KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II B.Sc MB

COURSE NAME: MEDICAL MICROBIOLOGY - PRACTICAL

COURSE CODE: 18MBU412 SYLLABUS BATCH-2018-2021

Semester - IV

I7MBU412 MEDICAL MICROBIOLOGY - PRACTICAL 4H – 2C

Instruction Hours / week: L: 4 T: 0 P: 4 **Marks:** Internal: **40** External: **60** Total: **100**

End Semester Exam: 9 Hours

SCOPE

- It provides the ability to characterize, isolate and identify different microbes. It includes a detailed study of characterization, etiology, pathogenicity, clinical systems, and laboratory diagnosis of disease causing Microorganisms.

OBJECTIVES

- To introduce the knowledge of the medically important microganisms, microbial morphology with the main focuses being the characterization, isolation and identification of different microorganism.

EXPERIMENTS

1. Identify bacteria (any three of *E. coli, Salmonella, Pseudomonas, Staphylococcus, Bacillus*) using laboratory strains on the basis of cultural, morphological and biochemical characteristics: IMViC, TSI, nitrate reduction, urease production and catalase tests.
2. Study of composition and use of important differential media for identification of bacteria: EMB Agar, McConkey agar, Mannitol salt agar, Deoxycholate citrate agar, TCBS, Salmonella Shigella/BSA Agar.
3. Study of bacterial flora of skin by swab method.
4. Antibacterial sensitivity assay by Kirby-Bauer method.
5. Determination of minimal inhibitory concentration (MIC) of an antibiotic.
6. Study symptoms of the diseases with the help of photographs: Polio, anthrax, herpes, chicken pox, HPV warts, AIDS (candidiasis), dermatomycoses (ring worms).
7. Study of various stages of malarial parasite in RBCs using permanent mounts.

SUGGESTED READINGS

1. Brooks G.F., Carroll K.C., Butel J.S., Morse S.A. and Mietzner, T.A. (2013). Jawetz, Melnick and Adelberg's Medical Microbiology. 26th edition. McGraw Hill Publication
2. Greenwood D, Slack R, Barer M, and Irving W. (2012). Medical Microbiology, 18th Edition. Churchill Livingstone.
3. Ryan KJ and Ray CG. (2014). Sherris Medical Microbiology, 6th Edition. McGraw-Hill Professional.
4. Ananthanarayan R. and Paniker C.K.J. (2009) Textbook of Microbiology. 8th edition, University Press Publication.
5. Madigan MT, Martinko JM, Dunlap PV and Clark DP. (2014). Brock Biology of Microorganisms. 14th edition. Pearson International Edition.
6. Goering R., Dockrell H., Zuckerman M. and Wakelin D. (2007). Mims' Medical Microbiology. 4th edition. Elsevier
7. Willey JM, Sherwood LM, and Woolverton CJ. (2013). Prescott, Harley and Klein's Microbiology. 9th edition. McGraw Hill Higher Education.

MEDICAL MICROBIOLOGY - PRACTICAL

SYLLABUS

1. Identify bacteria (any three of *E. coli, Salmonella, Pseudomonas, Staphylococcus, Bacillus*) using laboratory strains on the basis of cultural, morphological and biochemical characteristics: IMViC, TSI, nitrate reduction, urease production and catalase tests.

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7. Study of various stages of malarial parasite in RBCs using permanent mounts**.:**

EXPERIMENT NO: 1

Identification of *Escherichia coli*

Aim

To identify *E.coli* from the given culture.

Introduction

E.coli is one of the most important gram negative bacilli of the family Enterobacteriaceae.This genus is named after **Escherich**who was the first to describe the colon bacillus under the name ***Bacterium coli commune(1885)***.Based on minor differences in biochemical characteristics,colon bacilli were described under various names but in view of the mutability of the biochemical properties in this group,they have all been included in one species *E.coli* which is further subdivided into biotypes and serotypes.Three other species have been described in the genus but they are of little medical importance.

Unlike other coliforms.*E. coli* is a parasite living only in the human or animal intestine.Voided in feces,it remains viable in the environment only for some days.Detection of *E.coli* in drinking water,therefore,is taken as evidence of recent pollution with human or animal feces.

E.coli is considered as a normal flora of healthy human until 1920s.Now it is considered to be a pathogen.On the basis of its pathogenicity five types *E.coli* are described.*E. coli* and Entero aggregative *E.coli* may cause gastroenteritis and urinary tract infection.

Materials required

Sample: Broth or slant culture.

Chemicals and media required:

Gram staining reagent
EMB agar
Mac Conkey agar
XLD agar
SS agar
RajHans agar
Hektoein enteric agar
Biochemical test media&r reagents
Glass slides, Petri plates, Test tubes etc.

Procedure

- Subject the isolated pure culture to simple stain,gram stain and catalase to know the nature of family of the isolates.
- Streak the isolate on Mac Conkey agar and incubate at 37°C for 24 hours.
- Subject the isolate to Motility test,Indole test and TSI test.It is helpful to understand the probable nature of the isolates.
- Perform MR,VP,Urease,Nitrate,Deaminase,Decarboxylase,Melonate,KCN and carbohydrate fermentation tests along with EMB media inoculation to confirm generic level identification of the isolates.
- Confirm probably identified bacterial isolate by streaking on Hektoein enteric agar,XLDagar,SS agar and RajHansmedium.Incubate all the medium at 37°C for 24 hours and observed the colony morphology.

Results

Based on the above observation the given bacteria is identified as ***Escherichia coli.***

IDENTIFICATION OF *Salmonella sp.*

AIM:

To identify *Salmonella sp.* from the given culture.

INTRODUCTION :

The genus *Salmonella* consists of bacilli that parasitisethe intestines of a large number of vertebrate species and infect human beings, leading to enteric fever, gastroenteritis, septicemia with or without focal suppuration, and the carrier state.

The most important member of the genus is *Salmonella typhi*, the causative agent of typhoid fever. The typhoid bacillus was first observed by Eberth (1880)in the mesenteric nodes and spleen of fatal causes of typhoid fever and was isolated by Gaffky (1884).It came to be known as the Eberth and Gaffky bacillus or *Eberthellatyphi*. Salmon and Smith (1885)described a bacillus which was believed to cause hog cholera (mistakenly, as it is a virus disease). This bacillus, later called *S. Choleraesuis*, was the first of a series of similar organisms to be isolated from animals and human beings the genus *Salmonella*. It was subsequently realisedthat the typhoid bacillus also belonged to this group, in spite of minor biochemical differences, and it was redesignated*S. typhi*, the genus *Eberthella*having been abolished.

Salmonellae currently comprise above 2000 serotypes or species, all of them potentially pathogenic. For practical purposes, they may be divided into two groups:(1) The enteric fever group, consisting of a typhoid and paratyphoid bacilli that are exclusively or primarily human parasites;and(2)The food poisoning group, which are essentially animal parasites but which can also infect human beings, producing gastroenteritis, septicemia or localisedinfections.

MATERIALS REQUIRED:

SAMPLE:Broth or stap or slant culture.

CHEMICALS AND MEDIA REQUIRED:

Gram staining reagent, Mac conkey agar, XLDagar, SS agar, RajHans agar, Hektoein enteric agar, Biochemical test media and reagents, Glass slides, petri plates, test plates etc.

PROCEDURE:

- Subject the isolated pure culture to simple stain, gram stain and catalase to know nature of the family of the isolate.
- Streak the isolate on Mac conkey agar and incubate at 37°C for 24 hours.
- Subject the isolate to motility test, indole test, TSI test. It is helpful to understand the probable nature of the isolate.
- Perform MR, VP, Urease, nitrate, deaminase, decarboxylase, melonate, KCN and carbohydrate fermentation tests.
- Confirm probably identified bacterial isolate by streaking it on Haektoein enteric agar, XLD agar, SS agar and RajHans medium. Incubate all the media at 37°Cfor 24 hours and observed the colony morphology.

RESULTS :

Based on the above observation the given bacteria is identified as *Salmonella sp.*

IDENTIFICATION OF PSEUDOMONAS AERUGINOSA

AIM:

To identify pseudomonas aeruginosa from the given culture

INTRODUCTION:

Pseudomonas are a large group of aerobic, nonsporing Gram negative bacilli, motile by polar flagella. They are ubiquitous, mostly Saprophytic, being found in water, soil or other moist environments. Some Of them are pathogenic to plants, insects and reptiles. A few cause human Infection, typically opportunistic.

Pseudomonas aeruginosa is opportunistic pathogens of humans. It's a gram negative, aerobic rod belonging to the family pseudomonadaceae. These bacteria are common inhabitants of soil and water. Almost all strains are motile by means of a single polar flagellum. Infection associated with Pseudomonas aeruginosa are Endocarditis, Pneumonia, Bactermia, Septicemia, meningitis and brain abscesses, otitis, kertitis, neonatal Ophthalmia, osteomyelitis, Urinary tract infection. Gastrointestinal infection Like pediatric diarrhea, typical gastroenteritis, and necrotizing enterocolitis, Skin infection like burns, trauma or dermatitis ;folliculitis, acne vulgaris like Symptoms.

MATERIYAL REQUIRED :

Sample : Broth or stap or slant culture

Chemical& Media Required :

Gram staining reagent, Mac conkey agar,

Cetrimide agar, biochemical test media & reagent, class slides, and petri plates, Test tubes etc……

PROCEDURE:

- Check the purity of the culture by streaking the culture on nutrient agar plate,

Incubator at 37°C for 24 hours and note colony morphology of the culture (Pure culture showed uniform morphology).

- Perform gram staining to look for garm's nature and catalase and oxidase.
- Inoculate test organism on menconkey agar plate and incubator it for 24 hours at 37°C aerobically.
- Perform motility test, insole test and TSI test. It's helpful to understand the probable Nature of the isolate and these will differentateentrobacteriacea from other aerobic Family of human pathogens.
- Perform MR, VP, Urease, Nitrate, Deaminease, Decarboxylase, Melonate, KCN and Carbohydrate fermentation tests along with EMB media inoculation to confirm generic level identification of the isolate
- Probably Identified bacterial isolate is confirmed by streaking it on cetrimide agar and Incubate at 37°C for 24 hours and observed the colony morphology.

  RESULT : Based on the above observation the given bacteria is identified as Pseudomonas aeruginosa.

EXPERIMENT NO: 2

Eosin Methylene Blue Agar

EMB Agar (Eosin Methylene Blue Agar) is recommended for the isolation and differentiation of gram negative enteric bacteria from clinical and nonclinical specimens.

Composition** Ingredients Gms / Litre Peptic digest of animal tissue 10.000 Dipotassium phosphate 2.000 Lactose 5.000 Sucrose 5.000 Eosin - Y 0.400 Methylene blue 0.065 Agar 13.500 Final pH (at 25°C) 7.2±0.2 **Formula adjusted, standardized to suit performance parameters Directions Suspend 35.96 grams in 1000 ml distilled water. Mix until suspension is uniform. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. AVOID OVERHEATING. Cool to 45-50°C and shake the medium in order to oxidize the methylene blue (i.e. to restore its blue colour) and to suspend the flocculent precipitate. (If EMB Agar is inoculated on the same day, it may be used without autoclave sterilization).

Precaution: Store the medium away from light to avoid photooxidation

Principle And Interpretation

Eosin Methylene Blue (EMB) Agar was originally devised by Holt-Harris and Teague (1) and further modified by Levine (2). The above medium is a combination of the Levine and Holt-Harris and Teague formulae which contains peptic digest of animal tissue and phosphate as recommended by Levine and two carbohydrates as suggested by Holt-Harris and Teague.

Methylene blue and Eosin-Y inhibit gram-positive bacteria to a limited degree. These dyes serve as differential indicators in response to the fermentation of carbohydrates. The ratio of eosin and methylene blue is adjusted approximately to 6:1. Sucrose is added to the medium as an alternative carbohydrate source for typically lactose-fermenting, gram-negative bacilli, which on occasion do not ferment lactose or do so slowly. The coliforms produce purplish black colonies due to taking up of methylene blue-eosin dye complex, when the pH drops. The dye complex is absorbed into the colony. Nonfermenters probably raise the pH of surrounding medium by oxidative deamination of protein, which solubilizes the methylene blue-eosin complex resulting in colourless colonies (3). Some strains of Salmonella and Shigella species do not grow in the presence of eosin and methylene blue. Further tests are required to confirm the isolates.

Peptic digest of animal tissue serves as source of carbon, nitrogen, and other essential growth nutrients. Lactose and sucrose are the sources of energy by being fermentable carbohydrates. Eosin-Y and methylene blue serve as differential indicators. Phosphate buffers the medium.

The test sample can be directly streaked on the medium plates. Inoculated plates should be incubated, protected from light. However standard procedures should be followed to obtain isolated colonies. A non-selective medium should be inoculated in conjunction with EMB Agar. Confirmatory tests should be further carried out for identification of isolated colonies.

Quality Control Appearance Light pink to purple homogeneous free flowing powder.

Gelling: Firm, comparable with 1.35% Agar gel**.**

Colour and Clarity of prepared medium Reddish purple coloured, opalescent gel with greenish cast and finely dispersed precipitate forms in Petri plates

Reaction Reaction of 3.6% w/v aqueous solution at 25°C**.**

pH : 7.2±0.2 pH 7.00-7.40

Storage and Shelf Life Store below 30°C in tightly closed container and the prepared medium at 2-8°C and away from the light. Use before expiry date on the label.

SS Agar (Salmonella Shigella Agar)

SS Agar (Salmonella Shigella Agar) is a differential selective media used for the isolation of Salmonella and some Shigella species from pathological specimens, suspected foodstuffs etc.

Composition** Ingredients Gms / Litre Proteose peptone 5.000 Lactose 10.000 Bile salts mixture 8.500 Sodium citrate 8.500 Sodium thiosulphate 8.500 Ferric citrate 1.000 Brilliant green 0.00033 Neutral red 0.025 Agar 13.500 Final pH (at 25°C) 7.0±0.2 **Formula adjusted, standardized to suit performance parameters

Directions Suspend 60.00 grams in 1000 ml distilled water. Boil with frequent agitation to dissolve the medium completely. DO NOT AUTOCLAVE OR OVERHEAT. Overheating may destroy selectivity of the medium. Cool to about 50°C. Mix and pour into sterile Petri plates.

Principle And Interpretation

SS Agar medium is recommended as differential and selective medium for the isolation of Salmonella and Shigella species from pathological specimens (1) and suspected foodstuffs (2, 3, 4, 5) and for microbial limit test (6). SS Agar is a moderately selective medium in which gram-positive bacteria are inhibited by bile salts, brilliant green and sodium citrate.

Proteose peptone, beef extract provide essential growth nutrients. Lactose is the fermentable carbohydrate. Brilliant green, bile salts and thiosulphate selectively inhibit gram-positive and coliform organisms. Sodium thiosulphate is reduced by certain species of enteric organisms to sulphite and H2S gas and this reductive enzyme process is attributed by thiosulphate reductase. Production of H2S gas is detected as an insoluble black precipitate of ferrous sulphide, formed upon reaction of H2S with ferric ions or ferric citrate, indicated in the centre of the colonies.

The high selectivity of Salmonella Shigella Agar allows the use of large inocula directly from faeces, rectal swabs or other materials suspected of containing pathogenic enteric bacilli. On fermentation of lactose by few lactose-fermenting normal intestinal flora, acid is produced which is indicated by change of colour from yellow to red by the pH indicator-neutral red. Thus these organisms grow as red pigmented colonies. Lactose non-fermenting organisms grow as translucent colourless colonies with or without black centres. Growth of Salmonella species is uninhibited and appears as colourless colonies with black centres resulting from H 2S production. Shigella species also grow as colourless colonies which do not produce H2S.

It is recommended to inoculate plates of less inhibitory media parallel to SS Agar, such as Hektoen Enteric Agar (M467) orDeoxycholate Citrate Agar (M065) for easier isolation of Shigella species .

Quality Control Appearance Light yellow to pink homogeneous free flowing powder

Gelling Firm, comparable with 1.35% Agar gel

Colour and Clarity of prepared medium Reddish orange coloured clear to slightly opalescent gel forms in Petri plates. **Reaction**Reaction of 6.0% w/v aqueous solution at 25°C. pH : 7.0±0.2 **pH** 6.80-7.20

Storage and Shelf Life Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label

MacConkey Agar

MacConkey Agar is recommended for selective isolation of Escherichia coli from pharmaceutical products and is in accordance with harmonized methodology of BP. It is also recommended for selective isolation and differentiation of lactose fermenting and lactose non fermenting enteric bacteria.

Composition**

Ingredients Gms / Litre Peptones (meat and casein) 3.000 Pancreatic digest of gelatin 17.000 Lactose monohydrate 10.000 Bile salts 1.500 Sodium chloride 5.000 Crystal violet 0.001 Neutral red 0.030 Agar 13.500 pH after sterilization(at 25°C) 7.1±0.2 **Formula adjusted, standardized to suit performance parameters

Directions

Suspend 49.53 grams of dehydrated medium in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes i.e. validated cycle. AVOID OVERHEATING. Cool to 45-50°C. Mix well before pouring into sterile Petri plates. The surface of the medium should be dry when inoculated.

Principle And Interpretation

MacConkey Agar is the earliest selective and differential medium for cultivation of coliform organisms (1, 2). Subsequently MacConkey Agar and Broth have been recommended for use in microbiological examination of foodstuffs (3) and for direct plating / inoculation of water samples for coliform counts (4). This medium is also accepted by the Standard Methods for the Examination of Milk and Dairy Products (5). British pharmacopoeia (6) has recommended this medium for the subculture and identification of Escherichia coli . It is also cited as Agar Medium H. It is also recommended by and in accordance with the harmonized method of USP/BP/EP/JP (7, 6, 8, 9). 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.

After enrichment of Escherichia coli in MacConkey Broth (M083B), it is then subcultured on MacConkey Agar. Gramnegative bacteria usually grow well on the medium and are differentiated by their ability to ferment lactose. Lactose fermenting strains grow as red or pink and may be surrounded by a zone of acid precipitated bile. The red colour is due to production of acid from lactose, absorption of neutral red and a subsequent colour change of the dye when the pH of medium falls below 6.8. Lactose non-fermenting strains, such as Shigella and Salmonella are colourless 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.

Quality Control

Appearance Light yellow to pink homogeneous free flowing powder

Gelling Firm comparable with 1.35% Agar gel.

Colour and Clarity of prepared medium

Red with purplish tinge coloured clear to slightly opalescent gel forms in Petri plates.

pH 6.90-7.30

Cultural Response

Growth Promotion is carried out in accordance with the harmonized method of BP. Cultural response was observed after an incubation at 30-35°C for 18-72 hours. Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar.

Growth promoting properties

Growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu (at 30-35°C for <=18-72 hours).

Indicative properties

Colonies are comparable in appearance and indication reaction to those previously obtained with previously tested and approved lot of medium occurs for the specified temperature for a period of time within the range specified inoculating <=100 cfu (at 30-35°C for 18-72hrs)

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Mannitol Salt Agar M118

Mannitol Salt Agar M118 Intended Use: Mannitol Salt Agar is used as a selective media for the isolation of pathogenic Staphylococci from clinical and non-clinical samples.

Composition**

Ingredients Gms / Litre

Proteose peptone 10.000

HM peptone B # 1.000

Sodium chloride 75.000

D-Mannitol 10.000

Phenol red 0.025

Agar 15.000

Final pH (at 25°C) 7.4±0.2

Directions Suspend 111.02 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. If desired, add 5% v/v Egg Yolk Emulsion (FD045). Mix well and pour into sterile Petri plates. Note : This product contains 7.5% Sodium chloride as one of its ingredients. On repeated exposure to air and absorption moisture sodium chloride has tendency to form lumps, therefore we strongly recommend storage in tightly closed containers in dry place away from bright light . Principle And Interpretation Staphylococci are widespread in nature, although they are mainly found on the skin, skin glands and mucous membranes of mammals and birds. The coagulase-positive species i.e Staphylococcus aureus is well documented as a human opportunistic pathogen. The ability to clot plasma continues to be the most widely used and accepted criterion for the identification of pathogenic staphylococci associated with acute infections (1). Staphylococci have the unique ability of growing on a high salt containing media (2). Isolation of coagulase-positive staphylococci on Phenol Red Mannitol Agar supplemented with 7.5% NaCl was studied by Chapman (3). The resulting Mannitol Salt Agar Base is recommended for the isolation of coagulasepositive staphylococci from cosmetics, milk, food and other specimens (1, 4-7). The additional property of lipase activity of Staphylococcus aureus can be detected by the addition of the Egg Yolk Emulsion (FD045). The lipase activity can be visualized as yellow opaque zones around the colonies (8). HM peptone B and proteose peptone supply essential growth factors and trace nutrients to the growing bacteria. Sodium chloride serves as an inhibitory agent against bacteria other than staphylococci. Mannitol is the fermentable carbohydrate, fermentation of which leads to acid production, detected by phenol red indicator. S.aureus ferment mannitol and produce yellow coloured colonies surrounded by yellow zones. Coagulase-negative strains of S.aureus are usually mannitol non-

fermenters and therefore produce pink to red colonies surrounded by red-purple zones. Presumptive coagulase-positive yellow colonies of S. aureus should be confirmed by performing the coagulase test [tube or slide](1). Lipase activity of S.aureus can be detected by supplementing the medium with egg yolk emulsion. A possible S.aureus must be confirmed by the coagulase test. Also the organism should be subcultured to a less inhibitory medium not containing excess salt to avoid the possible interference of salt with coagulase testing or other diagnostic tests (e.g. Nutrient Broth) (M002) (9). Few strains of S.aureus may exhibit delayed mannitol fermentation. Negative results should therefore be re-incubated for an additional 24 hours before being discarded

Type of specimen Clinical samples: pus; Food and dairy samples, water samples. Specimen Collection and Handling For clinical samples follow appropriate techniques for handling specimens as per established guidelines (10,11). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6,12). After use, contaminated materials must be sterilized by autoclaving before discarding. Warning and Precautions In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/ eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

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Storage and Shelf Life

Store below 30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.Product performance is best if used within stated expiry period. Disposal User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques

Deoxycholate Citrate Agar M065

Intended Use:

Recommended for the isolation of enteric pathogens particularly Salmonella and Shigella species.

Composition**

Ingredients Gms / Litre

HI solids # 10.000

Proteose peptone 10.000

Lactose 10.000
Sodium deoxycholate 5.000
Neutral red 0.020
Sodium citrate 20.000
Ferric ammonium citrate 2.000
Agar 13.500
Final pH (at 25°C) 7.5±0.2
**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 70.52 grams in 1000 ml of distilled water. Heat to boiling to dissolve the medium completely. DO NOT
AUTOCLAVE. Avoid excessive heating as it is detrimental to the medium. Cool to 45-50°C. Mix well and pour into sterile
petri plates

Principle And Interpretation

Deoxycholate Citrate Agar is prepared as per the modified formula of Leifson (1). This medium is used for the isolation and maximum recovery of intestinal pathogens belonging to *Salmonella* and *Shigella* groups from foods (2). However, it is recommended to use less inhibitory medium when *Shigellae* have to be isolated (3). The selectivity of this medium permits the use of fairly heavy inocula without danger of overgrowth of *Shigella* and *Salmonella* by other microflora. For the routine examination of stool and urine specimens, it is suggested that other media such as MacConkey Agar (M082), Bismuth Sulphite Agar (M027) etc. be used in conjunction with this medium.

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 grampositive bacteria. Citrate salts, in the concentration included in the formulation, are inhibitory to gram-positive bacteria and most other normal intestinal organisms. HI solids is a source of carbon and nitrogen and this ingredient is used because the inhibition of coliforms produced is greater than when an extract or simple peptone is used. 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 colour 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 (1). 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.

Citrate and iron (Fe) combination has a strong hydrolyzing effect on agar when the medium is heated, producing a soft and unelastic agar. If autoclaved the agar becomes soft and almost impossible to streak (1). *Salmonella* Gallinarum is inhibited if sodium deoxycholate concentration is

increased to 0.1 % or greater (1). Surface colonies of non-lactose fermenters often
absorb a little colour (pinkish) from the medium and organisms may be mistaken for coliforms
Type of specimen
Clinical- faeces
Specimen Collection and Handling
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).
After use, contaminated materials must be sterilized by autoclaving before discarding.
Warning and Precautions
In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/
eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard
precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be
referred in individual safety data sheets.
Limitations
1. Further Biochemical identification is required for confirmation of species.
2. Due to nutritional variations some organisms may show poor growth.

TCBS Agar M189 Intended Use: TCBS Agar is recommended for the selective isolation and cultivation of Vibrio cholerae and other enteropathogenic Vibrio's causing food poisoning from clinical and food specimen.
Composition**
Ingredients Gms / Litre
Proteose peptone 10.000
Yeast extract 5.000
Sodium thiosulphate 10.000
Sodium citrate 10.000
Bile 8.000
Sucrose 20.000
Sodium chloride 10.000
Ferric citrate 1.000
Bromo thymol blue 0.040
Thymol blue 0.040
Agar 15.000
Final pH (at 25°C) 8.6±0.2
**Formula adjusted, standardized to suit performance parameters
Directions
Suspend 89.08 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Mix well and pour into sterile Petri plates.
Principle and Interpretation
TCBS Agar was developed by Kobayashi et al (1), who modified the selective medium of Nakanishi (2). Although this medium was originally designed for the isolation of V.cholerae and V. parahaemolyticus, most Vibrios grow to healthy large colonies with many different colonial

morphologies. TCBS Agar is also recommended by APHA for the selective isolation of V. cholerae and V.parahaemolyticus (3,4). Enrichment in Alkaline Peptone Water (M618), followed by isolation on TCBS Agar is routinely used for isolation of V.cholerae

Proteose peptone and yeast extract provide nitrogenous compounds, vitamin B complex and other essential growth nutrients. Bile, a derivative of bile salts and sodium citrate inhibit gram-positive bacteria and coliforms (8). Sodium thiosulphate serves as a good source of sulphur, which in combination with ferric citrate detects the production of hydrogen sulphide. For the metabolism of Vibrios, sucrose is added as a fermentable carbohydrate. Vibrio that is able to utilize sucrose will from yellow colonies. Bromothymol blue and thymol blue are the pH indicators. The alkaline pH of the medium improves the recovery of V.cholerae. Strains of V.cholerae produce yellow colonies on TCBS Agar because of fermentation of sucrose. V.alginolyticus also produce yellow colonies. V.parahaemolyticus is a sucrose non-fermenting organism and therefore produces blue-green colonies, as does V.vulnificus

Type of specimen Clinical : faeces ; Food samples; Water samples Specimen Collection and Handling For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,12). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,11,13) For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(4) After use, contaminated materials must be sterilized by autoclaving before discarding

Warning and Precautions In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/ eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets. Limitations 1.The medium should be inoculated heavily with faecal specimens because growth of few species may be inhibited on the medium due to fermentation of sucrose and accumulation of acids. 2..However, occasional isolates of Pseudomonas and Aeromonas may also form blue green colonies on TCBS Agar (9). 3.Proteus species that are sucrose-fermenters may form yellow colonies (9). 4.TCBS Agar is not a suitable medium for oxidase testing of Vibrio species (10). 5.A few strains of V. cholerae may appear green or colourless on TCBS Agar due to delayed sucrose fermentation (9). 6.TCBS Agar is highly selective for Vibrio species. Any H2S negative colony of TCBS Agar can be considered presumptive positive for Vibrio

. 6. Further biochemical and serological tests must be carried out for complete identification

SS Agar (Salmonella Shigella Agar)

SS Agar (Salmonella Shigella Agar) is a differential selective media used for the isolation of Salmonella and some Shigella species from pathological specimens, suspected foodstuffs etc.

Composition** Ingredients Gms / Litre Proteose peptone 5.000 Lactose 10.000 Bile salts mixture 8.500 Sodium citrate 8.500 Sodium thiosulphate 8.500 Ferric citrate 1.000 Brilliant green 0.00033 Neutral red 0.025 Agar 13.500 Final pH (at 25°C) 7.0±0.2 **Formula adjusted, standardized to suit performance parameters

Directions Suspend 60.00 grams in 1000 ml distilled water. Boil with frequent agitation to dissolve the medium completely. DO NOT AUTOCLAVE OR OVERHEAT. Overheating may destroy selectivity of the medium. Cool to about 50°C. Mix and pour into sterile Petri plates.

Principle And Interpretation

SS Agar medium is recommended as differential and selective medium for the isolation of Salmonella and Shigella species from pathological specimens (1) and suspected foodstuffs (2, 3, 4, 5) and for microbial limit test (6). SS Agar is a moderately selective medium in which gram-positive bacteria are inhibited by bile salts, brilliant green and sodium citrate.

Proteose peptone, beef extract provide essential growth nutrients. Lactose is the fermentable carbohydrate. Brilliant green, bile salts and thiosulphate selectively inhibit gram-positive and coliform organisms. Sodium thiosulphate is reduced by certain species of enteric organisms to sulphite and H2S gas and this reductive enzyme process is attributed by thiosulphate reductase. Production of H2S gas is detected as an insoluble black precipitate of ferrous sulphide, formed upon reaction of H2S with ferric ions or ferric citrate, indicated in the centre of the colonies.

The high selectivity of Salmonella Shigella Agar allows the use of large inocula directly from faeces, rectal swabs or other materials suspected of containing pathogenic enteric bacilli. On fermentation of lactose by few lactose-fermenting normal intestinal flora, acid is produced which is indicated by change of colour from yellow to red by the pH indicator-neutral red. Thus these organisms grow as red pigmented colonies. Lactose non-fermenting organisms grow as translucent colourless colonies with or without black centres. Growth of Salmonella species is uninhibited and appears as colourless colonies with black centres resulting from H 2S production. Shigella species also grow as colourless colonies which do not produce H2S.

It is recommended to inoculate plates of less inhibitory media parallel to SS Agar, such as Hektoen Enteric Agar (M467) orDeoxycholate Citrate Agar (M065) for easier isolation of Shigella species .

Quality Control Appearance Light yellow to pink homogeneous free flowing powder

Gelling Firm, comparable with 1.35% Agar gel

Colour and Clarity of prepared medium Reddish orange coloured clear to slightly opalescent gel forms in Petri plates. **Reaction**Reaction of 6.0% w/v aqueous solution at 25°C. pH : 7.0±0.2 **pH** 6.80-7.20

Storage and Shelf Life Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label

Bismuth Sulphite Agar

Intended Use: Bismuth Sulphite Agar is recommended for the selective isolation and preliminary identification of Salmonella Typhi and other Salmonellae from pathological materials, sewage, water supplies, food etc. Composition** Ingredients Gms / Litre Peptone 10.000 HM Peptone B # 5.000 Dextrose (Glucose) 5.000 Disodium phosphate 4.000 Ferrous sulphate 0.300 Bismuth sulphite indicator 8.000 Brilliant green 0.025 Agar 20.000 Final pH (at 25°C) 7.7±0.2 **Formula adjusted, standardized to suit performance parameters

Directions Suspend 52.33 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT STERILIZE IN AUTOCLAVE or by fractional sterilization since overheating may destroy the selectivity of the medium. The sensitivity of the medium depends largely upon uniform dispersion of precipitated bismuth sulphite in the final gel, which should be dispersed before pouring into sterile Petri plates.

Principle And Interpretation The Salmonellae constitute the most taxonomically complex group of bacteria among Enterobacteriaceae (1). Human Salmonella infections are most commonly caused by ingestion of food, water or milk contaminated by human or animal excreta. Humans are the only reservoirs of S. Typhi (2). Four clinical types of Salmonella infections may be distinguished (3) namely gastroenteritis, bacteremia or septicemia, enteric fever and a carrier state. Of the various media employed for the isolation and preliminary identification of Salmonellae, particularly

Salmonella Typhi; Bismuth Sulphite Agar is the most productive (4). Bismuth Sulphite Agar is a modification of original Wilson and Blair Medium (5-7). It is also recommended by various Associations (8-13) for the isolation and preliminary identification of Salmonella Typhi and other Salmonellae from pathological materials, sewage, water, food and other products. S. Typhi, S. Enteritidis and S. Typhimurium typically grow as black colonies with a surrounding metallic sheen resulting from hydrogen sulphide production and reduction of sulphite to black ferric sulphide. Salmonella Paratyphi A grows as light green colonies. Bismuth Sulphite Agar may be inhibitory to some strains of Salmonella species and therefore should not be used as the sole selective medium for these organisms. Also this medium favors use of larger inoculum as compared to other selective media, as it has unique inhibitory action towards gram-positive organisms and coliforms. Peptone and HM Peptone B serve as sources as carbon, nitrogen, long chain amino acids, vitamins and essential growth factors. Dextrose is the carbon source. Disodium phosphate maintains the osmotic equilibrium. Bismuth sulphite indicator along with brilliant green inhibits the intestinal gram-positive and gram-negative bacteria. Ferrous sulphate aids in detection of hydrogen sulphide production. Clinical samples can be directly used to inoculate Bismuth Sulphite Agar. In case of food samples, pre enrichment of the sample is done prior to inoculation.

- Equivalent to Beef Extract

Type of specimen

Clinical samples : faeces, urine, blood and other pathological material , Foodstuff , water samplesSpecimen Collection and Handling For clinical samples follow appropriate techniques for handling specimens as per established guidelines (16,17). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (10,12,15). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(9) After use, contaminated materials must be sterilized by autoclaving before discarding. Warning and Precautions In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/ eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations 1. DO NOT AUTOCLAVE OR OVERHEAT THE MEDIUM, as it destroys the selectivity of the medium. 2. S.Typhi and S.Arizonae exhibit typical brown colonies , with or without metallic sheen. 3.This medium is highly selective and must be used in parallel with less selective media for isolation. 4. With certain Salmonella species, typical black colonies with metallic sheen is observed near heavy inoculation and isolated colonies may show green colonies. 5. Shigella species are mostly inhibited on this medium; exceptions being S. flexneri and S. sonnei (14) 6. Some Salmonella like S. Sendai, S. Berta, S. Gallinarum, S. Abortus-equi are also inhibited (14).

Performance and Evaluation Performace of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control Appearance Light yellow to greenish yellow homogeneous free flowing powder

Gelling Firm, comparable with 2.0% agar gel.

Colour and Clarity of prepared medium Greenish yellow coloured, opalescent with flocculent precipitate forms in Petri plates.

Reaction reactionof 5.23% w/v aqueous solution at 25°C. pH : 7.7±0.2 pH 7.50-7.90

Storage and Shelf Life Store below 30°C in a tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after

tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label. Product performance is best if used within stated expiry period.
Disposal User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques

EXPERIMENT NO: 3

Study of bacterial flora of skin by swab method.

Aim

To isolate and identify the bacterial flora present on the skin surface using swab method.

Principle

The skin is the human body's largest organ, colonized by a diverse milieu of microorganisms, most of which are harmless or even beneficial to their host. Colonization is driven by the ecology of the skin surface, which is highly variable depending on topographical location, endogenous host factors and exogenous environmental factors. The cutaneous innate and adaptive immune responses can modulate the skin microbiota, but the microbiota also functions in educating the immune system. The development of molecular methods to identify microorganisms has led to an emerging view of the resident skin bacteria as highly diverse and variable. An enhanced understanding of the skin micro biome is necessary to gain insight into microbial involvement in human skin disorders and to enable novel promicrobial and antimicrobial therapeutic approaches for their treatment. The normal bacterial flora of the human skin has been a problem of interest to investigators for many years, yet it is difficult to find a satisfactory description of the usual bacertial population of ordinary human skin. Textbooks of bacteriology usually list staphylococci, diphtheroids, and a variety of saprophytic rods as occurring on skin and are vague as to the relative numbers of these organisms.

Sterile swabs can be used to test the level of microbial contamination on various surfaces such as air conditioning units, kitchen equipment, hospital wards, spas or any other place. Swab samples can be analyzed for total viable counts (usually referred to as colony forming units) or specific indicator organisms for food spoilage or sewage contamination. Swab samples are easy to collect.

Collection of Swab Samples

- Wear gloves
- Select a sampling area of about 10 cm X 10 cm.
- Break the seal round the tube containing the swab
- Remove the swab from the tube and rub and roll it firmly several times across the sampling area.
- Return the swab into the tube and label the sample
- Send the sample to the laboratory for analysis.
- Plated on to nutrient agar for bacteria incubated at 37^0 C and Rosebengal agar for Fungi incubated at room temperature.

If one is sampling a dry surface, it is recommended that a wet or moistened swab is used. If swab samples are collected for culture analysis, they should be sent to the laboratory within 24 hours after collection. If the analysis of the swab samples involves enumeration of the microbial contaminants, the size of the area sampled should be provided to the lab.

Result

The colonies on the plates were observed macroscopically and microscopically

EXPERIMENT NO: 4

ANTIBIOTIC SENSITIVITY TEST - KIRBY BAUER METHOD

AIM:

To become acquainted with the Kirby bauer technique for the evaluation of antimicrobial activity of chemotherapeutic agents.

PRINCIPLE:

Available chemotherapeutic agents very in the scope of antimicrobial activity. Some limited spectrum of activity being effective agent only one group of the micro organisms. Others exhibits a broad spectrum of activity against a wide range of many pathogens but it is some time necessary to use several agents to determine the drug of choice.

A standardised filter paper disc agar diffusion procedure, known as kirby bauer method is frequently used to determine the drug susceptibility of micro organisms. Filter paper disc of uniform size were in pregnated and then placed on the surface of agar plate that have been uniformly inoculated with actively growing log phase culture of the test organisms (lawn culture).

The confluent growth on the plate except at the zone of clearance around the antibiotic disc which inhibits the growth of the organism indicates the sustainability of the organism. The medium of choice is Muller Hinton agar with a pH of 7.2-7.4 which is poured into the plate to a uniform depth of 5mm and 25-20mm left for solidification.

MATERIALS REQUIRED:

- Culture: 0.85% saline suspension of *E. coli* and *S.aureus*
- Media: Muller Hinton agar
- **Antibiotic disc:**

a) Penicillin G : 10 µg
b) Streptomycin : 10 µg
c) Tetracycline : 30 µg
d) Gentamycin : 10 µg

- **Others**: forceps, sterile cotton swab, glass wares, marking pencil and Zone measuring scale

PROCEDURE:

- Muller Hinton agar plate were prepared and sterilized.

- Then the agar surface was inoculated with the test organisms by lawn culture (pipette out 0.1 µL of culture and spread using sterile swab).
- Using forceps (sterile) antibiotic disc were placed on the surface of the agar plates with required distance the disc were gently pressed on the agar surface.
- All the culture plate were inverted and incubated after 24 hours at 37 degree Celsius.
- A zone of clearance was observed and the diameter of the zone of clearance was measured in millimetre.

RESULT:

There was growth up to the disc on the Muller Hinton Agar plate while some of had a definite zone of inhibition around the disc. Based upon the diameter of zone sensitive, resistance and intermediate of the antibiotic were recorded and tabulated.

DISCUSSION:

The agar plates that had growth rate up to the disc shows resistance of the organism white those with a definite zone of inhibition around the disc shows sensitive susceptibility of the organism to a drug is determined by the size of zone. Which itself is depends on the visible such as,

- The antibiotic and rate of diffusion of the antibiotic into the medium and its interaction with test organisms.
- Concentration of organism in the culture.
- The growth rate of organism in the culture.
- The moisture rate and incubation conditions organism.

ANTIBIOTIC SUSCEPTIBILITY TEST

ANTIBIOTIC DISC	ZONE OF INHIBITION IN MILLIMETER	SUSCEPTABILITY
Streptomycin		
Kanamycin		
Bacitracin		
Penicillin		
Erythromycin		

EXPERIMENT NO: 5

Determination of Minimal Inhibitory Concentration (MIC) of an antibiotic

AIM:

To determination the smallest amount of antibiotic required to inhibit the growth of the organism by in vitro methods. This amount is refunded as minimal inhibitory concentration (MIC)

PRINCIPLE:

The effect of chemotherapeutic agent varies with target species some idea of effectiveness of chemotherapeutic agent against a pathogen can be obtained from minimal inhibitory concentration. MIC is the lower concentration drug that prevents pathogens growth. A pathogen should have MIC value low enough to be destroyed by the drugs. A pathogen with to high MIC value is resistant to the concentration.

MATERIALS REQUIRED:

Nutrient broth, inoculation loop, Streptomycin, pipette, spirit lamp, Test tube, log phase of *Staphylococcus* sps.

PROCEDURE:

- Antibiotic to be tested were prepared at various concentration.
- 5ml of nutrient broth was taken and1 mg of Streptomycin was added in first tube.
- Second tube contains 5ml of nutrient broth and 0.5mg of Streptomycin.
- Third tube contains 5ml of nutrient broth and 0.25mg of Streptomycin.
- Each tube with One drop of staphylococcus culture was incubated at 37°c for 24 hours.
- Turbidity was Measured of optical density (OD) a tube was prepared between the antibiotic and turbidity.

RESULT:

The Antibiotic concentration which shows_____inhibition (MIC) for the given organism is **_____µg/5ml** it is tabulated

Organism	**Streptomycin**	
	MIC (µg/5ml)	**SUSCEPTABILITY**
S.aureus		

EXPERIMENT NO: 6

Study symptoms of the diseases with the help of photographs: Polio, anthrax, herpes, chicken pox, HPV warts, AIDS (candidiasis), dermatomycoses (ring worms)

Polio

Polio is a contagious viral illness that in its most severe form causes nerve injury leading to paralysis, difficulty breathing and sometimes death. The Centers for Disease Control and Prevention (CDC) advises taking precautions to protect yourself from polio if you're traveling anywhere there's a risk of polio.Adults who have been vaccinated who plan to travel to an area where polio is occurring should receive a booster dose of inactivated poliovirus vaccine (IPV). Immunity after a booster lasts a lifetime.

Symptoms

Illustration of a nerve cell (neuron), showing axon and dendrites
Nerve cell (neuron)
Although polio can cause paralysis and death, the majority of people who are infected with the virus don't get sick and aren't aware they've been infected.

Nonparalytic polio

Some people who develop symptoms from the poliovirus contract a type of polio that doesn't lead to paralysis (abortive polio). This usually causes the same mild, flu-like signs and symptoms typical of other viral illnesses.
Signs and symptoms, which can last up to 10 days, include:
Fever, Sore throat, Headache, Vomiting, Fatigue, Back pain or stiffness, Neck pain or stiffness, Pain or stiffness in the arms or legs, Muscle weakness or tenderness

Paralytic polio

This most serious form of the disease is rare. Initial signs and symptoms of paralytic polio, such as fever and headache, often mimic those of nonparalytic polio. Within a week, however, other signs and symptoms appear, including:
Loss of reflexes
Severe muscle aches or weakness
Loose and floppy limbs (flaccid paralysis)
Post-polio syndrome
Post-polio syndrome is a cluster of disabling signs and symptoms that affect some people years after having polio. Common signs and symptoms include:
Progressive muscle or joint weakness and pain
Fatigue, Muscle wasting (atrophy), Breathing or swallowing problems, Sleep-related breathing disorders, such as sleep apnea, Decreased tolerance of cold temperatures

Causes

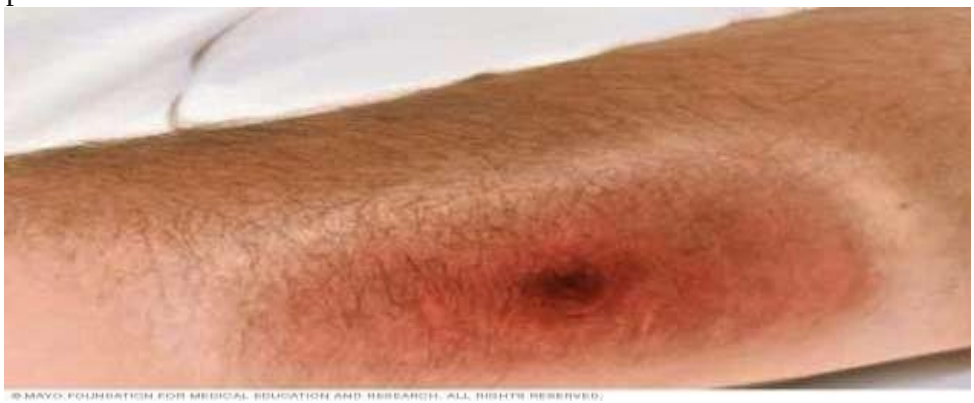
Poliovirus can be transmitted through direct contact with someone infected with the virus or, less commonly, through contaminated food and water. People carrying the poliovirus can spread the

virus for weeks in their feces. People who have the virus but don't have symptoms can pass the virus to others.

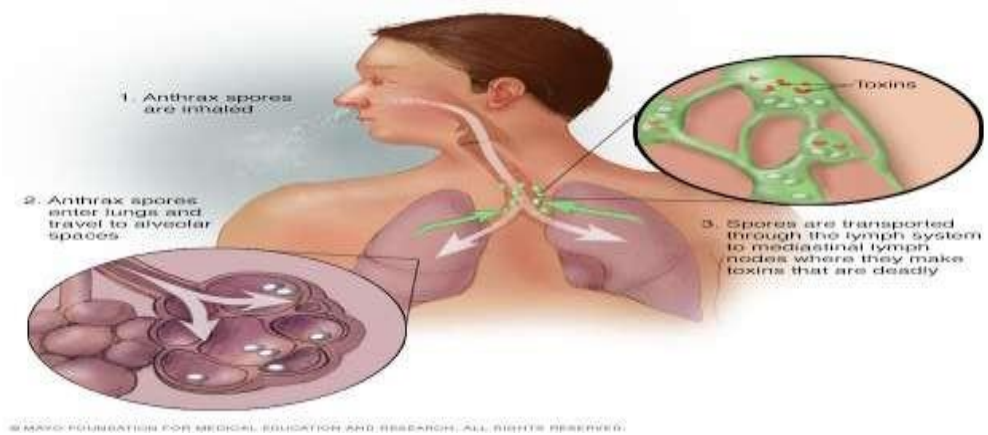
Anthrax

Anthrax is a rare but serious illness caused by a spore-forming bacterium, Bacillus anthracis. Anthrax mainly affects livestock and wild game. Humans can become infected through direct or indirect contact with sick animals. There's no evidence that anthrax is transmitted from person to person, but it's possible that anthrax skin lesions may be contagious through direct contact. Usually, anthrax bacteria enter the body through a wound in the skin. You can also become infected by eating contaminated meat or inhaling the spores. Signs and symptoms, which depend on how you're infected, can range from skin sores to vomiting to shock. Prompt treatment with antibiotics can cure most anthrax infections. Inhaled anthrax is more difficult to treat and can be fatal.

Symptoms



- **Cutaneous anthrax**



Inhalation anthrax

There are four common routes of anthrax infection, each with different signs and symptoms. In most cases, symptoms develop within seven days of exposure to the bacteria. The one exception is inhalation anthrax. After exposure to inhalation anthrax, it may take weeks before symptoms appear.

Cutaneous anthrax

A cutaneous anthrax infection enters your body through a cut or other sore on your skin. It's by far the most common route the disease takes. It's also the mildest — with appropriate treatment, cutaneous anthrax is seldom fatal. Signs and symptoms of cutaneous anthrax include:

- A raised, itchy bump resembling an insect bite that quickly develops into a painless sore with a black center
- Swelling in the sore and nearby lymph glands

Gastrointestinal anthrax

This form of anthrax infection begins by eating undercooked meat from an infected animal. Signs and symptoms include:

- Nausea
- Vomiting
- Abdominal pain
- Headache
- Loss of appetite
- Fever
- Severe, bloody diarrhea in the later stages of the disease
- Sore throat and difficulty swallowing
- Swollen neck

Inhalation (pulmonary) anthrax

Inhalation anthrax develops when you breathe in anthrax spores. It's the most deadly way to contract the disease, and even with treatment, it is often fatal. Initial signs and symptoms of inhalation anthrax include:

- Flu-like symptoms, such as sore throat, mild fever, fatigue and muscle aches, which may last a few hours or days
- Mild chest discomfort
- Shortness of breath
- Nausea

- Coughing up blood
- Painful swallowing

As the disease progresses, you may experience:

- High fever
- Trouble breathing
- Shock
- Meningitis — a potentially life-threatening inflammation of the brain and spinal cord

Injection anthrax

This is the most recently identified route of anthrax infection. It's contracted through injecting illegal drugs and has been reported only in Europe so far. Initial signs and symptoms of injection anthrax include:

- Redness at the area of injection (without an area that changes to black)
- Significant swelling

As the disease progresses, you may experience:

- Shock
- Multiple organ failure
- Meningitis

Causes

Anthrax spores are formed by anthrax bacteria that occur naturally in soil in most parts of the world.

The spores can remain dormant for years until they find their way into a host. Common hosts for anthrax include wild or domestic livestock, such as sheep, cattle, horses and goats.

Herpes

Genital herpes is a common sexually transmitted infection caused by the herpes simplex virus (HSV). Sexual contact is the primary way that the virus spreads. After the initial infection, the virus lies dormant in your body and can reactivate several times a year. Genital herpes can cause pain, itching and sores in your genital area. But you may have no signs or symptoms of genital herpes. If infected, you can be contagious even if you have no visible sores. There's no cure for genital herpes, but medications can ease symptoms and reduce the risk of infecting others. Condoms also can help prevent the spread of a genital herpes infection.

Symptoms

Genital herpes

Most people infected with HSV don't know they have it because they don't have any signs or symptoms or because their signs and symptoms are so mild.

When present, symptoms may begin about two to 12 days after exposure to the virus. If you experience symptoms of genital herpes, they may include:

- **Pain or itching.** You may experience pain and tenderness in your genital area until the infection clears.
- **Small red bumps or tiny white blisters.** These may appear a few days to a few weeks after infection.
- **Ulcers.** These may form when blisters rupture and ooze or bleed. Ulcers may make it painful to urinate.
- **Scabs.** Skin will crust over and form scabs as ulcers heal.

During an initial outbreak, you may have flu-like signs and symptoms such as swollen lymph nodes in your groin, headache, muscle aches and fever.

Differences in symptom location

Sores appear where the infection entered your body. You can spread the infection by touching a sore and then rubbing or scratching another area of your body, including your eyes.

Men and women can develop sores on the:

- Buttocks and thighs
- Anus
- Mouth
- Urethra (the tube that allows urine to drain from the bladder to the outside)

Women can also develop sores in or on the:

- Vaginal area
- External genitals
- Cervix

Men can also develop sores in or on the:

- Penis
- Scrotum

Recurrences are common

Genital herpes is different for each person. The signs and symptoms may recur, off and on, for years. Some people experience numerous episodes each year. For many people, however, the outbreaks are less frequent as time passes.

During a recurrence, shortly before sores appear, you may feel:

- Burning, tingling and itching where the infection first entered your body
- Pain in your lower back, buttocks and legs

However, recurrences are generally less painful than the original outbreak, and sores generally heal more quickly.

Causes

Two types of herpes simplex virus infections can cause genital herpes:

- **HSV-1.** This is the type that usually causes cold sores or fever blisters around your mouth. HSV-1 is often spread through skin-to-skin contact, though it can be spread to your genital area during oral sex. Recurrences are much less frequent than they are with HSV-2 infection.
- **HSV-2.** This is the type that commonly causes genital herpes. The virus spreads through sexual contact and skin-to-skin contact. HSV-2 is very common and highly contagious, whether or not you have an open sore.

Because the virus dies quickly outside of the body, it's nearly impossible to get the infection through

contact with toilets, towels or other objects used by an infected person

Chickenpox

Chickenpox (varicella) is a viral infection that causes an itchy rash with small, fluid-filled blisters. Chickenpox is highly contagious to people who haven't had the disease or been vaccinated against it. Before routine chickenpox vaccination, virtually all people had been infected by the time they reached adulthood, sometimes with serious complications. Today, the number of cases and hospitalizations is down dramatically.

For most people, chickenpox is a mild disease. Still, it's better to get vaccinated. The chickenpox vaccine is a safe, effective way to prevent chickenpox and its possible complications.

Symptoms

Chickenpox infection appears 10 to 21 days after exposure to the virus and usually lasts about five to 10 days. The rash is the telltale indication of chickenpox. Other signs and symptoms, which may appear one to two days before the rash, include:

- Fever
- Loss of appetite
- Headache
- Tiredness and a general feeling of being unwell (malaise)

Once the chickenpox rash appears, it goes through three phases:

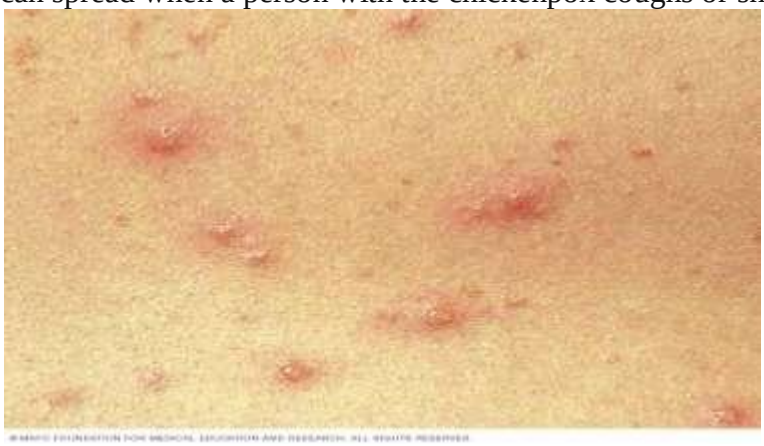
- Raised pink or red bumps (papules), which break out over several days
- Small fluid-filled blisters (vesicles), forming from the raised bumps over about one day before breaking and leaking
- Crusts and scabs, which cover the broken blisters and take several more days to heal

New bumps continue to appear for several days. As a result, you may have all three stages of the rash — bumps, blisters and scabbed lesions — at the same time on the second day of the rash. Once infected, you can spread the virus for up to 48 hours before the rash appears, and you remain contagious until all spots crust over.

The disease is generally mild in healthy children. In severe cases, the rash can spread to cover the entire body, and lesions may form in the throat, eyes and mucous membranes of the urethra, anus and vagina. New spots continue to appear for several days.

Causes

Chickenpox infection is caused by a virus. It can spread through direct contact with the rash. It also can spread when a person with the chickenpox coughs or sneezes and you inhale the air droplets.



(HPV). Warts

Genital warts are one of the most common types of sexually transmitted infections. Nearly all sexually active people will become infected with at least one type of human papillomavirus (HPV), the virus that causes genital warts, at some point during their lives. Women are somewhat more likely than men to develop genital warts.

As the name suggests, genital warts affect the moist tissues of the genital area. Genital warts may look like small, flesh-colored bumps or have a cauliflower-like appearance. In many cases, the warts are too small to be visible.

Like warts that appear elsewhere on your body, genital warts are caused by the human papillomavirus (HPV). Some strains of genital HPV can cause genital warts, while others can cause cancer. Vaccines can help protect against certain strains of genital HPV.

Symptoms

In women, genital warts can grow on the vulva, the walls of the vagina, the area between the external genitals and the anus, the anal canal, and the cervix. In men, they may occur on the tip or shaft of the penis, the scrotum, or the anus. Genital warts can also develop in the mouth or throat of a person who has had oral sexual contact with an infected person.

The signs and symptoms of genital warts include:

- Small, flesh-colored or gray swellings in your genital area
- Several warts close together that take on a cauliflower-like shape
- Itching or discomfort in your genital area
- Bleeding with intercourse

Genital warts may be so small and flat that they can't be seen with the naked eye. Sometimes, however, genital warts may multiply into large clusters.

Causes

The human papillomavirus (HPV) causes warts. There are more than 40 different strains of HPV that specifically affect the genital area. Genital HPV is spread through sexual contact. In most cases, your immune system kills genital HPV and you never develop signs or symptoms of the infection.

AIDS (candidiasis)

Candida are a group of common fungi that live on the skin and in the mouth. They are normally controlled by the immune system. However, if your immune system is weakened, *Candida* can grow on mucous membranes (the linings of body passages) or elsewhere in your body, causing symptoms known as candidiasis, candida or thrush.

HIV-negative people may develop candidiasis when their immune systems are temporarily depressed by factors such as stress, smoking or alcohol, or medical conditions such as diabetes. People taking antibiotics may have symptoms of candidiasis, because the antibiotic temporarily kills some of the harmless bacteria that inhabit the body, creating an imbalance that allows *Candida* to take their place. Using a type of medicine called inhaled corticosteroids (used to treat asthma and other conditions) can have the same effect.

Women often get candidiasis in the vagina, and men under the foreskin or the head of the penis, causing itching, burning or pain. Women are more at risk of candidiasis when they are pregnant. Candidiasis can be passed on from mother to baby during labour. It is also possible to have candidiasis in the mouth (oral thrush) or on the skin.

Among people with HIV, mild candidiasis in the mouth is relatively common even with a relatively high CD4 cell count (below 500). Women with HIV seem to get vaginal candidiasis more frequently than HIV-negative women.

Candidiasis becomes more common with lower CD4 cell counts. Recurrent infections can be a sign of HIV disease progression. Being on effective HIV treatment will prevent further damage to your immune system and make it much less likely you will have serious problems with candidiasis infections.

Symptoms

On the gums, tongue, inner cheek and/or upper throat (oropharyngeal candidiasis),*Candida* grows in white clumps that can be scraped away, or causes red patches called erythema.
Vaginal candidiasis can cause a discharge that can either be thick and yellowy-white, or clear and watery. It may sting when you urinate (pee).
It is possible for men to have genital thrush without having any symptoms. If you do have symptoms, they can include:

- irritation, burning, itching, redness or red patches under the foreskin or on the tip of your penis
- a white, curd-like discharge under the foreskin. You may also notice an unpleasant smell.

Candidiasis on the skin can cause a red, painful and itchy rash, which can then scale over and cause a white or yellow curd-like discharge.

Dermatomycoses (ring worms)

Dermatophytosis, also known as **ringworm**, is a fungal infection of the skin. Typically it results in a red, itchy, scaly, circular rash. Hair loss may occur in the area affected. Symptoms begin four to fourteen days after exposure Multiple areas can be affected at a given time.

About 40 types of fungi can cause ringworm. They are typically of the *Trichophyton*, *Microsporum*, or *Epidermophyton* type. Risk factors include using public showers, contact sports such as wrestling, excessive sweating, contact with animals, obesity, and poor immune function.[3][4]Ringworm can spread from other animals or between people Diagnosis is often based on the appearance and symptoms. It may be confirmed by either culturing or looking at a skin scraping under a microscope

Symptoms and Stages of a Ringworm Infection

The specific symptoms of ringworm depend on the location of the infection. They typically include:

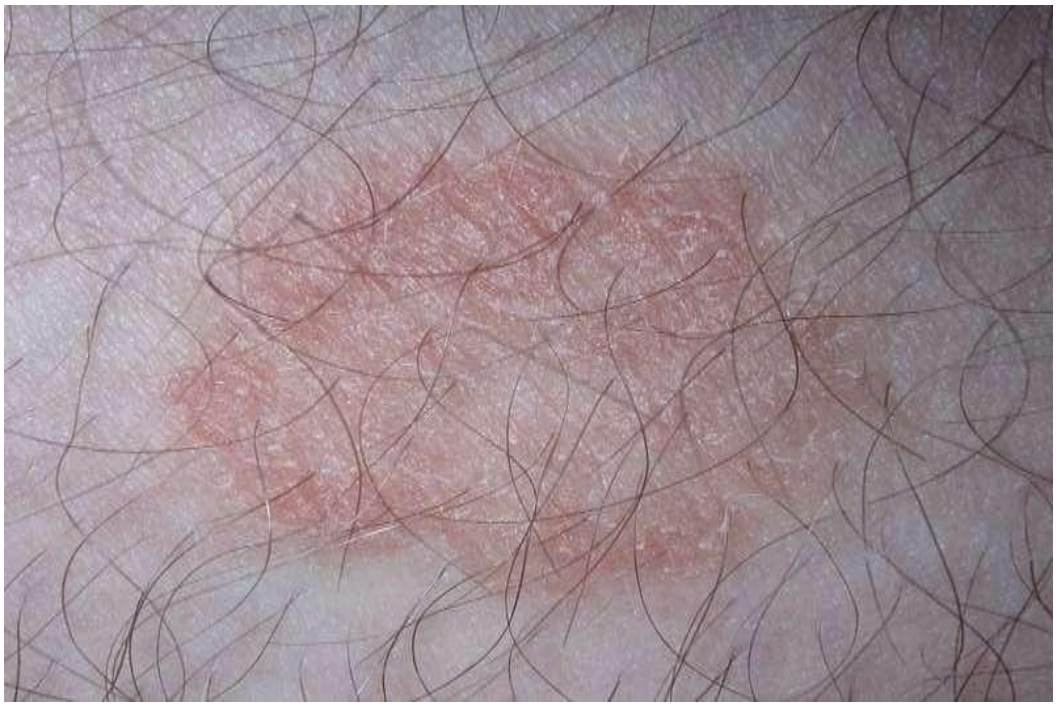
- Itchy skin
- Red, scaly, or cracked skin
- A ring-shaped rash (from which ringworm gets its name)
- Hair loss in the affected area (if hair was present)

The symptoms tend to appear between 4 and 14 days after the skin comes into contact with the fungi that cause ringworm. (1)
Ringworm typically starts out as red or pink skin patches (or spots) that may be either flat or slightly raised. In this initial stage, the sores may be moist, but more often they're dry, scaly, and itchy.
Over time, the rash will increase in size. Next, the center of the rash will start to clear up, leaving a ring-shaped infection with a red, raised border and a healthy-looking center (although the center may remain scaly and red).
If you scratch the rash you may break the skin, which could lead to a bacterial infection.
And if you immediately touch other areas of your body after scratching, you may also inadvertently spread the ringworm infection. So treating at the first sign of infection is vital.
Symptoms of ringworm can be different depending on the specific part of the body that's affected:

- **On the feet (tinea pedis, or "athlete's foot")**: Skin may become swollen, red, and itchy between the toes. The soles and heels of the feet may also be affected. In severe cases, blistering of the skin can occur.

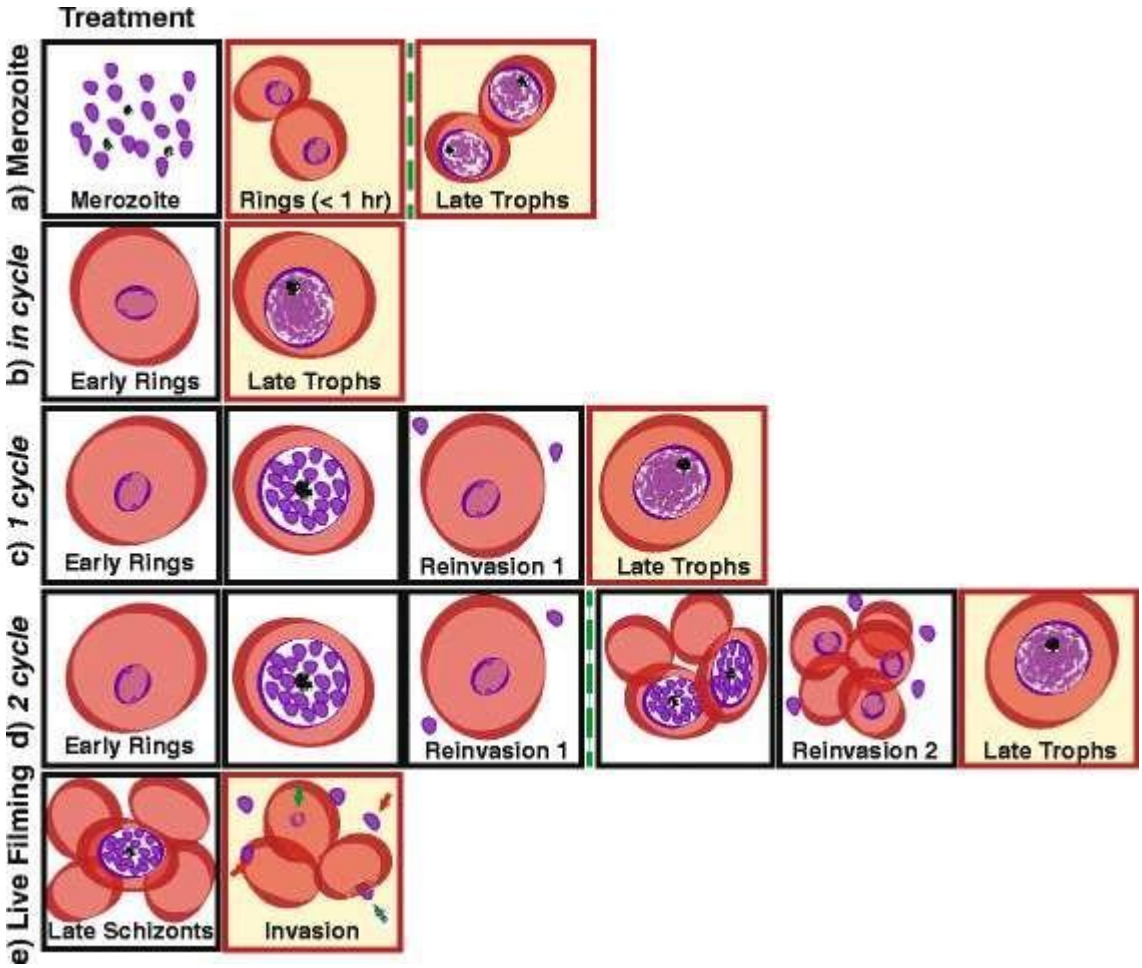
- **In the groin area (tinea cruris, or "jock itch")**: Itchy, scaly red spots usually appear on the inner thighs.
- **On the scalp (tinea capitis)**: Ringworm can look like an itchy, scaly, inflamed bald spot, and it can grow in size. Scalp infections are more common in children than adults.
- **In the beard (tinea barbae)**: Itchy red spots are visible on the cheeks, chin, and upper neck. The spots may become crusty and may cause hair that's affected to fall out.

On fingernails and toenails (onychomycosis): Nails become thick and abnormal in shape and color, and infection can spread from nail to nail. Onychomycosis often occurs in people who have athlete's foot for a prolonged period.



EXPERIMENT NO: 7

Study of various stages of malarial parasite in RBCs using permanent mounts



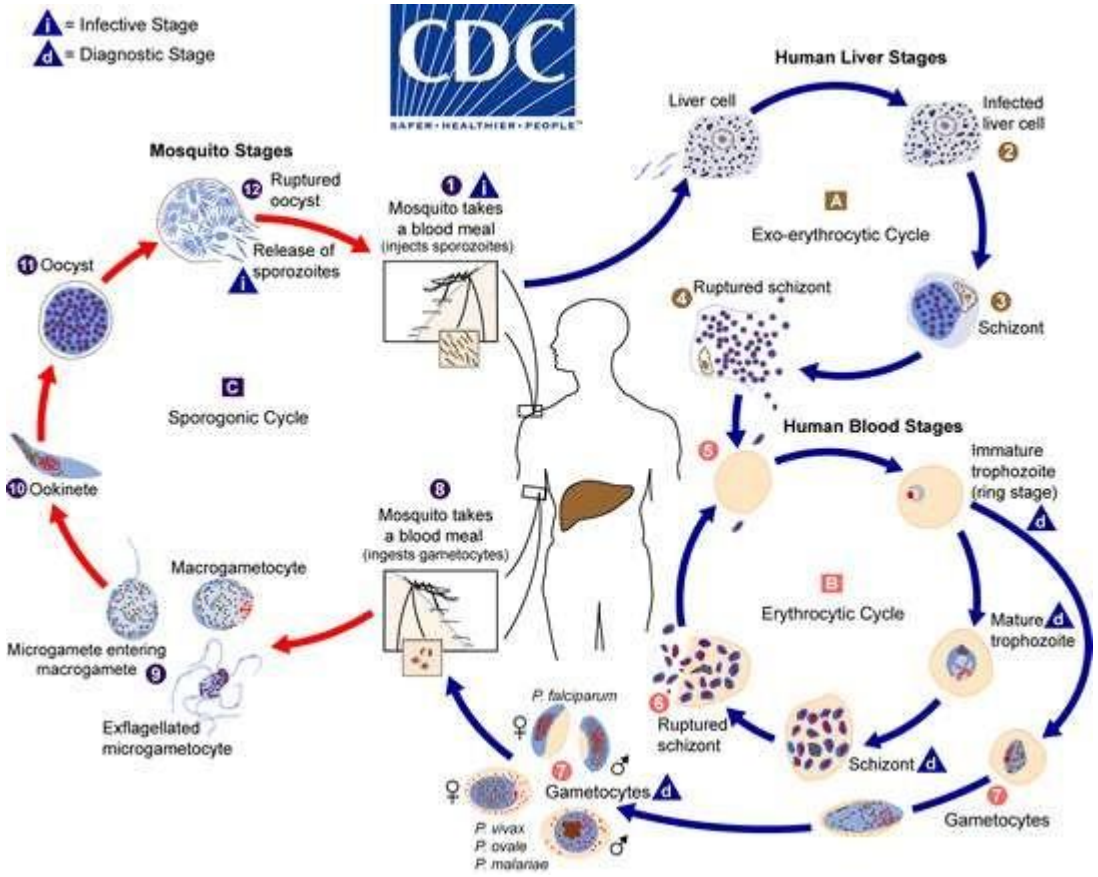
Lifecycle

The natural history of malaria involves cyclical infection of humans and female *Anopheles* mosquitoes. In humans, the parasites grow and multiply first in the liver cells and then in the red cells of the blood. In the blood, successive broods of parasites grow inside the red cells and destroy them, releasing daughter parasites ("merozoites") that continue the cycle by invading other red cells.

The blood stage parasites are those that cause the symptoms of malaria. When certain forms of blood stage parasites (gametocytes, which occur in male and female forms) are ingested during blood feeding by a female *Anopheles* mosquito, they mate in the gut of the mosquito and begin a cycle of growth and multiplication in the mosquito. After 10-18 days, a form of the parasite called a sporozoite migrates to the mosquito's salivary glands. When the *Anopheles* mosquito takes a blood

meal on another human, anticoagulant saliva is injected together with the sporozoites, which migrate to the liver, thereby beginning a new cycle.

Thus the infected mosquito carries the disease from one human to another (acting as a "vector"), while infected humans transmit the parasite to the mosquito, In contrast to the human host, the mosquito vector does not suffer from the presence of the parasites.



The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host ❶ . Sporozoites infect liver cells ❷ and mature into schizonts ❸, which rupture and release merozoites ❹. (Of note, in *P. vivax* and *P. ovale* a dormant stage [hypnozoites] can persist in the liver (if untreated) and cause relapses by invading the bloodstream weeks, or even years later.) After this initial replication in the liver (exo-erythrocytic schizogony Ⓐ), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony Ⓑ). Merozoites infect red blood cells ❺. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites ❺. Some parasites differentiate into sexual erythrocytic stages (gametocytes) ❼. Blood stage parasites are responsible for the clinical manifestations of the disease. The gametocytes, male (microgametocytes) and female

(macrogametocytes), are ingested by an *Anopheles* mosquito during a blood meal ❽. The parasites' multiplication in the mosquito is known as the sporogonic cycle **C**. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes ❾. The zygotes in turn become motile and elongated (ookinetes) ❿ which invade the midgut wall of the mosquito where they develop into oocysts ⓫. The oocysts grow, rupture, and release sporozoites ⓬, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites ❶ into a new human host perpetuates the malaria life cycle.

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