aerococcus*.
Blood agar can be made selective for certain pathogens by the addition of antibiotics, chemicals or dyes. Examples includes crystal violet blood agar to select *Streptococcus pyogens* from throat swabs, and kanamycin or neomycin blood agar to select anaerobes from pus.

Composition of Blood Agar

- **0.5% Peptone**
- **0.3% beef extract/yeast extract**
- **1.5% agar**
- **0.5% NaCl**
- **Distilled water**

(Since Blood Agar is made from Nutrient Agar, above is the composition of Nutrient Agar)

- **5% Sheep Blood**
- **pH should be from 7.2 to 7.6 (7.4)**

Uses of Blood Agar

1. Blood Agar is a general purpose enriched medium often used to grow fastidious organisms
2. To differentiate bacteria based on their hemolytic properties (β-hemolysis, α-hemolysis and γ-hemolysis (or non-hemolytic)).

Tuberculosis lab

EPTB: - extra pulmonary tuberculosis

Extrapulmonary tuberculosis (EPTB) is an infectious disease caused by *Mycobacterium tuberculosis* that occurs in organ systems other than the lungs.

Epidemiologic risk factors include birth in high TB-prevalent countries, exposure at place of residence/work in an institutional setting, and homelessness.

Diagnosis may be delayed as a result of nonspecific clinical manifestations that progress slowly and the low sensitivity of acid-fast bacilli (AFB) smear on extrapulmonary specimens.

Microbiologic proof is the key to diagnosis and treatment, and tissue biopsy is frequently required. Other supportive findings are granulomas and positive AFB stain on pathology, and chest x-ray findings.

Initial therapy is a 4-drug regimen of isoniazid, rifampin, pyrazinamide, and ethambutol; treatment lasts for at least 6 months

Organs other than lungs e.g plura, lymph nodes, genitourinary tract, skin, joints, bones and meninges.

Line probe assay: -

The line probe assay (LPA), based on strip technology was used to diagnose TB and detect RIF as well as Isoniazid (INH) resistance due to mutations in *rpoβ*, and both *inhA* and *katG* genes. The test was performed according to the manufacturer's protocol (Hain Life Science GmbH, Nehren, Germany). The method involved three processes: DNA extraction, multiplex PCR amplification, and reverse hybridization.

Acid-Fast Stain

It is the differential staining techniques which was first developed by Ziehl and later on modified by Neelsen. So this method is also called ***Ziehl-Neelsen staining*** techniques. Neelsen in 1883 used Ziehl's carbol-fuchsin and heat then decolorized with an acid alcohol, and counter stained with methylene blue. Thus Ziehl-Neelsen staining techniques was developed.
The main aim of this staining is to differentiate bacteria into acid fast group and non-acid fast groups.

This method is used for those microorganisms which are not staining by simple or Gram staining method, particularly the member of genus *Mycobacterium*, are resistant and can only be visualized by acid-fast staining.

Principle of Acid-Fast Stain

When the smear is stained with carbol fuchsin, it solubilizes the lipoidal material present in the Mycobacterial cell wall but by the application of heat, carbol fuchsin further penetrates through lipoidal wall and enters into cytoplasm. Then after all cell appears red. Then the smear is decolorized with decolorizing agent (3% HCL in 95% alcohol) but the acid fast cells are resistant due to the presence of large amount of lipoidal material in their cell wall which prevents the penetration of decolorizing solution. The non-acid fast organism lack the lipoidal material in their cell wall due to which they are easily decolorized, leaving the cells colorless. Then the smear is stained with counterstain, methylene blue. Only decolorized cells absorb the counter stain and take its color and appears blue while acid-fast cells retain the red color.

Procedure of Acid-Fast Stain

1. *Prepare bacterial smear on clean and grease free slide, using sterile technique.*
2. *Allow smear to air dry and then heat fix.*
   Alcohol-fixation: This is recommended when the smear has not been prepared from sodium hypochlorite (bleach) treated sputum and will not be stained immediately. M. tuberculosis is killed by bleach and during the staining process. Heat-fixation of untreated sputum will not kill M. tuberculosis whereas alcohol-fixation is bactericidal.
3. *Cover the smear with carbol fuchsin stain.*
4. *Heat the stain until vapour just begins to rise (i.e. about 60 C). Do not overheat. Allow the heated stain to remain on the slide for 5 minutes.*
   Heating the stain: Great care must be taken when heating the carbol fuchsin especially if staining is carried out over a tray or other container in which highly fiammable chemicals have collected from previous staining. Only a small fiame should be applied under the slides using an ignited swab previously dampened with a few drops of acid alcohol or 70% v/v ethanol or methanol. Do not use a large ethanol soaked swab because this is a fire risk.
5. *Wash off the stain with clean water.*
   Note: When the tap water is not clean, wash the smear with filtered water or clean boiled rainwater.
6. *Cover the smear with 3% v/v acid alcohol for 5 minutes or until the smear is sufficiently decolorized, i.e. pale pink.*
   Caution: Acid alcohol is fiammable, therefore use it with care well away from an open fiame.
7. *Wash well with clean water.*
8. *Cover the smear with malachite green stain for 1–2 minutes, using the longer time when the smear is thin.*
9. *Wash off the stain with clean water.*
10. *Wipe the back of the slide clean, and place it in a draining rack for the smear to air-dry (do not blot dry).*
11. *Examine the smear microscopically, using the 100 X oil immersion objective.*

Sputum smear fluorescence microscopy:-

PRINCIPLE Mycobacteria retain the primary stain even after exposure to decolorizing with acidalcohol, hence the term “acid-fast”. A counter-stain is employed to highlight the stained organisms for easier recognition. Potassium permanganate is used as counter-stain and it helps prevent non-specific fluorescence. With auramine staining, the bacilli appear as slender bright yellow luminous rods, standing out clearly against a dark background. The identification of the mycobacteria with auramine O is due to the affinity of the mycolic acid in the cell walls for the fluorochromes. In fluorescent microscopy, light rays of shorter wave length pass through smear stained by a fluorescent dye, such as auramine O, which have the property of absorbing light rays of shorter wave length and emitting light rays of longer wave length. A mercury vapour lamp is used as a source of light and by means of suitable filter only light rays of shorter wave lengths are allowed to emerge and these rays are used for microscopy. The condenser of the microscope is made of quartz which will not absorb ultra-violet rays.

Staining procedure: -

- Place the slides on a staining rack, with the smeared side facing up, the slides not touching each other
- Flood the slides with freshly filtered auramine-phenol. Let stand for 7-10 minutes
- Wash well with running water, taking care to control the flow of water so as to prevent washing away the smear
- Decolorize by covering completely with acid-alcohol for 2 minutes, twice
- Wash well with running water, as before to wash away the acid alcohol
- Counterstain with 0.1% potassium permanganate for 30 seconds
- Wash as before with water and slope the slides to air dry

Trueprep AUTO v2: - Universal Cartridge Based Sample Prep Device

Testing for infectious diseases by detecting the pathogens nucleic acids using nucleic acid ® amplification methods is a highly specific and sensitive diagnostic tool. Molbio's Truelab micro PCR System is a nucleic acid amplification platform that works on Real Time Polymerase Chain Reaction (PCR) technology that enables near patient diagnosis through disposable, disease ® specific micro PCR chips and a portable, automated Truelab Real Time micro PCR analyser. The Trueprep AUTO v2 Universal ® Cartridge Based Sample Prep Device together with Trueprep AUTO v2 Universal Cartridge Based Sample Prep Kit provides an easy method of nucleic acid extraction and purification. Trueprep AUTO v2 Universal Cartridge Based Sample Prep Device is light weight and portable and operates on mains and/or re-chargeable battery. It is capable of performing upto 16 sample extractions with one recharge and is fully automatic, with minimal hands on time. The cartridge based extraction process is quick, reliable, and efficient and does not require highly skilled personnel to carry out the extraction process. All the waste from processing of the sample is contained within the cartridge dump area thus posing no risk from potentially biohazardous material. The device has a universal protocol and can work with all kinds of samples such as sputum, BAL, whole blood, serum, plasma, tissue, stool, urine, CSF, pleural fluid, lymph node aspirate, pus, peritoneal fluid, saliva, swab specimens and culture specimen.

Principle: -

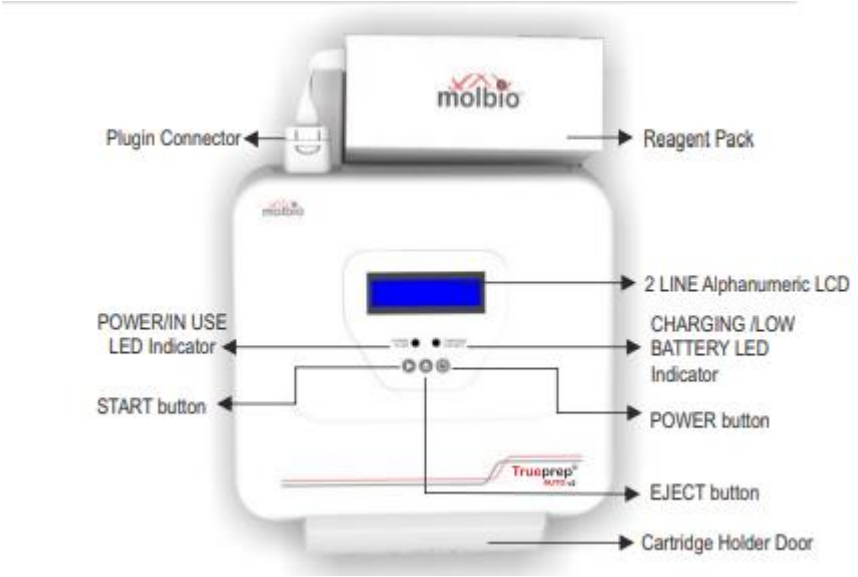
The Trueprep AUTO v2 is an electromechanical system pre-programmed to sequentially heat, mix and add reagents to the contents of the cartridge placed in the cartridge holder and has a 2 - line LCD screen that displays the status. Specimen pre-treated with lysis reagent is added to the sample chamber of the cartridge which is then placed in the cartridge holder of the device. Sample processing is initiated upon pressing the start button on the device, through an automatic pre-programmed process wherein nucleic acids released by chemical and thermal lysis of cells bind to the proprietary matrix in the matrix chamber. In subsequent steps, the captured nucleic acids are washed with buffers to remove the PCR inhibitors and finally eluted from the matrix using the elution buffer. At the end the cartridge is automatically ejected and the elute containing purified nucleic acids is then collected from the elute chamber for further analysis.

Environmental Requirement:-

The Trueprep AUTO v2 has been designed to operate safely within the following environment specifications: Room temperatures (between 15°C to 45°C) Relative Humidity (RH) between 10% –90% (non-condensing) the unit should be stored on a flat, dry surface.

Specifications:-

Trueprep® AUTO v2 Device	
Principle	Proprietary matrix-based extraction
Operation	Fully Automatic
Display Screen	2 line alphanumeric LCD
Power	Rechargeable Lithium Ion Battery Pack 7.4 V, 8.7 Ah External AC/DC adaptor: Input 1.5A, 100/240V, 50/60 Hz; Output 10V, 4.5A
Weight	2.75 kgs
Size	215 x 235 x 115 mm
Software	Proprietary firmware
Operating environment	Temperature 15 - 45°C, RH: 10 -90%



Materials Required: -

Contents: -

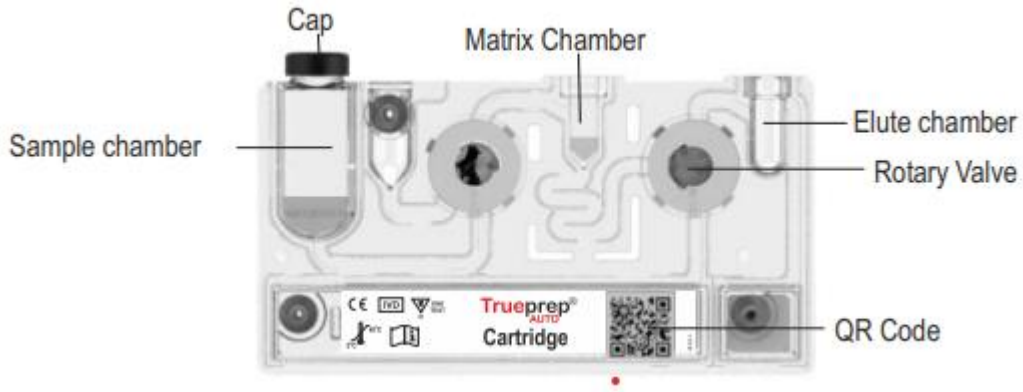
Trueprep AUTO v2 Universal Cartridge Based Sample Prep Device (REF603042001) AC Adapter (SMPS) with power cord to power -1No. ® Trueprep Replaceable tray for cartridge holder(REF603090001)- 25 Nos. ® Trueprep Cartridge Stand (REF603100001) - 1No. ® Trueprep Reagent Pack holder (REF603160001) - 1No. ® Trueprep AUTO v2 Plug-in connector for 25TReagent Bottle Pack/Flush (REF604090001) - 1No. User Manual -1No.

Materials Required but not provided with Device: -

Trueprep AUTO v2 Universal Cartridge Based Sample Prep Device compatible with : ® Trueprep AUTO/AUTO v2 Universal Cartridge Based Sample Prep Kit (REF60203AR25/ REF60207AR50) consisting of Universal Reagent Pack (25/50 tests) Universal Cartridge Pack (25/50 Nos.) Disposable transfer pipette graduated (25x3ml/50x3ml) Package insert (1 No.)

The Cartridge: -

Samples are processed on disposable, single-use cartridges that are inserted into the cartridge holder. These cartridges come pre-loaded with Internal Positive Control and only require the ® addition of pre-treated sample to the sample chamber for processing on the Trueprep AUTO v2.



Truenat MTB

Chip-based Real Time PCR Test for Mycobacterium tuberculosis

INTENDED USE

Truenat MTB (REF 601030005/601030020/601030025/601030050/ 601030100/601030200) is a chip-based Real Time Polymerase Chain Reaction (PCR) test for the quantitative detection and diagnosis of Mycobacterium tuberculosis (MTB) in human pulmonary and EPTB specimen and aids in the diagnosis of infection with MTB. Truenat MTB runs on the Truelab Real Time micro PCR Analyzers.

2. INTRODUCTION Tuberculosis (TB) is an infectious disease caused predominantly by the bacillus

Mycobacterium tuberculosis. It typically affects the lungs (pulmonary TB) but can affect other sites as well (extra pulmonary TB), Tuberculosis (TB) is the second largest killer worldwide, after HIV and is the leading cause of death in HIV patients. Pulmonary TB spreads through air and is highly contagious. Over 80% of TB infections are pulmonary and if left untreated, a pulmonary TB patient can infect up to 10-15 other people through close contact over the course of a year. Due to the highly infectious nature of pulmonary TB, it is important to diagnose and treat the disease very early. Despite the availability of highly effective treatment for decades, TB remains a major global health problem mainly because of poor case detection. The most common method for diagnosing pulmonary TB worldwide is sputum smear microscopy. However sensitivity of direct smear microscopy is low and estimates range from 30% to 70%. It is even lower in case of HIV-infected patients. Culture is more sensitive than microscopy and is considered the current gold standard. Culture requires specialized and controlled laboratory facility and highly skilled manpower and takes 3 to 6 weeks to provide the result. Molecular techniques such as polymerase chain reaction (PCR) or Real Time PCR are much

more sensitive than microscopy and culture. However PCR or Real Time PCR tests have so far been restricted to centralized reference laboratories as they require skilled manpower and Elaborate infrastructure. Also the turnaround time for results could take a few days. The Truelab Real Time micro PCR System enables decentralization and near patient diagnosis of MTB by making real time PCR technology rapid, simple, robust and user friendly and offering "sample to result capability even at resource limited settings. This is achieved through a combination of light weight, portable, mains/battery operated Truelab Real Time micro PCR Analyzer and Trueprep AUTO/AUTO v2 Sample Prep Device and room temperature stable Truenat micro PCR chip and Trueprep AUTO/AUTO v2 Sample Prep kits so that even the peripheral laboratories with minimal infrastructure and minimally trained technicians can easily perform these tests routinely in their facilities and report PCR results in less than an hour. Moreover, with these devices PCR testing can also be initiated in the field level, on site. Truenat MTB is a disposable, room temperature stable, chip based Real Time PCR test with dried MgCl, in reaction well and freeze dried PCR reagents in microtube for performing Real Time PCR test for detection of Mycobacterium tuberculosis and runs on the Truelab Real Time micro PCR Analyzer. It requires only six (6) µL of purified DNA to be added to the reaction well for the analysis. The intelligent chip also carries test and batch related information including standard values for quantitation. The Truenat MTB chip-based Real Time PCR test also stores information of used test to prevent any accidental re-use of the test

3. PRINCIPLE OF THE TEST

Truenat MTB works on the principle of Real Time Polymerase Chain Reaction based on Taqman chemistry. The DNA from the patient sample is first extracted using Trueprep AUTO/AUTO v2 Universal Cartridge Based Sample Prep Device and Trueprep AUTO/AUTO v2 Universal Cartridge Based Sample Prep Kit. The Truenat MTB chip is placed on the chip tray of the Truelab Real Time micro PCR Analyzer. Six (6) µL of the purified DNA is then dispensed using the provided micropipette and tip into the microtube containing freeze dried PCR reagents and allowed to stand for 30-60 seconds to get a clear solution. A No mixing by tapping, shaking or by reverse pipetting should be done. Six (6) µL of this clear solution is thenpipetted out using the same pipette and tip and dispensed into the reaction well of the Truenat MTB chip and the test is started. A positive amplification causes the dual labeled fluorescent probe in the Truenat MTB chip-based Real Time PCR test to release the fluorophores in an exponential manner which is then captured by the built-in opto-electronic sensor and displayed as amplification curve on the analyzer screen, on a real time basis during the test run. The Cycle threshold (Ct) is defined as the number of amplification cycles required for the fluorescent signal to cross the threshold (ie. exceed the background signal). Ct levels are inversely proportional to the amount of target nucleic acid in the sample. (ie. the lower the Ct level the greater is the amount of target nucleic acid in the sample). In the case of negative samples, amplification does not occur and a horizontal amplification curve is displayed on the screen during the test run. At the end of the test run, a MTB "DETECTED" or "NOT DETECTED result is displayed and in positive cases, quantitative values is also displayed on the screen. Based on the Ct of the internal positive control (IPC), the validity of the test run is also displayed. The IPC is a full process control that undergoes all the processes the specimen undergoes from extraction to amplification thereby validating the test run from sample to result. Absence of or shift of IPC Ct beyond a pre-set range in case of negative samples invalidates the test run. While IPC will co-amplify in most positive cases also, in some specimen having a

high target load, the IPC may not amplify, however the test run is still considered valid. The results can be printed via Bluetooth using the Truelab micro PCR printer or transferred to the lab computer/or any remote computer via Wifi network or 3G/GPRS network. Upto 20,000 results in Truelab Uno Dx/ Duo/Quattro can be stored on the analyzer for future recall and reference

Serology lab

Dengucheck combo:-

DEVICE

Dengucheck™ Combo is a rapid, qualitative immunochromatographic test system for the detection of Dengue NS 1 (Dengue Non-Structural Protein-1) antigen and differential detection of IgG & IgM antibodies to Dengue virus in human serum or plasma. The test system can be used as a screening test for Dengue viral infection and as an aid for differential diagnosis of the self-limiting primary Dengue infections and the potentially fatal secondary Dengue infections in conjunction with other criteria.

SUMMARY

Dengue virus (serotypes 1-4) belongs to the family of Flaviviridae, which is widely distributed in the epidemic and endemic areas throughout tropical and subtropical regions of the world. Dengue virus infection is considered significant in terms of morbidity, mortality and economic cost associated with it an estimated 100 million cases of dengue fever occurring throughout the world yearly. Dengue virus is transmitted in nature principally by the Aedes aegypti and Aedes albopictus mosquitoes. The mosquito vector is highly domesticated and an urban species. Dengue presents typically as a fever of sudden onset with headache, retro-orbital pain, pain in the back and limbs (break-bone fever), and lymphadenopathy and maculopapular rash. Primary dengue virus infection is characterized by elevation in dengue virus specific NS 1 antigen level in patient's blood stream from 1-6 days after onset of symptoms. Patients diagnosed with dengue infection in endemic areas generally have secondary infection, whereas patients in non-endemic areas are usually diagnosed with primary infection. Specific antibody response to Dengue virus enables serodiagnosis and differentiation between primary and secondary dengue infections and detection of potentially life-threatening conditions such as DHF and DSS.

Dengucheck™ Combo is a new generation rapid Immunochromatographic test system for detection of dengue virus infection in very early stage and differential diagnosis of dengue virus infection (primary or secondary), simultaneously.

PRINCIPLE:-

Dengucheck Combo test kit consists of two devices held in a tray, one device for detection of Dengue NS 1 antigen and second device for differential detection of IgG & IgM antibodies to dengue virus in human serum/ plasma specimen. In both the devices -Dengue NS 1 antigen and IgG/IgM to dengue virus, the detection system utilizes the principle of agglutination of antibodies/ antisera with respective antigen in immuno-chromatography format along with use of nano gold particles as agglutination revealing agent. In NS 1 detection device, as the test sample flows through the membrane assembly of the device, the highly specific Agglutinating sera for dengue NS 1-colloidal gold conjugate complexes with dengue NS 1 antigen present in the sample and travels on the membrane due to capillary action. The complex moves further on the membrane to the test region T where it is immobilized by another specific Agglutinating sera for dengue NS 1 coated on the membrane leading to the formation of a pink-purple band. Absence of this colored band in the test region indicates a negative test result for dengue NS 1 antigen. In IgG/IgM detection device, as the test sample flows through the membrane assembly within the test device, the Dengue specific antigen colloidal gold conjugate complexes with specific antibodies (IgG and/ or IgM) to Dengue virus, if present in the sample. This complex moves further on the membrane to the test region where it is immobilized by the specific Agglutinating sera for human IgG and/or Agglutinating sera for human IgM coated on the membrane leading to formation of colored band/s which confirms a positive test result. Absence of these colored bands in the test region indicates at negative test result for IgG & IgM antibodies to dengue virus.

In each NS 1 & IgG/IgM device; a built-in control band in the control area marked 'C' appears when the test has been performed correctly, regardless of the presence or absence of the dengue NS 1 antigen and/ or 'anti-Dengue virus' antibodies in the specimen. It serves to validate the test performance of each device.

One step rapid test for HCV MERISCREEN HCV

INTENDED USE

MERISCREEN HCV is a single test device for the qualitative desection of HCV antibodies (IgM, IgG & IgA) in human Serum/Plasme/Whole blood samples by healthcare professionals

INTRODUCTION:

Hepatitis C virus (HCV) is now recognised as the primary cause of transfusion associated hepatitis. HCV is a single stranded positive-sense RNA virus and is globally present in acute presentation of HCV infection patients may develop jaundice, others may go on to develop chronic hepatitis with life threatening conditions such as cirrhosis and hepatocellular carcinoma Diagnosis of HCV is mainly done by either direct Selection of viral RNA by PCR or by detection of anti-HCV antibodies. Recombinant DNA techniques have been used to develop structural and non-structural proteins derived from HCV RNA with utility for antibody screening. Anti-HCV assays have evolved as from first generation products, which used C-100-3 peptide. Second generation assay used recombinant viral proteins, Core, NS3 and NS4. Whereas third generation anti HCV assay uses antigens from Core (structural), NS3

protease/helicase (non-structural), NS4 (non-structural) and ISS replicase (non-structural) proteins, which provides greater sensitivity and specificity.

PRINCIPLE:

MERISCREEN HCV a qualitative rapid test based on immuno chromatography principle employs double antigen sandwich site immunoassay on nitrocellulose membrane. As the test sample flows through the membrane assembly of the test device, the recombinant Hepatitis C Virus antigens (Core, NS3, NS4 & NSS)-colloidal gold conjugate forms a complex with HCV specific antibodies in the sample. This complex moves further on the membrane to the test region where it is immobilized by the recombinant HCV antigens (Core, NS3, and NS4 & NS5) coated on the membrane leading to the formation of a reddish purple

Coloured band at the test region "T' which confirms a positive test result Absence of this coloured band in test region 'T' indicates a negative test result. Control band will appear irrespective to the sample status. This control band serves to validate the test results by turning from pale blue to reddish purple color indicating the proper test performance completion. Control band will appear irrespective to the sample status. Control band is the procedural control and it has nothing to do with the intensity of test band(s).

One Step test for HBsAg MERISCREEN HBsAg: -

INTENDED USE:

MERISCREEN HBsAg is a rapid, qualitative sand-witch immunoassay for the detection of Hepatitis B surface antigen (HBsAg) a marker for Hepatitis B infection in human serum of plasma by trained competent person. This kit is designed for primary screening of Hepatitis B virus

INTRODUCTION:

Hepatitis B Virus (HBV) is the most common cause of persistent viremia and the most important cause of chronic liver disease and hepatocellular carcinoma. Clinically apparent HBV infections may have been extent for several millennia. It is estimated that there are 300 million chronic carriers of HBV in the world. The carrier rates vary from as little as 0.3% (Westem countries) to 20% (Asia, Africa). HBV is a hepatotropic DNA virus. The core of the virus contains a DNA polymerase, the core antigen (HBcAg) and the "e" antigen (HBeAg). The core of HBV is enclosed in a coat that contains lipid, proteins and carbohydrate and expresses an antigen terms Hepatitis B surface antigen (HBsAg).

HBsAg is the first marker to appear in the blood in acute hepatitis B, being detected 1 week to 2 months after exposure and 2 weeks to 2 months before the onset of symptoms. Three weeks after the onset of acute hepatitis almost half of the patients will still be positive for HBsAg. In the chronic carrier state, the HBsAg persists for long periods (6-12 months) with no sero-conversion to the corresponding antibodies. Therefore, screening for HBsAg is highly desirable for all donors, pregnant women and people in high risk groups.

PRINCIPLE:

MERISCREEN HBsAg is a rapid test based on immunochromatography principle. HBsAg specific antibody is immobilized on to the test region of nitrocellulose membrane. The sample is dispensed into the sample well. HBsAg if present in the test sample binds to monoclonal anti-HBsAg coupled with colloidal gold. The antigen antibody complex moves along the membrane and gets captured by anti-HBsAg antibody immobilized on nitrocellulose membrane, which is visualize by reddish purple band on the test region. At control region Goat anti-mouse IgG is immobilized and it binds to un-reacted colloidal gold conjugate to give coloured band at Control region by turning pale blue to reddish purple colour. Control band will appear irrespective to the sample status. Control band is the procedural control and it has nothing to do with the intensity of test band.

HAV IgM Rapid Test – Cassette (serum/plamsa/white blood)

INTENDED USE

The HAV IgM Rapid Test is a lateral flow immunoassay for the qualitative detection of IgM antibodies to hepatitis A virus (HAV) in human serum, plasma or whole blood. It is intended to be used by professionals as a screening test and provides a preliminary test result to aid in the diagnosis of infection with HAV any interpretation or use of this preliminary test result must also rely on other clinical findings as well as on the professional judgment of health care providers. Alternative test method(s) should be considered to confirm the test result obtained by this device.

SUMMARY AND EXPLANATION OF THE TEST

HAV a positive-sense RNA virus, is a unique member of the family Picomaviridae Its transmission depends primarily on serial transmission from person-to-person by the fecal-oral route. Although hepatitis A is not ordinarily a sexually transmitted disease, the infection rate is high among men who have sex with men as a result of oral-anal contact The presence of specific anti-HAV IgM in blood samples suggests an acute or recent HAV infection Anti-HAV IgM rapidly increases in liter over a period of 4-6 weeks post infection and then declines to non-delectable levels within 3 to 6 months in most patients The HAV IgM Rapid Test is a lateral flow immunoassay for the qualitative detection of anti-HAV IgM in serum, plasma or whole blood. It can be performed within 15 minutes by minimally skilled personnel without the use of laboratory equipment.

TEST PRINCIPLE

The HAV IgM Rapid Test is a lateral flow chromatographic immunoassay The test Specimen 10 Cool Live Test Line Buffer Wall cassette consists of: 1) a colored conjugate pad containing HAV antigen conjugated with colloidal gold (HAV Ag conjugates) and a control antibody conjugated with colloidal gold and 2) a nitrocellulose membrane strip containing a test line (T line) and a control line (C line) The T line is pre-coated with mouse anti-human IgM, and the C line is pre-coated with a control line antibody. When an adequate volume of test specimen and sample diluent are dispensed into the sample and buffer wells of the cassette, respectively, the specimen migrates by capillary action across the cassette. Anti-HAV IgM, if present in the specimen, will bind to the HAV Ag conjugates. The immunocomplex is then captured on the membrane by the pre-coated mouse anti-human loM forming a colored T line, indicating an IgM anti-HAV positive test result. Absence of the T line suggests an anti-HAV IgM negative test result The test contains an interal control (C line) which should exhibit a colored line of the immunocomplex of control line antibodies regardless of any color development on the T line it the Cine does not develop, the test result is invalid, and the specimen must be retested with another device

Dengucheck: -

Rapid test system for the detection of Dengue NS 1 antigen and IgG/IgM antibodies to Dengue virus in human serum/plasma

INTENDED USE Dengucheck" Combo

Non-Structur rapid, qualitative immunochromatographic test system for the detection of Dengue NS 1 (Dengue tein-1) antigen and differential delection of IgG & IgM antibodies to Dengue virus in human serum or can be used as a screening test for Dengue viral infection and as an aid for differential diagnosis of the sary Dengue infections and the potentially fatal secondary Dengue infections in conjunction with other criteria.

SUMMARY

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Dengucheck" Combo is a new generation rapid Immunochromatographic test system for detection of dengue virus infection in very early stage and differential diagnosis of dengue virus infection (primary or secondary), simultaneously.

PRINCIPLE

Despucheck Combo test kit consists of two devices held in tray, one device for detection of Dengue NS 1 antigen and second device for differential detection of IgG & IgM antibodies to dengue virus in human serum/ plasma specimen. In both the devices-Dengue NS 1 antigen and IgG/IgM to dengue virus, the detection system utilizes the principle of agglutination of antibodies/ antisera with respective antigen in immuno-chromatography format along with use of nano goid particles as agglutination revealing agent. in NS 1 detection device, as the test sample flows through the membrane assembly of the device, the highly specific Agglutinating sera for dengue NS 1-colloidal gold conjugate complexes with dengue NS 1 antigen present in the sample and travels on the membrane due to capillary action. The complex moves further on the membrane to the test region T where it is immobilized by another specific Agglutinating sera for dengue NS 1 coated on the membrane leading to the formation of a pink-purple band Absence of this colored band in the test region indicates a negative test result for dengue NS 1 antigen, in IgG/IgM detection device, as the lest sample flows through the membrane assembly within the test device, the Dengue specific antigen colloidal gold conjugate complexes with specific antibodies (IgG and/ or IgM) to Dengue virus, if present in the sample. This complex moves further on the membrane to the test region where it is immobilized by the specific Agglutinating sera for human IgG and/or Agglutinating sera for human igM coated on the membrane leading to formation of colored band's which confirms a positive test result. Absence of these colored bands in the test region indicates a negative test result for igG & IgM antibodies to dengue virus. in each NS 1& IgG/IgM device; a built-in control band in the control area marked "C" appears when the test has been performed correctly, regardless of the presence or absence of the dengue NS 1 antigen and/ or 'anti-Dengue virus' antibodies in the specimen. It serves to validate the test performance of each device.

Widal test

Principle of Widal test

Bacterial suspension which carry antigen will agglutinate on exposure to antibodies to *Salmonella* organisms. Patients' suffering from enteric fever would possess antibodies in their sera which can react and agglutinate serial doubling dilutions of killed, colored *Salmonella* antigens in a agglutination test.
The main principle of widal test is that if homologous antibody is present in patients serum, it will react with respective antigen in the reagent and gives visible clumping on the test card and agglutination in the tube. The antigens used in the test are "H" and "O" antigens of *Salmonella* Typhi and "H" antigen of *S.* Paratyphi. The paratyphoid "O" antigen are not employed as they cross react with typhoid "O" antigen due to the sharing of factor 12. "O" antigen is a somatic antigen and "H" antigen is flagellar antigen.

Syphilis:-

The VDRL reagent test kits (Venereal disease research laboratory) contain stabilised uses VDRL antigen. The VDRL antigen detects Reagin antibodies, which are antibodies produced by the human body, against substances released by cells (such as the heart and the liver) that are damaged by the Syphilis organism.

The RPR (Rapid Plasma Reagin) Syphilis test uses RPR Carbon antigen which is an ethanolic solution containing cholesterol, bovine heart cardiolipin, and lecithin and microparticulate carbon. The RPR Carbon antigen aggregates in the presence of Reagin antibodies in serum or plasma The RPR test enables confirmation of an active infection before starting treatment,. RPR tests are also commonly used to check the on-going treatment of a Syphilis infection.

The TPHA test for syphilis (Treponema pallidum haemagglutination assay) employs preserved avian erythrocytes coated with antigens of T. pallidum, which bind with TP antibodies present in patient's serum or plasma. TPHA is an indirect hemagglutination test for qualitative and semi-quantitative detection of TP antibodies, no agglutination generally indicates absence of TP antibodies. Reagents are supplied at optimal dilution.

Hiv lab:-

Acquired immunodeficiency syndrome (AIDS) is a chronic, potentially life-threatening condition caused by the human immunodeficiency virus (HIV). By damaging your immune system, HIV interferes with your body's ability to fight infection and disease.

HIV is a sexually transmitted infection (STI). It can also be spread by contact with infected blood and from illicit injection drug use or sharing needles. It can also be spread from mother to child during pregnancy, childbirth or breastfeeding. Without medication, it may take years before HIV weakens your immune system to the point that you have AIDS.

There's no cure for HIV/AIDS, but medications can control the infection and prevent progression of the disease. Antiviral treatments for HIV have reduced AIDS deaths around the world, and international organizations are working to increase the availability of prevention measures and treatment in resource-poor countries.

Symptoms

The symptoms of HIV and AIDS vary, depending on the phase of infection.

Primary infection (Acute HIV)

Some people infected by HIV develop a flu-like illness within 2 to 4 weeks after the virus enters the body. This illness, known as primary (acute) HIV infection, may last for a few weeks.

Possible signs and symptoms include:

- Fever
- Headache
- Muscle aches and joint pain
- Rash
- Sore throat and painful mouth sores
- Swollen lymph glands, mainly on the neck
- Diarrhea
- Weight loss
- Cough
- Night sweats

These symptoms can be so mild that you might not even notice them. However, the amount of virus in your bloodstream (viral load) is quite high at this time. As a result, the infection spreads more easily during primary infection than during the next stage.

Clinical latent infection (Chronic HIV)

In this stage of infection, HIV is still present in the body and in white blood cells. However, many people may not have any symptoms or infections during this time.

This stage can last for many years if you're receiving antiretroviral therapy (ART). Some people develop more severe disease much sooner.

Symptomatic HIV infection

As the virus continues to multiply and destroy your immune cells — the cells in your body that help fight off germs — you may develop mild infections or chronic signs and symptoms such as:

- Fever
- Fatigue
- Swollen lymph nodes — often one of the first signs of HIV infection

- Diarrhea
- Weight loss
- Oral yeast infection (thrush)
- Shingles (herpes zoster)
- Pneumonia

Progression to AIDS

Access to better antiviral treatments has dramatically decreased deaths from AIDS worldwide, even in resource-poor countries. Thanks to these life-saving treatments, most people with HIV in the U.S. today don't develop AIDS. Untreated, HIV typically turns into AIDS in about 8 to 10 years.

When AIDS occurs, your immune system has been severely damaged. You'll be more likely to develop diseases that wouldn't usually cause illness in a person with a healthy immune system. These are called opportunistic infections or opportunistic cancers.

The signs and symptoms of some of these infections may include:

- Sweats
- Chills
- Recurring fever
- Chronic diarrhea
- Swollen lymph glands
- Persistent white spots or unusual lesions on your tongue or in your mouth
- Persistent, unexplained fatigue
- Weakness
- Weight loss
- Skin rashes or bumps

CD4 cell count machine was there to count the T lymphocytes

Hematology lab

Blood Smear

A blood smear is a sample of blood that's spread on a glass slide which is treated with a special stain. In the past, all blood smears were examined under a microscope by laboratory professionals. Now automated digital systems may be used to help examine blood smears.

The purpose of examining a blood smear is to check the size, shape, and number of three types of blood cells:

- **Red blood cells**, which carry oxygen from your lungs to the rest of your body
- **White blood cells**, which fight infection
- **Platelets**, which help your blood to clot

Other names: peripheral smear, peripheral blood film, smear, blood film, manual differential, differential slide, blood cell morphology, blood smear analysis

A blood smear is used to help diagnose and monitor many conditions, such as blood disorders, sudden kidney failure, and treatment for certain cancers.

You may need a blood smear if you have abnormal results on a complete blood count (CBC). A CBC is a routine test that measures many different parts of your blood.
Your health care provider may order a blood smear if you have symptoms of a blood disorder, such as:

- Fatigue
- Jaundice, a condition that causes your skin and eyes to turn yellow
- Unusual bleeding, including nosebleeds
- Fever that lasts, or comes and goes
- Bone pain
- Anemia
- Easy bruising
- A spleen that's larger than normal

Red blood cell results that aren't normal, it may be a sign of:

- Anemia
- Sickle cell anemia
- Hemolytic anemia, a type of anemia in which the body destroys red blood cells faster than they are replaced
- Thalassemia
- Bone marrow disorders
- Liver disease
- Cancer that has spread to the bone

White blood cell results that aren't normal may be a sign of:

- Infection or inflammation

- Allergies
- Leukemia
- Bone marrow disorders

Platelet results that aren't normal may be a sign of:

- Thrombocytopenia, a condition in which your blood doesn't have enough platelets, which increases the risk of bleeding
- Inherited platelet disorders (uncommon), such as Bernard-Soulier syndrome

If you have been very ill or stressed, or you have had a blood transfusion, the shape and number of your blood cells may be different than usual. So, a blood smear may not provide enough information for your provider to make a diagnosis. If any of your blood smear results are not normal, your provider will likely order more tests. Talk with your provider to learn more about your results.

Learn more about laboratory tests, reference ranges, and understanding results.

Is there anything else I need to know about a blood smear?

A blood smear may be used to help find certain types of parasites in your blood which cause diseases, such as:

- Malaria, spread by bites from infected mosquitos
- Babesiosis, spread mainly by bites from infected ticks
- Chagas disease, spread mainly by bites from "kissing bugs" (triatomine)

Staining Blood Smears

Stain only one set of smears, and leave the duplicates unstained. The latter will prove useful if a problem occurs during the staining and/or if you wish later to send the smears to a reference laboratory.

Wright's stain: -

Wright's stain is a commonly used dye for staining blood smears. The basic dye in Wright's stain is azure B and the acidic dye is eosin Y. The dyes form ionic complexes with cellular constituents. The cationic azure B binds to anionic proteins such as DNA and RNA and imparts a purple color to these cellular components. The anionic eosin Y forms ionic bonds with the cationic proteins such as hemoglobin and stains them an orange-red color. Other cellular components such as cell cytoplasm and granules stain according to the ionic properties they hold.

When staining smears with Wright's stain, the slides are flooded with the stain. It is left on for two minutes. The solvent is methanol which acts as a fixative. An equal volume of sodium phosphate buffer at pH 6.4 is then added and mixed with the stain until a metallic scum forms on the upper surface. This is left for five minutes before washing with a gentle stream of water. The time in which the smear is exposed to the stain and buffer can be adjusted according to the cellular staining characteristics. Once the smear is air-dried, it can be viewed microscopically. Permanent mounting of the slide with a coverslip is optional. Red blood cells should appear pink to salmon pink in color. Nuclei of leukocytes should appear deep purple, and the cytoplasm of lymphocytes should be pale to sky blue.

Complete Blood Count (CBC)

A complete blood count, or CBC, is a blood test that measures many different parts and features of your blood, including:

- **Red blood cells**, which carry oxygen from your lungs to the rest of your body.
- **White blood cells**, which fight infections and other diseases. There are five major types of white blood cells. A CBC test measures the total number of white cells in your blood. A different test called a **CBC with differential** measures the number of each type of these white blood cells.
- **Platelets**, which stop bleeding by helping your blood to clot.
- **Hemoglobin**, a protein in red blood cells that carries oxygen from your lungs to the rest of your body.
- **Hematocrit**, a measurement of how much of your blood is made up of red blood cells.
- **Mean corpuscular volume (MCV)**, a measure of the average size of your red blood cells.

Other names for a complete blood count: CBC, full blood count, blood cell count

A complete blood count is a common blood test that is often part of a routine checkup. Complete blood counts can help detect a variety of disorders including infections, anemia, diseases of the immune system, and blood cancers.

The following are expected complete blood count results for adults. The blood is measured in cells per liter (cells/L) or grams per deciliter (grams/dL).

Red blood cell count	**Male:** 4.35 trillion to 5.65 trillion cells/L **Female:** 3.92 trillion to 5.13 trillion cells/L
Hemoglobin	**Male:** 13.2 to 16.6 grams/dL (132 to 166 grams/L) **Female:** 11.6 to 15 grams/dL (116 to 150 grams/L)
Hematocrit	**Male:** 38.3% to 48.6% **Female:** 35.5% to 44.9%
White blood cell count	3.4 billion to 9.6 billion cells/L
Platelet count	**Male:** 135 billion to 317 billion/L **Female:** 157 billion to 371 billion/L

- **Red blood cell count, hemoglobin and hematocrit.** The results of these three are related because they each measure a feature of red blood cells.

  Lower than usual measures in these three areas are a sign of anemia. Anemia has many causes. They include low levels of certain vitamins or iron, blood loss, or another medical condition. People with anemia might feel weak or tired. These symptoms may be due to the anemia itself or the cause of anemia.

  A red blood cell count that's higher than usual is known as erythrocytosis. A high red blood cell count or high hemoglobin or hematocrit levels could point to a medical condition such as blood cancer or heart disease.

- **White blood cell count.** A low white blood cell count is known as leukopenia. A medical condition such as an autoimmune disorder that destroys white blood cells, bone marrow problems or cancer might be the cause. Certain medicines also can cause a drop in white blood cell counts.

A white blood cell count that's higher than usual most commonly is due to an infection or inflammation. Or it could point to an immune system disorder or a bone marrow disease. A high white blood cell count also can be a reaction to medicines or hard exercise.

- **Platelet count.** A platelet count that's lower than usual is known as thrombocytopenia. If it's higher than usual, it's known as thrombocytosis. Either can be a sign of a medical condition or a side effect from medicine. A platelet count that's outside the typical range will likely lead to more tests to diagnose the cause.

Reticulocyte Count

Reticulocytes are red blood cells that are still developing. They are also known as immature red blood cells. Reticulocytes are made in the bone marrow and sent into the bloodstream. About two days after they form, they develop into mature red blood cells. These red blood cells move oxygen from your lungs to every cell in your body.

A reticulocyte count (retic count) measures the number of reticulocytes in the blood. If the count is too high or too low, it can mean a serious health problem, including anemia and disorders of the bone marrow, liver, and kidneys.
Other names: retic count, reticulocyte percent, reticulocyte index, reticulocyte production index, RPI

A reticulocyte count is most often used to:

- Diagnose specific types of anemia. Anemia is a condition in which your blood has a lower than normal amount of red blood cells. There are several different forms and causes of anemia.
- See if treatment for anemia is working
- See if bone marrow is producing the right amount of blood cells
- Check bone marrow function after chemotherapy or a bone marrow transplant

You may need this test if:

- Other blood tests show your red blood cell levels are not normal. These tests may include a complete blood count, hemoglobin test, and/or hematocrit test.
- You are being treated with radiation or chemotherapy
- You recently received a bone marrow transplant

You may also need this test if you have symptoms of anemia. These include:

- Fatigue
- Weakness
- Shortness of breath

- Pale skin
- Cold hands and/or feet

Sometimes new babies are tested for a condition called hemolytic disease of the newborn. This condition happens when a mother's blood is not compatible with her unborn baby. This is known as Rh incompatibility. It causes the mother's immune system to attack the baby's red blood cells. Most pregnant women are tested for Rh incompatibility as part of routine prenatal screening.

If your results show a lower than normal amount of reticulocytes, it may mean you have:

- **Iron deficiency anemia**, a type of anemia that happens when you don't have enough iron in your body.
- **Pernicious anemia**, a type of anemia caused by not getting enough of certain B vitamins (B12 and folate) in your diet, or when your body can't absorb enough B vitamins.
- **Aplastic anemia**, a type of anemia that happens when the bone marrow isn't able to make enough blood cells.
- **Bone marrow failure**, which may be caused by an infection or cancer.
- **Kidney disease**
- **Cirrhosis**, scarring of the liver

Osmotic Fragility Test

An osmotic fragility test is a blood test which works to see if red blood cells have a tendency to break apart easily.

Two conditions that can cause this to happen are called thalassemia and hereditary spherocytosis (HS). These conditions cause the red blood cells to be more likely to break and become a smaller size.

Both thalassemia and HS may lead to hemolytic anemia. This is a type of anemia where you have a low count of red blood cells because your body is destroying them too fast.

Red blood cells are tested to see how likely they are to break, or rupture. This is done by putting them in a hemodiluted or hypotonic solution, which makes the cells swell and burst.

The edges of red blood cells are made of proteins which keep the cell together. If one of these proteins are weak, the edges are more likely to break when placed in the water-based solution.

An osmotic fragility test is often used to confirm red blood cell disorders.

Sickle cell test: - A sickle cell test is a simple blood test used to determine if you have sickle cell disease (SCD) or sickle cell trait. People with SCD have red blood cells (RBCs) that are abnormally shaped. Sickle cells are shaped like a crescent moon. Normal RBCs look like doughnuts.

The sickle cell test is part of routine screening performed on a baby after they're born. However, it can be used on older children and adults when needed.

Peripheral smear. This is a blood test that looks at the shape and number of your red and white blood cells. Some of your blood is put on a slide and examined by computer or under a microscope to identify sickle-shaped cells. This test is usually done for children and adults along with a CBC.

A peripheral smear is also called a blood smear, peripheral blood film, manual differential, or manual diff.

Hemoglobin electrophoresis. This is a blood test that looks at the types of hemoglobin in your blood. A current is applied to the blood sample, which separates blood into bands of hemoglobin. There are lots of different types of hemoglobin, but hemoglobin S and some others are associated with sickle cell disease.

This is a routine test for newborn screening but is also done for children and adults.

Sickle turbidity test. Also called hemoglobin solubility, this is an inexpensive test where blood is added to a solution. If hemoglobin S is present, the test turns cloudy, or turbid, as it is exposed to the solution.

Sickle turbidity testing can often have false positives. So when a sickle turbidity test is positive, another test is usually done to double-check the results.

Erythrocyte Sedimentation Rate (ESR)

An erythrocyte sedimentation rate (ESR) is a blood test that that can show if you have inflammation in your body. Inflammation is your immune system's response to injury, infection, and many types of conditions, including immune system disorders, certain cancers, and blood disorders.
Erythrocytes are red blood cells. To do an ESR test, a sample of your blood is sent to a lab. A health care professional places the sample in a tall, thin test tube and measures how quickly the red blood cells settle or sink to the bottom of the tube. Normally, red blood cells sink slowly. But inflammation makes red blood cells stick together in clumps. These clumps of cells are heavier than single cells, so they sink faster.

If an ESR test shows that your red blood cells sink faster than normal, it may mean you have a medical condition causing inflammation. The speed of your test result is a sign of how much inflammation you have. Faster ESR rates mean higher levels of inflammation. But an ESR test alone cannot diagnose what condition is causing the inflammation.

Other names: ESR, SED rate sedimentation rate; Westergren sedimentation rate

Histopathology lab

- Sample collection: Use fresh, non-autolyzed samples for best results. Place in fixative immediately. **
- Include both lesional and normal tissue if possible.
- Most tissues should be cut so that each section is no thicker than ¼ inch (6 mm).
- For the following samples-
  - Brain: Submit either without sectioning or with coronal (divides into front and back) sections at 1-2 inch intervals.
  - Spinal cord: Submit either intact (with the dura mater opened) or in segments at least one inch in length.
  - Intestines: Submit in segments 1-2 inches in length.
  - Lymph nodes: Section once in sagittal plane (divides into left and right halves).
  - Large masses: Incise to allow penetration by formalin.
  - Excision specimens (surgical biopsies), where whole organs or affected areas are removed at operation
  - Incisional biopsy specimens, where tissue is removed for diagnosis from within an affected area
  - Punch biopsies, where punches are used to remove a small piece of suspicious tissue for examination (often from the skin)
  - Shave biopsies, where small fragments of tissue are "shaved" from a surface (usually skin)
  - Curettings, where tissue is removed in small pieces from the lining of the uterus or cervix
  - Core biopsies, where a small tissue sample is removed using a special needle, sometimes through the skin (percutaneously)

Fixation

Fixation is a crucial step in preparing specimens for microscopic examination. Its objective is to prevent decay and preserve cells and tissues in a "life-like" state. It does this by stopping enzyme activity, killing microorganisms, and hardening the specimen while maintaining sufficient molecular structure to enable appropriate staining methods to be applied (including those involving antigen-antibody reactions and those depending on preserving DNA and RNA). The sooner fixation is initiated following the separation of a specimen from its blood supply, the better the result will be. The most popular fixing agent is formaldehyde, usually in the form of a phosphate-buffered solution (often referred to as "formalin"). Ideally, specimens should be fixed by immersion in formalin for six to twelve hours before they are processed.

Grossing

Grossing, often referred to as "cut-up", involves a careful examination and description of the specimen that will include the appearance, the number of pieces, and their dimensions. Larger specimens may require further dissection to produce representative pieces from appropriate areas. For example, multiple samples may be taken from the excision margins of a tumour to ensure that the tumour has been completely removed. In the case of small specimens, the entire specimen may be processed. The tissues selected for processing will be placed in cassettes (small perforated baskets), and batches will be loaded onto a tissue processor for processing through to wax.

Histopathology sample processing: -

- Sample undergoes grossing followed by labelling by doctors
- Samples is stored in the cassettes (square, rectangular, circular)
- Cassettes are stored in the formalin (for breast samples strong formalin is used due to their urgent report need)
- Samples are kept in the formalin for 5 days for breast and the urgent samples they are kept for two days
- Then sample cassettes are kept in the diluted alcohol jars (4 to 5)
- Sample cassettes are kept there in 1^{st} jar, after one hour in 2^{nd} jar and then in 3^{rd} jar for overnight, on the next day the cassettes are shifted to the 4^{th} jar and then 5^{th}
- Then the cassettes bucket is then shifted to the acetone jar and after an hour to the next jar
- Then the jar is shifted to the xylene jar 1 and then after an hour the 2^{nd} jar
- Sample is kept in the first wax bath for an hour and then in the next wax bath for the whole night
- On the next day, samples are proceeded for the wax blocking

Blocking: -

- Paraffin wax is melted and poured in the moulds then fixed tissue samples are kept inside the molten wax and kept for cooling after cooling the wax block is cut into two blocks and labelled and are sticked properly
- Blocks are trimmed and thin sections of 2 to 5 microns are removed and put on the water bath (55 to 60oc) this sections are then taken on the slides and slides are held on burner for few seconds to melt wax.
- Slides are labelled and kept in incubator for overnight about 37oc
- After that on the next day the slides are sent for staining

Histopathology staining: -

Stains used: - haematoxylin

- Incubated slides were kept in the xylene for 10 mis for the removal of the wax
- Wipe slides and keep in absolute alcohol for the 10 mins
- Wash slides with the tap water 2 to 3 mins
- Keep slides in hematoxylin for 20 mis
- Wash excess stain in 1% acid alcohol (HCL is used)
- Wash under tap water till the tissue sections become blue (approax. 2 mins)
- Keep slides into eosin stain for 1 to 2 mins after that wash with alcohol
- Wipe the slide from dorsal and ventral slide
- Keep in xylene for few minutes
- Remove from xylene and put drop of DP x mount on the slide
- Cover the slide with the coverslip and label the slide

REFERENCEES: -

- **https://www.mountsinai.org/health-library/tests/rpr-test#:~:text=RPR%20(rapid%20plasma%20reagin)%20is,the%20back%20of%20the%20hand.**
- **https://microbiologyinfo.com/widal-test-introduction-principle-procedure-interpretation-and-limitation/**
- **https://www.mayoclinic.org/diseases-conditions/hiv-aids/symptoms-causes/syc-20373524#:~:text=Acquired%20immunodeficiency%20syndrome%20(AIDS)%20is,to%20fight%20infection%20and%20disease**
- **https://microbiologyinfo.com/acid-fast-stain-principle-procedure-interpretation-and-examples/**
- **https://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-019-4470-9#:~:text=The%20line%20probe%20assay%20(LPA,GmbH%2C%20Nehren%2C%20Germany).**
- **https://www.who.int/publications/i/item/9789240046665**
- **https://microbiologyinfo.com/blood-agar-composition-preparation-uses-and-pictures/**
- **https://microbiologyinfo.com/triple-sugar-iron-tsi-test/**
- **https://microbiologyinfo.com/catalase-test-principle-uses-procedure-result-interpretation-with-precautions/**
- **https://www.mdpi.com/2075-4418/10/10/832#:~:text=According%20to%20traditional%20guidelines%2C%20blood,routine%20urine%20cultures%20%5B8%5D.**
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