

I N D E X

Name ARASH . H . N . Std 11 Sec

Roll No. _____ Subject MICROBIOLOGY School/College _____

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INFECTIOUS DISEASES



① TYPHOID ★

① causative organism .

Salmonella Typhi (g-bacteria) . & P Typhi

② signs & symptoms

- * slow rise in temperature .
- * headache
- * abdominal pain .
- * splenomegally
- * weight loss
- * diarrhoea
- * constipation

③ Mode of Transmission

- * Faecal oral route
- * close contact with the patient
- * contaminated water & food .
- * Flies & cockroaches .

④ Diagnosis

- * Blood culture .
- * urine & stool culture
- * specific serologic test includes , WIDAL TEST .

⑤ prevention

- * wash hands frequently
- * drink hot water
- * Avoid eating raw vegetables
- * chase hot food

⑥ Treatment

- * Ciprofloxacin → 400mg through IV every 12 hrs .
- * Ceftriaxone → 2g through IV everyday .

② MALARIA

① causative organism.

plasmodium falciparum, vivax, oval, malariae (protozoal parasites)

② Signs & Symptoms

- * slow rise in temperature
- * headache
- * arthralgia.
- * hepatomegally
- * shivering.
- * vomiting

③ mode of Transmission

- * mosquito bites
- * mother to unborn child.
- * Blood transfusion.
- * By sharing needles.

④ Diagnosis.

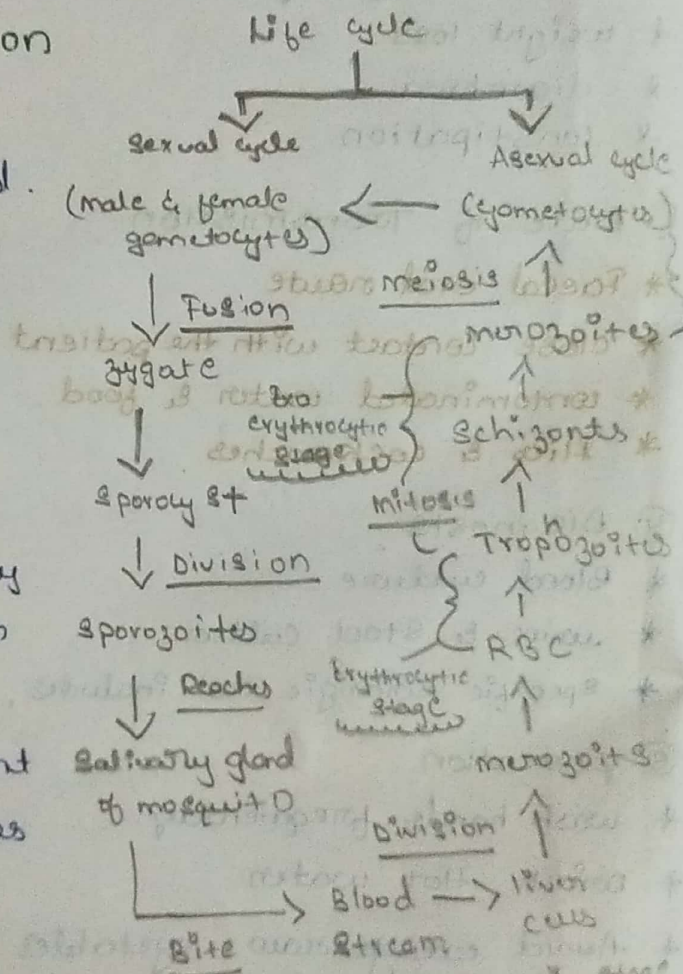
- * PCR Test.
- * Antigen detection
- * Fluorescent microscopy
- * malaria blood smear

⑤ prevention

- * use mosquito repellent
- * wear protective clothes
- * use bed nets

⑥ Treatment.

- * primaquine is ^{the} std medicine for malaria in the liver
- * chloroquine for malaria in blood.
- * some type of malaria are chloroquine resistant, so quinine is used.



② TUBERCULOSIS

① Causative organism.

Mycobacterium tuberculosis. (Narrow cut nor eg-)

② Signs & Symptoms

- * Fever
- * Cough
- * Fatigue
- * weight loss.
- * Night Sweats

③ mode of Transmission

- * By Inhaling sneeze droplets from infected individual.

Genetogony

④ Diagnosis.

- * Mantoux test
- * Acid fast Bacilli test (AFBT)
- * Interferon gamma release assay (IGRA)

⑤ prevention

- * Try ^{to} cover mouth during sneezing.
- * use N-95 mask.
- * Take BCG vaccine.

⑥ Treatment.

- * Isoniazid
- * Rifampin
- * pyrazinamide
- * Ethambutol
- * Streptomycin.

④ SYPHILIS

① Causative organism

Treponema pallidum (γ-bacteria)

② Signs & Symptoms

- * painless ulcers
- * sores
- * itching
- * fatigue
- * swollen lymph nodes
- * weight loss

③ Mode of Transmission

- * Sexual contact
- * blood transfusion
- * mother to unborn child

④ Diagnosis

- * Dark field microscopy
- * Venereal disease research laboratory test (VDRL)
- * Direct fluorescent antibody (DFA)

⑤ Prevention

- * use condoms with water based lubricants
- * use dental dams for oral sex
- * just have sex with one partner
- * having regular STD checkups

⑥ Treatment

- * penicillin → 2.4 MU IM
- * If allergic to penicillin go for Tetracyclines - 100mg orally 4 times a day
- * Ceftriaxone → 1g orally 14 days

① GONORRHEA

① causative organism

Neisseria gonorrhoea (g-bacteria)

② Signs & Symptoms

- * Rectal discharge
- * Urethral Infection
- * Endocervical Infection
- * Pharyngeal Infection

③ Mode of Transmission

- * sexual contact with infected person
- * Infected blood transfusion
- * Materno-fetal transmission

④ Diagnosis

- * Gram stained smear
- * ELISA
- * DNA probe techniques
- * Nucleic Acid Amplification Technique (NAAT)

⑤ Prevention

- * use condoms with water based lubricants
- * use dental dams for oral sex
- * just have sex with one partner
- * having regular STD checkups

⑥ Treatment

- * Ceftriaxone - 125 mg IM once
- * Cefepodoxin - 500 mg PO once
- * Cefixime - 400 mg PO once

⑥ CHOLERA

① Causative organism.

Vibrio cholerae (γ-bacteria)

② Signs & Symptoms.

- * Fever
- * Abdominal pain
- * Headache
- * Fatigue
- * Vomiting
- * Diarrhoea

③ Mode of Transmission

- * Faecal oral route
- * Contaminated water & food
- * From poorly cooked food

④ Diagnosis.

- * Stool culture
- * PCR technique
- * Dark field microscopy
- * Identification of *V. cholera* in stool

⑤ Prevention

- * Immunization by Sd cholera vaccine
- * Boiled water & cooked food should be taken
- * Good sanitation condition

* ⑥ Treatment

- * Ciprofloxacin → 500 mg 2 times / day
- * Norfloxacin → 400 mg 2 times / day
- * Tetracycline → 500 mg 2 times / day
- * Ampicillin or amoxicillin are generally for children

① AIDS

- ① causative organism
- Human Immunovirus

② Signs & Symptoms

- * Fever
- * Vomiting
- * Head ache
- * Diarrhoea
- * Sore throat
- * Lymphadenopathy
- * Weight loss
- * Night sweats
- * Nausea

③ Mode of Transmission

- * Sexual contact with infected person
- * Infected blood transfusion
- * Materno-faetal transmission

④ Diagnosis

- * ELISA
- * Western blot technique
- * Recommended DNA techniques
- * Viral Load
- * CD₄ count

⑤ Prevention

- * Use condoms with water based lubricants
- * Use dental dams for oral sex
- * Just have sex with one partner
- * Having regular STD checkups

⑥ Treatment

Regimen I

- * Zidovudine + Lamivudine + Nevirapine

Regimen II

- * Zidovudine + Lamivudine + Efavirenz

⑧ LEPROSY

① Causative organism

Mycobacterium leprae (G+ bacteria)

② Signs & Symptoms

- * Numbness
- * painless ulcers
- * Skin lesions
- * Eye damage
- * loss of digits

③ Mode of Transmission

- * Close contact with infected person
- * Spread through nasal secretion, droplets

④ Diagnosis

- * Nerve biopsy
- * Ziehl-Neelsen stain
- * Lepromin test
- * Liver Function test

⑤ Prevention

- * Avoid contact with droplets & nasal secretion
- * BCG vaccine along with *M. leprae* organism

⑥ Treatment

- * DAPSONE
- * Rifampin
- * Clofazimine

① HEPATITIS ★

① causative organism.

① 'Herpes virus'

Cytomegalus virus [CMV].

Eppstein Barr virus [EBV].

② Hepatotrophic virus.

Hepatitis A virus [HAV].

Hepatitis B virus [HBV].

Hepatitis C virus [HCV].

Hepatitis delta virus [HDV].

Hepatitis E virus [HEV].

② signs & symptoms

* Fever

* Headache.

* abdominal pain

* anorexia.

* fatigue.

* Nausea

* vomiting.

③ Mode of Transmission

* fecal-oral route

* by blood transfusion.

* iv drug abuse.

* tattooing.

* ear piercing

* sharing of razors.

* Sexual transmission.

④ Diagnosis

- * elevated alkaline phosphatase, γ -glutamyl transferase
- * elevated bilirubin levels
- * Elevated AST, ALT
- * positive test for IgM anti-HAV
- * RIA
- * ELISA
- * liver biopsy

⑤ Prevention

- * Maintaining hygiene
- * Immunization through vaccines
- * By Avoiding Infection

⑥ Treatment

① Non pharmacological

- * proper Sanitation, healthy diet, Avoiding hepatotoxic drugs & Alcohol
- * Hepatotoxic drugs includes quinine, cimetidine, paracetamol

② pharmacological treatment

- * verapamil
- * Sulphonamides
- * vaccine such as HBV vaccine
- * Antitherpes agents Includes Idoxuridine, Acyclovir, valacyclovir

10. AMOEBIASIS

① Causative organism

- * *Entamoeba histolytica* (protozoan parasite)

② Signs and symptoms

(i) Intestinal amoebiasis.

- * abdominal discomfort.

- * abdominal cramps

- * flatulence

- * diarrhoea

(ii) Hepatic amoebiasis

- * Fever

- * weight loss

- * Hepatomegaly

- * Jaundice

(iii) cerebral amoebiasis

- * Nausea.

- * vomiting

- * headache

③ mode of Transmission

- * contaminated soil or water

- * Faecal-oral route

- * Anal Sexual practise

④ Diagnosis

- * Stool test

- * Antibody tests

- * chest radiography

- * Liver function tests.

⑤ prevention.

- * wash hands thoroughly with soap and hot running water for at least 10 sec
- * wash your hands after using the toilet or changing baby's diaper and before handling food
- * clean bathroom and toilets often, pay particular attention to toilet seats and taps
- * Avoid sharing towels or face washers
- * Avoid raw vegetables in endemic area, as they are fertilized using human feces
- * Boil water or treat with Iodine tablets
- * Avoid eating street foods especially in public places
- * good Sanitary practice
- * Filtration of water supply

⑥ Treatment

- * metronidazole
- * Tinidazole
- * Nitroimidazole
- * Chloroquine.

10 mark important concepts



① classify bacteria on the basis of morphological features. Add a note on composition and function of cell wall.

Morphological classification.

① Spherical

* These are smallest bacteria which are spherical in shape

→ Micrococci :- when they occur singly.



Eg :- micrococci

→ Diplococci :- when they occur in pair



Eg :- Diplococcus pneumoniae

→ Streptococci :- when they occur in chain.



Eg :- Streptococcus lactis

→ Staphylococci :- when they are found in group.

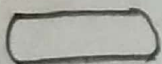


Eg :- Staphylococcus Aureus

② Cylindrical

* The no of rod shaped bacteria is far greater than spherical due to fact that surface area to volume is higher in rods

→ Bacillus :- when only one rod shaped structure represents Bacteria

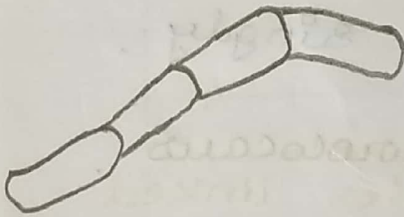


Eg :- Lactobacillus

-> Diplobacillus :- when rod shaped structure occurs in pair



-> Streptobacillus :- when rod shaped structure occurs in chain

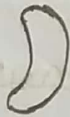


Ex :- Bacillus anthracis.

③ Spiral

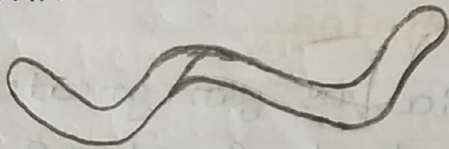
* Spiral bacteria have one or more twists, they are never straight.

-> vibrio :- They are comma shaped bacteria which cause cholera



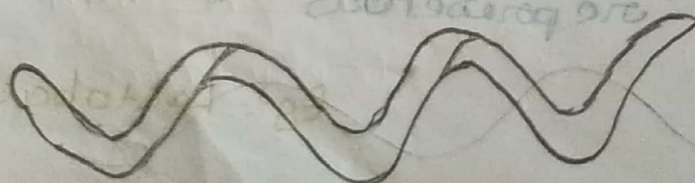
Ex :- vibrio cholerae

-> Spirilla :- These are longer rigid rods with several curves.



Ex :- Spirillum aurum

-> Spirochetes :- unlike spirilla, which use flagella to move, spirochetes move by means of an axial filament.



Ex :- Treponema pallidum.

* It is a flexible organism having several coils.

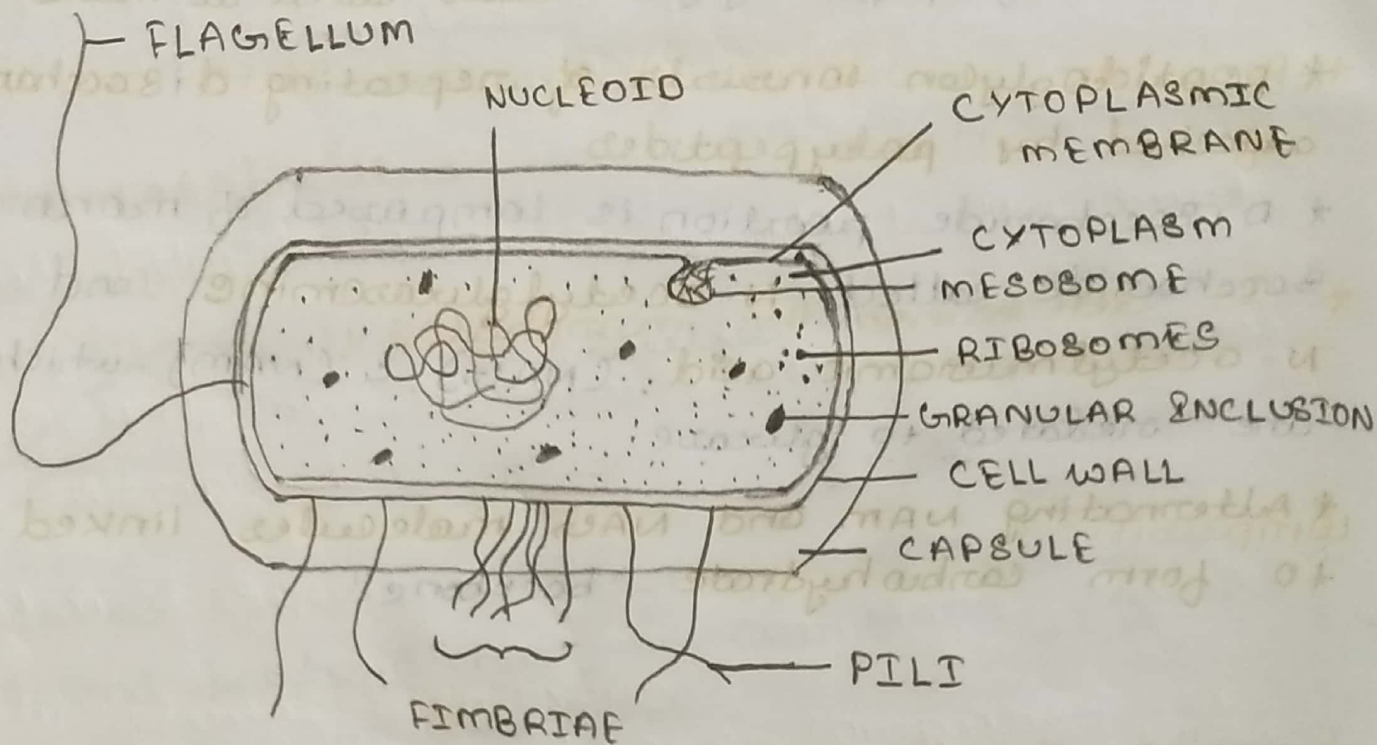
Composition of cell wall

- * The bacterial cell wall is composed of network of peptidoglycan (also called as murein)
- * peptidoglycan consists of repeating disaccharide attached by polypeptides
- * Disaccharide portion is composed of mono-saccharide called N-acetylglucosamine and N-acetylmuramic acid (NAG) & (NAM), which are related to glucose.
- * Alternating NAM and NAG molecules linked to form carbohydrate "backbone"

Function of cell wall

- * protects cells against osmotic shock and physical damage
- * Regulates substance transport into and out of cells
- * mineral storage of cells
- * It determine the morphology

② Draw ultra structure of typical Bacteria
write composition and function of its organelles



External Structures

① cell wall

Composition

- * The bacterial cell wall is composed of network of peptidoglycan (also called as murein)
- * peptidoglycan consists of repeating disaccharide attached by polypeptides.
- * Disaccharide is composed of monosaccharide called N-acetylglucosamine (NAG) & N-acetyl-muramic acid (NAM) which are related to glucose
- * Alternating NAM and NAG molecules linked to form carbohydrate "back bone"

Function

- * protects cell against osmotic shock and physical damage
- * Regulates substance transport into and out of the cell
- * mineral storage of cells
- * It determines the morphology.

② Capsule.

Composition.

- * Capsule is composed of 98% water, 2% polysaccharide or poly peptide

Function.

- * Capsule resist phagocytosis by WBC's.
- * It prevents the cell from desiccation.

③ Flagella.

Composition.

- * Flagella is composed of flagellin protein.

Function.

- * It helps in motility of the bacteria

④ pili

Composition

* pili is composed of pilin protein

Function

* pili helps the bacteria to attach the host cells

* pili helps in transfer of DNA from donor to recipient

Internal Structure

① Cytoplasmic membrane

* Cytoplasmic membrane composed of phospholipids and proteins

Function

* It maintains intracellular osmotic pressure

* It consists of enzymes capable of catalyzing chemical reaction.

② Cytoplasm

Composition

* Cytoplasm contains 90% water, protein, lipids, inorganic ions

* DNA and Ribosomes are the major structure in the cytoplasm

Function

- * It helps to distribute water, oxygen and other substances throughout the cell.

③ Mesosomes

- * It is a folds in cytoplasmic membrane.

Function,

- * Septal mesosomes participate in DNA Replication and cell division.
- * Non-septal mesosomes participate in secretion.

④ Ribosomes

Composition.

- * Each Ribosomes consists of smaller sub-unit and large subunit. Each subunit consists of protein and rRNA.

Function.

- * It helps in protein synthesis.

⑤ NUCLEOID

Composition

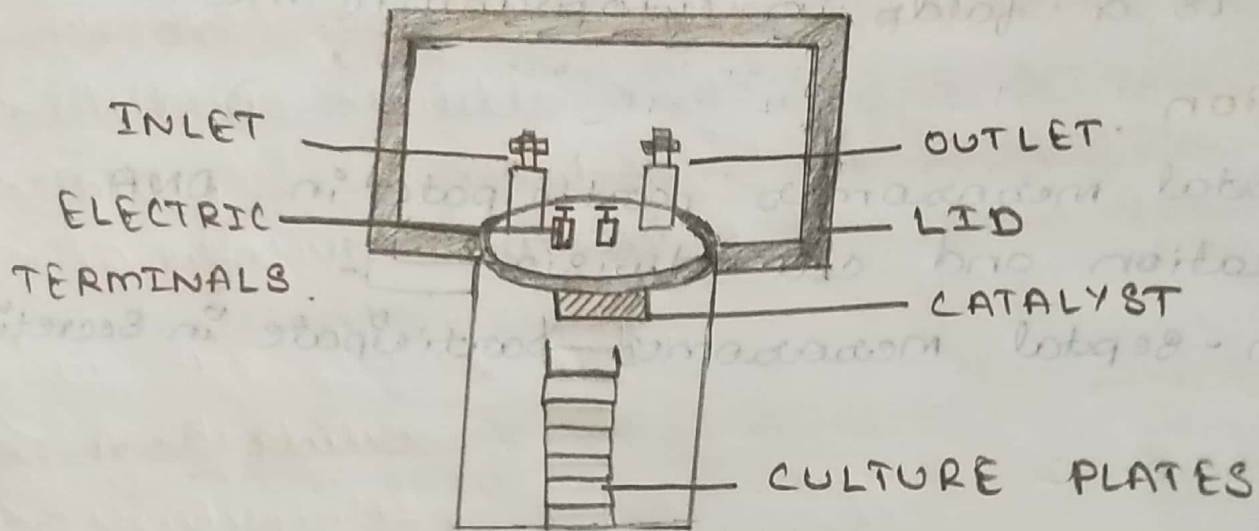
- * It contains circular molecule of double stranded DNA.

Function

- * It controls all the activities of the cell.
- * It stores hereditary information of the cell.

③ Explain any four efficient methods of cultivation of anaerobic bacteria.

① McIntosh and Fildes Anaerobic Jar



- * It consists of metal jar with metal lid which can be clamped with screw.
- * The lid has two tubes, one acts as gas inlet and the other one has outlet.
- * Lid has two terminals which can be connected to electrical supply.
- * Culture plates are placed inside the jar.
- * The outlet tap is closed and inlet tube is connected with Hydrogen gas cylinder.
- * After filling Hydrogen gas electrical terminals are connected and palladium is heated.
- * Palladium acts as catalyst for combination of Hydrogen and residual oxygen. It ensures complete anaerobiosis.

② GNASPAK.

- * This method consists of envelope and jar
- * GNASPAK envelope contains 3 tablets
 - ① citric acid
 - ② sodium carbonate
 - ③ sodium borohydride.
- * It generates hydrogen and CO_2 on addition of water
- * Hydrogen combines with oxygen to produce water and thus creation of anaerobiosis.

③ candle jar method.

- * Culture plates are placed in air tight containers. Light candle is kept before lid is sealed
- * Burning candle use up all the oxygen before it get extinguished and create anaerobiosis

④ Robertson's cooked meat medium

- * It consists of fat free, minced, cooked meat in broth.
- * It permits the growth of even strict anaerobes and indicates their proteolytic activities by meat turned black.

④ Classify bacteria on the basis of oxygen
PH, temperature requirements, Add note
on effect of hyper and hypotonicity of
bacteria?

* on the basis of oxygen Requirement.

- obligate aerobes
- obligate anaerobes
- Facultative anaerobes

* on the basis of PH

- Acidophiles
- Neutrophiles.
- Alkalophiles

* on the basis of temperature.

- Thermophiles
- mesophiles
- psychrophiles

* Effect of Hypertonicity.

- If the environment is hypertonic, the water concentration is greater inside the cell the solute concentration is higher outside.
- The Interior of the cell is hypotonic to the surrounding hypertonic environment so water goes out of the cell.

* Effect of Hypotonicity.

→ If the environment is hypotonic, the water concentration is greater outside the cell and the solute concentration is higher inside.

→ The interior of the cell is hypertonic to the hypotonic surroundings. Water goes into the cell.

⑤ Differentiate blue gram positive and gram negative cell wall. Add a note on principle & procedure of gram staining technique & Acid fast staining technique.

GRAM POSITIVE BACTERIA

- * It retains crystal violet during gram staining

- * Lipid content is low in cell wall

- * Peptidoglycan layer is thick.

- * Periplasmic space is absent

- * Cell wall is smooth

GRAM NEGATIVE BACTERIA

- * It does not retain crystal violet during gram staining

- * Lipid content is high in cell wall

- * Peptidoglycan layer is thin

- * Periplasmic space is present

- * Cell wall is wavy.

Gram's Staining

Reagents: Gram's Iodine, Ethyl alcohol

Stains: Crystal violet, Safranin.

Principle

* Gram's staining technique divides bacteria into two groups.

- (i) Gram positive: These which retain primary stain
- (ii) Gram negative: These which lose primary stain on application of decolouriser and take the colour of counterstain.

Reagents

(1) primary stain (Crystal violet).

* It stains all the cells

(2) mordant (Gram's Iodine)

* It forms crystal violet - Iodine - Mg Ribonucleate complex in Gram positive bacteria

* This complex is not formed in Gram negative bacteria as Mg-Ribonucleate is absent in cell wall.

(3) Decolourising agent (Ethyl alcohol).

* On application of decolourising agent to Gram positive cell wall which contains less lipid results in shrinkage of cell wall. Thus CV-I-Mg ribonucleate complex is retained in the cell.

* Decolourising agent extracts lipid from Gram negative bacteria, hence there is increase in permeability. Due to this CV-I complex is extracted and cell gets decolourised.

(4) Counterstain (safranin)

* This is used to stain those cells that have been previously decolourised.

procedure

- * prepare a smear from bacterial suspension
Allow to Air dry then heat fix it.
- * cover the smear with crystal violet and keep for 1 min
- * wash the smear with water and cover it with Gram's Iodine for 1 min
- * wash the smear with water and decolourise with Ethyl alcohol
- * wash the smear with water and counterstain with safranin
- * wash the smear with water allow it to dry.
- * put a drop of oil on the smear and examine under oil - immersion objective.

Acid-fast staining

Stains: Carbol fuchsin, methylene blue.

Reagents: Acid - Ethyl alcohol.

Principle

- * Some bacteria resist decolourisation by both acid and alcohol and hence they are referred as acid-fast organisms
- * Acid-fast cells have high lipid content in its cell wall hence it is difficult to stain. so penetration of dye is facilitated with α phenol once the cell is stained it is difficult to decolourise.

Reagents

① Primary stain (Carbol fuchsin)

- * Carbol fuchsin, a phenolic stain that is soluble in lipoidal material
- * All cells appear red after application of primary stain.

② Decolourising agent (acid-ethyl alcohol)

- * on application of decolourising agent, acid fast cells show resistance to decolourisation.
- * Non-acid fast cells show decolourisation due to lack of cellular waxes.

③ Counter stain (methylene blue)

- * Non-acid fast bacteria absorb the counter stain and appear blue.
- * acid fast cells retain the red colour of primary stain.

Procedure

- * prepare a smear from bacterial suspension
- Allow to Air dry and then heat fix it
- * Cover the smear with carbol fuchsin and heat for 5 min.
- * wash the smear with water and decolourise with Acid-alcohol
- * wash the smear with water and counter-stain with methylene blue.
- * wash the smear with water & allow it to dry.
- * put a small drop of oil on the smear and examine under oil immersion objective.

⑥ Mention methods used for identification of Bacteria. Explain biochemical tests.

Staining Techniques.

- ① Monochrome staining
- ② Negative staining
- ③ Gram staining
- ④ Acid fast staining

Biochemical Tests

- ① Indole production Test ^{Kovacs's}
- ② Citrate utilisation Test ^{citrate blue}
- ③ Methyl Red Test ^{(MRVP) tests}
- ④ Voges-Proskauer Test ^{Barritt's}
- ⑤ Starch hydrolysis
- ⑥ Gelatin hydrolysis

IMViC Test.

* It is used to differentiate gram negative intestinal bacteria on the basis of enzymatic reaction.

Indole production Test.

Culture: Nutrient broth culture of *Escherichia coli* and *Enterobacter aerogenes*

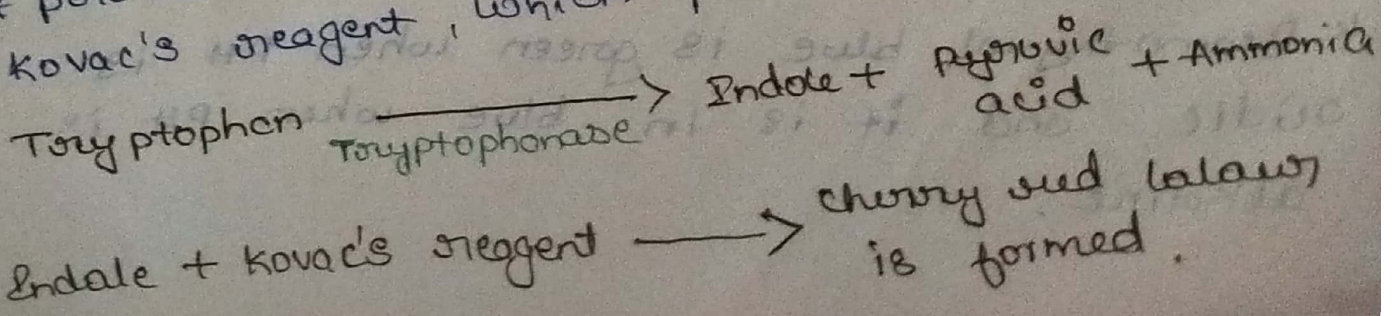
media: - SIM broth

Reagent: KOVAC'S Reagent

Principle:

* Tryptophan, an essential amino acid present in SIM Broth, is oxidized by some bacteria by the enzyme Tryptophanase resulting in formation of Indole, Pyruvic acid, Ammonia

* production of Indole is detected by adding Kovac's reagent, which produces cherry red colour



procedure

- * prepare SIM broth medium and inoculate with *E. coli* and *Enterobacter aerogenes*
- * Incubate the tubes at 37°C for 48 hours
- * After Incubation, Add 1ml of Kovac's Reagent

Observation & Result

- * Examine the colour in each test tube
- * Development of cherry red colour in *E. coli* tube is Indole positive.
- * Absence of cherry red colour in *Enterobacter aerogenes* is Indole negative.

Citrate utilization Test

Culture:- Nutrient broth culture of *E. coli* and *Enterobacter aerogenes*

media:- Simmons Citrate broth.

Reagents:- Bromothymol blue.

Principle:-

- * Citrase is an enzyme produced by microorganisms out on citrate to yield pyruvic acid and CO_2
- * CO_2 combines with sodium and water to form sodium carbonate (alkaline product)
- * Bromothymol blue is green when pH is acidic and it is in blue when pH is alkaline.

Citric acid $\xrightarrow{\text{citrase}}$ pyruvic acid + CO_2

$\text{CO}_2 + 2\text{Na}^+ + \text{H}_2\text{O} \longrightarrow \text{Na}_2\text{CO}_3$ (alkaline product)

procedure

- * prepare Simmon's citrate broth and inoculate with *E. coli* and *Enterobacter aerogenes*.
- * Incubate the tubes at 37°C for 48 hours
- * After incubation add 1ml of Bromothymol blue

observation & Result

- * Examine the colour in each test tube.
- * Development of blue colour in *Enterobacter aerogenes* tube is citrate positive.
- * Absence of blue colour in *E. coli* tube is citrate negative.

Methyl-Red Test

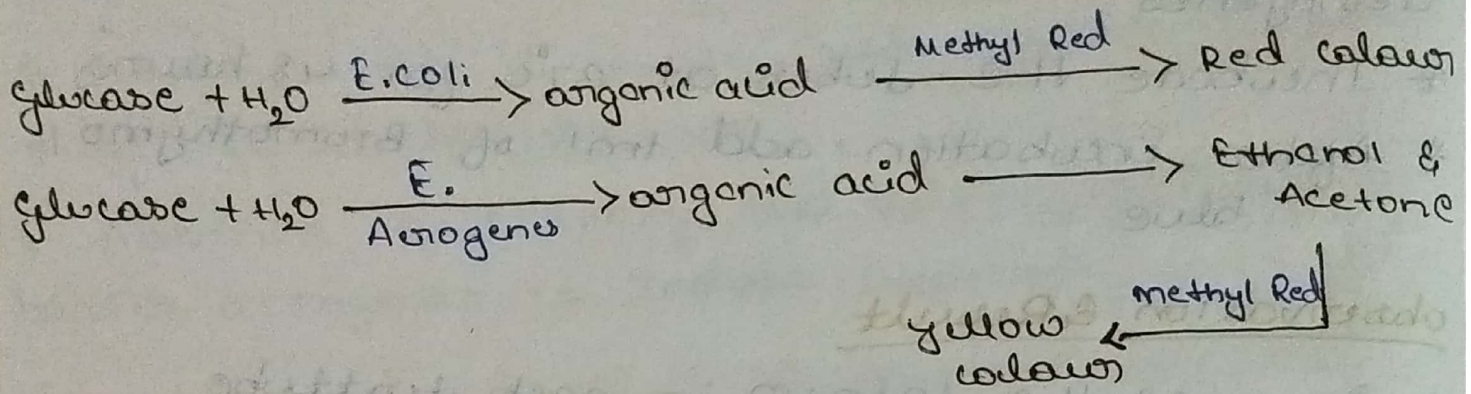
Culture: Nutrient broth culture of *E. coli* and *Enterobacter aerogenes*

media: MR-VP broth

Reagents: methyl red indicator.

Principle:

- * Glucose is oxidized by *E. coli* and *Enterobacter aerogenes* to produce organic acids.
- * The low acidic pH (4) is maintained by *E. coli*, but *Enterobacter aerogenes* enzymatically converts these acids into non-acids like ethanol and acetone, resulting in elevated pH (6).



Procedure

- * Prepare MR-VP broth and inoculate with *E. coli* and *Enterobacter aerogenes*.
- * Incubate the tubes at 37°C for 48 hours.
- * After incubation add 1 ml of methyl red.

Observation & result

- * Examine the colour in each test tube.
- * Development of red colour in *E. coli* tube at pH (4) is a positive test.
- * Development of yellow colour in *Enterobacter aerogenes* tube at pH (6) is a negative test.

Voges Proskauer Test

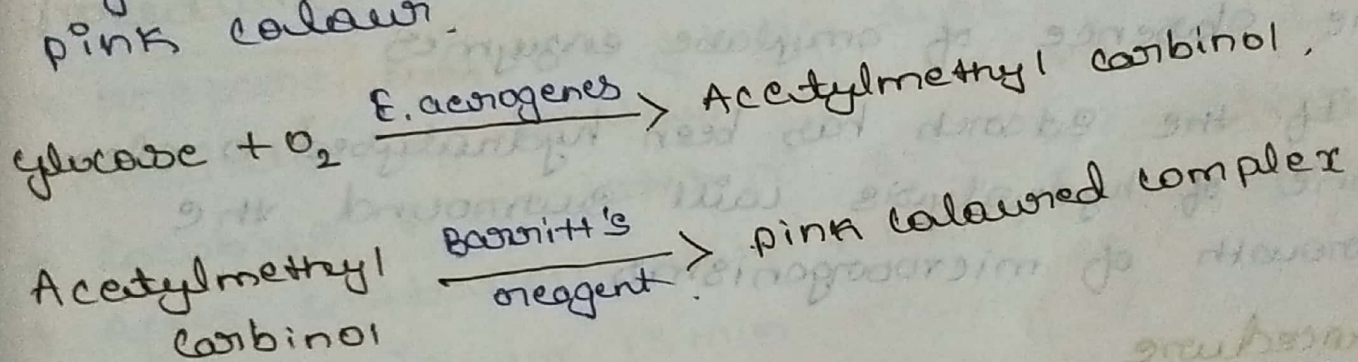
Culture: Nutrient broth cultures of *E. coli* and *Enterobacter aerogenes*

Media: MR-VP broth.

Reagent: Barritt's reagent

Principle.

- * Glucose is oxidized by some microorganisms to yield neutral product Acetylmethyl carbinol
- * production of Acetylmethyl carbinol is detected by adding Barritt's reagent, which produces pink colour.



procedure

- * prepare MR-VP broth and inoculate with *E. coli* and *Enterobacter aerogenes*
- * Incubate the tubes at 37°C for 48 hours
- * After incubation add 1 ml of Barritt's reagent.

Observation & results.

- * Examine the colour in each test tube.
- * Development of pink colour in *E. aerogenes* tube is a positive test.
- * Absence of pink colour in *E. coli* tube is Acetylmethyl carbinol negative.

Starch Hydrolysis

Culture: Nutrient broth culture of *Bacillus Subtilis* and *Escherichia coli*

media: Starch agar

Reagent: Gram's Iodine solution

Principle.

- * Starch Agar is used to demonstrate the hydrolytic activities of amylase to yield maltose and glucose
- * Starch in presence of Iodine will form blue/black colour to the medium. Indicating the absence of amylase enzymes
- * If the starch has been hydrolysed a clear zone of hydrolysis will surround the growth of microorganisms

procedure

- * prepare starch agar plate, make streaks
- * Inoculation of *Bacillus Subtilis* & *E. coli*
- * Incubate the plates at 37°C for 48 hrs
- * After incubation, Flood the plates with Gram's Iodine solution

observation & result.

- * Observe the colour in each plate
- * Absence of blue or black colour in *Bacillus Subtilis* tube is amylase positive
- * presence of blue or black colour in *E. coli* tube is amylase negative.

Gelatin hydrolysis

culture: Nutrient broth culture of *Bacillus subtilis* and *Escherichia coli*

media: Nutrient gelatin tubes

principle:

- * Nutrient gelatin is used to demonstrate the hydrolytic activities of gelatinase to yield amino acids
- * once degradation occurs, even at low temperature, it will not restore the gel.

procedure.

- * Prepare Nutrient gelatin tube, make stab inoculation of *Bacillus subtilis* and *E. coli*
- * Incubate the plates at 37°C for 48 hrs
- * After incubation, place tubes in refrigerator at 4°C for 15 min.

observations and Result.

- * observe the state of medium in each tube.
- * Absence of liquified gelatin in *E. coli* tube is gelatinase negative.
- * presence of liquified gelatin in *Bacillus subtilis* is gelatinase positive.

⑦ what is pure culture ? write in detail about Isolation of pure culture.

Pure culture

* It is a group of organism, all of which are progeny of single organism.

① Surface plating

* It is the method employed in clinical bacteriology.

* It enables the Isolation of distinct colonies which may be picked out for further purification and study.

② pretreatment with bacteriocidal substances

* pure culture may be obtained by pretreatment of specimens with appropriate bacteriocidal substances which destroy the unwanted bacteria.

* It is the standard practise for the Isolation of tubercle bacilli from sputum by treatment with alkali, acid or other substances.

③ optimum temperature.

* Separation of bacteria on the basis of different optimum temperature can be done by incubating at different temperatures.

* only thermophilic bacteria grow at 60°C .

④ Selective filter method

- * Bacteria of different sizes may be separated by selective filters.
- * It is used to separate virus from bacteria.

⑤ Coraigie's tube method,

- * Separation of motile and non-motile bacteria can be done using coraigie's tube.
- * It contains narrow tube opens at both ends which contain agar is placed in the medium in such a way that it projects above the level of medium.
- * The mixture is inoculated in the tube.
- * On incubation, the motile bacteria alone travels the agar and appear on top of the medium.

⑧ Enumerate methods for Total counting & viable counting. Explain any 2 methods for Total counting and viable counting.

for Total counting
membrane filtration

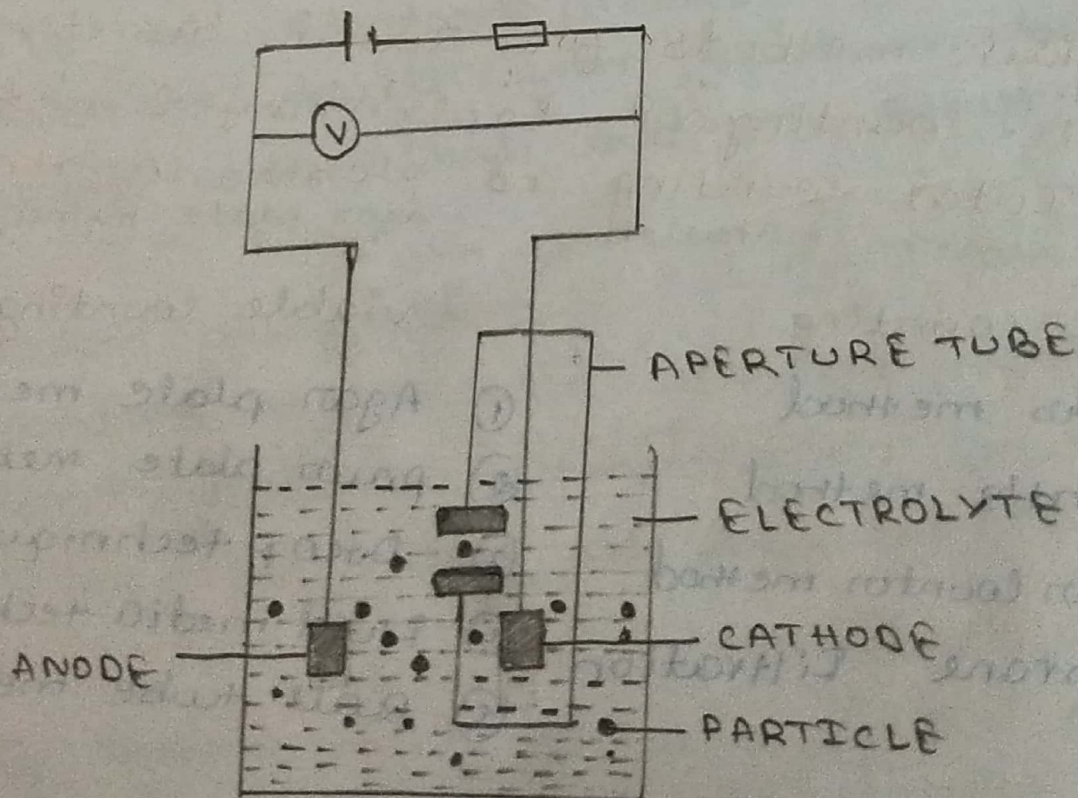
viable counting.

- Total counting
- ① Breed's method
 - ② Wright's method
 - ③ Coulter counter method
 - ④ membrane filtration

- ① Agar plate method
- ② pour plate method
- ③ Drop technique
- ④ Fluid media technique
- ⑤ Roll tube method

① Coulter counter method

- * particles are suspended in an electrically conductive fluid
- * Suspension flows through a suitable aperture with an immersed electrode on either side
- * particle concentration is arranged so that only one particle travels through the aperture at a time
- * As the particle passes through the aperture, some electrolyte is displaced and changes the resistance b/w the electrodes which causes a pulse in the voltage.
- * The magnitude of the pulse will be proportional to the volume of the particle.



② membrane filtration

- * provided the suspension is sufficiently dilute the membrane filtration method can be employed for counting.
- * The organisms retained on the filter are stained, dried and treated with immersion oil to make it transparent.
- * The organisms are counted on microscope
- * The no of bacteria collected on the filter from known volume of suspension can be calculated from the knowledge of field area and filtration area of the membrane.

③ Roll tube method

- * Roll tubes are prepared by adding diluted organism to test tube containing small volume of nutrient agar.
- * Test tube should be rotated almost horizontally under cold water until the medium set in a thin film around inside of the tube.
- * After Rolling, bottles are incubated and inverted.

① Agar plate method

④ Drop technique

- * suspension is diluted to contain about 80 organisms per cm^3
- * using dropping pipette, two drops of each dilution are dropped on to each plate
- * After 20 min the drops have been absorbed by the medium and then the plates are incubated inverted
- * counts can be examined in shorter time

⑤ Explain principle, procedure, application, demerits and diagram of sterilization using autoclave.

principle.

- * moist heat is the saturated steam under pressure.
- * Microorganisms are destroyed by moist heat due to coagulation of proteins in the cell
- * upon heating, wet protein release free -SH group and form smaller peptide chain, which in turn form new complexes different from original protein.

procedure.

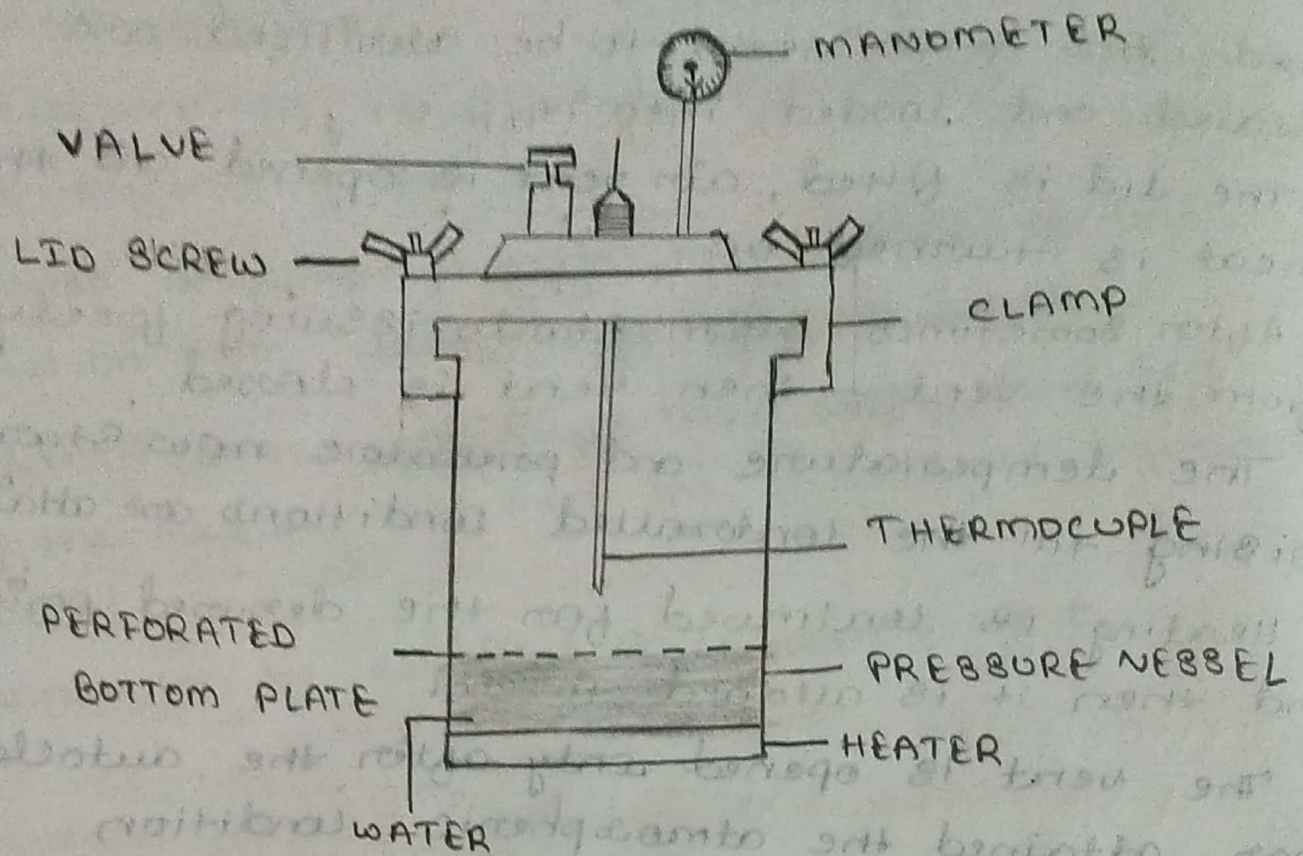
- * Two litres of water is placed in the vessel and the containers to be sterilized are sealed and loaded into it.
- * The lid is fitted, air vent is opened and the heat is turned on.
- * After sometimes steam starts issuing freely from the vent, then vent is closed.
- * The temperature and pressure now start rising till the controlled conditions are attained.
- * Heating is continued for the desired period and then it is allowed to cool.
- * The vent is opened only after the autoclave has attained the atmospheric condition.
- * The lid is opened and the autoclave is unloaded.

Application.

- * used in sterilizing culture media, Instruments
- * Sterilize syringes, Transfusion equipments
- * used in sterilizing cotton wool, cloth wrappers.

Demerits.

- * Some plastic wares melts in high temperature.
- * Many chemicals breakdown during sterilization.
- * Sterilizing large containers requires extra time.
- * It is unsuitable for powders, oils.



10). Explain, principle, procedure, application, demerits, mechanism of sterilization, heat transfer of sterilization using Hot air oven?
 principle [mechanism of sterilization].

- * Sterilizing by dry heat is accomplished by conduction.
- * The heat is absorbed by the surface, then passes towards the centre.
- * Eventually temperature reach Entirely required for sterilization.
- * Dry heat does most of the damage by oxidizing molecules. The essential cell constituents are destroyed and the organism dies.

* The temperature is maintained for almost an hour to kill the Resistant species.

procedure.

* The articles to be sterilized are placed in the hot air oven and the door is closed.

* The heaters and fans are switched on.

* Once the oven has reached the sterilization temperature, the variation of temperature should not exceed $\pm 5^{\circ}\text{C}$.

The temperature variation is temperature difference between centre and any other points on chamber.

* Thermocouples are fitted in the article. These thermocouples indicate the temperature at the centre of the articles.

* After hot air oven has been maintained for the required holding time, current is withdrawn and the oven is allowed to cool.

* The door of the oven is not opened for unloading until oven temperature attain the atmospheric condition.

Application

* Employed in sterilizing glasswares, metalwares

* In sterilization of powders, oil substances

* used to sterilize surgical instruments,

Non absorbable Sutures.

Demerits

- * Dry heat Sterilization cannot be used for surgical dressing, as the natural moisture present in them evaporates.
- * Rubber, plastic cannot withstand the high temperature.
- * periodic validation is required.
- * The penetration power of dry heat is low.

Process of Heat transfer -

① Conduction

- * The process of heat transfer along the perforated shelves is limited.
- * Because there is Insufficient contact between the shelves and the loads.

② Convection

- * Air is a poor conductor of heat. Hence considerable proportion of air must pass over the load.
- * In the absence of fan, there is restricted movement of air and uneven heat distribution. Thus a fan is fitted at the back of oven.

③ Radiation

- * heaters are fitted in the oven walls, the radiation transfer heat to the articles placed near the oven walls.
- * This causes loads near the wall being heated to greater extent when compared to loads at the centre.

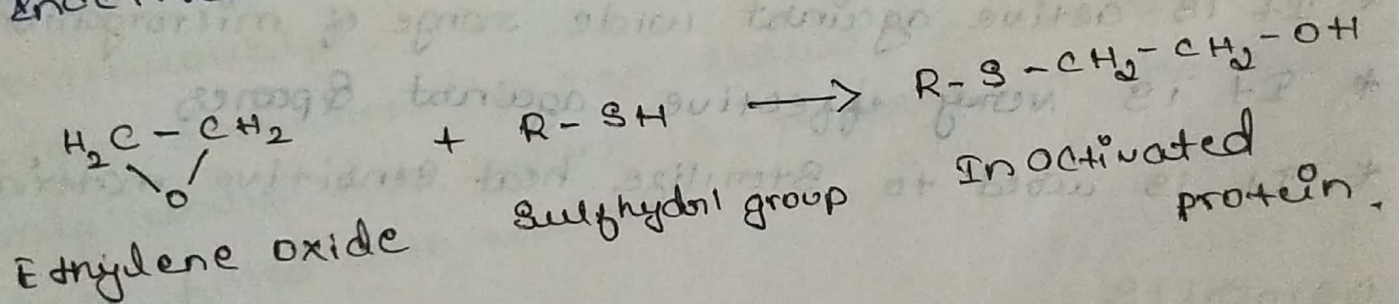
① Explain mechanism of action, principle, procedure, applications, factors affecting sterilization, merits & demerits of sterilization using EO?

principle [mechanism of action]

* EO attacks the functional group of proteins in microorganisms

* There is an exchange of H-atom of sulphhydryl group with one alkyl group of ethyleneoxide radical, This results in alkylation

* The modified protein of microorganism gets inactivated resulting in microbial death.



procedure

* The material is placed in modified autoclave

* moisture is introduced to provide relative humidity.

* Ethylene oxide is then admitted, pressure required is maintained throughout the exposure period.

* gas is then exhausted, filtered air is introduced into the chamber until atmospheric pressure is attained.

Applications

This method employed to sterilize

- * medical devices
- * biological preparation
- * Catgut
- * antibiotics
- * Hospital beddings
- * plastic materials

Merits

- * Ethylene oxide has good penetrating power.
- * It is effective even at low temperature
- * It is active against wide range of microorganisms
- * It is very effective against spores
- * It is used to sterilize heat sensitive materials

Demerits

- * It has relatively slow action upon microorganisms
- * It requires aeration to remove residual ethylene oxide

Factors affecting sterilization by EO

- (i) Concentration of EO
- (ii) Humidity of the sterilization
- (iii) Temperature of sterilization
- (iv) Time of exposure
- (v) physical nature and permeability of load
- (vi) Type & No of microorganism

⑫ Explain source, mechanism of sterilization, merits, demerits, application of sterilization using gamma radiation?

Source of gamma Radiation

Radionuclides i.e Cobalt-60, cesium-137.

mechanism of sterilization.

- * The unstable atoms of Cobalt-60 on disintegration emits two gamma rays in series.
- * The articles to be irradiated are packed and passed on conveyor belt to radiation chamber.
- * The radiation source emits γ -rays continuously.
- * The radiation source should be elevated only under use which is present in hollow tank filled with water.

merits

- * packaged pharmaceutical products can be irradiated
- * Radiation of a material is not depend on its thermal conductivity.
- * Energy output is reliable
- * Maintenance cost of Radiation source is less.

Demerits

- * Due to high penetrating power high shielding of workers is required.
- * The radiation source emits continuously which cannot be switched off.
- * Radiation scatters in all directions, resulting in reduced efficiency.

Application of Sterilization

Gamma rays are used in sterilization of:

- (i) Surgical Instruments
- (ii) Catgut
- (iii) Transfusion Equipment
- (iv) Surgical dressing like adhesive, bandages
- (v) Vaccines
- (vi) Packing materials

- ⑬ Explain principle, procedure, merits, demerits of sterilization by membrane filtration?

Principle

- * This technique involves filtering through special sterile filter.

- * These filters are made of cellulose and have 0.5μ diameter pores.
- * A grid pattern is typically printed to facilitate colony counting.
- * When the sample is filtered, bacteria in the sample are trapped on the surface of the filter.
- * The filter is then carefully removed and examined.

procedure

- * membrane filters are available in the form of circular disks.
- * glass holders are used for supporting the membrane filter.
- * Small volume are filtered under vacuum, large volumes are filtered under pressure provided by nitrogen gas.
- * After the filtration, the microorganisms which are collected on the membrane are examined.

merits

- * Insignificant adsorption of medicament.
- * pressure can be applied up to 7 bars.
- * chance of contamination is less.
- * microorganisms can be collected & studied.
- * Heat labile bacteria can be separated by membrane filters rather than sterilization by heat.

* membrane particles do not penetrate from membrane to filtrate

Demerits

- ① They get block very often
- ② membrane get dissolved in organic solvents like ketones and esters

14. Add a note on sterility test methods

? classify sterilization methods or steps involved in sterility testing

Sterility test methods

Method - A membrane Filtration

* This method is preferred when substance under Examination is.

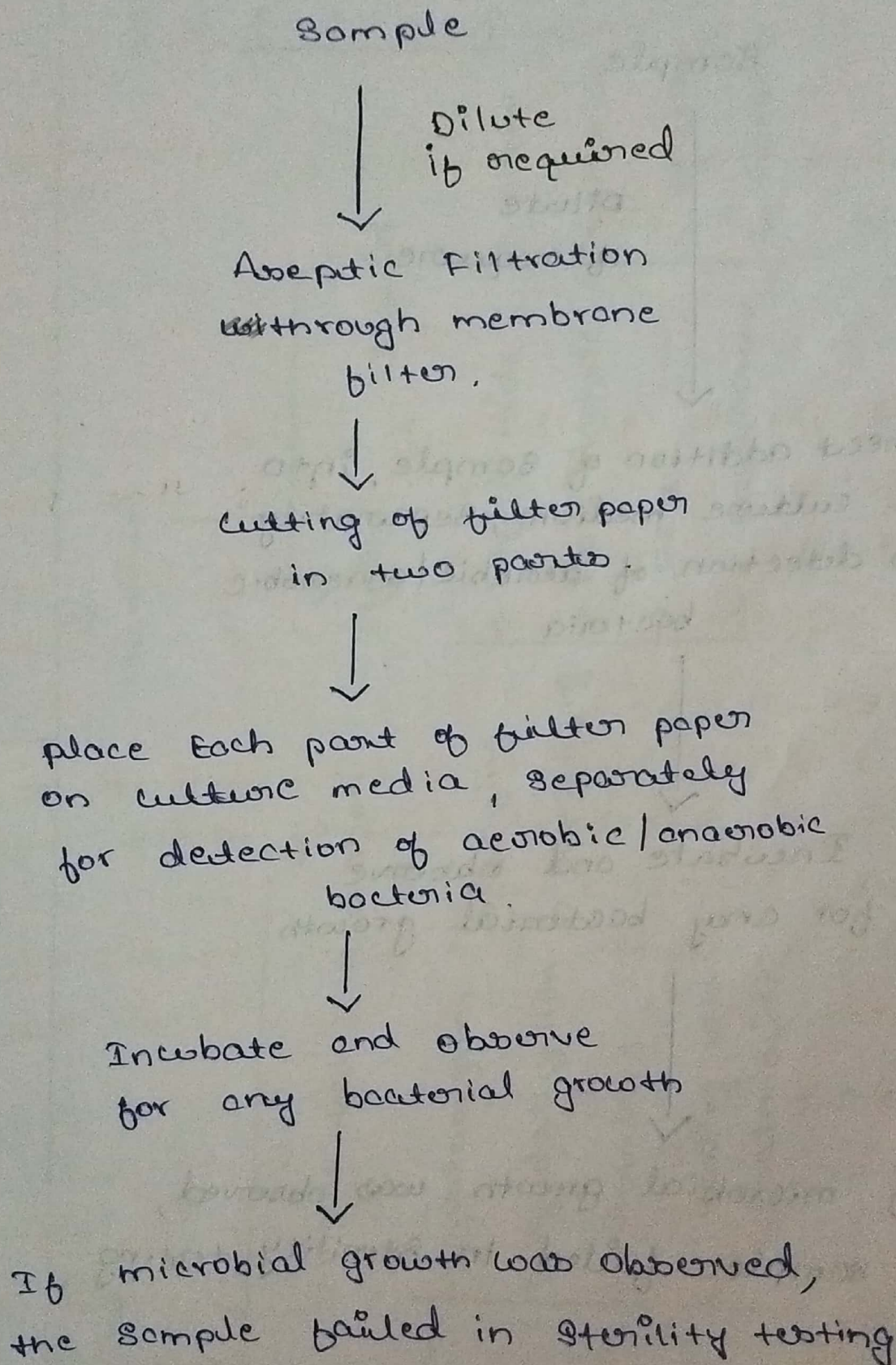
- ① an oil
- ② an ointment that can be put into solution
- ③ a non-bacteriostatic solid
- ④ a soluble powder or a liquid.

method - B Direct Inoculation

* This method is preferred when substance under Examination is

- ① an oils and oily solution.
- ② For aqueous solution
- ③ For liquids immiscible with vehicles.
- ④ For sterile devices.

Method A - membrane Filtration.



Method B - Direct Inoculation

Sample

dilute

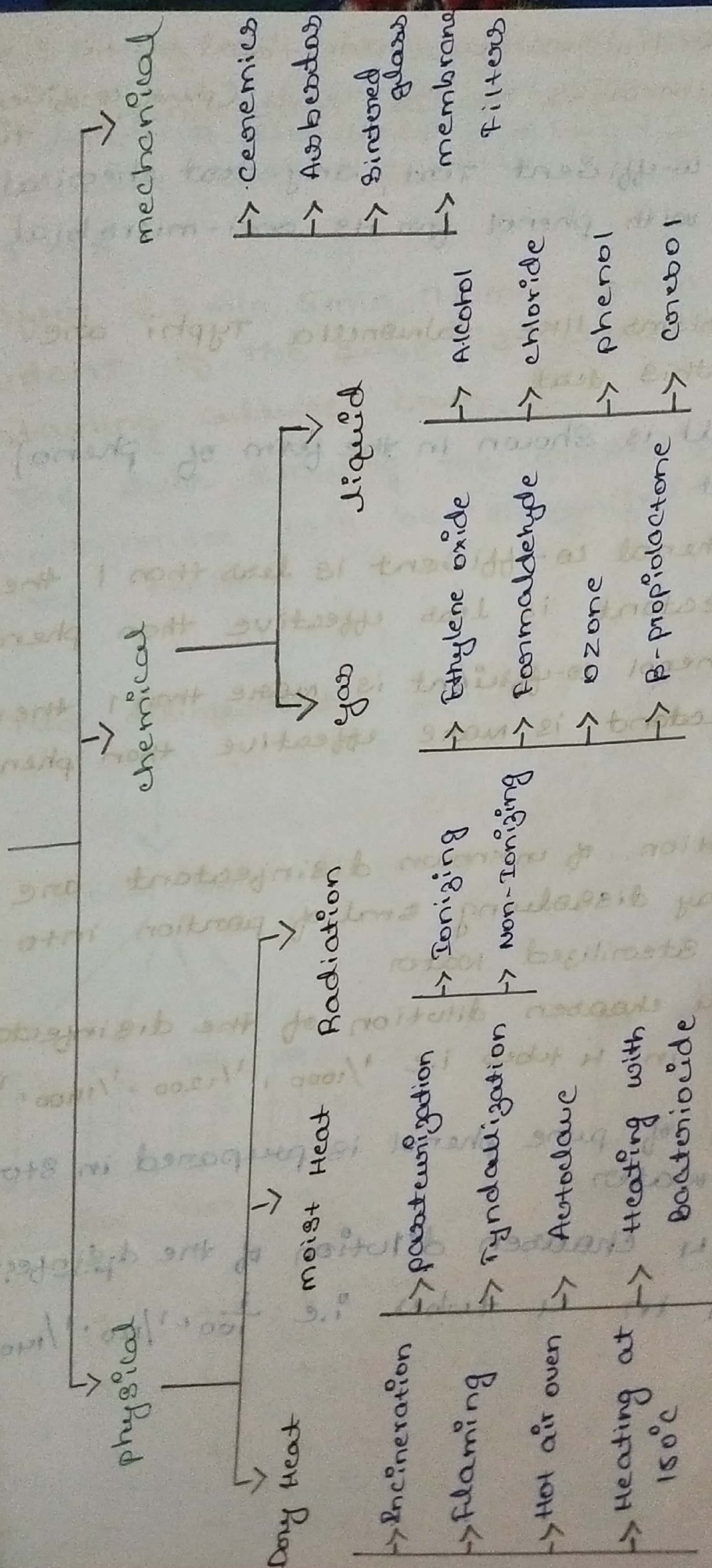
if require

Direct addition of sample into
the culture medium, separately
for detection of aerobic/anaerobic
bacteria.

Incubate and observe
for any bacterial growth

If microbial growth was observed,
the sample failed in sterility testing

sterilization methods

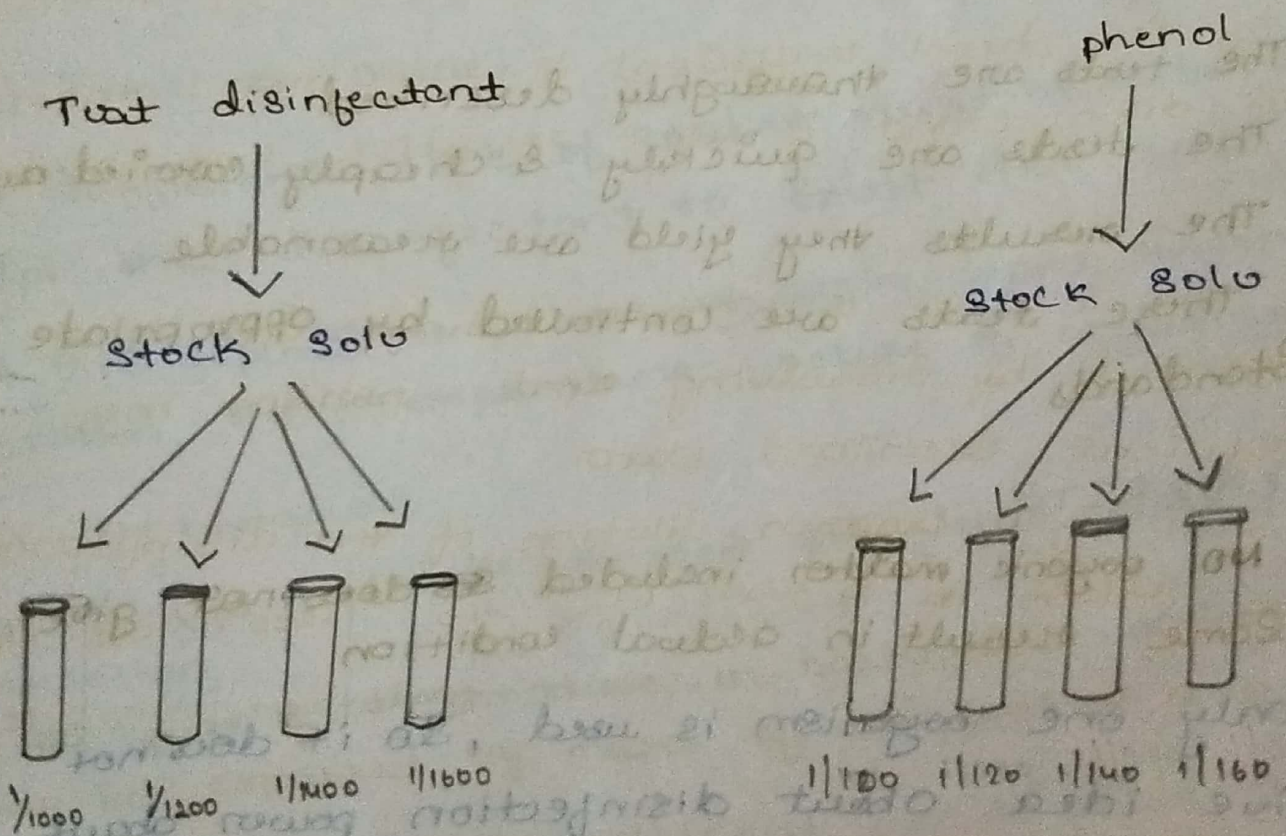


15. Describe Redial Walker test. List out merit & Demerits of the test. (phenol-coefficient (Evaluation of Disinfectants) bacteriocidal Test)
- * In phenol co-efficient test, any test chemical is compared with phenol for its anti-microbial activity.
 - * Microorganisms like *Salmonella Typhi* are used for this test.
 - * The result is shown in the form of phenol co-efficient.
 - * If the phenol co-efficient is less than 1 then test disinfectant is less effective than phenol.
 - * If the phenol co-efficient is more than 1 then test disinfectant is more effective than phenol.

Procedure.

- * Test dilution of unknown disinfectant are prepared by dissolving 5ml portion into sufficient sterilized water.
- * 5ml of a chosen dilution of the disinfectant are taken in 4 tubes i.e. $1/1000$, $1/1200$, $1/1400$, $1/1600$.
- * 5% w/v sol of pure phenol is prepared in sterile distilled water.
- * 5ml of a chosen dilution of the phenol is taken in 4 tubes i.e. $1/100$, $1/120$, $1/140$, $1/160$.

- * 0.2 ml of Test microorganisms are added in Test disinfectant tubes & phenol tubes
- * Incubate both the sets at 37°C for 48 hrs.
- * After 2.5 min, 5 min, 7.5 min, 10 min loopful of content of the tube is added to the tube containing culture broth.
- * The tube showing the growth of the test microorganism will be recognised by turbidity of the broth.



+	+	+	+	After 2.5 min	+	+	+	+
-	+	+	+	After 5 min	-	+	+	+
-	-	+	+	After 7.5 min	-	-	+	+
-	-	-	+	After 10 min	-	-	-	+

R 10
10-efficient = Dilution of unknown which kills in
7.5 but not in 5min

Dilution of phenol which kills in
7.5 but not in 5min

$$= \frac{1200}{120} = 10$$

* This Indicates that disinfectant is 10
times more efficient than phenol

MERITS

- * The tests are thoroughly described
- * The tests are quickly & cheaply carried out
- * The results they yield are reasonable
- * These tests are controlled by appropriate

Standards

DEMERITS

- * No organic matter included so does not give
some result in actual condition
- * Only one organism is used, so it does not
give idea about disinfection power about
other organism.
- * Slower acting disinfectants are not tested
by this method.
- * used to evaluate phenolic type disinfectant
only.

16. Explain different factor affecting disinfection

① Concentration.

* It is expected that concentrated disinfectant should act rapidly while diluted one should take more time

* Effectiveness is generally related to concentration exponentially.

* There is optimum concentration of phenol, 1% beyond this conc' disinfectant effectiveness becomes less.

② Time of contact

* Sufficient time of contact must be allowed for the disinfectant to exert its action.

* This depends on nature of disinfectant, concentration, pH, Temperature, nature of organism.

③ Temperature.

* Disinfection can be treated as first order reaction.

* The effect of temperature on bactericidal activity, may be expressed by means of temperature co-efficient

* The temperature co-efficient for 10° rise in temperature is expressed by Q_{10} values

$$Q_{10} = \frac{\text{Time to kill at } T^{\circ}}{\text{Time to kill at } (T+10)^{\circ}}$$

④ Type, no & condition of organism present

* In general disinfectants have greater effect on gram (+) bacteria.

* The endospore of bacteria are also highly resistant to disinfectant.

* Large population of bacteria affects disinfectant action.

⑤ presence of organic matter & other Inactivation

* The presence of organic matter may reduce the effect of a disinfectant by absorbing or inactivating it.

⑥ PH

* The bacterial growth is optimal in the pH range 6-8, and growth declines outside this range.

* Change in PH affects.

→ Rate of growth of inoculum

→ potency of disinfectant.

→ The ability of disinfectant to combine with cell

⑦ SURFACE TENSION.

* The contact of disinfectant with the cell is facilitated by its surfactant properties.

* phenol and related compounds have lower surface tension. Thus it is combined with soap & kerosene oil.

17. what are sterility Indicators ? mention Indicators used for various sterilization methods.

Ans: Sterility Indicators are those whose job is to indicate the efficiency of any sterilisation process.

physical Indicators

- * Display on the sterilizer. Shows the parameters like time, temp, pressure associated with each sterilization cycle for each load, such Indicators are considered as physical Indicator.
- * correct reading of the physical Indicator do not guarantee sterilization. but incorrect reading can be first indication of a problem

method

device

① Dry heat, Moist heat
gasous Sterilization.

Temperature Recording
chart.

② Radiation Sterilization.

Recording charts

③ Filtration Sterilisation.

Bubble point pressure
test.

Chemical Indicators

* The chemical Indicators that are used in dry or moist heat Sterilization, may melt or change its colour when satisfactory condition for sterilisation is attained

* In case of gaseous Sterilization, Rayoch Socket is used, it is an Indicator paper, its job is to show a colour change and to indicate the condition.

Biological Indicators

* The biological Indicators are actually bacterial Spores used in the form of Suspension in culture media that are kept in Sterilizer.

* The bacterial Spores are selected as per the process being used for Sterilization

Method

Microorganisms

① Dry heat Sterilization

Bacillus Subtilis

② moist heat Sterilization

Bacillus thermophilus

③ gaseous Sterilization

Bacillus Subtilis

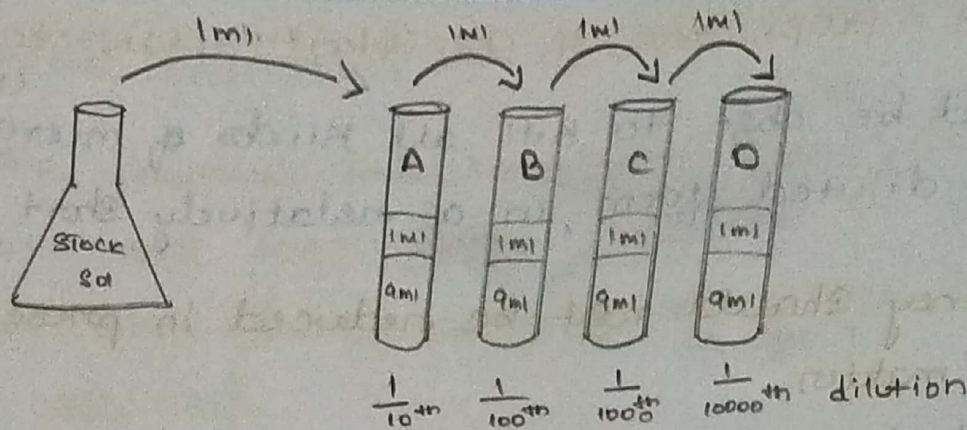
18. List the properties of an Ideal disinfectant (5m)

- * It should be able to kill all kinds of microbes in a very diluted form, in a relatively short time
- * Its efficiency should not be reduced in presence of organic matter
- * It should be non-toxic to humans, non-staining and non-offensive
- * It should be soluble in water and have a reasonable shelf life
- * It should have good wetting and penetrating properties.
- * It should not separate on standing
- * It should not corrode instruments
- * Finally it should be cheap & easily available

19. Explain different methods for evaluation of bacteriostatic activity (bacteriostatic)

① Serial dilution fluid media method. (MIC) ^{minimum inhibitory conc.}

- * MIC measures the lowest concentration of the disinfectant that inhibits the growth of known strain of organism



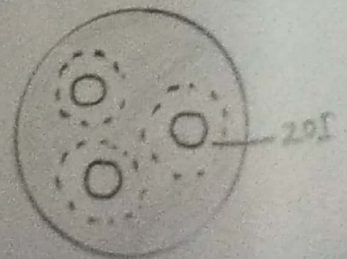
Method

- ① Add 9 ml of broth to each test tube and prepare the dilution by adding 1 ml Test Sol into A tube mix well, then add 1 ml from A to B, and then 1 ml from B to C & C to D.
- ② Graded concentration of test substance in nutrient medium are inoculated with the test organism and incubated.
- ③ The minimum concentration preventing growth is taken as measure of bacteriostatic activity.

② Cup plate method

* In this method the agar is melted, cooled, suitably, inoculated with test organism and poured into sterile petri dish.

* When the inoculated agar is solidified, holes about 8mm in diameter are cut in the medium with sterile cork borer.



* In all the cases zones of inhibition may be observed.

* The diameter of the zones gives rough indication of activities of anti-microbial substance.

③ Ditch - plate method.

* An agar is poured in a petridish, allow to solidify, ditch cut is made on agar.

* A solution of antimicrobial substance is filled into the ditch.

* Loopful of test organism is streaked on the agar surface.

* organism resistant to antimicrobial grow upto ditch where as susceptible organism show a zone of inhibition.

* The width of the zone gives rough indication of activities of antimicrobial substance.

5 mark important concepts



① Describe methods of reproduction in fungi

- * Fungi reproduce by the production of spores. Spores can be either asexual or sexual.
- * Filamentous fungi can reproduce asexually by fragmentation of their hyphae.

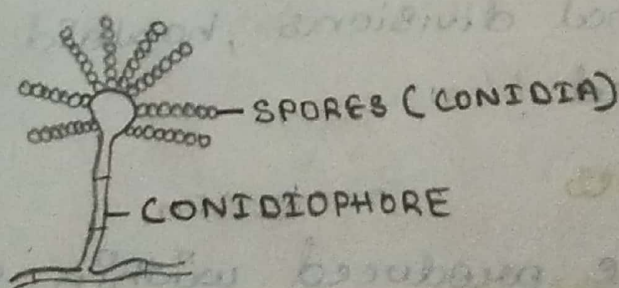
Asexual spores.

- * Asexual spores are called conidia.
- * When these spores germinate, they become organism that are genetically identical to the parent cell.
- * There is no fusion of the nuclei of cells.

Major type of Asexual spores.

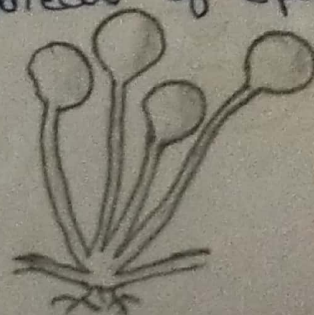
① Conidiospores.

- * Spores are produced in a chain at the end of conidiophores.
- * Spores are not enclosed in a sac.



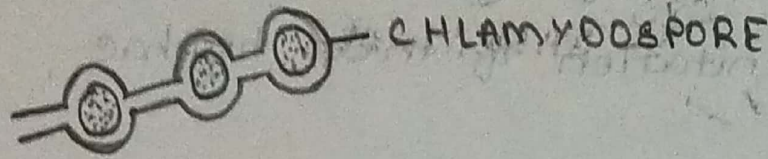
② Sporangiospores.

- * Spores are formed within the sporangium.
- * Sporangium contains hundreds of sporangiospores.



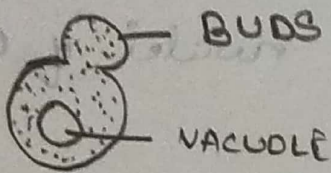
③ Chlamydoconidia

- * Hyphae divide into spore like cells with large food reserve



④ BLASTOSPORES

- * It is produced by budding from parent cell
- * It is found in yeasts



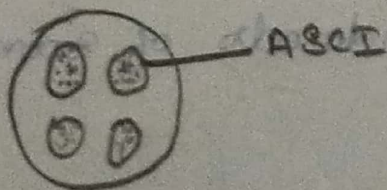
Major types of sexual spores

- * Sexually opposite cells unite, when the cells unite, the nuclei fuse to form diploid nucleus

- * After several divisions, haploid state is reestablished.

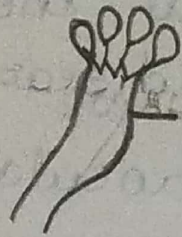
① Ascospores

- * Spores are produced within spherical cells called asc:



② BASIDIOSPORE

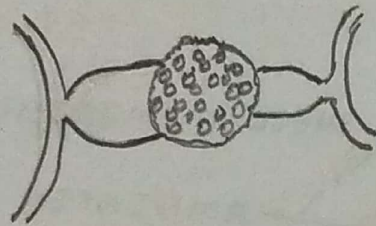
* Spores produced externally on a structure called basidium



BASIDIUM

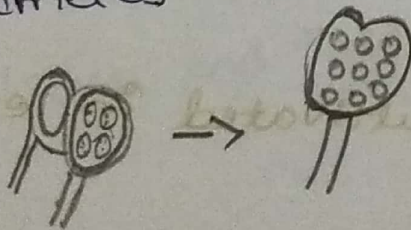
③ Zygosporer

* Spores produced by the fusion of similar appearing gametes formed at the tips of hyphae.



④ OOSPORES.

* Spores resulting from the mating of two unlike gametes



② write a note on cultivation of viruses

* There are 3 methods used for their cultivation

① Animals inoculations

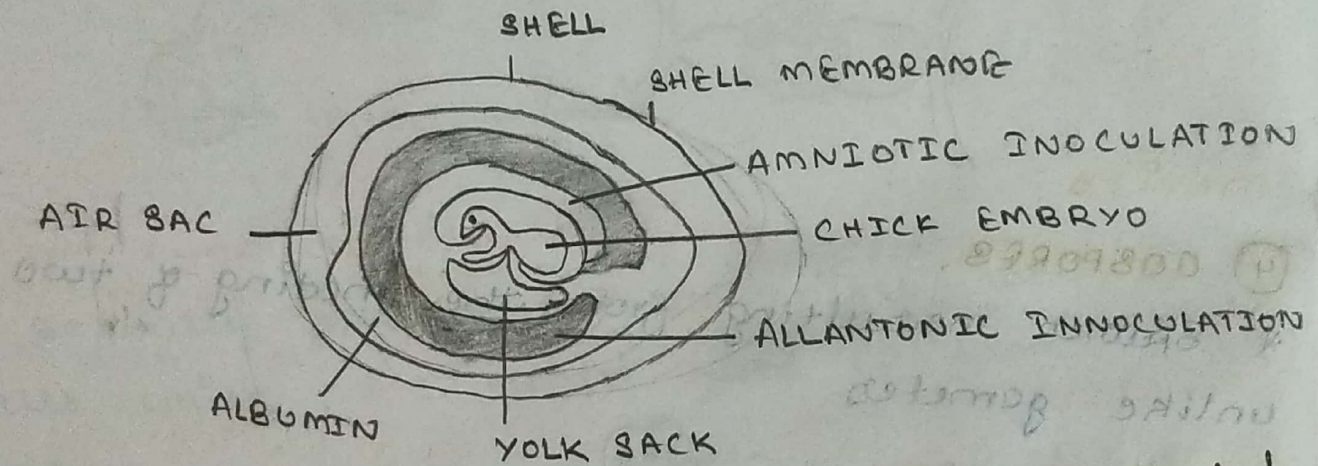
② Chick Embryo

③ Tissue culture

① Animal Inoculation

- * one of the oldest method for the cultivation of virus.
- * polio virus after Intraspinal Inoculation in monkey causes paralytic disease
- * Cox-Sackie virus after Inoculation in mice causes paralysis.
- * growth of virus in animals can be known by disease or death.

② chick Embryo.



- * virus can also be cultivated in embryonated egg
- * A hole is drilled in the shell of the embryonated egg and a viral suspension is injected into fluid of egg. It is exactly injected to membrane of the egg.
- * viral growth is indicated by the death of the embryo or by the formation of lesions on egg membranes.

③ Cell Culture.

- * cells are suspended in growth medium and distributed in petridishes
- * The cells adhere to glass surface and grow out to form a monolayer sheet. They are called as primary cell culture.
- * They are useful for isolation and cultivation of viruses for vaccine production.

③ write about merits and demerits of viral cultivation techniques

① Animal Inoculation.

merits.

- * Diagnosis, pathogenesis and clinical symptoms are determined.
- * useful in studying immune response, epidemiology and onogenesis.

Demerits.

- * Expensive and difficulties in maintenance of animals
- * Antibodies are produced.
- * There will be major handling problems.

② Chick embryo

Merits

- * ~~Least~~ effective and maintenance is much easier.
- * NO antibodies are formed

Demerits

- * This method is limited to few viruses
- * Eggs may be contaminated with mycoplasma which may interfere with the growth of virus.

③ Cell culture

Merits

- * Simple and Inexpensive
- * NO antibodies are formed

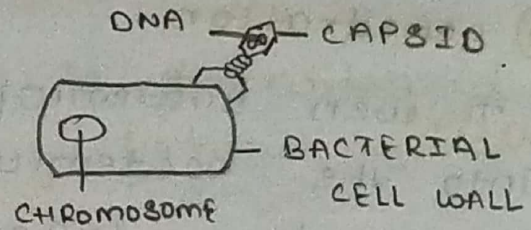
Demerits

- * This method is limited to few viruses
- * Technically difficult
- * Unsuspected virus may be present

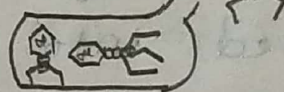
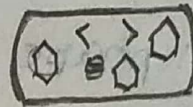
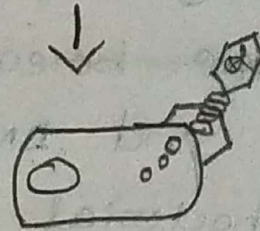
④ Describe steps involved in Replication of virus

- * Virus multiply only intracellularly.

① Attachment



② Penetration



③ Biosynthesis

④ Maturation

⑤ Release

① Attachment

* Attachment site on the virus attaches to complementary receptor site on the bacterial cell

* Attachment is the chemical interaction in which weak bonds are formed between the attachment site and Receptor site.

② Penetration

- * T. even bacteriophage injects its DNA into the bacterium by releasing an enzyme phage lysozyme from its tail
- * Lysozyme breaks a portion of the cell wall and DNA from the bacteriophage enters bacterial cell.

③ Biosynthesis

- * In this phase phage DNA uses ribosomes, enzymes and amino acids of host cell for translation

④ Maturation

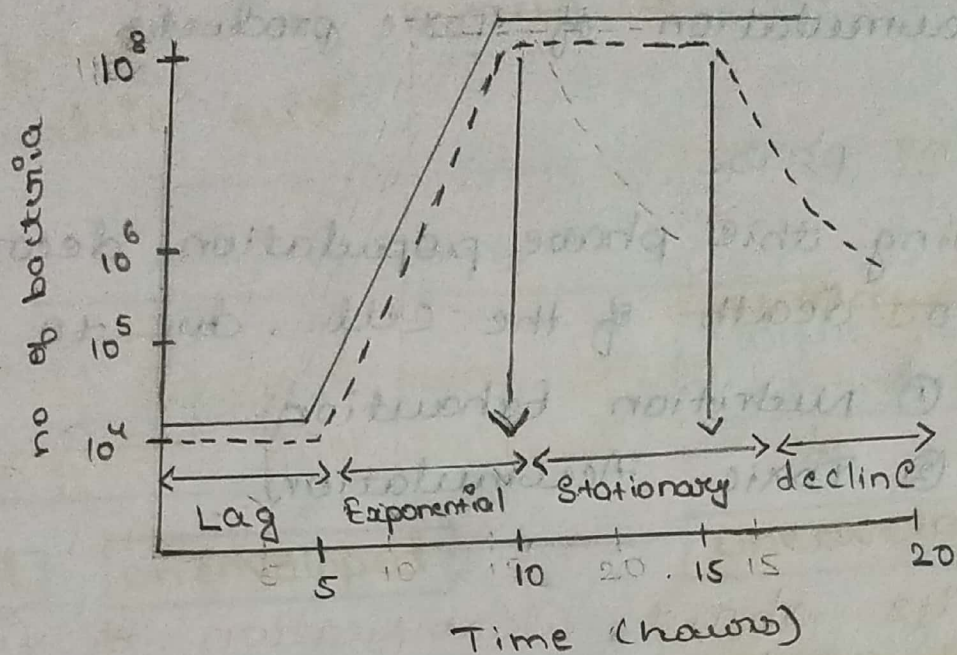
- * Bacteriophage DNA and capsids are assembled into complete virions
- * Viral components assembled into viral particle spontaneously
- * Tails are separately assembled from protein subunits

⑤ Release

- * Release of virions from the host cell is the final stage of viral multiplication.
- * Lysozyme synthesized within the cell causes the bacterial cell wall to break down, and newly produced bacteriophages are released from host cell.

⑤ Describe bacterial growth curve

* when dormant bacteria is transferred into fresh medium, they exhibit typical growth curve.



① Lag phase.

During this phase

- ① There is increase in size of the cell
- ② There is increase in metabolic rate
- ③ Adaptation with macromolecular synthesis

② Log phase

* After Adaptation cell division proceeds at log rate

* The no of bacteria doubles with each generation time. Because no of bacteria increases exponentially.

③ Stationary phase

* It is the stage where rate of multiplication and death becomes almost equal, due to

- ① Depletion of nutrient
- ② Accumulation of Toxic products

④ Decline phase

* During this phase population decreases due to death of the cell, due to

- ① Nutrition Exhaustion
- ② Toxic Accumulation

⑥ write about classification of virus

* Classification of viruses on the basis of host

① Zoophagineae (Animal viruses)

* They usually have DNA but may also have RNA

* These viruses infect man, dog, cow and Arthropods

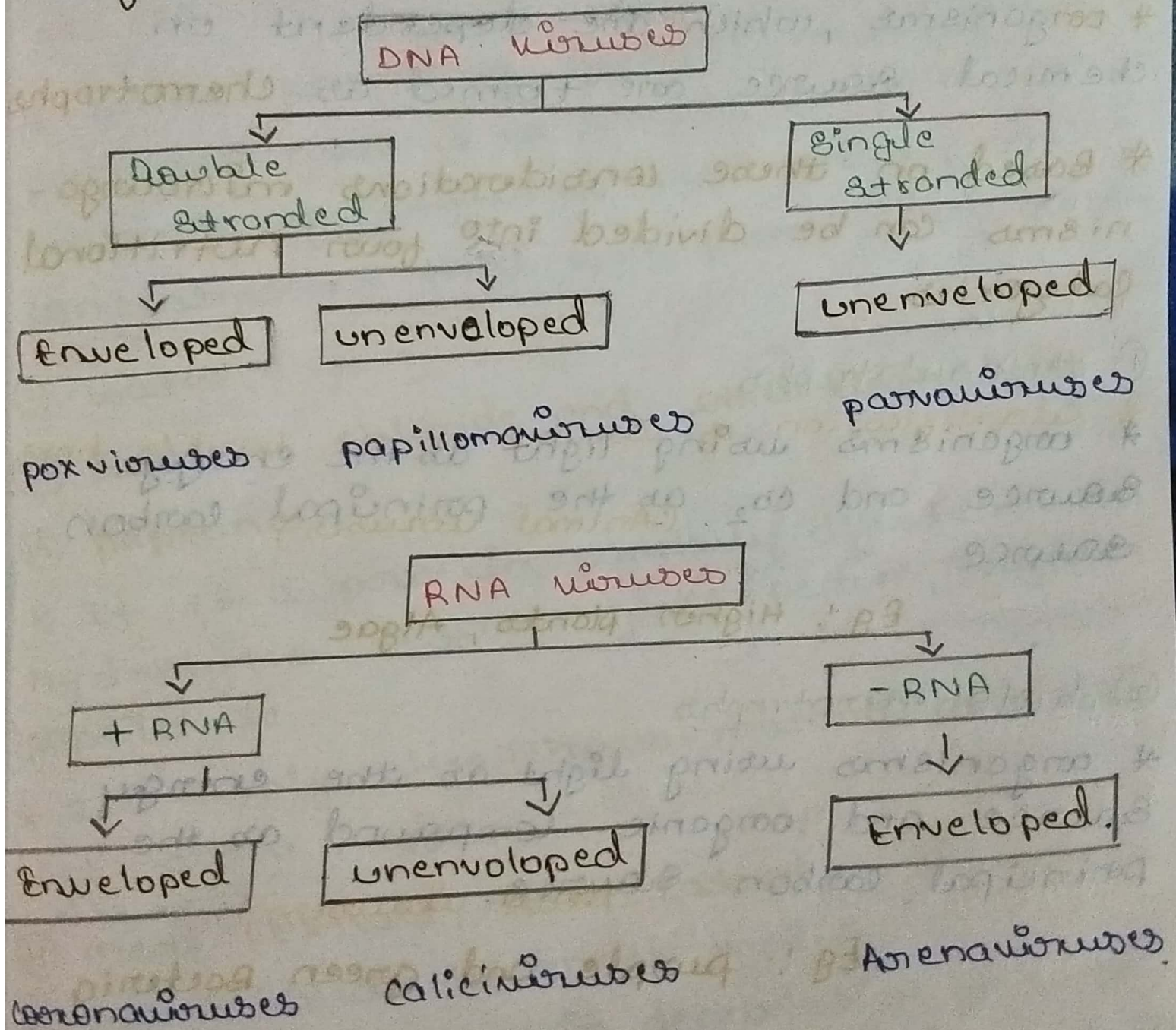
② Phytophagineae (Plant viruses)

* They have RNA and infect angiosperms like potato, sugarcane and other higher plants.

③ Bacterial viruses (phagineae)

* They have DNA and called as Bacteriophages or simply phages

* classification of virus on the basis of nucleic Acid.



⑦ Classify bacteria on the basis of nutritional requirements.

Energy.

* With respect to Energy source, organisms that are able to use light as an energy source are termed as phototrophs.

* Organisms, which are dependent on chemical source are termed as chemotrophs.

* Based on these considerations microorganisms can be divided into four nutritional categories.

① photoautotrophs

* organisms using light as the energy source and CO_2 as the principal carbon source.

Eg: Higher plants, Algae

② photoheterotrophs

* organisms using light as the energy source and organic compound as the principal carbon source.

Eg: purple and green Bacteria

③ chemoautotrophs

* organisms using chemicals as the energy source and CO_2 as the principal carbon source.

④ chemoheterotrophs -

* organisms using chemicals as the energy source and organic substance as the principal carbon source

② write a note on raw materials used for preparation of culture media

* while preparing culture medium, the primary goal is to provide a balanced mixture of required nutrients, at concentrations that will permit good growth

① water.

② sodium chloride and other electrolytes.

③ peptone

* It is a protein partially digested with hydrolytic enzymes like pepsin, trypsin, papain etc.

* It supply nitrogenous material and also act as a buffer.

④ Meat Extract, Yeast Extract.

* It is used to Enrich media.

* It contain protein degradation products, carbohydrates

* It contains inorganic salts and growth factors

⑤ Blood

- * usually defibrinated horse blood is used
- * serum from other animals are also used.

⑥ Agar

- * It is a carbohydrate derived from seaweed
- * It does not provide any nutrition to bacteria. It acts only as solidifying agent

⑦ Define and classify culture media with examples

Culture media

It is a substance that gives artificial environment stimulating natural conditions necessary for growth of bacteria

Classification

① Based on their consistency

- ① Solid medium. Ex: Nutrient Agar
- ② Liquid medium. Ex: Nutrient Broth
- ③ Semi solid medium. Ex: SIM

② Based on the constituents

- ① Simple medium. Ex: Nutrient Agar
- ② Complex medium

② Synthetic medium Ex: peptone water

④ Special media

③ special media

① Enriched media Ex: chocolate Agar.

② Enrichment media Ex: Alkaline peptone water

③ selective media Ex: Mac Conkey's medium

④ Indicator media Ex: Blood Agar

⑤ differential media Ex: Mac Conkey's medium

⑥ Transport media Ex: Stuart's medium

④ Based on oxygen requirement.

- Aerobic media

- Anaerobic media Ex: Thioglycolate medium

⑩ write salient features of differential and selective media along with Examples and explain salient features of each media with examples

① solid medium

* It is used to study colonies of individual bacteria

* Agar and gelatin are the important constituents of solid media.

Ex: Nutrient Agar

② Liquid media

- * It is used as enriched media before plating on solid media
- * It is not suitable for Isolation of organism in a pure culture

Ex': Nutrient Broth.

③ Simple media

- * It is also called as basal media
- * It consists of meat Extract, Sodium chloride, water, Agar.

Ex': Nutrient Agar

④ Complex media

- * Media other than basal media
- * They have added Ingredients provide special nutrients.

⑤ Synthetic media X

- * Media prepared from pure chemical
- * It is used for Research purpose

Eg': peptone water

⑥ Enriched media

- * Substance like blood, serum are added to basal medium
- * It is used to grow bacteria which have complicated Nutritional requirements

Ex': Chocolate Agar, blood Agar

⑥ Enrichment media

- * It is used to Isolate pathogens from mixed culture by using growth media that favours the growth of particular organisms
- * It is incorporated with Inhibitory substance to suppress the unwanted organism.

Ex': Alkaline peptone water.

⑦ Selective media

- * Inhibitory substance is added to solid media.

Ex': MacConkey's medium - gram⁺ve bacteria
U medium - M. Tuberculosis
TCBS - V. cholerae
Wilson and Blair medium - S. typhi

⑧ Differential media

- * A media which has substances incorporated in it which distinguish bacteria

Ex': MacConkey's medium

⑨ Indicator media

- * These media contain an indication which changes its colour when bacteria grows in them

Ex': Blood Agar.

⑩ Transport media

- * media used for transporting the sample
 - * Delicate organisms may not survive the time while for transporting the specimen without transport media
- Ex: Stuart's medium

⑪ Anaerobic media

- * These media are used to grow anaerobic organisms

Ex: Thioglycolate medium

⑫ write a note on physical factors affecting growth of bacteria

Temperature

- * It determines rate of growth, multiplication, survival and death of all living organisms
- * High temperature causes membrane damage, denaturing enzymes, proteins
- * At low temperature enzymes do not function properly

Types of Temperature

① Minimum growth temperature

- * It is the lowest temperature at which organisms grow.

② Maximum growth temperature

* It is the highest temperature at which organisms grow

③ optimum growth temperature

* It is the temperature at which rate of multiplication is most rapid.

PH

* It refers to negative logarithm of hydrogen ion concentration.

* microbial growth is strongly affected by the PH of the medium.

* drastic change in cytoplasmic PH causes membrane damage and inhibit the activity of enzyme

Osmotic pressure

* For the survival and growth, micro-organisms prefer isosmotic pressure.

* Both higher and lower osmotic pressure environments are used for killing or inhibiting the bacterial growth.

Light

* Bacteria grow well in the dark. They are sensitive to UV light and other

Radiations

* culture die if it is exposed to sunlight.

⑫ Explain mechanism of action, procedure application, demerits of uv radiation?

Mechanism of action

- * The microbial nucleic acids contain pyrimidine exhibit high selectivity to uv radiation

- * This causes bonding b/w two neighbouring pyrimidines results in formation of pyrimidine dimer

- * It results in Inhibition of DNA Replication

procedure

- * uv radiation is produced by passing low current through mercury lamp

- * Sterilization of air is done by mounting uv lamps on walls or fixing them to vessels.

Application

- * Sterilization of air

- * uv lamps are mounted on walls, to prevent local infection from patients

- * Destroys virus & bacteria from vaccines.

Demerits.

- * Due to poor penetrating power, it is limited to surface destruction of microorganisms

- * uv lamps are made ineffective by dust

- * Cannot be used for sterilizing packed articles

- * Certain microorganisms exhibit resistance towards uv.

- * unreliable method.

⑬ write a note on heating with bactericide?

* The Sterilization method is suitable for injectables and is an official method which has now replaced by tyndallization.

* This method is based on the principle that bactericidal agents exhibit greater efficiency at higher temperatures.

* The bactericidal agent used for sterilization should possess following properties.

→ It should not be toxic

→ It should be stable under condition of sterilization.

→ It should be compatible with other active ingredient

→ It should possess bactericidal activity over wide range of pH value.

* For Injections

Chlorocresol

0.2% w/v

phenylmercuric acetate

0.002% w/v

* The procedure involves heating the parenteral solution in their final containers at 100°C for 30 min in a steamer by adding bactericidal agent.

* This method is unsuitable for parenterals used for Intracutaneous, Intrathecal and Intravenous administration, as toxicity can be exhibited by bactericidal agent.

14) Classify disinfectants with ex.
class:

- ① Acids :- Boric acid, Benzoic acid.
- ② Alcohol :- Ethanol, Isopropyl alcohol
- ③ Aldehydes :- Formaldehyde, glutaraldehyde
- ④ Dyes :- Gentian violet, brilliant green
- ⑤ Halogens :- Iodine, Chlorine
- ⑥ phenol derivative :- phenol, cresol
- ⑦ Surfactants :- Soaps, Cetrinide
- ⑧ Oxidizing agents :- Hydrogen peroxide, potassium permanganate
- ⑨ Metallic salts :- Silver nitrate, Zinc compounds.

15) Classification, MOA and uses of each class of disinfectants.

(i) phenolic disinfectants

(ii) aldehydes.

(iii) Halogens.

① phenolic disinfectants

classification.

- ① cresol
- ② xylenols
- ③ chloroxylenols
- ④ phenol

Mechanism of Action.

- ① disruption of cells.
- ② precipitation of cell protein.
- ③ Inactivation of enzymes
- ④ leakage of amino acids from the cells

uses.

- ① Bactericidal Disinfectant
- ② Fungicidal Disinfectant
- ③ Mouth wash
- ④ Topical antiseptic.
- ⑤ throat Lozenges.
- ⑥ Mycobactericidal.

① Aldehyde.

classification

- ① Formaldehyde.
- ② glutaraldehyde

Mechanism of action

① Alkylation

→ Transfer of alkyl group from one molecule to another in protein & nucleic acids

② Disruption of cells

③ precipitation of cell protein

④ Inactivation of enzymes

⑤ Leakage of amino acids from cells.

uses

- ① Disinfectant
- ② preservative
- ③ Antiseptic
- ④ Ayer

Halogens

classification

- (i) Fluorine .
- (ii) chlorine .
- (iii) Bromine .
- (iv) Iodine .

Mechanism of Action

- (i) disruption of cell .
- (ii) precipitation of cell protein .
- (iii) Inactivation of enzymes
- (iv) leakage of amino acids from cell

uses_

- (i) Disinfectant
- (ii) Antiseptic .
- (iii) Tincture
- (iv) Fungicide
- (v) virucide

16. ~~various~~ principle and method of microbiological assay of vit B₁₂ & B₂ by titrimetric method

principle

- * The basis of this assay is to measure the ability of test organism to utilize the substance being assayed under proper nutritional condition.
- * The growth of test organisms is directly proportional to amount of factor added to medium.
- * For the assay of vit B₁₂ *Lactobacillus leichmannii* is used as test microorganism and basal media for growth of culture.

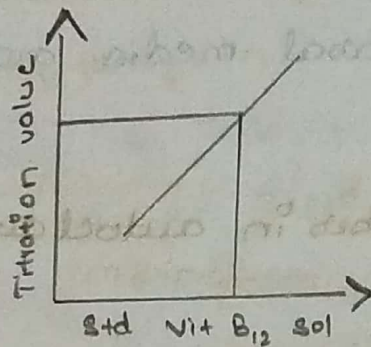
procedure

- * Clean ten test tubes & add 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 & 5 ml of std cyanocobalamin sol.
- * To each add 5 ml basal medium and adjust to 10 ml by water.
- * In another 4 test tube add 1, 2, 3, 4 ml of test solution which is to be assayed.
- * To each add 5 ml basal medium and adjust to 10 ml by water.
- * Sterilize all Test tubes in autoclave at 121°C for 15 min.
- * Inoculate drop of Inoculum prepared of *Lactobacillus leichmannii*.
- * Incubate the test tubes for 70 hours at temp range of 30-37°C.

* After incubation period titrate content of each test tube with 0.05 N NaOH. using bromothymol blue as indication until green colour appear.

Interpretation of results.

* Draw a smooth curve and determine the conc of test sol by interpolating



Vitamin B₂.

principle:-

* The basis of this assay is to measure the ability of test organism to utilize the substance being assayed under proper nutritional condition.

* The growth of test organisms is directly proportional to amount of factor added to medium.

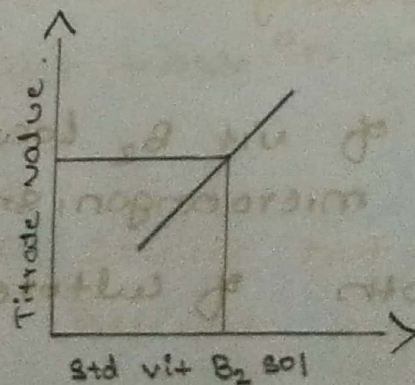
* For the assay of vit B₂ *Loate bacillus cerei* is used as Test microorganism and basal media for growth of culture.

procedure:

- * Clean 7 test tubes and add 0, 0.5, 1, 1.5, 2, 2.5, 3 ml of Std Riboflavin sol.
- * To each add 5 ml basal media and adjust to 5 ml by water.
- * In another 5 test tubes add 0.5, 1, 1.5, 2, 2.5 ml of Test Sample which is to be assayed.
- * To each add 5 ml basal media and adjust to 5 ml by water.
- * Sterilize all test tubes in autoclave at 121°C for 15 min.
- * Inoculate drop of Inoculum prepared of *Lacto-bacillus casei*
- * Incubate the test tubes for 70 hours at temp range of $30-35^{\circ}\text{C}$
- * After Incubation period titrate content of each test tube with 0.05 N NaOH to PH 6.8

Interpretation of results

- * Draw a Smooth curve and determine the conc' of test sol by Interplotting.



17. write principle & method of microbial assay of streptomycin by cup plate method & penicillin.

principle

* It is based upon comparison of Inhibition of microorganisms by measured concentration of antibiotics.

* Cup plate method is generally used for assay of streptomycin.

* For the assay of streptomycin, *Bacillus subtilis* is used as test microorganism in Antibiotic assay medium.

procedure

* Take 1ml of streptomycin sulphate and dilute with 100ml of sterile water.

* By using this stock sol, prepare 10, 12, 14, 16 & 18 units/ml of standard stock sol.

* prepare sol of unknown sample of antibiotic and dilute it upto level of standard sol.

* Sterilize antibiotic assay medium by autoclave and prepare a petri plates.

* Spread the microorganisms on surface of petriplate.

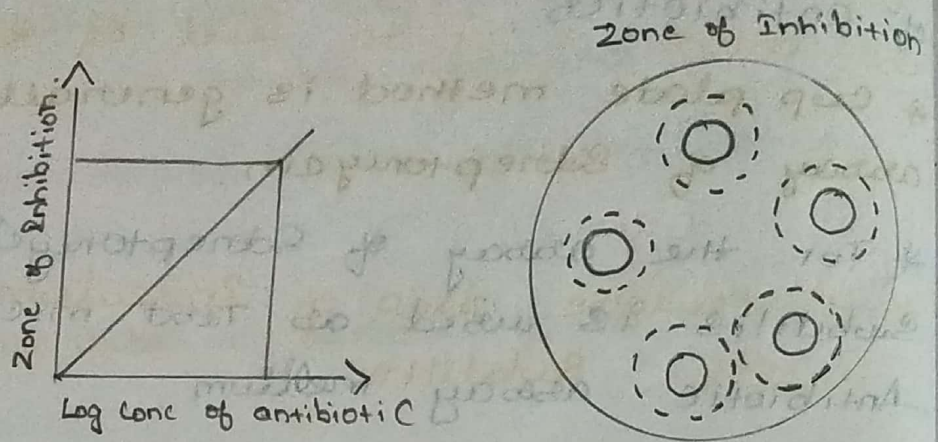
* prepare cavities on plate using cork borer.

* Standard and test dilute antibiotic sol are added in each labelled cavity

* Incubate the test tubes for 70hrs at temp range of 30-35°C.

observation & results.

- * measure diameter of zone of inhibition
- * plot graph of diameter of zone of inhibition against log conc of antibiotic and determine the conc of unknown antibiotic sample



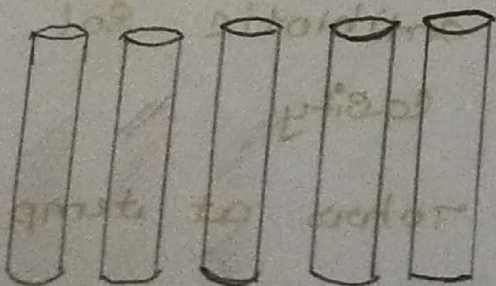
18. write principle and method of microbiological assay of Streptomycin by tube assay method

principle

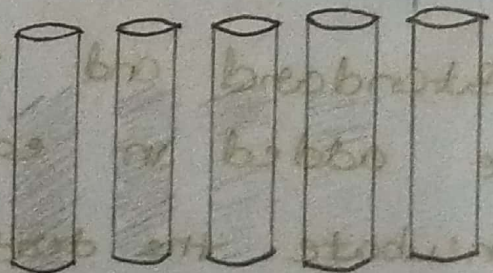
- * It is based upon comparison of inhibition of microorganisms by measured concentration of antibiotics

- * For the assay of Streptomycin, *Bacillus subtilis* is used as Test microorganism in Antibiotic assay medium.

Procedure:



TT for std antibiotics



TT for Test antibiotics

- * Take 1ml of Streptomycin Sulphate and dilute with 100ml of Sterile water.
- * By using this stock sol, prepare 10, 12, 14, 16 & 18 units 1ml of std stock sol.
- * prepare sol of unknown sample of antibiotic and dilute it up to level of Standard sol.
- * Sterilize all test tubes in autoclave at 121°C for 15 min.
- * Inoculate drop of inoculum prepared of *Bacillus Subtilis*.
- * Incubate the test tube for 70 hours at temp range of $30 - 35^{\circ}\text{C}$.
- observation & Result.
- * Turbidity in the test tube indicates the growth of microorganism.
- * clear TT indicates the action of antibiotic at particular conc.

(19) principle, procedure & application of Schick's test

principle

* Schick test detects immunity to diphtheria, either acquired through previous infection or vaccination

procedure

* A small amount of diphtheria toxin in 0.2ml is injected intradermally into the left forearm

* An equivalent amount of heat inactivated toxin in 0.2ml is injected intradermally into the right forearm

* The test is read at 1-4 days

* Schick negative will have no skin reaction due to sufficient antitoxin being present to neutralize the toxin.

→ Schick negative person is immune and need not to be immunized.

* Schick positive will develop positive erythematous reaction which persists for 7 days, These individuals are susceptible to diphtheria

Application

* To detect immunity to diphtheria.

②0 pri, pro & application of QBC Test

principle

① Fluorescent stain

* stain Acridine orange (AO) is used to improve the visibility of malaria parasites, when excited by blue light

② Centrifugation

* Centrifugation makes parasite easier to locate within the tube

* when tube is centrifuged, blood components and malaria parasites separate based on density and concentrate in distinct layer.

procedure

* QBC capillary - blood tubes are pre-coated with anticoagulants, Acridine orange and are filled with blood directly from a finger puncture.

* After filling, tubes are sealed with rubber stopper.

* Tube is centrifuged with 11,000 - 12,000 rpm for

5 min

* on centrifugation, blood components and malaria parasites separate based on density and concentrate in distinct layer.

Application,

* diagnosis of malaria.

(21) pri, pro, appli of Mantoux test?

principle

- * Small amount of tuberculin is injected Intradermally
- * It reacts with sensitized T-cells and produces hypersensitivity reaction.
- * Redness appearing around the injected part is called erythema
- * The characteristic skin reaction develops fully after 48-72 hrs.

procedure

- * Select preferred site of injection and disinfect with 70% alcohol
- * With the help of 26 G needle, draw 0.1 ml of 5 Tuberculin units (TU), without trapping any airbubbles
- * penetrate superficial layer of skin with an angle of 30°
- * Read the result after 72 hrs, measure the diameter of erythema

application

- * In diagnosis of Tb

② pr^o, pro, applic of ELISA ?

* It is the technique used to detect the Antigen present in serum sample

Types

① Single antibody method.

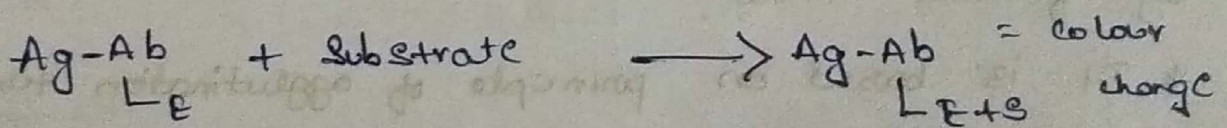
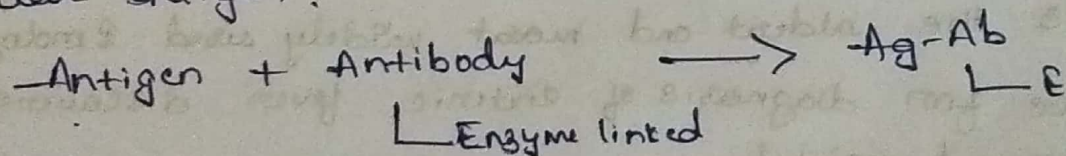
② Double antibody method.

principle

* Enzyme linked antibody is added to Antigen in serum sample, on reaction give rise to antigen-antibody complex.

* Then Substrate added will react with Enzymes which is attached to antibody, ultimately Substrate - enzyme reaction takes place and it will show colour change

* changed colour will be analysed under colorimeter in a specified wavelength, to get quantity of particular antigen.



procedure.

* Samples are loaded into ~~duplicate~~ wells of microplate strips, Incubated for 5 min

* Add primary antibody to all wells, Incubate for 5 min, & then Rinse

③ Add enzyme linked secondary antibody to all wells. Incubate for 5 min. Rinse.

④ Add enzyme substrate to all wells & Incubate for 5 min

Application

* used to estimate hormones like T_3 , T_4 , insulin, renin, aldosterone, FSH, LH in serum sample

* used to estimate tumour markers like PSA & AFP

* used for the assay of antibody in serum

* used for the assay of autoantibody
Eg, anti-DNA

23 - principle, principle, appli of WIDAL TEST?

principle

* widal test is used to detect serum antibody

* It is the oldest and most widely used serological technique for diagnosis of enteric fever discovered by jernand widal.

* It is based on principle of agglutination test

* antigens used for this technique are

- O & H antigens of *Salmonella typhi*
- H antigens of *Salmonella paratyphi* A, B
- If the antibodies are present in the serum it will agglutinate.

Method.

* Take GTT, add 0.9 ml saline in 1st tube, 0.5 ml normal saline in other 5 tubes

* Add 0.1 ml serum to 1st tube so it becomes 1 ml, 1 part of serum & 9 part of saline becomes 1 in 10 dilution ($\frac{1}{10}$) of serum

* now transfer 0.5 ml from 1st tube to 2nd tube which contains 0.5 ml of saline. so it becomes 1 ml of 1 in 20 dilution ($\frac{1}{20}$). so doubling dilution is done with all test tube, as 0.5 ml is discarded

* Add 0.5 ml of diluted antigen to all test tube, incubate it, then observe for agglutination.

* Highest dilution of serum that give agglutination is titre

	1:20	1:40	1:80	1:160	1:320
Sal typhi O	+	+	-	-	-
Sal typhi H	+	+	+	-	-
Sal paratyphi AH	-	-	-	-	-
Sal paratyphi BH	-	-	-	-	-

Agglutination > 80 is significant for O antigen

Agglutination > 160 is significant for H antigen.

Application

* used for the diagnosis of Typhoid

24. prin, proc, appli of western Blotting Technique

Principle.

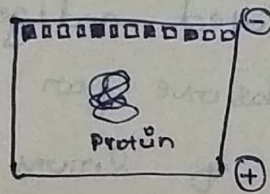
* western blotting is an Immunoblotting technique which relies on specificity of binding b/w molecule of interest and a probe to allow detection of molecule of interest.

* In western blotting, the molecule of interest is a protein and the probe is typically an antibody raised against that particular protein.

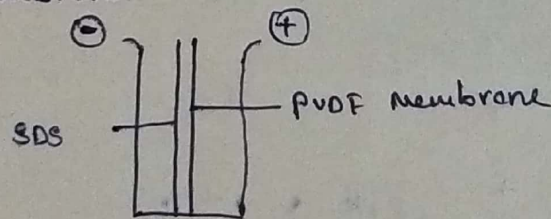
procedure.

* Load & separate protein sample on SDS-PAGE

*



* Electrophoretically transfer fractionated proteins onto PVDF membrane



* Add primary Antibody Specific to target protein

* Add HRP labelled Secondary antibody specific to primary antibody

* Add HRP Substrate and expose to film

Appli:

* Confirmatory test for Hep-B

* En analysis of Biomarkers such as hormones, growth factors & cytokines

25. principle, principle, principle of Southern blotting technique

- * It is a hybridization technique for identification of particular size of DNA
- * DNA fragments are separated based on size by gel electrophoresis
- * desired DNA is detected using specific DNA probe that is complementary to desired DNA
- * Hybridization probe is short, single stranded DNA, The probes are labelled with markers, so that they can be detected after hybridization

procedure

- * The DNA is fragmented by using suitable restriction enzyme
- * desired fragment is separated by gel electrophoresis
- * separated strands of DNA is transferred to nylon membrane by the process of blotting.
- * The DNA bound to membrane is treated with labeled probe, which contains complementary sequence
- * hybridized regions are visualized by Autoradiogram

Appl:

- * Identification & Isolation of desired DNA
- * Identification of mutations.

2 mark important concepts



① state Koch's postulates

- * Specific organism must be present in the lesion of a disease
- * Specific organism can be isolated and grown in pure culture under laboratory condition
- * Isolated organism when inoculated into healthy host must show some symptom of disease
- * Some microorganisms can be reisolated from the experimental animals and cultured again. The cultured organism must show some characteristics as that of disease causing organism.

② write any four application of Microbiology.

- * Microbes in pharmaceutical Industry
 - > Antibiotics and Steroid production is carried out with the help of microorganisms
 - Eg: penicilline is prepared by penicillium notatum
- * Microbes in chemical Industry.
 - > Commercially valuable solvents, enzymes are produced by microorganisms
 - Eg: proteases, Invertase
- * Microbes in Vaccine production
 - > Microbes are used as Immunizing agents to produce vaccine.

* Microorganisms in Beverage Industry
 → certain microorganisms carry out fermentation process where they change glucose to alcohol.

③ write four pharmaceutical use of microorganisms

* Antibiotics and Steroid production is carried out with the help of microorganisms
 Eg: penicillin is prepared by penicillium notatum

* Insulin and Interferons are produced by microbes

* Microbes are used in production of Antifungal drugs

* Microbes are used in production of Antistatin

④ Name the major divisions of microbial world

- ① Bacteria
- ② Viruses
- ③ Fungi
- ④ Algae
- ⑤ Protozoa
- ⑥ Archaebacteria

⑤ write contributions of Antony van Leeuwenhoek

- * He was the first person to describe the spermatozoa.
- * He discovered blood cells.
- * He discovered microscopic animals.
- * He identified many prokaryotes.

⑥ write contribution of Edward Jenner

- * He introduced vaccination against smallpox.
- * He was successful in terminating cholera.
- * He introduced antiseptic surgery.
- * He carried out experiments on human blood.

⑦ write contribution of Robert Koch

- * Identification of Tuberculosis
- * Development of pure cultures
- * Isolation of causative microorganisms
- * Description of spores

⑧ write contribution of Louis Pasteur

- * He laid down foundation for germ theory of disease.
- * Disapproval of spontaneous generation.
- * Described the term Aerobic and Anaerobic.
- * Development of vaccines.

- (9) Write contribution of Alexander Fleming
- * Accidental discovery of penicillin
 - * Discovery of bacteriolytic component in human tissue
- (10) Write the concept of Spontaneous generation

Abiogenesis or Spontaneous Generation

- * It states that living organisms could develop from non-living material
- * This theory was rejected with the advancement in biosciences
- * This theory is rather unscientific and lacked evidence

(11) Media for cultivation of fungi?

- * Malt Extract Agar
- * Potato Dextrose Agar
- * Brain heart Infusion Agar

(12) Merits of Embryonic cultivation of virus?

- * Cost effective and maintenance is much easier
- * No antibodies are formed

(13) What is capsid?

- * Depending on the virus nucleic acid may be linear or circular
- * The protein coating surrounding the nucleic acid core is called capsid

14. what is envelope in virus?
- * Virus capsids are covered by an envelope which usually consists of lipids, proteins, carbohydrates
 - * Depending on the virus, envelopes may or may not be covered by spikes

15. classify fungi

- * Ascomycota
- * Basidiomycota
- * Zygomycota
- * Chytridiomycota

16. pharmaceutical use of fungi

- * Important antibiotics, such as penicillin & cephalosporins can be isolated from the fungi
- * Recombinant DNA technology, which includes fungi as hosts
- * used in industrial fermentation process

17. write str of typical virion

HIV virus diagram.

18. mycelium?

- * Mycelium is the mass of branched, tubular filaments of fungi
- * Mycelium makes up undifferentiated body called thallus
- * Mycelium may be microscopic in size or developed into visible structures.

19. ⁶viruses are obligate parasites?

- * Because it cannot function or replicate without a host
- * The lack metabolic machinery of their own to generate energy or to synthesise protein

20. d/w Flagella & Fimbriae

Fimbriae

- * Fimbriae is a bristle like short fibres
- * Fimbriae enables cell to stick to the surface
- * Fimbriae are solid str without a lumen.

Flagella

- * Flagella is a whip like filamentous str
- * Flagella assists in locomotion
- * Flagella is hollow tubular str with lumen

21. d/w Enriched & Selective media

Enriched

- * used to enhance the growth of specific organism

Ex: chocolate Agar,
Blood Agar

Selective

- * contains agent that inhibit growth of other organisms except the needed one

Ex: McConkey's medium
Lysine medium

22. Mesosomes

- * It is a folds in cytoplasmic membrane
- * Septal Mesosomes participate in DNA Replication & cell division

* Non-Septal mesosomes participate in secretion

23 D/W prokaryotes & Eukaryotes

prokaryotes	Eukaryotes
* Circular DNA in cytoplasm	* Linear DNA in nucleus
* Non membrane bound nucleoid	* Membrane bound nucleus
* Plasma membrane lack receptors	* Plasma membrane possess receptors
* Lack cytoskeleton	* Possess cytoskeleton
* Asexual reproduction	* Sexual reproduction

24 d/w Bacteria & Virus

Bacteria	Virus
* Bacteria don't need host organisms for replication	* Virus need host organism
* Considered as living organism	* Non living
* Visible under light microscope	* Visible under electron micro-
* Localized Infection	* Systemic Infection
* Infections can be prevented by antibiotics	* Spread can be prevented by vaccines
* Unicellular	* No cells

25. Alw Bacteria & Fungi

Bacteria

Fungi.

- * Prokaryotes
- * unicellular
- * Some use flagella to move
- * cell wall is made up of peptidoglycan

- * Eukaryotes
- * Multicellular
- * Immobile organism
- * cell wall is made up of chitin

26. Alw phototrophes & chemotrophes

phototrophes

chemotrophes

- * organisms using light as energy source & CO_2 as principle carbon source

- * organisms using chemical as energy source and CO_2 as principle carbon source

27. functions of leptoplasmic membrane

- * It maintains intracellular osmotic pressure
- * It consists of enzymes capable of catalyzing chem. cal. reaction.

28. what are plasmids

- * A plasmid is small DNA molecule present in the cell that is physically separated from chromosomal DNA and can replicate independently.
- * They carry small number of gene, some associated with antibiotic resistance.

29. Micronutrients

- * Micronutrients are essential elements required for the organisms to carry out the physiological function.
- * Micronutrient deficiency can easily be recognized and treated.
- * Micronutrient requirement differ b/w organism.

30. Composition of peptidoglycan

- * peptidoglycan consists of repeating disaccharides attached by polypeptides
- * Disaccharide portion is composed of monosaccharide called N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), which are related to glucose.
- * Alternating NAM & NAG molecule linked to form carbohydrate backbone.

31. Pleomorphism

- * It is the ability of the microorganisms to alter their morphology, biological function or reproductive modes in response to environmental condition.

Helicobacter pylori - which exists as Helix or coccoid form

32. Swollen form

- * Swollen forms of bacteria in ageing culture, especially in high salt concentration.

Ex: plague bacillus

33. Intracytoplasmic Inclusions 9. ex

* Cytoplasmic Inclusions are diverse Intracellular non-living substances that are not bound by membranes

Ex: glycogen, lipids, pigments

34. Function of capsule

* Capsules resist phagocytosis by WBC

* It prevents the cell from desiccation

35. Mesophilic, psychrophilic, thermophilic

* Mesophiles are microorganisms which grow at moderate temperature 10°C and 40°C

Ex: E. coli, Staphylococcus aureus

* Psychrophiles are microorganisms which grow at cold temperature

Ex: Vibrio, Pseudomonas

* Thermophiles are microorganisms which grow at warmer temperature

Ex: Streptococcus thermophilus

36. Fastidious bacteria

* These are group of bacteria which requires special nutritional supplements and conditions to grow

37. Selective media

- * It is the media which used to grow the microorganisms, where inhibitory substances are added to solid media to get selective organism.

Ex: MacConkey's medium, U medium

38. pour plate method

- * It is the method of choice for counting the bacteria.
- * Inoculum is placed in the center of petri dish, then molten agar is then poured into the petri dish.

uses: determining no of microbes/mL

39. log phase & decline phase

log phase

- * No of bacteria doubles with each generation time.
- Becomes no of bacteria $\uparrow$ exponentially

Decline phase

- * ^{ring} Due to this phase population decreases due to death of the cell.

40. Facultative anaerobes

- * Facultative aerobes are org^s that makes ATP by aerobic respⁿ if oxygen is present, but capable of switching to fermentation if oxygen is absent.

obligatory anaerobes

- * obligate aerobes die in the presence of oxygen

Turbidostat

- 41 Chemostat
- * Chemical composition is kept constant at a controlled level for the culture of microorganism.
 - * Chemical composition of the medium is constant.
 - * Fresh medium is continuously added.
- * Feedback is maintained b/w the turbidity and the dilution rate.
- * Turbidity of the medium is constant.
 - * Fresh medium is automatically added.

42 Organotrophs Lithotrophs

- * Energy from organic compound.
- * Energy from inorganic compounds.

Ex: Sulphur bacteria
Hydrogen bacteria

Ex: prokaryotes
Eukaryotes

43 Autotrophs

- * Food is prepared from CO_2 , water & sunlight.
- * chlorophyll is required

* Food is generally in day time

Heterotrophs

- * Food is obtained from other organisms.
- * chlorophyll is not required

* Food can be obtained at all time

44. What is anaerobic media? Give ex.

* The culture media that include anaerobic blood agar plates enriched with substances such as brain-heart infusion, yeast extract.

* The media which are used to grow anaerobic bacteria is called anaerobic media.
Ex: Thioglycolate medium

45. Basal media? Give ex.

* A media which promote the growth of many type of microorganisms, which don't require any special nutrient supplements.

Ex: nutrient broth, nutrient Agar

46. Synchronous growth.

* Synchronous growth is the growth of bacteria such that all the bacteria are at the same stage in their growth cycle.

selective.

47. Transport, Enriched media, & differential media with ex.

(i) Transport media is used for the transporting the sample.

(ii) Delicate microorganisms may not survive while transporting the specimen without transport media.

Ex: Stuart medium.

(i) It is used to isolate pathogens from mixed culture by using growth media that favours the growth of particular organisms.

Ex: Alkaline peptone water.

① Selective media is the media used for the growth of microorganisms where it is incorporated with inhibitory substance to suppress the unwanted organism

Ex: McConkey's agar medium $\rightarrow$ E. coli
W medium $\rightarrow$ M. Tuberculosis

② A media in which has substance incorporated in it, which distinguish bacteria

Ex: McConkey's medium

48. Role of agar in culture media

- * Solidifying agent
- * provide nutrient without getting metabolized by bacteria
- * Supports the growth of wide range of bacteria

49. arrangement based classification of cocci

① Spherical

- * These are smallest bacteria which are spherical in shape

$\rightarrow$ Micrococci $\rightarrow$ when they occur singly

Ex: Micrococcus

$\rightarrow$ Diplococci $\rightarrow$ when they occur in pair

Ex: Diplococcus pneumoniae

$\rightarrow$ Streptococci $\rightarrow$ when they occur in chain

Ex: Streptococcus lactis

$\rightarrow$ Staphylococci $\rightarrow$ when they are found in group

Ex: Staphylococcus aureus

Mean generation time

* It is the average interval b/w birth of individual & the birth of offspring

* mean generation time is the amount of time required for the concⁿ of cell to double during log stage

* Increase in population is always 2^n where n is the no of generation.

Q1. Mention chemical used in gasper method

(i) Citric acid.

(ii) Sodium carbonate.

(iii) Sodium borohydride.

Q2. Spirochetes, *Sickeltoia*

* Spirochetes are gram negative, motile, spiral bacteria.

* Unlike *Spirilla*, which use flagella to move, Spirochetes move by means of an axial filament.

* It is a flexible organism having several coils.

Ex: *Treponema pallidum*

Q3. Reagents used in acid-fast staining?

Stains: - Carbol fuchsin, methylene blue.

Reagents: - Acid - Ethylalcohol.

54. Mordant

Decolorizing agent

- * It forms a complex in gram positive bacteria
- * It leads to cell shrinkage in γ^+ cell, & extracts lipids from γ^- cell and makes the cell decolorized.

55. Acid fast vs Non-Acid fast

- * It contains mycolic acid which resist staining
- * It does not contain mycolic acid
- * If once stained difficult to destain
- * It undergoes destain due to lack of lipid layer

56. uses of preservation technique

- * usually involves preventing the growth of bacteria & fungi
- * Retarding the oxidation of fats which cause rancidity

57. Micromanipulators

- * Micromanipulators are device intended to translate macroscopic movement of the human hand into microscopic movement of a fine tool held in its grasp

uses:- designed to move small instruments & tools.

58. principle & uses of Coulter Counter Instrument.

* As the particle passes through the aperture, some electrolyte is displaced and changes the resistance b/w the electrode and causes the pulse in the voltage.

* The magnitude of the pulse will be proportional to volume of the particle.

uses: This method is used in total counting of bacteria.

59. Cryoprotective agents? give ex.

* Cryoprotectant is a substance used to protect biological tissue from freezing damage.

* Antarctic organisms create ~~pro~~ cryoprotectants in their bodies to minimize freezing damage during cold winter.

✓ * Cryoprotective agents are substances used in frozen storage of microorganisms which includes simple & complex chemical compounds.

ex: glycerol, skimmed milk, methanol

60. principle of Starch hydrolysis test?

* Starch Agar is used to demonstrate the hydrolytic activities of amylase to yield maltose & glucose.

* Starch in presence of Iodine will form blue/black color to the medium. Indicating the absence of enzyme.

* If the starch has been hydrolysed a clear zone of hydrolysis will surround the growth of microorganisms.

61 carbohydrate utilization test?

* Indole production test.

* Citrate utilisation test.

* Methyl Red Test

* Voges-Proskauer test

* Starch Hydrolysis

* Gelatin Hydrolysis

62 Define Sterilization?

* It is the process in which inactivation or elimination of all viable organisms is achieved.

63 pasteurization? application

* It is the process of heating up milk to a high temperature for a short time and then cooling it down again quickly.

* The std temp used is 70°C for 30 min.

Application

(i) Dairy Industry

(ii) Food processing industry to achieve food preservation & food safety.

64 what is incineration?

* It is the method used to sterilize anatomical waste and microbiological waste at a temperature of 1000°C to ash & gas.

65. Red hot sterilization process

* In this method items to be sterilized are held almost vertically in a Bunsen flame until red hot

Items to be sterilized are

- Inoculating wire loops
- points of forceps

66. Application of UV radiation as sterilant

* Sterilization of air.

* UV lamps are mounted on walls, to prevent cross infection from patients

* Destroys viruses & bacteria from vaccines

67. Ionizing Non ionizing radiations used for ster

- | | |
|-----------------|----------------------|
| (i) X-rays | (i) Microwaves |
| (ii) gamma rays | (ii) Radionuclides |
| (iii) UV light | (iii) Infrared waves |

68. Media used for sterility testing?

- (i) peptone broth.
- (ii) glucose peptone broth.
- (iii) Liver broth.
- (iv) Cooked meat medium.

69. List and Biological Indicators

Spores of *Bacillus subtilis*

Spores of *Bacillus thermophilus*

Q70 why are positive & negative controls are used in sterility testing?

* positive control is an experiment that involves the repetition of the test. ~~using~~

* It is the duplicate experiment which helps the analyst confirms the correctness of the results

* Negative control is an experiment in which microbiologist knows that there will be negative outcome

* It involves testing the experiment with something that will have no effect on it.

Q71 applications of membrane filtrations

(i) Reverse osmosis :- concentration of solution by removal of water.

(ii) ultrafiltration :- concentration of large and macromolecules.

(iii) Microfiltration :- Separation of heat labile bacteria by membrane filters

Q72 appl & limitations of formaldehyde.
Appl.

(i) It has been used for fumigation of rooms & hospital blankets since long limit

(ii) proteins & organic matter readily inactivate formaldehyde.

73. What are HEPA filters?

* The HEPA filters removes particulates such as microorganisms, from the air.

* The HEPA filters does not remove vapours or gases

* HEPA filters used in biosafety cabinets should have minimum filtration efficiency 99.99%.

74. Time & temperature of incubation for sterility testing

Culture media :- } 121°C for 20 min
Diluting fluids :- }

75. Disinfection

* Disinfection is the process of destruction or removal of pathogenic microorganisms and the object is said to be disinfected.

Ex: Alcoholic, phenolic Disinfectants

76. Antisepsis

* If the object is animate such as human body tissue, then the chemical used for destruction or removal of pathogenic microorganisms are Antisepsis

Ex: Iodine, Alcohol

77. Classify Disinfectants with ex

① Halogens :- Iodine, Chlorine

② Alcohol :- Ethanol, Isopropyl Alcohol

③ phenol derivative :- phenol, cresol

④ metallic salts :- Silver nitrate, zinc compounds

78. Ex for water disinfectant

Ans Chlorine, Chlorine dioxide, ozone.

79. Aerial disinfectants

Ans Aldehyde :- Formaldehyde, glutaraldehyde

80. What is MIC?

Ans MIC measures the lowest concentration of the disinfectant that inhibits the growth of known strain of organism on incubation.

81. Zone of Inhibition?

Ans It is an area of media where bacteria are unable to grow, due to presence of drug that prevents their growth.

82. Disc Diffusion method?

* paper disc impregnated with antimicrobial agent are placed on agar medium uniformly seeded with test organism.

* If the bacteria are susceptible to particular antibiotic, a zone of inhibition is formed.

eg for

83. Determination of Radial walkers Co-efficient?

RW = $\frac{\text{Dilution of unknown which kills}}{\text{Dilution of phenol which kills}}$
Co-efficient = $\frac{\text{in 7.5 but not in 5 min}}{\text{in 7.5 but not in 5 min}}$

D/O Bacteriostatic & Bacteriocidal agent

Bacteriocidal

* It refers to antibiotic that kill Bacteria

* Action is Irreversible

* Inhibit cell wall formation of bacteria

Ex: Cephalosporins
Vancomycin

Bacteriostatic

* It refers to antibiotics that prevent the growth of bacteria

* Action is Reversible

* Inhibit DNA Replication & protein Synthesis

Ex: Tetracyclines
Sulfonamides

85. what are haptens?

Ans: Antigens which have the ability to react specifically with the appropriate antibodies are called haptens

86. Epitope & paratopes

(i) Epitope is the part of an antigen molecule to which an antibody attaches itself.

(ii) It binds to paratope on antibodies and receptors on both B & T cells

(iii) It is also known as antigenic determinant

(i) paratope is the part of an antibody molecule that binds to antigen

(ii) It binds to epitope

(iii) It is also known as antigen binding site

87. Characters of antigen-antibody reactions?

- (i) Agglutination
- (ii) precipitation
- (iii) complement fixation
- (iv) Enzyme linked Immunosorbent assay
- (v) Fluorescent Antibody technique

88. what are mixed vaccine? Give ex.

* To simplify administration, more than one immunizing agent may be included in vaccine. These are known as "combined" vaccine.

Ex: Diphtheria, whooping cough, tetanus

89. live vaccine

* Live vaccine contains Bacteria/Viruses which have been mutated & weakened. So that they create protective immune response but do not cause disease in healthy people.

~~inactivated~~ ^{killed} vaccine
* Inactivated vaccine contain whole bacteria or viruses which have been killed or small part of bacteria or virus such as protein or sugars which cannot cause disease.

90. Active Immunity vs passive immunity

* produced inside of the body

* Introduced from outside of the body

* Results from

* Results from

(i) Direct infection

(i) Breast milk

(ii) vaccination

(ii) Injection

(iii) Mother to baby through placenta

* overtime effect

* Immediate effect

* long term effect

* Short term effect

91 Vaccine

Sera

- * Vaccine is an antigenic substance used to provide immunity against disease
- * It is a clear, pale yellow liquid that separated from the clotting factors
- * Contains dead bacteria or weak bacteria
- * Does not contain bacteria or toxins
- * Stimulates the body to make antibodies
- * Contains anti-bodies formed in another animal

92 Toxoids

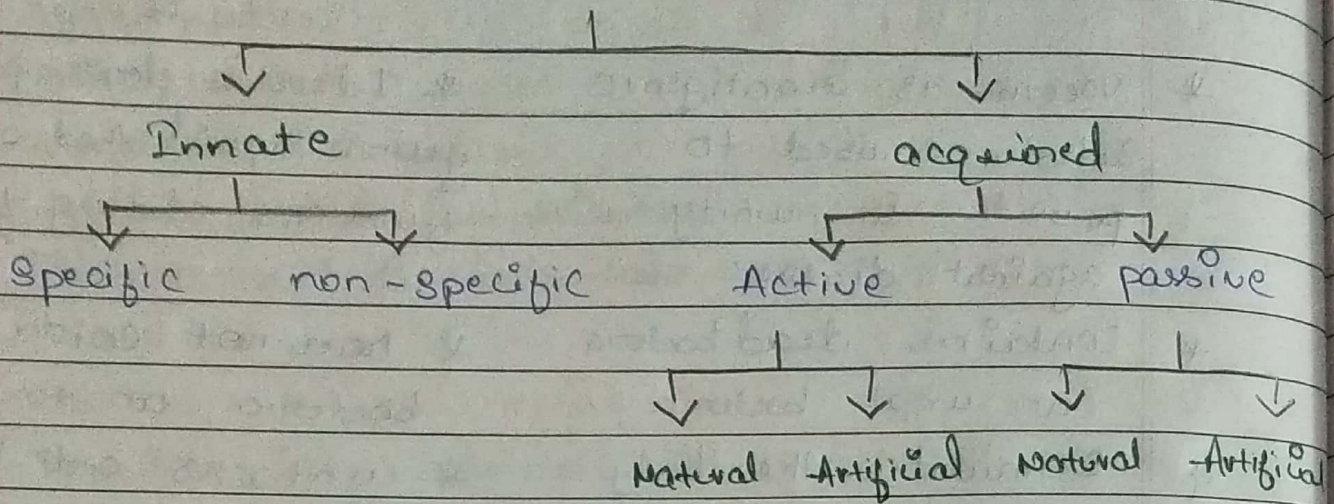
- * Toxoid is an inactivated toxin whose toxicity has been suppressed either by chemical or heat treatment where immunogenicity is maintained
- * Toxins are secreted by bacteria whereas toxoids are altered form of toxins

93 Application of ELISA

- * Serum Antibody Concentrations
- * Detecting potential food Allergies
- * Diagnosis of HIV, STD, Hepatitis
- * Detection of antigens
- * Detection of antibodies

94

Immunity



95.

Agglutination

precipitation

- * process of forming a solid mass from the particles present in the solution
- * particles present in the sol are the starting material
- * useful in blood grouping
- * process of forming a insoluble solid mass from ions present in the solution
- * Ions present in the sol are the starting material
- * useful in quantitative analysis

96

Different types of Immunoglobulins

① IgA, IgD, IgE, IgG, IgM.

97

Antitoxin

- * An Antitoxin is an antibody with the ability to neutralize specific toxin
- * Antitoxin are produced by certain animals, plants and bacteria in response to toxic exposure.

98. General Storage Condition for vaccine & sera,

Inactivated vaccine — $2^{\circ}\text{C} - 8^{\circ}\text{C}$ or 5°C
 Sera — 45°C

99. Define vaccine with ex?

* Vaccine is any preparation intended to produce immunity to a disease by stimulating the production of antibodies

Ex: Cholera vaccine
 BCG vaccine.

100. Define Immunity.

* It is the ability of an organism to resist a particular infection or toxin by the action of specific antibodies or sensitized white blood cells

101. Organisms used for assay of Streptomycin.

* *Streptococcus aureus*

* *Bacillus Subtilis*

* *Klebsiella pneumonia*

For Vit B_{12} —> *Lactobacillus leichmanni*

Vit B_2 —> *Lactobacillus casei*

102. Anti-microbial Sensitivity? method of determination

* It is the measurement of the susceptibility of bacteria to antibiotics

- | | |
|--------------------------------------|-------------------------|
| ① cup plate method | } agar diffusion method |
| ② ditch plate method | |
| ③ Serial dilution fluid media method | |

103. Causative organism & diagnostic test for malaria

Causative organism

- * Plasmodium vivax
- * Plasmodium falciparum
- * Plasmodium malariae

Diagnostic test

- * Fluorescent microscopy
- * PCR
- * Malaria blood smear
- * Serology

preventive measures of malaria

- * always using bed net
- * using mosquito repellent
- * wearing long sleeves
- * pour any left over water into ground

Mode of transmission of malaria

- * bite of infective female Anopheles mosquito
- * Transfusion of blood from infected mosquito to person
- * use of contaminated needle & syringe
- * mother to unborn child

104. Signs & Symptoms & prevention of Dengue

Sign & Symptoms

prevention

- | | |
|----------------------|----------------------------|
| (i) Headache | * always using bed net |
| (ii) Eye pain | * using mosquito repellent |
| (iii) Rash | * wearing long sleeves |
| (iv) Muscle pain | * pour any left over water |
| (v) Joint pain | into ground |
| (vi) Nausea/vomiting | |

attenuated vaccine ? give ex
 vaccine contains Bacteria/viruses which have
 been mutated & weakened, so that they
 create protective immune response but
 they do not cause disease in healthy people