# Virtual University of Pakistan

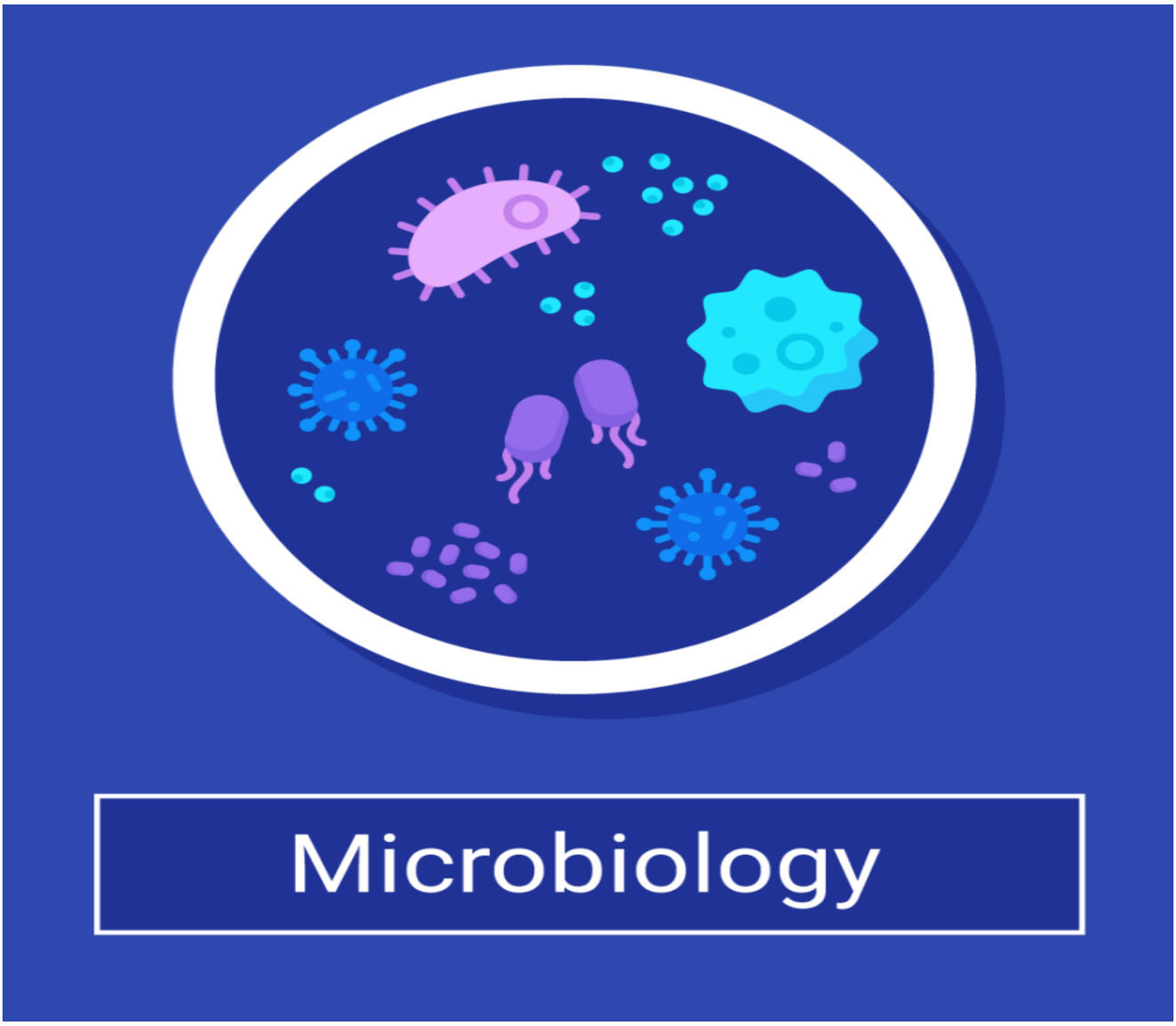
## Federal Government University

# MSC-ZOOLOGY

## PAST SOLVED UPDATED PAPER 2022

# BT102-Microbiology

| QUIZ 1 | QUIZ 2 | QUIZ 3 | GRAND QUIZ | MID TERM | FINAL TERM |
|---|---|---|---|---|---|





# MUHAMMAD IMRAN

# MUHAMMAD IMRAN

## BT102 - Microbiology

## Quiz No.1 2021

1. Which of the following contains mycolic acid? _____Mycobacterium and Nocordi
2. After gram staining, gram positive bacteria appear. _____purple
3. Rod shaped bacteria are called. _____bacilli
4. Energy is required in. _____active movement
5. Which of the following structure is involved in the locomotion of bacteria? _____flagella
6. Gram positive bacteria produced. _____exotoxins
7. The basal body of flagellum of gram positive bacteria consist of rings. _____2
8. 70s ribosome consist of two subunits. _____50S and 30s
9. Are used for twitching motility. _____pili
10. Integral protein are imbedded in the cell membrane and are insoluble in water.
11. Which of following pH is acidic?_____ all of the given option
12. Bacteria get nitrogen from____. _____all of the given option
13. Which of the following is the characteristic of coenzyme?_____ all of the given option
14. Bacteria get sulfur from____._____ all of the given option
15. Which of the following is the physical source of growth? _____All of the given options
16. What are the physical requirements of bacteria? _____All of the given options
17. Which of the following bacteria formed endospore?_____ all of the given option
18. In anaerobic respiration, final electron acceptor is inorganic substance other than O2 is _____All of the given options
19. Which of the following is required for aerobic respiration? _____None of the given options
20. Psychrophiles are extremophilic organisms that are capable of growth and reproduction in temperatures around_____ -10 to 20
21. O2 has no effect on _____. _____Aerotolerant aerobes
22. Which of the following is not present in bacteria?___ both histone and nuclear membrane
23. Phosphate ions_____ Phosphate ions
24. Bacteria utilize oxygen for _____. _____aerobic respiration
25. Specialized resting cells are known as ……._____ Endospores
26. Which of the following is used to maintain the desired pH?_____ buffers
27. Photoautotrophs use ..... as a source of energy. _____Light
28. NADH and FADH2 during Krebs cycle oxidized via Electron transport chain and generate ATP molecules_____38
29. Which of the following compounds are needed for nucleic acid synthesis?_____ _____ _____ _____Sulfur and phosphorus
30. Which of the following group of microbes strictly need oxygen for growth?__ __Obligate Aerobes
31. Which of the following enzymes catalyze the conversion of atmospheric oxygen into hydrogen peroxide? _____Superoxide dismutase
32. Bacteria need…. for the synthesis of protein and nucleic acids. _____Nitrogen
33. Which of the following is hyperthermophile in nature? _____Archaea
34. Removal and gain of electrons from an atom is known as ....._____Redox Reaction
35. Helicobacter pylori is a _____aerotolerant aerobe
36. Which of the following is the cellular energy for most of the microbes? _____Carbon
37. Microorganisms belonging to the group, do not use O2 but can tolerate it fairly well _____Aerotolerant Aerobes
38. Movement against the concentration gradient is known as..._____Active movement
39. Biological oxidations involve the loss of hydrogen atoms, they are also called….reactions._ _____Dehydrogenation
40. All organic compounds contain_____ carbon
41. Cytoplasm contains... percent water._____80%
42. Cold loving bacteria are known as..._____Psychrophiles
43. Chemoheterotrophs use Carbon from_ _____Carbohydrates
44. Removal of electrons from an atom is known as_ _____Oxidation
45. Aerobes that require O2 in low amounts are called as a_____Microserophiles
46. Besides water, is the most important requirement for microbial growth._ _____Carbon
47. Oxidation of glucose to pyruvic acid with the production of some ATP and energy-containing NADH is known as _____Glycolysis

48. Microorganisms that use light as a source are known as ___._____**Photoautotrophs**
49. Which of the following is used for catalase test? _____**Hydrogen peroxide**
50. Which of the following is anoxygenic?_____**Rhodopseudomanas**
51. Chemoautotrophs use Carbon from_____**co2**
52. Which of the following condition in which water moves into the cell? _____**Hypotonic**
53. NADH and FADH? are oxidized during -_____**Electron transport chain**
54. Which of the following initiate the spore activation? _____**Heating**
55. Bacteria need for the synthesis of nucleic acids._____**Sulfer phosphorus**
56. Facilitated diffusion is done with the help of ……_____ **Integral Proteins**
57. Which of the following is the smallest bacterium?_____ **Mycoplasma**
58. The optimum temperature of mesophiles is ___:_____ **37°C**
59. Which of the following is responsible for destruction of cell membrane?_ **All of the given options**
60. Hyperthermophiles lives in ____._____**Hot springs**
61. Archea don't have cell wall or have pseudomurein and behave like_____ **Gram negative bacteria**
62. The basic shapes of bacteria are:_____ **All of these**
63. Glycolysis is the____ with the production of some ATP and energy-containing NADH.__ _____**oxidation of glucose to pyruvic acid**
64. The major difference between Gram positive and Gram negative bacteria is of:_____ **Cell wall**
65. The bacteria which don't possess flagellum are called_____: **Atrichous**
66. Flagella move ……. for forward thrust._____- **Both counter clockwise & run/swim**
67. Which of the following is the type of membrane protein?___ **Both peripheral & integral proteins**
68. Water molecules can pass through lipid bilayer by *simple diffusion* or though water channels called____:_____ **Aquaporins**
69. The ____ contains hydrolytic enzymes and binding proteins for nutrient processing and uptake._ _____ **gram-negative bacteria**
70. The ____ do not use hydrogen from water but from $H_2S$ or $H_2$ to reduce $CO_2$.__**Photoautotrophs**
71. Which of the following is the backbone of all organic matter?_____ **Carbon**
72. A viscous and gelatinuous secretion that surrounds the bacterial cell is called ____:__**Glycocalyx**
73. Teichoic acid is made up of …….. _____**All of the given options**
74. Which of the following is not a part of the flagella?_____ **None of the given options**
75. Which of the following structure used for moving?_____ **Flagella**
76. Hair like small appendages on G-neg. cells are known as ……._____**Fimbrae**
77. When the virus is outside of a cell in extracellular state, it is called_____**Virion**
78. Viroids only infects _____**Plants**
79. Virus is surrounded by a ____ coat called capsid._____ **Protein**
80. Cell wall of algae is made up of ……._____**Cellulose**
81. According to the five kingdom of classification bacteria belongs to ____ kingdom._____**Monera**
82. Which of the following is a basic dye?_____ **Crystal violet**
83. In which of the following kingdoms microbes are placed?_____ **Monera**
84. Three domain classification was proposed by _____**Carl Woese**
85. Which of the following is the advantage of staining the microbes?_____ **All of the given options**
86. The theory of spontaneous generation was proposed by ……_____**Aristotle**
87. Which of the following stain is used as simple stain?_____ **Methylene Blue**
88. Which of the following are microbial products?_____**All of these**
89. Robert Koch discovered that _____ is the cause of anthrax:_____ ***Bacillus anthracis***
90. Decolorizing agent used in Gram staining is:_____ **Alcohol**
91. According to the latest system of classification, organisms are classified into ____ kingdoms.___ **5**
92. Which of the following is the dye for negative staining?_____ **Eosin**
93. Which of the following is required for treatment of diseases and cancer?_____ **Antibodies**
94. The first drawing of microbes was published by _____._____**Robert Hooke**
95. Eyepiece is also known as ……..._____**Ocular Lens**
96. Which of the following is the reason for changes in the classification?___ **All of the given options**
97. There are …… Kingdoms in domain Eucarya._____ **4**
98. Eukaryotes have distinct …… as point of difference to the prokaryotes._____ **Nucleus**
99. Refractive index is the____:_____**Ability of a medium to bend the light**
100. The experiments conducted by _____ ended the spontaneous generation controversy._____ _____**Louis Pasteur**
101. The study of pandemics, endemics and epidemics is done in:_____ **Epidemiology**
102. In 1665, first drawing was published of microbes by:_____ **Robert Hooke**
103. The light source in fluorescent microscopy is_____:_____**UV light**
104. Classification of microbes is important to____:_____**All of these**

105. Mycology is branch of biological science which deals with the study of ……__**Fungi**
106. Which is the correct order of Gram Staining?_____ **Crystal Violet > Gram Iodine > Decolorizer > Safranin**
107. In the golden age of microbiology:_____ **All of these**
108. A colony or a drop of broth culture can be picked by using __ for making smear._____ **Platinum loop**
109.

# GRAND QUIZ

1. The bacteria who don't retain the color of crystal violet in Gram staining:_**Gram negative bacteria**
2. Which of the following is required to see microorganisms?_____ **All of the above options**
3. Monitoring of diseases in populations is known as ……_____ **Epidemiology**
4. Which of the statement is not correct for passive movement?_____ **none of these**
5. Half of the dry weight of microbes is___:_____ **Carbon**
6. Antony Van Leeuwenhoek used the term _____ for microbes firstly:_____ **animalcules**
7. Which of the following organisms mostly live in extreme conditions?_____ **Archea**
8. In oil immersion lens, the image is magnified ____ times:_____ **100 times**
9. Which of these is a type of differential staining?_____ **Gram staining**
10. The drum stick shaped endospore is identified in____:_____ **Clostridium tetani**
11. ____ is used by bacteria for DNA transfer._____ **pili**
12. Phenol and Phenolics compound First widely used antiseptics for disruption_____ **plasma membranes**
13. In Gram positive bacteria, layer of ____ is thick._____ **Peptidoglycan**
14. Microscopy that is required for unstained specimens is known as _____ **Darkfield Microscopy**
15. Photoautotrophs use Carbon from _____._____ **CO2**
16. In catalase test, hydrogenperoxide is converted into ___._____**water and Oxygen**
17. The oil immersion lens of microscope is of …… power._____**100x**
18. Salts composed of a negative and positive ions are known as …….._____ **Stains**
19. The man behind the cell theory was _____._____ **Robert Hooke**
20. Which of the following bacteria has atypical cell wall?_____ **Mycoplasmas**
21. Antigen – Antibody reaction is known as ……_____ **agglutination**
22. How we can fix the smear?_____ **both Physical and Chemical Methods**
23. Special staining includes:_____ **All of these**
24. The study of microbes is known as ……. _____**Microbiology**
25. Mordant is used to_____._____ **Decolorize the smear**
26. The scientist who concluded that, "Air is the vital force" is:_____ **Spallanzani**
27. Naming of the organisms was first introduced by_____._____ **Linnaeus**
28. The ____ use light as a source of energy and organic compounds as carbon source._____**Photoheterotrophs**
29. The cell wall of Archea is composed of special _____._____ **Lipids**
30. Which of the following is acidophiles?_____-_____ **Both of them**
31. Which of the following is used for plasma membrane disruption?___ **Both phenol and bisphenol**
32. The term "animalcules" for microbes was firstly used by ……. **Antony Van Leeuwenhoek**
33. The unicellular form of fungi is known as ……_____ **Yeast**
34. Mycobacterium tuberculosis causes _____:_____- **leprosy**
35. The main reason discovered by Pasteur of souring of beer and wine was:_____ **Bacteria grow in presence of oxygen and form acetic acid**
36. Which of the following is the most important structure in prokaryotes?_____ **Cell Wall**
37. When cocci joins in chain like structure then the shape is known as ……_____**Streptococci**
38. Prokaryotes and Eukaryotes are ……. Similar_____ **Chemically**
39. LPS stands for ……_____ **Lipopolysaccharide**
40. Bacteria named as *Eschericia coli* is named after scientist:_____ **Theodor Escherich**
41. Spirochetes bacteria move by ……..._____ **Axial Filaments**
42. Chemoautotrophs and Photoautotrophs use ___ as carbon source._____ **$CO_2$**
43. Substances that absorb short wavelength of light and emit longer wavelength are known as …….._____ **Fluorochromes**
44. Which of the following is used for gene mapping?_____ **Conjugation**
45. The branch of microbiology that deals with monitoring diseases in a population and implementation of appropriate control measures is called_____ **Epidemiology**

46. Which of the following disease caused by spirochetes?_____ **All of the given options**
47. Before staining, the smear is ____._____ **Fixed**
48. Gram Staining falls into which of the following category?_____ **Capsule Staining**
49. Pasting of sample on glass slide is known as ……..._____ **All of the given options**
50. Boiled extracts of hay or meat can give rise to microorganisms, this statement was proposed by _____. **John Needham**
51. Usually bacteria are stored in _____ **solutions**.
52. When two round shape bacteria joins then it is known as ……_____ **Cocci**
53. Prokaryotes divide by ____:_____ **Binary fission**
54. *Spirillum minus* causes ____:_____- **Rat bite fever**
55. The___ antigen is used for identification of serovar in Gram negative bacteria._____ **H antigen**
56. Soluble antigen binds with antibodies is known as ……_____ **Precipitation test**
57. A resistant dormant structure within a cell is called____._____ **Endospore**
58. Psychrophiles are extremophilic organisms that are capable of growth and reproduction in temperatures around_____ **15 centigrade**
59. Which of the following are too small to be seen with the unaided eye ?_____ _____ _____ **Both germs & microorganisms**
60. *Nocardia* is an example of:_____ **Acid fast bacteria**
61. Prokaryotes have no ……_____ **All of the given options**
62. Which of the following is correct about E. coli?_____**Facultative Anaerobes**
63. Oil is used for _____ lens._____ _____**100X**
64. Extrachromosomal DNA is known as ……_____ **Plasmid**
65. Who is first to publish the extensive and accurate observations of microbes?_____ _____ ____ **Antony van Leeuwenhoek**
66. Which of the following is correct about Mycobacterium tuberculosis?_____ **Obligate Aerobes**
67. Which of the following contain mycolic acid?_____ **Mycobacterium and Nocordia**
68. Lipopolysaccharide thick layer is present in:_____ **Gram positive bacteria**
69. E.coli was discovered by a scientist named_____:_____ **Theodor Escherich**
70. Flagella is responsible for ……_____ **Locomotion**
71. Pasteur found that yeast ferment sugar to ____._____ **Alcohol**
72. Which of the following is used for microbial control through chemicals?_____ **Phenols**
73. Which of the following are the first living organisms on our planet?_____ **Bacteria**
74. Who discovered the first vaccine?_____ **Louis Pasteur**
75. The light source in microscope is called ____:_____**Illuminator**
76. Anthrax is caused by ……_____ **Bacillus anthracis**
77. Which of the following organisms act as protozoa like at one stage and fungus like in another?__ _____ **Slime mold**
78. Immunology deals with the study of_____:_____ **All of these**
79. Which of the following organisms are motile and unicellular organisms?_____ **Protozoa**
80. Peptidoglycan is made up of ……._____ **Lipids**
81. Ability of a medium to bend the light is called……_____ **Refractive index**
82. The addition of phosphate in a chemical compound is called ___._____ **Phosphorylation**
83. Lipid A is responsible for ……..._____ **All of the given options**
84. Dichromatic mirror reflects short wavelengths and transmits longer wavelengths in _____.__ _____**Fluorescent microscopy**
85. Which of the following staining is done for Acid Fast Bacteria?_____- **Endospore Staining**
86. Which of the following directs the light through the specimen?_____ **Condenser**
87. ……. is composed of pilin_____ **Fimbriae**
88. Which of the following is the dye for positive staining?_____ **All of the given options**
89. Which of the following is the physical method of microbial control?_____ **All of the given options**

## Quiz No.2 Topics 46 to 56

1. Protein could be detected by _____**Gel Electrophoresis**
2. Bacterial cell wall made up of_____**peptidoglycan**
3. Proteins from microbes are separated by_____ **Gel electrophoresis**
4. ……. is Transfer of DNA by a virus called bacteriophages_____ **Transduction**
5. The Halogens Iodine and chlorine Inhibit …….. Function _____ **protein**
6. Transformation is the horizontal gene transfer between two_____ **bacteria**
7. Which of the not a bacterial gene transfer method? _____ **Transfection**

8. The primary stain gram method is _____ **Crystal violet**
9. The alcohols ethanol isopropanol are used for the denatured of _____**protein**
10. Antigens antibodies reactions is a …….. method for microbial identification_____ **serological**
11. The halogen which alter protein synthesis and membranes is _____._____**Iodine**
12. Zinc chloride are commonly used in _____. _____**Mouth washes**
13. Pasteurization is done at _____.**63℃ for 30 mins**
14. Which of the following is used as antimicrobial agent?_____ **All of the given options**
15. The primary stain of Gram's method is ----_____**Crystal Violet**
16. The antibiotic which prevent the spoilage of cheese is ____._____**Natamycin**
17. The lowest temperature at which all cells in a culture are killed in 10 min is called_____ _____ _____ _____**Thermal Death Point**
18. Alcohols _____ proteins and dissolve lipids._____ **Denature**
19. Time during which all cells in a culture are killed at a given temperature is called _____ _____ __.**Thermal Death Time**
20. The structure containing DNA that physically carries hereditary information is called ____ _____. **Chromosome**
21. Which of the following is used for inhibition of protein synthesis?__ **None of the given options.**
22. Which of the following is the moist heat sterilization?_____ **Autoclaving**
23. Which of the following is not the characteristic of an ideal chemical?_____ **high temprature**
24. Phenol and Phenolic compounds are first widely used antiseptics for disruption of___ ___ _____**.Plasma membrane**
25.

## Quiz No.2 Topics 62 to 72.

1. ……are Normal microbiota that can cause infection under stress conditions._____ _____ **Opportunistic pathogen**
2. Lichens are classified on the basis of _____ **fungi**
3. Virus attach to_____ **cell membrane**
4. Reverse transcriptase is an enzyme that transcribes _____**RNA to DNA**
5. ……..cell cultural can be used for the cultivation of viruses._____**all of above**
6. Neurodegenerative diseases are caused by_____ **prions**
7. Virusoids are also known as_____ **satellite viruses**
8. Lichen is a …….relationship_____ **Symbiotic**
9. Capsomeres are composed of_____ **protein**
10. Phaeophyta is……..in color_____ **Brown**
11. Plaque formation is a method of viral _____ **Enumeration**
12. Cytopathic effects refers to…….change in host cells that are caused by viral invasion___ _____ _____ **structural**
13. Range of viral infectivity is determined by _____ **Host cell receptors**
14. Toga virus get entry to host cell by _____ **pinocytosis**
15. Papovavirus is a ……..virus_____ **DNA**
16. There are ….. epidemiological method to study the occurrence and transmission of disease in a population._____ **three**
17. Lichen is a combination of cyanobacteria and _____ **fungi**
18. Location of algae in oceans depends on the availability of _____**All of the above options**
19. ……. is a competitive exclusion by Competition for nutrients or by chemicals_____ _____**Microbial antagonism**
20. Which of the following methods are used in epidemiological studies?_____**All of these**
21. The area where the virus infection has lysed the cells is called___.____ _____**Plaque**
22. ……. Is permanent residence but do not cause diseases under normal conditions.___ _____ _____**Normal microbiota**
23. The time between infection and disease is called……_____.**Incubation period**
24. Microbial communities that live in or on humans is known as ……_____**Microbiome**
25. The number of people affected by disease in relation to the total population in the given time period is called____._____**Morbidity rate**
26. The Sites where organisms of infections remain viable and act as source of infection is termed as …… _____**Reservoir**

1. Peripheral proteins_____ ___**loosely connected and soluble in water**
2. Plasmid_____ _____**extrachromosomal circular DNA**
3. Mesophils optimum temp_____ _____**is 37**
4. Unicellular fungi is _____**yeast**
5. Asexual spores are formed by_____ **mitosis**
6. Mycobiont is_____ _____**fungi**
7. ELISA is used for_____ _____**Antigen and antibody**
8. Western blotting is used for_____ _____**proteins**
9. Consistently present but with low incidence_____ _____**endemic**
10. Which is not belong to innate immunity_____ _____**specific**
11. Which is not lymphocyte_____ _____**monocytes**
12. Which is not belong to ELISA_____ _____**Probe**
13. Extreme halophiles require high salt concentration_____**30%**
14. pH of mold is _____ **5-6**
15. which do not grow in the presence of o2_____ **Obligate anaerobes**
16. the process in hot air sterilization is _____ **oxidation**
17. which is not a method of direct count_____ **Spectrophotometer**
18. resolution of microscope is_____ **0.2µm**
19. persisitent infection _____**Viruses replicate slowly**
20. cell wall is absent in_____ _____**mycoplasma**
21. Resolution of Electron Microscope _____**0.2nm**
22. Ribosomes made up of _____**mRNA and protein**
23. Rings of basal body of gram negatives_____ **4**
24. Gram negative appears on stain _____**red**
25. Basic dye methylene _____**blue**
26. Rat bite fever caused by _____ __**Spirillum minus**
27. High salt concentration used by obligat halophiles_____**30%**
28. Acellular... _____**viruses**
29. Resolution of Electron Microscope...._____**0.2nm**
30. Ribosomes made up of... _____**mRNA nd protein**
31. Rings of basal body of gram negatives....._____**4**
32. Gram negative appears on stain..._____**red**
33. Basic dye.... _____**methylene blue**
34. Rat bite fever caused by.... _____**Spirillum minus**
35. High salt concentration used by obligat halophiles.... _____**30%**
36. Direct count method..._____**membrane filter**
37. Study of fungi..._____**mycology**
38. Standard condition for hot air oven...._____**170*C for 2hours**
39. Autoclaving at... ?_____**121*C 15psi 120min**
40. EtO do not use..._____**1500mg/ml**
41. Optimum pH for molds is..._____**5 -6**
42. Peripheral proteins----------_____**-loosely connected and soluble in water**
43. Plasmid--------_____**extrachromosomal circular DNA**
44. Mesophils optimum temp. is _____**37**
45. Unicellular fungi is _____**yeast**
46. Asexual spores are formed by mitosis
47. Mycobiont is--------_____**-fungi**
48. ELISA is used for _____**Antigen and antibody**
49. Western blotting is used for _____**proteins**
50. which do not grow in the presence of $O_2$ Obligate anaerobes the process in hot air sterilization is _____**oxidation**
51. which is not a method of direct count Spectrophotometer persisitent infection --------_____ _____**Viruses replicate slowly**

# MID TERM

## 1. Function of cell membrane factors of bacterial cell death. 5 marks

cell membrane is composed of a phospholipid. molecules arranged tail to tail with heads facing away from each other. This makes lipid molecules to appear as a bilayer. So all membranes enclosing the organelle in the cell or the cell itself look as bilayer structures. This is the basic design of the plasma membrane. Protein and carbohydrates are also inserted into the plasma membrane.

There are some basic function of cell membrane in bacteria.

1. Selective permeable barrier
2. Passive and Active Transport
3. Respiration in microbes (bacteria)
4. Photosynthesis in microbes
5. Lipid synthesis
6. regulates the transport of substances in and out of the cell.
7. receives chemical messengers from other cell.
8. cell mobility, secretions, and absorptions of substances

- Cell wall parts are transported by a molecule called bactoprenol which is present in the plasma membrane.
- PM has many receptors in it with which various ligands can bind and initiate signals for gene expression etc.

Cell membrane lipids can be disrupted by alcohols, quaternary ammonium compounds and polymixins.
So, these compounds are used as disinfectants or for controlling microbial growth.

## 2. Oxidative phosphorylation and mechanism of phosphorylation.3marks

**Oxidative Phosphorylation**

a. Electrons captured from foods are transferred to co-enzymes such as NAD+ or FAD etc.

b. Then these electrons pass through a series of electron carriers and then ultimately to the last electron acceptor molecules such as O2 or other inorganic compounds (nitrate, sulphate, carbonate etc) through a series of these electron carriers in system called electron transport chains. c. During these events, ATP is generated by chemiosmosis.

**Photophosphorylation**

a. This occurs in plants and chlorophyll containing microbes or photosynthetic cells. In these cells, light energy is finally converted into ATP. An electron transport chain is also involved here. This is summarized in the accompanying diagram above.

## 3. Dry heat method of sterilization.3 marks

Form of dry heat include:

1. Dry heat (hot-air oven),
2. Flaming (Platinum loop sterilization in the flame of Bunsen burner),
3. Incineration (burning to ashes).

Similarly, moist heat can also be grouped into autoclaving (15psi), boiling at $100_oC$, and Tyndallization ($100_oC$ for 3 consecutive days to sterilize sugar solution that can be degraded by autoclaving) and pasteurization (usually at low temperature than $100_oC$).

## 4 F+cell.2 marks.

In E. coli, fertility factor (F factor) plasmid was the first plasmid observed to be transferred from one organism to the other, hence those bacteria that have this plasmid are called $F_+$ cells to differentiate from those that do not have one ($F_-$). However, once $F_-$ cells acquire $F_+$ plasmid, they also become $F_+$.

## 5. Western boltting.5 marks

**Western Blotting:** We can use this technique for the detection of antigen in the serum. Microbial proteins can be separated on SDS-PAGE by electrophoresis and the presence of these proteins can be detected by enzyme-tagged antibodies specific to those proteins. A color band will be seen where the specific protein (antigen) is present on the gel. Please remember that proteins in the gel are first transferred to a paper strip before they could be detected by specific antibodies as seen in the accompanying figure.

## 6. Function of cell wall in bacteria .5 marks

Cell wall is the most important layer in bacteria. It is rigid layer just outside the plasma membrane.
Most important structure in prokaryotes It provides shape to the organism.

- It provides protection from osmotic lysis.
- It is involved in pathogenicity (ability of the organism to cause the disease).
- Several antibiotics act on it.

Each bacterium is enclosed by a rigid cell wall composed of peptidoglycan, a protein-sugar (polysaccharide) molecule. The wall gives the cell its shape and surrounds the cytoplasmic membrane, protecting it from the environment. It also helps to anchor appendages like the pili and flagella, which originate in the cytoplasm membrane and protrude through the wall to the outside. The strength of the wall is responsible for keeping the cell from bursting when there are large differences in osmotic pressure between the cytoplasm and the environment.

## 7. What is vertical and horizontal gene transfer and also write type of lateral gene transfer.3marks

**Vertical Gene Transfer**

This is a normal way of transferring genes from parents to offspring. This happens when a cell divides. Each daughter cell receives exactly what its parent cell has.

**Horizontal Gene Transfer**

When genes are transferred from cells to cells within the same species, the process is called horizontal gene transfer. This can happen between cells of the same species, or across different species of organisms. Horizontal gene transfer involves a donor and a recipient cell. The recipient cell then incorporates received DNA into its own genome and this genome becomes a recombinant molecule or recombinant DNA. The cell that has this recombinant DNA in it is called a recombinant cell. Three processes are known by which genes can be transferred horizontally from one cell to the other, and they include:

- Transformation
- Conjugation
- Transduction

## 8. Osmotic pressure.osmtic layer and plasmolysis . 3 marks

**Osmotic pressure** is the force caused by a solution passing through a semi permeable surface by **osmosis**, which is equal to the force required to resist the solution from passing back through the surface. An example of **osmotic pressure** is the process to filter water.

**plasmolysis**

If cells are placed in hypertonic solutions, water leaves the cells shrinking the cells and damaging them. This process is called plasmolysis.
**Plasmolysis** is the process in which cells lose water in a hypertonic solution

## 9. What is sporogenesis OR sporulation .2 marks

Formation of spores takes place within a vegetative cell and the process is called sporulation or sporogenesis. It is initiated when nutrients become unavailable.

## 10. Why 70% alcohol is more effective the 100% alcohol.2 marks.

Ethanol, isopropanol are examples. Alcohols denature proteins, and dissolve lipids. Alcohols require water for being more effective. This is the reason that seventy percent alcohol is more effective than 100%. Please note that alcohols can effectively kill vegetative form of bacteria, and fungi but not spores. Alcohols are not very effective on wounds. Commonly used in hand sanitizers.

## 11. L-form bacteria.2marks

Some cells, called **L-form** organisms, are naturally found without the cell wall. In other words, normally, they have cell wall but under certain conditions, they may lack a cell wall. These cells can be created when grown in the presence of penicillin which inhibits cell wall synthesis.

## 12. What is HRF and function.2 marks

If F factor gets integrated into the chromosome of the recipient cells, the bacterium becomes high frequency of recombination cell (Hfr).

- Conjugation can be used for gene mapping on the chromosomes.
- conjugation_mapping.html
- High frequency of recombination is also a kind of conjugation.

Can be used for mapping genes.
high frequency of recombination

In some cells that carry $F_+$, $F_+$ (Fertility factor) gets incorporated in the chromosomal DNA which converts $F_+$ cells to high frequency of recombination cells (Hfr cells). When conjugation occurs between an Hfr cell and an $F^-$ cell, the Hfr cell's chromosome (with its integrated F factor) replicates, and a parental strand of the chromosome is transferred to the recipient cell. Since this transfer starts from the middle of F factor gene, and most of the time, this transfer is not complete; the whole chromosome of Hfr cell is not transferred. However, some genes can be transferred during this process. When these genes become integrated into the genomic DNA of the recipient cells, the recipient cells acquires new versions of genes that were not part of its genome previously. It may be noted that $F^-$ cells remains $F^-$ negative cell because F factor is not transferred completely. This whole process is illustrated in the accompanying diagram. Also remember that these newly acquired genes can be mapped easily with respect to the time they get transferred to $F^-$ cells.

So, conjugation can be used to map the genes on the bacterial chromosome. Video clip in the lecture illustrates this mapping very well.

## 14. Pilli and its functions.5 marks

These are hair-like structure composed of pilin, usually one to ten in number.

a. Longer than fimbriae

b. Used for attachment to:

- Host cells
- Bacteria

c. Used for DNA transfer from on bacterium to another:

Conjugation (Sex pili)

d. Also function in twitching Motility Gliding Motility is also the function of the pili.

## 15. Process of fermentation.

Fermentation: Initial steps for glucose oxidation by fermentation are the same as they occur in glycolysis for respiration. However, when pyruvate is generated through glycolysis, electrons are also captured by NADH and this NADH needs to be regenerated into $NAD_+$ for recycling. If the final electron acceptor is one of the end products such as ethanol, lactic acid, acetic acid etc, the process is called fermentation. Both anaerobic respiration and fermentation do not use oxygen during these processes, and during fermentation, there are no electron transport chain involved as you can see in the accompanying diagram. Examples of fermentation are given in the accompanying diagram:

## 16. Process of germination of spore.

Spore germinates when it finds a conducive environment for its growth. Germination has 3 phases or stages:

**Activation:** It prepares the spore for germination. Heat can activate the spore when appropriate moisture and nutrients are present in the environment.

**Germination:** Spore starts swelling and loosing its coats etc. It becomes metabolically active.

**Outgrowth**: New components are made.

## 17. Characterstic of magnetosome.

Inclusions of iron oxide

Surrounded by invaginations of plasma membrane

Present in G negative bacteria

Act like a magnet. Bacteria can stick to iron containing rocks for nutrition.

Decompose $H_2O_2$ which is toxic for cells.

## 18. Four bacterial growth curve phase.

There are four distinct phases of this curve.

1. **The Lag Phase:** cells prepares for growth in this phase. No growth is observed during this period or phase, however. Cells are metabolically very active during this phase.
2. **The Log Phase:** During this phase, organisms multiply exponentially or logarithmically. Generation time becomes constant during this phase and that is the reason, the log graph will show a straight line. Cells are in the most active stage during this phase of growth curve. For commercial applications such as vaccine production, cells have to remain in this phase in order to reproduce most efficiently resulting in increased cell mass or number. Another application of this phase of growth is to determine the generation time.
3. **The Stationary Phase:** This is also called a period of equilibrium as microbial deaths equal production of new cells. In other words, organisms start dying during this phase, however, the number of dead organisms is replaced by new organisms because there is still replication of cells going on. So, overall number of organisms does not change. This is the reason, it is called a stationary phase.
4. **The Death Phase:** The number of deaths exceeds the number of new cells formed during this phase. In other words, overall number decreases. It is also called logarithmic decline phase. Why there is a decline phase or death phase. The reason is simple: Nutrients are depleted and waste products which are toxic to the cells accumulate suppressing the growth and killing the cells.

## 20. Taxonomic Hierarchy 5 marks

Microbes are placed in groups based on similarities that they share with each other. All organisms can be grouped into a series of subdivisions that make up the taxonomic hierarchy. A bacterial **species** represents —a monophyletic and genomically coherent cluster of individual organisms that show a high degree of overall similarity with respect to many independent characteristics, and is diagnosable by a discriminative phenotypic propertyǁ (definition taken from the internet). A **genus** consists of various specie; however, these species differ from each other in certain ways, although these are related by descent with each other. Related genera make up a **family**. A group of similar families constitutes an **order,** and a group of similar orders makes up a **class.** Related classes, in turn, make up a **phylum.** All phyla that are related to each other make up a **kingdom**, and related kingdoms are grouped into a **domain**.

Domain
➢ Kingdom
▪ Phylum
• Class Order
➢ Family
▪ Genus
• Species

## 21. Inclusion bodies 3 marks

Inclusions are reserved deposits of nutrients. Bacteria accumulate nutrients when nutrients are plentiful.

Concentrating nutrients in the form of inclusions avoids the increase in osmotic pressure due to accumulation of nutrients in one place. Not all organisms accumulate them. So, inclusions vary from bacteria to bacteria. They can also serve as markers for bacterial identification as some are limited to specific organisms. The followings are some of the important inclusions: • Metachromatic granules

• Polysaccharide granules

• Lipid inclusions
• Sulfur granules etc.

## 22. Antisepsis, sepsis, sterilization definition.3 marks

**Antisepsis**: Removing pathogens from living tissue

**Sepsis:** Microbial contamination
A toxic inflammatory condition arising from the spread of an organism from a focus of infection.
**Sanitization**: Lowering microbial counts on eating utensils

## 23. Oxidation and reduction 2 marks

**Oxidation** = loss of electrons, or gain of oxygen, or loss of hydrogen .
**Reduction** = gain of electrons, or loss of oxygen, or gain of hydrogen

## 24. Three character of algae.

These are simple eukaryotic cells. Some are unicellular, others are multicellular (thallus); however, they lack tissues such as roots, stem and leaves typically seen in plants. Algae absorb nutrients from water through their surfaces and are mostly photoautotrophs; however, a few are chemoheterotrophs. They are responsible for 80% atmospheric $O_2$ on the face of the earth. Microscopic exam is needed to identify unicellular and filamentous algae. However, multicellular algae that are commonly known as seaweeds are macroscopic in nature and can be identified morphologically without the help of a microscope. Four groups of such algae include blue-green algae, green algae, brown algae and red algae. These algae are located in the sea at various locations and absorb light of various wavelengths, hence red algae are located far from the surface and can use blue light from the sun as blue light is of shorter wavelength and can penetrate deep in the sea. Please see the accompanying figure (above) for a better understanding of how wavelength relates with various algae. Also remember, blue-green algae need magnification in order to be correctly identified, although they are not microscopic. o Body of multicellular alga such as seaweed is called a thallus which consists of branched holdfasts (anchor alga to rock) stemlike hollow stipes and leaflike blades. There is no vascular tissue in these algae. Also, the stipe is not lignified or woody, so it does not provide support to the weed. Surrounding water provides the support for the thallus. Some algae have a gas filled body inside them which keeps them floating in the water. This gas filled structure is called pneumatocyst or float.
These are eukaryotes. Their cell wall consists of cellulose. These are photosynthetic and produce oxygen. They are usually unicellular, but multicellular algae are also common. Seaweeds and pond scum are some of the examples.

## 25. Thermal death point and thermal death rate.

**Thermal Death Point:** It is the lowest temperature at which all cells in a culture are killed in 10 min. It will be a specific temperature for a specific species of organism.

**Thermal Death Time:** Time (minimum) during which all cells in a culture are killed at a given temperature. This will vary from temperature to temperature for the same organism. Obviously, higher temperatures will take less time to kill the organisms than low temperatures.

## 26. How bacteria can be identified.

Microbes especially bacteria (disease causing) can be identified by three methods:

A. Classical or Conventional Method
B. Serological Methods
C. Nucleic Acid based Methods

Classical methods of microbial identification involve differential staining of the sample before it its culturing, culturing the sample onto nutrient agar, blood agar and MacConkey's agar, purifying the culture (colonies expected to be involved in the disease) and detection of various enzymes that belong to various metabolic pathways. Classically, such methods used to take a long time to perform (3 days at least); however, rapid identification methods have become available now which use preformed media that can be used for testing the presence of metabolic enzymes. Such a kit is shown in the figure above (previous page).

Serological methods involve antibodies and antigen interactions. Antibodies are produced by B lymphocytes against any foreign antigens such as bacteria and their toxins that enter the body of animals or humans. Antibodies are very specific in their interaction. In other words, antibodies made against E. coli do not bind or interact with Staphylococci or vice versa. Although, there are many serological techniques that can be successfully used for identification of microbial infections, we will focus on only a few of them. The main advantage of using serological method is the speed and economy. In other words, serological methods are quick to do (take only about an hour) and can work directly on the sample (sample does not have to be cultured and purified as is needed in conventional methods of identification). Serological methods can be performed on cultured microbes as well which again speed up the diagnosis. Also remember that in all these serological tests, either the serum or the antigen should be known to us.

## 27. Catalase and its protocol.

When $H_2O_2$ is produced, it gives rise to peroxide anions which are toxic to the cells. Cells have evolved to deal with this toxic $H_2O_2$ by an enzyme called catalase as under:

Reduction = gain of electrons, or Loss of oxygen, or gain of hydrogen

**Oxidation**: Removal of electrons from an atom

1. • **Reduction**: Gain of electron • Oxidation and reduction reactions are coupled.

## 28. write names of 5 kingdom system (5)

In 1969, five kingdom classification was proposed by Robert Whittaker as under:

1. Monera: Bacteria
2. Plantae: plants
3. Animalia: Animals
4. Fungi: Yeasts, molds and mushrooms
5. Protista: These are unicellular eukaryotes. Organisms that do not fit into any other category are placed in Protista. They are larger than prokaryotes. They include algae, protozoa, slime molds and water molds.

**Five Kingdom Classification of Organisms**

1.Animalia
2.Plantae
3.Fungi
4.Monera
5.Protista

Living organisms are subdivided into 5 major kingdoms, including the Monera, the Protista (Protoctista), the Fungi, the Plantae, and the Animalia. Each kingdom is further subdivided into separate phyla or divisions. Generally "animals" are subdivided into phyla, while "plants" are subdivide.

## 29.Using of ionization radiation 2 marks.

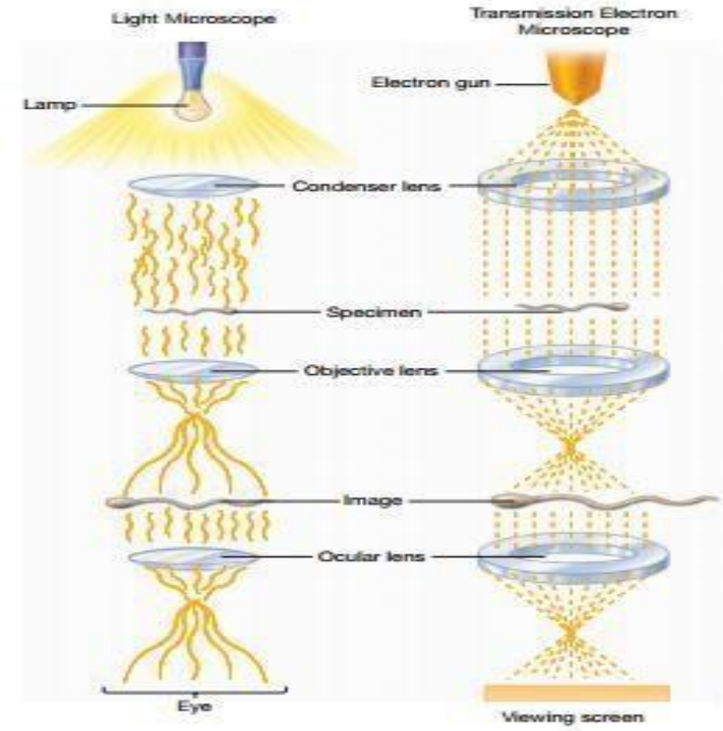
include gamma rays, X rays, or high-energy electron beams. The ionizing radiation possesses a wavelength shorter than that of nonionizing radiation, less than about 1 nm. All these short wavelengths cause ionization of water generating high reactive hydroxyl radicals which are damaging to the cells as they bind to DNA and results in mutations. Medical plastic supplies, medicines and meat products can be sterilized by radiation.

## 30 Three characteristics of integral prtein.3marks

**Integral proteins are inserted in the membrane or embedded in the membrane.**

integral proteins are not static in position in the membrane. They can diffuse laterally and change positions in the membrane from time to time. So, plasma membrane is like water pond and integral proteins are like plastic bags people throw in the pond. These plastic bags swim and move by air currents. Membranes are much like that. Integral proteins act as channels or carriers in facilitated diffusion. Integral proteins are called transporters or permeases.

## 31. Labelling of electron transmission microscope 5 marks




## 35. Physical and chemical method of making of smear 3marks

Staining starts with making a smear using a glass slide.

- Using a platinum loop, a colony of a drop of broth culture can be smeared into a thin film on a glass slide for making a smear.
- The specimen is spread into a thin film (smear).
- Smear is air-dried.
- Smear is fixed (attached) to the slide before staining.
- **Heating** the slide is one way of fixing the smear.
- **Methyl alcohol** can be used.

## 36. Difference between oxidation and reduction 2 marks

**Oxidation** is the process of loss of electrons whereas **Reduction** is the process of gain of electrons. **Oxidation** can also be gaining of oxygen atoms whereas **Reduction** is loss of Oxygen atoms.

## 37. Buffer

Buffers are used to maintain desired pH o Peptones and amino acids are used as buffers o Phosphates are also used for buffers **.**

A buffer is a solution that can maintain a nearly constant pH if it is diluted, or if relatively small amounts of strong acids or bases are added. A buffer solution can be made by mixing a weak acid with one of its salts OR mixing a weak base with one of its salts.

## 38. Five name of bacteria which grow on optimal temperature.

Organisms are basically classified into three groups based on the temp requirements:

1. **Psychrophiles:** These are further divided into strict psychrophiles and psychrotrophs:
   **Psychrotrophs:** Cold loving: 15 $_0$C o
   **Psychrotrophs:** Optimum temp is 20-30 $_0$C. Food spoilage bacteria that can spoil food during refrigeration.
2. **Mesophiles:** 25 – 40 $_0$C: These are the ones that cause diseases in animals and humans.
   o Moderate temp loving organisms o Optimum: 37 $_0$C as this is the body temperature of humans and animals.
3. **Thermophiles:** 50 – 60 $_0$C o Heat loving o Important in organic compost piles o These are further divided into hyperthermophiles that grow optimally at 80 $_0$C. They live in hot springs.

## 39. Branches of mycology 2 marks

**Mycology** is the branch of biology concerned with the study of fungi, including their genetic and biochemical properties, their taxonomy and their use to humans as a source for tinder, traditional medicine, food, and entheogens, as well as their dangers, such as toxicity or infection.

- Medical mycology
- Agricultural mycology
- Ecological mycology – Decomposers of woods, leaves, feces and other organic matters

## 40. Difference between precipitation and agglutination test 2 marks

41. **Agglutination Test:** Let's first talk about a few definitions that will make our life easier. There are two words we must know about them: They are agglutination and precipitation.

**Agglutination** is a process in which soluble antibodies interact with a particulate antigen.

**Precipitation** is a process in which soluble antibodies interact with a soluble antigen. Now let's talk about a few examples of agglutination tests:

**Blood Grouping:** This is a typical agglutination test which involves antibodies (that are always soluble in nature) and RBCs (that are particulate in nature).

**Rose Bengal Plate Agglutination Test:** This test is used to diagnose Brucellosis in animals in which serum of animals suspected for Brucellosis is mixed with Brucella antigen (bacterim). The resulting positive interaction is seen as flocculation as are shown in the accompanying figure:

**Widal Test:** This test involves Salmonella cells (Antigen) and Salmonella antibodies.

**Coombs Test:** This involves antibodies coated RBCs (antigen) and antiantibodies.

**agglutination tests** are qualitative **tests** used to detect the presence of antibodies in serology laboratories and blood banks. Treated red blood cells or colored latex beads, coated with antigen, clump in the presence of antibody to the antigen.

**Precipitation Tests:** These involve soluble antigens and soluble antibodies. Examples include:

o **Agar Gel Diffusion Test:** This test is performed in agarose gel which is poured in a petri dish and wells are created which are used to place antigens and antibodies for interactions with each other. Antigens and antibodies diffuse from their respective wells and, where these molecules meet each other, a precipitation line is formed as shown in the accompanying figure.

**Precipitation of proteins:** This method can also be used to isolate a specific

protein from cells by using protein specific antibodies which precipitate out that protein from a mixture of cellular proteins.

A **precipitation reaction** is a **test** which is involved in the serology, for the detection of antigens and antibodies. ... **Precipitation test** is used to quantify both antigen and antibody, and therefore its specificity also depends upon the concentration of both the reactants

## 42. Briefly explain conjugation .transformation,transduction,

a. **Conjugation** is the process by which one **bacterium** transfers genetic material to another through direct contact. During **conjugation**, one **bacterium** serves as the donor of the genetic material, and the other serves as the recipient
   Genes transfer process can be mediated by plasmids. Plasmids are extra chromosomal circular DNA fragments that replicate independently of bacterial chromosome. Conjugation requires direct cell to cell contact and cells have to be opposite mating types.
b. **Transduction** is the process by which foreign DNA is introduced into a bacterial cell by a virus or viral vector. An example is the viral transfer of DNA from one bacterium to another and hence an example of horizontal gene transfer
   Transferring of a gene from a bacterium to another bacterium via a virus is called transduction. o When a bacteriophage infects a bacterium such as E. coli, it replicates inside E. coli and also produces a protein coat (which is called a capsid) in which viral DNA is packed before the virus is released.
c. **Bacterial transformation** is a process of horizontal gene transfer by which some **bacteria** take up foreign genetic material (naked DNA) from the environment. ... The prerequisite for **bacteria** to undergo **transformation** is its ability to take up free, extracellular genetic material. Such **bacteria** are termed as competent cells.
   Naked DNA in solution is transferred from one bacterium to another during this process.

## 43. Characteristics Antibiotic chemicals.

Although antibiotics are used to kill organisms in disease states, some antibiotics are not very effective for this purpose; however, these antibiotics could be used in food to prevent food spoilage. Nisin and natamycin prevent spoilage of cheese.

## 44. Define culture , culture medium, inoculum

a. **What is culture.**
   This term is used to define growth of microbes. In other words, microbial growth in the lab is called a culture.
b. **What is a culture medium?**
   o A nutrient material that supports the growth of microbes in the lab is called a culture medium.
   A culture media is a special medium used in microbiological laboratories to grow different kinds of microorganisms. A growth or a culture medium is composed of different nutrients that are essential for microbial growth.
c. **Inoculum** Microbes introduced into a culture medium that initiate growth of organisms.

**inoculum** A small amount of material containing **bacteria**, viruses, or other microorganisms that is used to start a culture

## 45. Advantage of staining

I. Most organisms appear colorless when seen under a microscope.
II. Staining emphasizes certain structures of the organisms.
III. Staining is just coloring with a dye.
IV. Staining increases visibility of microbes because staining increases contrast.
V. Shape, size and arrangements of the organisms can be readily seen.
VI. Purity or contamination of a culture could be determined.

VII. Differentiation and classification of microbes is possible. For example, microbes can be categorized into Gram positive or Gram negative groups.

VIII. Structures such as flagella, capsule and spores etc. of bacteria can be detected with staining.

1. It gives quick results when examining infections.
2. It is simple and cost-effective.
3. It helps with determining appropriate treatments for infection.
4. It allows for various methods of testing.
5. It is basically a key procedure in identifying bacteria.

## 46. Five layer of endospores.

1) **Exosporium:** A thin delicate outermost covering of the spore
2) **Spore coat:** 2nd layer underneath the exosporium. It is thick and composed of several protein layers. Resistant to chemicals .It contains enzymes for germination. Germination of spores into vegetative form occurs when environment becomes favorable for their growth.
3) **Cortex:** It is the 3rd layer from outside in. It has peptidoglycan in it.
4) **Spore cell wall or core wall:** Surrounds the protoplast or spore core
5) **Spore core:** Contains nucleoid and ribosomes

## 47. Name three buffers used for controlling PH of culture medium? 3

I. Peptones
II. amino acids
III. PBS

## 48. Three shapes of bacteria? 3

- Coccus These are circular or spherical in shape.
- Bacillus These rod shaped bacteria.
- Spiral These are curved shaped bacteria. They are further divided into 3 more subgroups.

- **Vibrio:** curved rods
- **Spirillum:** Helical but **rigid**
- **Spirochete:** Helical but **flexible**
- Spirochetes move by axial filaments which are enclosed by the outer membrane.

## 1. What is flagellum? Give its functions?

Long filamentous structure that propels bacteria is called flagellum. It helps bacteria to move.

## 2. What is phosphorylation?

The addition of an inorganic phosphate group to a chemical compound is called phosphorylation. e.g addition of P to ADP for synthesis of ATP during metabolic reactions.

## 3. Name two basic dyes?

In basic dye the color is in the positive ion. e.g

I. 1-Crystal violet
II. 2-Methylene
III. 3-blue Malachite green
IV. 4-Safranin

## 4. Difference between viable count and direct count?

### Viable count:

Viable count is a method used in cell culture to determine the number of living cells in a culture. This is different from other cell counting techniques because it makes a distinction between live and dead cells

### Direct count:

Direct counting methods include microscopic counts using a hemocytometer or a counting chamber. The hemocytometer works by creating a volumetric grid divided into differently sized cubes for accurately counting the number of particles in a cube and calculating the concentration of the entire sample.

## write the name of three steps of glucose respiration.

Respiration of glucose takes place in three general steps:

- **Glycolysis:** Oxidation of glucose to pyruvic acid
- **Krebs cycle:** Oxidation of acetyl CoA to CO2
- **Electron Transport Chain**: Coenzymes that carry electrons from Krebs cycle or glycolysis are oxidized to create ATP.
- **Respiration is further divided into two types:**

**Aerobic Respiration:** If in the electron transport chain, the final electron acceptor is oxygen, it is called an aerobic respiration.

**Anaerobic Respiration:** If in the electron transport chain, the final electron acceptor is any inorganic compound other than oxygen, it is called an anaerobic respiration.
This final electron acceptor may be a nitrate ion, a sulfate ion, or a carbonate ion.

## 7. what is fluorochromes?

O Fluorochromes are substances that absorb short wavelength of light and emit longer wavelength (visible light spectrum). The fluorochromes are used in fluorescent microscopy. These are attached to antibodies that are specific to organisms. So, if an organism is present in the specimen, we can use specific antibodies to determine the presence of those organisms by use of fluorochrome tagged antibodies.

## 9. What is resolution?

O Resolution is the ability of the lenses to distinguish between two closely lying objects as separate. See the accompanying diagram for visual concept of resolution.
O Light microscope resolving power is 0.2 μm.

$d = \frac{0.5\lambda}{n \sin \theta}$ o n = refractive index, 1.5 for immersion oil o Resolution (d) in the formula given in the diagram is directly proportional to the wavelength of the light used. As resolution is the smallest distance between two closely lying points, use of light with smaller wavelength gives better resolution. That is why it is find blue filters for blue light as illumination for the microscope, as blue light has the shortest wavelength in the visible spectrum.

## 10. Why solid medium is good than liquid medium for the growth of bacteria

Solidification of the medium is done for purifying organisms from each other as solid medium provides surface for individual colonies to grow well separated from each other. A well isolated colony is assumed to be the progeny of a single cell. Purification of organisms is done by streaking the organisms. Secondly, the solid media are used to study the colony characteristics.

## Q.5 Difference Between simple staining and differential staining (2 marks)

Simple staining:

- A single stain is used.
- Stain is washed off.
- A mordant is added, sometime.
- Microbes seen under the microscope.

Examples of simple stain:

- Methylene Blue and crystal violet Differential staining:
  - Differential stains react differently with different kinds of bacteria.
- Most common is Gram staining.
- 1884, by Hans Christian Gram Crystal Violet is added.
- It is primary stain
- Crystal violet is washed off.
- Iodine is added to enhance binding.
- Iodine is mordant.
- The slide is washed off with alcohol.
- Decolorizing agent

Examples of differential staining:

- Fuchsin or safranin

## 16. . What is refractive index

It is the ratio of the velocity of light in a vacuum to its velocity in a specified medium. Refractive Index: Ability of a medium to bend the light. Refractive index as that of the glass (1.5).

**Refractive Index**: Ability of a medium to bend the light

• Light rays move in a straight line through a single medium

• To increase contrast, we stain the specimen.

## 17. Parts of flagella.

A flagellum consists of the following 3 parts as shown in the accompanying diagram below:
Filament: It is made of a protein called flagellin, which makes H antigen that can be used for serovar identification in gram negative bacteria.

Hook: A different protein • Basal Body: The most complex

**18. Define Ribosome?**

Ribosomes are non-membranous organelles present in both prokaryotes and eukaryotes and involved in protein synthesis. Their composition in prokaryotes is as follow

- Composed of proteins + ribosomal RNA
- 2 subunits
- 70S ribosomes (when two units are combined into one unit)
- 50S: one rRNA
- 30S: two rRNA
- Several antibiotics act on ribosomes and inhibit protein synthesis • Streptomycin attach to 30S

21. **what is chemotaxic and phototaxic**

Movement of organism in response a chemical is called chemotaxix. Movement of organism in response to light stimulus is called phototaxix.

22. **What is endospore?**

A resistant dormant structure formed within a bacterial cell to pass unfavorable conditions.

• Specialized resting cells • Highly durable, dehydrated forms of bacteria • Formed inside a bacterium

•

Survive extremes of temperatures; resistant to heat; needs to be autoclaved in order to be killed.

• Survive lack of water; can survive in high salt concentrations. Clostridia spores can survive in honey and that is one reason, honey is not recommended for infants, because spore can cause tetanus in babies.

• Survive radiation danger •

Survive in the toxic environment as well.

• Mostly formed by Gram positive bacteria
- Genus Clostridium
- Genus Bacillus

• One gram negative: *Coxiella burnetii* also has it.

• Formation of spores takes place within a vegetative cell and the process is called sporulation or sporogenesis. It is initiated when nutrients become unavailable.

23. **Write name of three passive movements.** Simple diffusion, Facilitated diffusion. Osmosis

24. **Metachromatic granules**

Also called volutin, they stain red with certain dyes such as methylene blue. That is why they are called metachromatic (stain in different color as methylene blue gives blue color but the color on these granules is red). • Large inclusions • These inclusions contain inorganic phosphates. Inorganic phosphates are used up in ATP synthesis. •

Characteristics of *Corynebacterium diphtheria: This bacterium can be identified by the presence of these granules in it.*

24. **Name 5 Kingdom**

Monera 2- Protista 3- Fungi 4- Plantea 5-Animalia

**26. Coenzyme** **coenzymes**

are organic nonprotein molecules that bind with the protein molecule (apoenzyme) to form the active enzyme (holoenzyme). Coenzymes such as NAD, NADP, FAD, FMN etc. are used as carriers of these electrons in oxidation reduction reactions.

**27. Subgroup of spiral bacteria**

Spirals are curved shaped bacteria. They are further divided into 3 more subgroups.

- **Vibrio:** curved rods
- **Spirillum:** Helical but **rigid**
- **Spirochete:** Helical but **flexible**
- Spirochetes move by axial filaments which are enclosed by the outer membrane

**28. Methods used for viable counting of microbes.**

- Pour plate method
- Spread plate method

**29. major two prokaryutes group?**

Domain Archea Domain eubacteria

**30. How bacteria got nitrogen??**

- By decomposing proteins
- From $NH_4^+$ ions
- From Nitrates
- Use Gaseous $N_2$ are nitrogen fixing bacteria. These

**31. What are microaerophiles (2)**

**Microaerophiles** need oxygen because they cannot ferment or respire anaerobically. However, they are poisoned by high concentrations of oxygen. They gather in the upper part of the test tube but not the very top

**32. Glycocalyx 2**

It is the outermost layer, viscous and gelatinous in nature that surrounds the cells. It is composed of polysaccharide and polypeptide or both.

If glycocalyx is organized and firmly attached to the cell, it is called a capsule.It
– Plays important role in virulence (degree of pathogenicity)
– Can also be a part of the vaccine against the bacteria to which it belongs.
– Can be demonstrated by negative staining technique.
– Capsulated organism make glistening colonies as seen in the accompanying diagram below:
- *Bacillus anthracic, streptococcus neumonia* and Klebsiella are examples of capsulated organisms.
- Thickness of the capsule depends upon the culture conditions.

Capsules are mostly are water soluble. Also remember that capsular organisms usually make the broth viscous and stringy.

**33. What is meant by Virulence Morbidity Morbidity rate Mortality Mortality rate Pathogenicity.**

O **Virulence:** The extent of pathogenicity
- **Morbidity:** Incidence of a disease
- **Morbidity rate:** Number of people affected in relation to the total population in a given time period
- **Mortality:** Deaths from a disease
- **Mortality rate:** Number of deaths from a disease in relation to the population in a given time
- **Pathogenicity:** The ability to cause disease

## What is a passive moment write its types?

Movement of substances with the concentration gradient. This means that substances will move from higher concentration of a substance to lower concentration. Gradient provides the force for movements of molecules and this happens with no energy expenditures.

- Passive Movement is divided into two groups.

1.. Simple Diffusion
2.. Facilitated Diffusion

- Passive Movement

Movement of substances with the concentration gradient. This means that substances will move from higher concentration of a substance to lower concentration. Gradient provides the force for movements of molecules and this happens with no energy expenditures.

- Passive Movement is divided into two groups:

### 1. Simple Diffusion;

Area of high concentration to low concentration. This continues until ions or molecules are evenly distributed. When this happens (equal distribution through), it is called a point of equilibrium. O2 and CO2 are examples of simple diffusion.

### 2. Facilitated Diffusion

i. Integral proteins act as channels or carriers in facilitated diffusion

i. Integral proteins are called transporters or permeases ii. No energy is required during transportation of substances through these integral proteins. iii. Two kinds of transporters are known:

i. Nonspecific transporters ii. Ions specific transporters 1. Change in shape 2. Sugars, vitamins iii. Examples of facilitated diffusion include:

i. Large molecules degraded by extracellular enzymes
ii. Smaller molecules then bind transporter
iii. Water molecules can pass through lipid bilayer by simple diffusion or though water channels, aquaporin's

**1. Three differences between prokaryotes and eukaryotes.** **1-**

Prominent nucleus is found in eukaryotes while it is absent in prokaryotes. 2-
membrane bound organelles are found in eukaryotes and absent in prokaryotes.
3-No histones are found in prokaryotes while present in eukaryotes.
4-Examples prokaryote bacteria and archea all organism found in domain Eukarya are eukaryotes.

- 

**Describe structural functions following: I. Ribosomes ii. cell wall iii. flagella. Ribosomes**

- Composed of proteins + ribosomal RNA
- 2 subunits
  - 70S ribosomes (when two units are combined into one unit)
- 50S: one rRNA
- 30S: two rRNA
- Several antibiotics act on ribosomes and inhibit protein synthesis
  - Streptomycin attach to 30S **ii.**

**Cell wall**

Cell wall is the most important layer in bacteria. It is rigid layer just outside the plasma membrane. Most important structure in prokaryotes It provides shape to the organism. It provides protection from osmotic lysis. It is involved in pathogenicity (ability of the organism to cause the disease). Several antibiotics act on it.

**iii. Flagella**

Long filamentous structure that propels bacteria. A flagellum consists of the following 3 parts.

• Filament: It is made of a protein called flagellin, which makes H antigen that can be used for serovar identification in gram negative bacteria. • Hook: A different protein • Basal Body: The most complex

6. **Describe 5 bacteria which need oxygen for growth?**

1-Nocardia asteroids 2-Myoacterium tuberculosis 3-Bacillus anthracis 4- Psedomonas aeurginosa 5-Neisseria gonorrhea

7. **What are physical requirements for the growth of bacteria.**3

- Temperature • pH • Osmotic Pressure

8. **Write down type of bacteria?**

Bacteria can be classified on different basis like photosynthetic saprotrophic heterotrophic on the basis of mode of obtaining food. aerobic and anaerobic on the basis of oxygen requirment. gram positive and nagative on the basis of cell wall composition. Fermentor and non fermentor on the basis of fermention carrying out ability and so on..

**What is function of i) illuminator ii) condenser iii) Objective lens**

- Illuminator: a light source
- Condenser: Directs the light through the specimen
- Objective Lenses: Close to the specimen

11. **Differentiate b/w phase contrast and dark field microscopy.**

**Dark field microscopy**: Arrangement of the condenser in dark field microscopy throws light rays at an oblique angle so that if the specimen is not present on the slide, there will be no image formed. However, if the the organisms are present on the glass slide, those organisms will reflect the light into the objective lens and we will see the cells as bright objects in a dark background much like we see stars in the sky at night. o The beauty of this microscope is that one does not have to stain the organisms to see them under the microscope. **Phase contrast microscopy:** This instrument is built on the concept of phases of light waves. The light ray that passes through an organism gets retarded about ¼ of its wavelengths than the light that passes outside the organism (through the glass slide only). To further retard the retarded light ray, a phase plate is inserted into the body of the microscope (above the objective lens) which is thicker in the center than the ends. This arrangement causes the

Dark field microscopy:

- This microscope makes use of a special condenser that provides illumination from the sides.
- The beauty of this microscope is that one does not have to stain the organisms to see them under the microscope.

Phase contrast microscopy:

- This instrument is built on the concept of phases of light waves.
- This method for visualizing organisms also does not involve staining.

12. **Characteristics of algae**

These are eukaryotes. Their cell wall consists of cellulose. These are photosynthetic and produce oxygen. They are usually unicellular, but multicellular algae are also common. Seaweeds and pond scum are some of the examples.

**14. function of cell membrane in bacteria**

- Selective permeable barrier • Passive and Active Transport • Respiration in microbes (bacteria) • Photosynthesis in microbes • Lipid synthesis • Cell wall parts are transported by a molecule called bactoprenol which is present in the plasma membrane. • PM has many receptors in it with which various ligands can bind and initiate signals for gene expression etc.

**Inclusions and its types**

Inclusions are reserved deposits of nutrients. Bacteria accumulate nutrients when nutrients are plentiful.

Concentrating nutrients in the form of inclusions avoids the increase in osmotic pressure due to accumulation of nutrients in one place. Not all organisms accumulate them. So, inclusions vary from bacteria to bacteria. They can also serve as markers for bacterial identification as some are limited to specific organisms. The followings are some of the important inclusions:

- Metachromatic granules
- Polysaccharide granules

• Lipid inclusions • Sulfur granules etc.

**3 parts of lipopolysaccharides.3 marks**

Lipopolysaccharide molecule consists of 3 parts:

1. Lipid A: endotoxin

– Fever, vasodilation and shock

2. Core polysaccharide

a. Structural support

3. O side chain

a. Antigenic much like teichoic acid in gram positive bacteria

**23. Name types of culture on the basis of function.**

1-Defined media. 2-Complex media. Defined and complex media can further be divided into **General purpose media, Selective media, Differential media, and Enrichment media.**

**24. What do you know about types of techoic acid?**

**25. Define antisepsis.**

**Antisepsis**: Removing pathogens from living tissue

**Differentiate between clone and strain?**

Clone: Population of cells derived from a single cell that are genetically identical.

**Strain:** A genetic variant of a clone is called a strain.

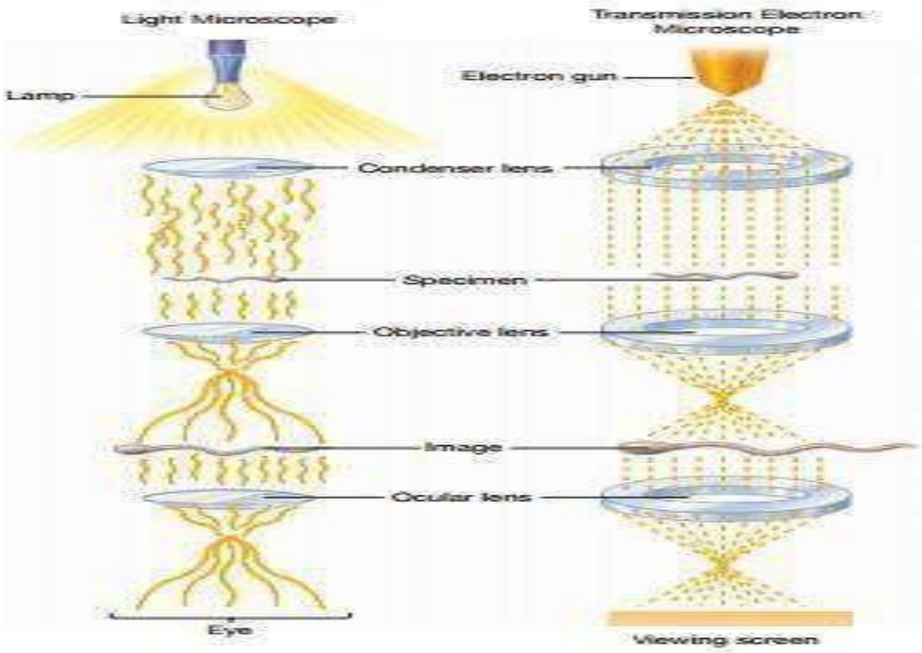
**4. Enlist five bacteria that need oxygen for growth (5)**

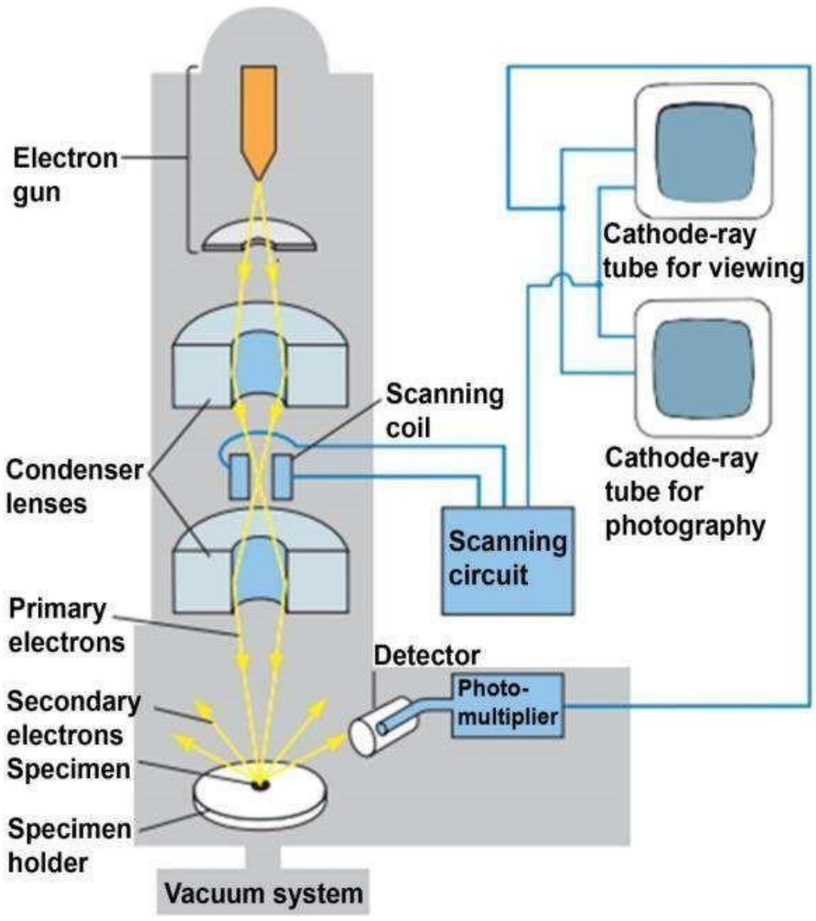
Characteristics of metachromatic granules5 • Also called volutin, they stain red with certain dyes such as methylene blue. That is why they are called metachromatic (stain in different color as methylene blue gives blue color but the color on these granules is red).

• Large inclusions • These inclusions contain inorganic phosphates.

Inorganic phosphates are used up in ATP synthesis. • Characteristics of *Corynebacterium diphtheria: This bacterium can be identified by the presence of these granules in it.*

**5. Label diagram of electron microscope.**






## 7. Explain methods of microbial control in detail.

These methods can be broadly classified into four groups: Physical, Mechanical, Chemical and Biological Physical methods:

**Heat:** It is the cheapest sources of all and easily available to control microbial growth. There are a few heat related concepts that we must appreciate. Heat denatures enzymes.

**Thermal Death Point:** It is the lowest temperature at which all cells in a culture are killed in 10 min. It will be a specific temperature for a specific species of organism.

**Thermal Death Time:** Time (minimum) during which all cells in a culture are killed at a given temperature. This will vary from temperature to temperature for the same organism. Obviously, higher temperatures will take less time to kill the organisms than low temperatures. Methods that use heat can further be classified into **dry and moist** heat methods.

- Form of dry heat include: 1. Dry heat (hot-air oven), 2. Flaming (Platinum loop sterilization in the flame of Bunsen burner), 3. Incineration (burning to ashes). Similarly, moist heat can also be grouped into autoclaving (15psi), boiling at $100_oC$, and Tyndallization ($100_oC$ for 3 consecutive days to sterilize sugar solution that can be degraded by autoclaving) and pasteurization (usually at low temperature than $100_oC$).
- **Pasteurization:** This technique typically employs low heat for killing pathogenic and food spoiling bacteria in milk. However, it does not kill thermoduric organisms. Thermoduric organisms are not pathogenic though. Products other than milk, such as ice cream, yogurt, and beer, all have their own pasteurization times and temperatures, which often differ considerably. Dairy industry tests phosphatase to determine if pasteurization of dairy product has occurred effectively because phosphatase present in raw milk gets inactivated with effective pasteurization temperatures. The following three equivalent heat treatments can be given to achieve pasteurization of milk: o 63°C for 30 min o High-temperature short-time: 72°C for 15 sec o Ultra-high-temperature: 140°C for 4 sec

**Other Physical Methods** o

Low temperature inhibits microbial growth by slowing down chemical reactions.

**Refrigeration:** Materials stored at 2-8°C can last for a day or two.

**Deep-freezing:** Long term storage of materials is possible at minus temperatures in the deep freezers.

**Lyophilization**: Freeze drying is another method for long term storage of food and other materials. o **High pressure** deshapes and denatures proteins: This technique is used for preserving juices and wines.

o **Desiccation** prevents metabolism: This method is very old and still in use today. Food stored in high concentrations of salt or sugar solution can keep for long.

o **Osmotic pressure** causes plasmolysis. High salt concentrations for example can take the water content of the cells out leaving them starved of water.

**PHYSICAL METHODS OF MICROBIAL CONTROL**

 **Filtration:** It is also a physical method. It can be used to filter air (using high efficiency particulate air filter) or liquid medium using membrane filters. **Radiation:**

 o Ionizing and non-ionizing radiation o

Ionizing Radiation: High energy waves that destroys microbes.

 Ionization radiations include gamma rays, X rays, or high -energy electron beams. The ionizing radiation possesses a wavelength shorter than that of nonionizing radiation, less than about 1 nm. All these short wavelengths cause ionization of water generating high reactive hydroxyl radicals which are damaging to the cells as they bind to DNA and results in mutations. Medical plastic supplies, medicines and meat products can be sterilized by radiation.

 Non -ionizing Radiation: wavelengths longer than 1nm fall into non-ionizing radiation. The best example is UV light. UV light causes mutations in the DNA by dimerization of thymidine bases. These *thymine dimers* inhibit correct replication of the DNA during reproduction of the cell. The most useful wavelength is 260 nm. This wavelength is specifically absorbed by DNA. UV radiation is used to control organisms in the air mostly. However, surfaces are also sterilized by UV radiation. UV light cannot penetrate deep into the fluids, so only surface bacteria are killed. See the range of UV light that is useful for microbial killing.

**Gases with microbial activity:** o **Ethylene Oxide:** Many heat-sensitive items such as disposable plastic, petri dishes and syringes, heart-lung machine components, sutures, and catheters are sterilized by ethylene oxide. Ethylene oxide is a strong alkylating agent that kills by reacting with functional groups of DNA and proteins to block replication and enzymatic activity. It rapidly penetrates packing materials, even plastic wraps. EtO is explosive, supplied in a 10% to 20% concentration mixed with either $CO_2$ or dichlorodifluoromethane. A clean object can be sterilized if treated for 5 to 8 hours at 38°C or 3 to 4 hours at 54°C when the relative humidity is maintained at 40 to 50% and the EtO concentration at 700 mg/L.

**Chemical methods:**

Chemicals are used to control microbial growth. Factors that influence the efficacy of a chemical disinfectant include the concentration of the disinfectant, presence of organic matter in the environment (matrix) where the disinfectant is being used, pH of the environment (disinfectants are more effective at acidic pH), and time of exposure to the disinfectant.

**Alcohols:** Ethanol, isopropanol are examples. Alcohols denature proteins, and dissolve lipids. Alcohols require water for being more effective. This is the reason that seventy percent alcohol is more effective than 100%. Please note that alcohols can effectively kill vegetative form of bacteria, and fungi but not spores. Alcohols are not very effective on wounds. Commonly used in hand sanitizers.

 **Heavy Metals and Their Compounds:** Silver, zinc, mercury and copper have biocidal and antiseptic activity. Heavy metals can be effective at very low concentration. This property is called oligodynamic action. Heavy metals denature proteins. o **Silver nitrate (1%):** Commonly used as a disinfectant. o **Silver and sulfadiazine combination:** used on burns.

- **Mercuric chloride:** It is bacteriostatic in nature. This compound is toxic though and its use is not favored very much. o **Copper sulfate:** A commonly used disinfectant for controlling green algae in water ponds.
- **Zinc chloride:** Commonly used in mouthwashes.

 **Surface Active Agents (Surfactants):** They decrease surface tension among molecules of a liquid. Soap and detergents fall into this category.

- **Soaps and Detergents:** These have no antiseptic activities. They cause mechanical removal of germs by emulsification. This mechanical removal is also called degerming activity.
- **Quaternary Ammonium Compounds (Quats**): The most common ones are cationic detergents. They do not kill endospores and mycobacteria though. They are more active against gram positive than gram negative bacteria. The affect plasma membrane, inhibit enzymes and denature proteins.

Benzalkonium chloride is an example of a Quat.

 **Chemical Food Preservatives:** These chemicals are used in the food to retard food spoiling bacteria. These chemicals are either simple organic acids or their salts (that are easily metabolized in the body if eaten, therefore are safe) and include sodium benzoate,

sorbic acid, and calcium propionate. Sodium nitrate and sodium nitrite are also used to preserve meat and meat products. ○ **Antibiotics:** Although antibiotics are used to kill organisms in disease states, some antibiotics are not very effective for this purpose; however, these antibiotics could be used in food to prevent food spoilage. Nisin and natamycin prevent spoilage of cheese.

 **Aldehydes:** These are the most effective antimicrobials. Two examples are formaldehyde and glutaraldehyde. They inactivate proteins by forming covalent cross-links with several organic functional groups on proteins (—$NH_2$, —OH, —COOH, and —SH).

## 9. Explain salient features of viruses, virions, viroids and prions.

**VIRUSES:**They are acellular structures that consist of a protein capsid enclosing either DNA or RNA (but never both) and sometimes may contain an extra membrane called envelope.

**VIROIDS:**They are composed only of circular single stranded (ss) RNA.

**VIRUSOIDS:**They contain circular ssRNA that need helper viruses for replication and encapsidation. They are also called satellite viruses.

**PRIONS:**These are basically infectious proteins formed inside the body. They behave like organisms as they can be transmitted from one individual to another. Extremely resistant to heat.

## 11. Describe five types of microscopy.

1. **Dark field microscopy:** This microscope makes use of a special condenser that provides illumination from the sides only as depicted in the diagram below: o This special arrangement of the condenser throws light rays at an oblique angle so that if the specimen is not present on the slide, there will be no image formed. However, if the the organisms are present on the glass slide, those organisms will reflect the light into the objective lens and we will see the cells as bright objects in a dark background much like we see stars in the sky at night. The beauty of this microscope is that one does not have to stain the organisms to see them under the microscope.
2. **Phase contrast microscopy**:This instrument is built on the concept of phases of light waves. The light ray that passes through an organism gets retarded about ¼ of its wavelengths than the light that passes outside the organism (through the glass slide only). To further retard the retarded light ray, a phase plate is inserted into the body of the microscope (above the objective lens) which is thicker in the center than the ends. This arrangement causes the retarded light to further slow down about ¼ of its wavelength, which when recombines with the un-retarded light creates a contrast which makes the cells/organisms visible. This method for visualizing organisms also does not involve staining.
3. **Fluorescent microscopy** ○ This microscope is equipped with UV light that provides the source of light for illumination. However, this light source does not pass through the slide, it instead falls over the slide (having specimen) and creates fluorescence. o Fluorochromes are substances that absorb short wavelength of light and emit longer wavelength (visible light spectrum)

○ The fluorochromes are attached to antibodies that are specific to organisms. So, if an organism is present in the specimen, we can use specific antibodies to determine the presence of those organisms by use of fluorochrome tagged antibodies. See the accompanying diagrams for details. Look for UV lamp (Mercury Lamp).

**4. Transmission electron microscope:**

○ Electron microscope (EM) makes use of electron beam as an alternative to light rays. Since electron beam has much shorter wavelength, resolution of EM is 0.1nm compared with light microscope which is 200nm. ○ Second difference of EM with light microscope is that focusing of electron beam is done not by glass lenses but by electromagnets that acts like lenses. ○ The third difference in EM is that the image of the specimen is formed on a fluorescent screen like a TV screen.

**5. Scanning electron microscope:**

This is a modified version of electron microscope. Electron beam is allowed to fall on to the specimen and the image is formed from the reflected electrons that are collected and assembled into an image by the detector.

## Q.2 Functions of cell membrane? (5marks)

- Selective permeable barrier
- Passive and active transport
- Respiration in microbes (bacteria)
- Photosynthesis in microbes
- Liquid synthesis
- Cell wall parts of transported by a molecule called bactoprenol which is present in the plasma membrane
- PM has many receptors in it which varies ligands can bind and initiate signals for gene expression etc.

## Q.3 what is coenzyme give its example? (3marks)

A coenzyme is an organic non protein compound that binds with an enzyme to catalyze a reaction. Coenzymes are often called cofactors, but they are chemically different.

A coenzyme is an organic non-protein compound that binds with an enzyme to catalyze a reaction. ... A coenzyme cannot function alone, but can be reused several times when paired with an enzyme. ... Without coenzymes or cofactors, enzymes cannot catalyze reactions effectively. Example: example is thiamine pyrophosphate (TPP).

Examples:

NAD, NADP, FAD, FMN etc.

## Q.4 Two basic stains? (2marks)

Basic dye: The color is in the positive ion

- Crystal violet
- Methylene blue

## Q: 6 Two benefits of using solid medium? (2marks)

Solidification of the medium is done for the purifying organisms from each other as solid medium provides surface for individual's colonies to grow well separated from each other.

A well isolated colony is assumed to be the progeny of a single cell.

The solid media are used to study the colony characteristics. (VU Biology Zone Talha & team)

## Q: 11 Components of polysaccharides granules?

Typically consists of glycogen or starch
Can be demonstrated by iodine which makes glycogen appear reddish brown while Starch appears blue.

## Q: 13 Structure and functions of ribosomes?

Composed of proteins + ribosomal RNA

2 subunits

• 70S ribosomes (when two units are combined into one unit) 50S: one rRNA

30S: two rRNA

Several antibiotics act on ribosomes and inhibit protein synthesis

• Streptomycin attach to 30S

Passive Movement is divided into two groups:

### 1. Simple Diffusion

• Area of high concentration to low concentration
• This continues until ions or molecules are evenly distributed. When this happens (equal distribution thought), it is called a point of equilibrium.
• O2 and CO2 are examples of simple diffusion.

### 2. Facilitated Diffusion

i. Integral proteins act as channels or carriers in facilitated diffusion

i. Integral proteins are called transporters or permeases ii. No energy is required during transportation of substances through these integral proteins.

iii. Two kinds of transporters are known:

Nonspecific transporters

Examples of facilitated diffusion include:
i. Large molecules degraded by extracellular enzymes ii. Smaller molecules then bind transporters

iii. Water molecules can pass through lipid bilayer by simple diffusion or though water channels, aquaporin's.

## Q.15 Types of microbes based on oxygen requirements?

1. Obligate Aerobes
2. Facultative Anaerobes
3. Obligate Anaerobes
4. Aero tolerant Aerobes
5. Obligate Aerobes

Oxygen must be present for their growth. These Organisms do not grow if O2 is not present in the medium. If you test these organisms in a tube containing nutrient agar, the growth will Remain confined to the surface only (see the diagram for the concept). Mycobacterium tuberculosis is an example of such an organism.

### Facultative Anaerobes:

These organisms prefer aerobic respiration; however, if oxygen is not available, they can use anaerobic respiration or fermentative modes for generating ATPs. These organisms will be Present throughout the tube; however, more growth will be seen on the Top (close to the surface) because these organisms prefer using oxygen if it is available.

### Obligate Anaerobes:

Although, these organisms do not use oxygen, they cannot grow in the presence of oxygen. The reason is that oxygen creates toxic) compounds which can kill these cells. Normally, oxygen

Toxic compounds are eliminated by the cells, however, strict anaerobe do not have a system to get rid of these toxic compounds when they are made in the cells.

### Aero tolerant Aerobes:

These organisms do not use oxygen and also are not bothered by the presence of oxygen. In other words, they have a Better system to dispose of toxic oxygen compounds.

## Q.17 Write characteristics of Agra? Characteristics of Agar (Solidifier of a medium):

- Agar is derived from a sea weed.
- It is a complex carbohydrate which is not used by bacteria during the growth.
- It liquefies at 850C and solidifies at 43C. • Agar could be used to solidify medium in Petri plates or slants.

## Q.18 Difference between Eukaryotes and prokaryotes?

Prokaryotes and eukaryotes are chemically similar. All made up of nucleic acids, protein, lipids, and carbohydrates.

### Prokaryotes have the following features:

- DNA is not enclosed in a nuclear membrane.
- Chromosome: mostly single, circular
- DNA not associated with histones
- No membrane enclosed organelles
- Cell wall has peptidoglycan, a complex carbohydrate.
- Divide by binary fission.

### Eukaryotes have the following features:

- DNA is enclosed in a membrane-Bound nucleus.
- DNA is found in multiple Chromosomes.

(V_U_B_i_o_l_o_g_y_Z_o_n_e_T_a_l_h_a_&_t_e_a_m)

- Chromosomes are linear, thread-Like structures.
- DNA is associated with histones.

## Q.18 Types of spiral bacteria? / Sub groups of spiral bacterium?

### Spiral:

These are curved shaped bacteria.

They are further divided into 3 more subgroups. Vibrio:

Curved rods

### Spirillum:

Helical but rigid

### Spirochete:

Helical but flexible. Spirochetes move by axial filaments which are enclosed by the outer membrane.

## Q.21 Function of pili?

- These are hair-like structure

Composed of pilin, usually one to ten in number.

- Longer than fimbriae • Used for attachment to:
  •Host cells
  •Bacteria
- Used for DNA transfer from on bacterium to another: Conjugation (Sex pili)
- Also function in twitching Motility
- Gliding Motility is also the function of pili

## Q.24: Five names of microscopy?

1. Dark field microscopy
2. Phase contrast microscopy
3. Fluorescent microscopy
4. Transmission electron microscopy
5. Scanning electron microscopy

## Q.25 what is microareophiles?

A microaerophile is a microorganism that requires oxygen to survive, but requires environments containing lower levels of oxygen than are present in the atmosphere.

Produce $O_2$ toxic compounds if exposed to more $O_2$

• Listeria, Vibrio

Q.26 How bacteria get nitration? By decomposing proteins

From $NH_4^+$

Ions

From Nitrates

Use Gaseous $N_2$

These are nitrogen fixing bacteria.

## Q.27 what is fimbriae also give location and function?

Hair-like small appendages on G-neg cells

Composed of pilin, a protein

Can be at one pole or around the entire cell

Used for attachment to surfaces or epithelial cells

Fimbria: Singular

Example
Neisseria gonorrhoeae

## Q.31 what is chemotoxic and phototoxic?

Phototrophs: If the electrons are derived from the light, the organisms are called phototrophs. Chemotrophs: If the electrons are extracted from the chemicals such as the food the organisms eat or use, the organisms are called chemotropism.

## Q.32 what is function and structure of the following a) ribosomes b) flagella

### Flagella structure:

A flagellum consists of the following 3 parts

- Filament: It is made of a protein called flagellum, which makes H antigen that can be used for server identification in gram negative bacteria.
- Hook: A different protein • Basal Body: The most complex
- Function:

Flagella are used for motility. Motility can be demonstrated by use of a cavity slide and a Coverslip by hanging drop method.

### Ribosomes:

Composed of proteins + ribosomal RNA 2 subunits

- 70S ribosomes (when two units are combined into one unit) 50S: one rRNA

30S: two rRNA
Several antibiotics act on ribosomes and inhibit protein synthesis • Streptomycin attach to 30S

## Q.33 Parts of flagella.

Flagellum: Long filamentous structure that propels bacteria

Filament: Flagellin

- Hook: A different protein
- Basal Body: The most complex

## Q.42 writes four phases of growth curve?

1. The Lag Phase:
2. The Log Phase:
3. The Stationary Phase:
4. The Death Phase:

## Q.42 Defines virulence?

Virulence is a pathogen's or microbe's ability to infect or damage a host. In the context of gene for gene systems, often in plants, virulence refers to a pathogen's ability to infect a resistant host. Salts OR mixing a weak base with one of its salts.

## Q.43 Write three components of LPS?

LPS consists of three components or regions: Lipid A, an R polysaccharide and an O polysaccharide. Region I. Lipid A is the lipid component of LPS. It contains the hydrophobic, membrane-anchoring region of LPS.

## Q. 43 write two prokaryotic groups?

Prokaryotic cells are divided into the domains Bacteria and Archaea.

## Q.45 write three names of bacteria classified on the basis of optimal temperature?

1. Psychrophiles:
2. Mesophiles: 25 – 40
3. Thermophiles: 50 – 60

## Q. 46 write methods of viable counting?

 The plate count 
Separate count.

Q.47 Three properties of integral proteins? (3marks)

## Q.49 major two prokaryotes group?

Bacteria

Archea

## Q.50 bacteria how to get nitrogen thier growth

How bacteria get N

- Decomposing proteins
- NH4+ ions
- Nitrates
- Gaseous N2

– Nitrogen fixation

## Q.55 Three shapes of bacteria? 3

- Coccus
- Bacillus
- Spiral

## Q.56 lassification of bacteria based on temperature? 5

- Psychrophiles: Cold loving: 15 0C • Mesophiles:
- 25 – 40 0C
- Moderate temp loving
- Optimum: 37 0C
- Thermophiles: 50 – 60 0C
- Heat loving
- Important in organic compost piles

## Q.58 Industrial microbiology?

It is a branch of biotechnology that applies microbial sciences to create industrial products in mass quantities. There are multiple ways to manipulate a microorganism in order to increase maximum product yields

Use of microbes or their enzymes for large scale production of biomolecules.

## Q.60 Glycocalyx and composition 2

It is the outermost layer, viscous and gelatinous in nature that surrounds the cells. It is composed of polysaccharide and polypeptide or both.

## Q.63 Vertical Gene Transfer

This is a normal way of transferring genes from parents to offspring. This happens when a cell divides. Each daughter cell receives exactly what its parent cell has.

## Classification of bacteria based on temperature? 5

**Psychrophiles:**

• Cold loving: 15 0C
**Mesophiles:**
• 25 – 40 0C

• Moderate temp loving •
Optimum: 37 0C

**Thermophiles: 50 – 60 0C**

• Heat loving

• Important in organic compost piles

## How bacteria get sulphur... 3

Ans:

• Sulfate ions

• Hydrogen sulfide

• Sulfur containing amino acids

## 3 differences between prokaryotes and eukaryotes.... 3

| Prokaryotes | Eukaryotes |
|---|---|
| **Cell type**<br>Mostly unicellular(some cyanobacteria may be multicellular) | **Cell type**<br>Mostly multicellular |
| **Nucleus**<br>True nucleus is absent. Nucleus lack nuclear membrane and nucleolus. Such nucleus is called nucleoid. | **Nucleus**<br>Nuclear membrane and nucleolus are present. |
| **Chromosome**<br>Usually single circular without histones. | **Chromosome**<br>Multiple linear with histones |
| | |

## Why solid medium is more better... 2

Ans:

• Pure culture is easy to obtain by streaking the organisms

• Colony characters can be studied

## Names of basic amino acids. 2

Ans:

1. Arginine (Arg)
2. Lysine (Lys)

## Flagella 2

• Flagellum: Long filamentous structure that propels bacteria

• Classification of bacteria based on flagella

• Atrichous: No flagellum

• Peritrichous: Distributed over the entire cell
• Polar:  Monotrichous  Amphitrichous  Lophotrichous

## • Vertical Gene Transfer o

This is a normal way of transferring genes from parents to offspring. This happens when a cell divides. Each daughter cell receives exactly what its parent cell has.

## • Horizontal Gene Transfer

When genes are transferred from cells to cells within the same species, the process is called horizontal gene transfer. This can happen between cells of the same species, or across different species of organisms. Horizontal gene transfer involves a donor and a recipient cell. The recipient cell then incorporates received DNA into its own genome and this genome becomes a recombinant molecule or recombinant DNA. The cell that has this recombinant DNA in it is called a recombinant cell. Three processes are known by which genes can be transferred horizontally from one cell to the other, and they include: 1. Transformation 2. Conjugation 3. Transduction

## Transformation

Naked DNA in solution is transferred from one bacterium to another during this process.

## Conjugation

Genes transfer process can be mediated by plasmids. Plasmids are extra chromosomal circular DNA fragments that replicate independently of bacterial chromosome. Conjugation requires direct cell to cell contact and cells have to be opposite mating types.

## Transduction

Transferring of a gene from a bacterium to another bacterium via a virus is called transduction. o When a bacteriophage infects a bacterium such as E. coli, it replicates inside E. coli and also produces a protein coat (which is called a capsid) in which viral DNA is packed before the virus is released.

## CLASSICAL METHODS OF BACTERIAL IDENTIFICATION

A classification system provides a basis for characterization and comparison that ultimately helps us in their identification as an individual. Although, the methods for classifying microbes may be different from the methods that are used for identification of microbes for treatment purposes, however, both share the same basic theme: grouping organisms based on their similarities with each other.

It may also be noted that large organisms such as plants and animals can be grouped on the basis of morphology alone; such a characteristic does not strictly apply to the bacteria which more or less have the same shape. In other words, morphology alone cannot help microbes in their classification or identification. That is the reason, classification and identification of microbes involves detection of enzymes of various metabolic pathways in these cells. So, biochemical testing is integral part of microbial (prokaryotes) classification and identification.

o Most of these identification schemes have a dichotomy. In other words, if an enzyme or a character is present, we call this as positive for that enzyme, and if the organism is negative with respect to the character under question, we place the organism into another group (negative group). This type of yes and no situations leads to a classification or identification scheme as is depicted in the accompanying figure for identifying selected genera of enteric bacteria.

- **Microbes especially bacteria (disease causing) can be identified by three methods:**
  - Classical or Conventional Method
  - Serological Methods
  - Nucleic Acid based Methods

- Classical methods of microbial identification involve differential staining of the sample before it its culturing, culturing the sample onto nutrient agar, blood agar and MacConkey's agar, purifying the culture (colonies expected to be involved in the disease) and detection of various enzymes that belong to various metabolic pathways. Classically, such methods used to take a long time to perform (3 days at least); however, rapid identification methods have become available now which use preformed media that can be used for testing the presence of metabolic enzymes.

## Economic use of fungi 3

o Saccharomyces are used for alcoholic beverages under anaerobic conditions for alcohol production (fermentative). However, if Saccharomyces are incubated aerobically (for bread making), they metabolize glucose to produce CO2 (some ethanol is also produced but gets evaporated during baking) which expands the dough causing it to rise.

o This yeast is also extensively used for molecular biology work and vaccine production. Vitamin C is obtained from Aspergilla's Niger; cellulase from Trichoderma, antibiotics from many fungi and anticancer Taxol from Taxomyces are some other examples of useful fungal products.

o Entomophaga (kills gypsy moth), hence is used as a biological pest control. Mushrooms can be cooked and eaten as a source of proteins for humans.

## Two name of basic stains?

1. Crystal violet
2. Methylene blue 3. Malachite green
4. Safranin.

**Negative Staining**

• Eosin • Acid fuchsin • Nigrosin.

### 1- Glycocalyx?

A viscous and gelatinous secretion that surrounds the cells – Polysaccharide and polypeptide or both • Capsule: Organized and firmly attached – Virulence – Vaccine • Slime: loosely attached. Bacillus anthracis • Streptococcus pneumoniae • Klebsiella. Glycocalyx as biofilm Bacteria secrete extracellular polymeric substance (EPS) • Protects cells within it. • Facilitates communication amongst cells • Enables the cells to attach to various surfaces for survival.

### 9- Chemical damage of plasma membrane? Name of Chemical that Cause Disrupt of Plasma membrane?

Phenol and Phenolics • First widely used antiseptics • Disrupt plasma membranes.

### 10- Shape of Bacteria?

Spiral – Vibrio: curved rods – Spirillum: Helical but rigid – Spirochete: Helical but flexible • Move by axial filamentDefine Culture, Culture Medium, Inoculum? Osmosis?

– Net movement of solvent • From high conc of solvent to low conc – OR low conc of solute to higher conc of solute. Solvent will move b/c of semipermeable membrane.

### 12- Endospore? Endospore Staining?

Endospore: A resistant dormant structure within a cell • Position of the endospore varies within the cell • Appear as a clear hallo in Gram stained smears. Malachite green: Primary stain • Heated to steaming for 5 min. • Washed with water. • Counterstained with safranin.

### 19- Function of Cell Membrane of bacteria?

Photosynthesis • Lipid synthesis • Cell wall parts • Receptors

### 21- Discus stage involve in the germination of spore?

**Activation**: Prepares spore for germination. – Heating can initiate it. • **Germination**: Spore starts swelling and loosing its coats etc. – Metabolically active • **Outgrowth**: New components are made.

### 22- Integral protein with example?

Integral proteins – Imbedded in the membrane – Insoluble in water – Amphipathic in nature • Some act as receptors. Integral proteins are not static in position • Can diffuse laterally and change position in the membrane.

### 23- Characteristic Metachromatic granules?

Metachromatic Granules: Also called volutin • Large inclusions • Stained red with methylene blue • Contain inorganic phosphates • Characteristics of Corynebacterium diphtheria.

### 24- Kind of Spiral shape of bacteria?

• Spiral – Vibrio: curved rods

### 27- Define lysosome?

An organelle in the cytoplasm of eukaryotic cells containing degradative enzymes enclosed in a membrane.

### 28- Name the counting method for cell?

A counting chamber,(also known as hemocytometer), is a microscope slide that is especially designed to enable cell counting ..... The separating distance between the chamber and the cover is predefined, thus the volume of the counted culture can be calculated and with it the concentration of cells.

### 29- Name Five Bacteria phase?

In autecological studies, the growth of bacteria (or other microorganisms, as protozoa, microalgae or yeasts) in batch culture can be modeled with four different phases: lag phase (A), log phase or exponential phase (B), stationary phase (C), and death phase (D).

### 30- Name Five Bacteria on the bases of oxygen growth? Classification of Bacteria on Oxygen requirement? On the bases of oxygen write name five bacteria group?

Aerobes. 1.1. require oxygen. 1.2. use oxygen as a final electron acceptor. ...
Microaerophiles. 2.1. require limited amount of oxygen. 2.2. ...
Obligate anaerobes. 3.1. require absence of oxygen. 3.2. ...

Aerotolerant anaerobes. 4.1. not use oxygen for growth. 4.2. ...
Facultative anaerobes. 5.1. can grow in present / absence of oxygen. 5.2

### 31- Sulfur Bacteria Source?

Sulfur-oxidizing bacteria produce effects similar to those of iron bacteria. They convert sulfide into sulfate, producing a dark slime that can clog plumbing. Sulfur-reducing bacteria (SRBs) live in oxygendeficient environment of the two types, sulfur-reducing bacteria are the more common.

### 32- Chemotaxis and Phototaxis Sporulation?

**Phototaxis** is a kind of taxis, or locomotory movement, that occurs when a whole organism moves towards or away from stimulus of light. ... This is analogous to positive **chemotaxis** except that the attractant is light rather than a chemical.

**Sporulation** Formation of spores takes place within a vegetative cell and the process is called sporulation or sporogenesis. It is initiated when nutrients become unavailable.

### 33- Prokaryotic and Eukaryotic Difference?

The main difference between prokaryotes and eukaryotes is this: eukaryotic cells contain membranebound organelles, such as the nucleus, while prokaryotic cells do not.

### 40- Formula of Compound Microscope?

me=−di′do′=−−367 mm44.0 mm=8.33 m e = − d i ′ d o ′ = − − 367 mm 44.0 mm = 8.33 . So the overall magnification is
m

= mome = (−30.0)(8.33) = −250. **(Internet)**

### 41- Name Two type of Technoice acid found in bacteria?

Gram-positive **bacteria** possess a thick cell wall containing many layers of peptidoglycan and teichoic **acids**. In contrast, gram-negative **bacteria** have a relatively thin cell wall consisting of a few layers of peptidoglycan surrounded by a **second** lipid membrane containing lipopolysaccharides and lipoproteins.
**Teichoic acids** are **found** within the cell wall of most Gram-positive bacteria such as species in the genera Staphylococcus, Streptococcus, Bacillus, Clostridium, Corynebacterium, and Listeria, and appear to extend to the surface of the peptidoglycan layer.

## 1. Names of the Five Kingdom Classification of Organisms (vip)

### Five Kingdom Classification of Organisms

- Monera: Bacteria and Archea
- Protista
- Fungi
- Plantae
- Animalia

## 2. Define gene phenotype and chromosomes ?

### Chromosome:

Structure containing DNA that physically carries hereditary information; the chromosomes contain the genes

### Phenotype:

Expression of the genes

## 3. Define plasmolysis osmotic pressure ?

### Plasmolysis :

Plasmolysis is the process in which cells lose water in a hypertonic solution.

### Osmotic Pressure

Pressure required to prevent movement of water into a solution containing some solute.

## 4. Why catalase test is used ?

The catalase test is a particularly important test used **to determine wheather a gram-positive cocci is a staphylococci or a streptococci**. Catalase is an enzyme that converts hydrogen peroxide to water and oxygen gas.If bubbles appear (due to the production of oxygen gas) the bacteria are catalase positive.

•

## 6. what is industrial microbiology (2) marks.

**Industrial Microbiology**

• Use of microbes or their enzymes for large scale production of biomolecules

## 7. w ho discover small pox in which year (3 marks )

Edward Jenner discovered small pox in 1796.

## 8. d/f between vertical horizontal transfer of gene .

| Vertical gene transfer | Horizontal |
|---|---|
| Parents to off springs | From bacteria to bacteria or<br>From cells to cells within the same generation |

## 9. five kingdom classification 5 marks

**Five Kingdom Classification of Organisms**

- ➢ Monera: Bacteria and Archea
- ➢ Protista
- ➢ Fungi
- ➢ Plantae
- ➢ Animalia
- ➢ Microbes placed in the first 3 kingdoms

### Reasons for Changes in the Classification

- ➢ Electron microscope
- ➢ Biochemical and physiological characteristics
- ➢ Nucleic acid and protein sequencing o Ribosomal RNA (rRNA)
- ➢ Bacteria and Archea two different groups

### Three Domain Classification (1977)

=

- ➢ Bacteria: True or eubacteria ➢ Archea:
- ➢ Eucarya

– Protists (slime molds, protozoa, algae)
– Fungi
– Plants
– Animals

### Domains & Kingdoms

- ➢ Domain: Bacteria, Kingdom Bacteria
- ➢ Domain: Archea, Kingdom Archea
- ➢ Domain: Eucarya Kingdom Protista Kingdom Fungi Kingdom Plantae Kingdom Animalia

### Bacteria

- ➢ Procaryotes
- ➢ Usually single-celled organisms
- ➢ Cell wall made up of peptidoglycan

**Archea**

- ➢ Procaryotes
- ➢ Cell wall has no peptidoglycan, but has special lipids.
- ➢ Vary in rRNA sequencing
- ➢ Live in extreme conditions – Thermophiles o Halophiles

**Eucarya**

1. Protists
2. Fungi
3. Animals
4. plants

## 10. Define gene ,chromosome and phenotype ?

Phenotype:

Expression of the genes

Gene

A segment of DNA that encodes a functional product, usually a protein

Chromosome

Structure containing DNA that physically carries hereditary information; the chromosomes contain the genes.

## 11. Brifely explain transfromation conjugation and transduction 3 marks

**Transformation**

## Transformation can occur in vitro

- In broth culture, live non-capsulated organisms cultured. Dead capsulated cells mixed with it. Capsulated bacteria were recovered from broth. Research revealed it to be DNA.
- Competent cells: that take up the DNA • Not all cells do it naturally.

Transformation is uptake of naked DNA by microbes

**Conjugation**

- ➢ Mediated by a plasmid
- ➢ Plasmid is an extra chromosomal circular DNA fragment. Replicates independently of chromosome. Genes of plasmids not important for bacterial growth.
- ➢ Requires direct cell to cell contact. Opposite mating types cells. Plasmids in G – ive cells have genes that code for sex pili. G +ve secrete sticky surface molecules
- ➢ These plasmids are transmissible from one bacterium to another during conjugation.
- ➢ Plasmid is replicated and a single copy of the plasmid is transferred to the recipient bacterium.
- ➢ Complimentary copy is then made.
- ➢ Fertility factor carrying plasmid was the first plasmid studied (F) **Transduction**

• Transfer of DNA by a virus called bacteriophages.

Phages can transfer DNA and the process is transduction

- ➢ Generalized
- ➢ Specialized

## 12. Factors influence death rate of bacteria.

: presence of organic matter in human wastes, etc. presence of organic matter in human wastes and bodily fluids inhibits death induced by antimicrobial growth. Factors that affect microbial death rate: temperature. some disinfectants work better at warm temperatures.

## 13. Five kindom system names ?

**Five Kingdom Classification of Organisms**

- ➢ Monera: Bacteria and Archea
- ➢ Protista
- ➢ Fungi
- ➢ Plantae
- ➢ Animalia

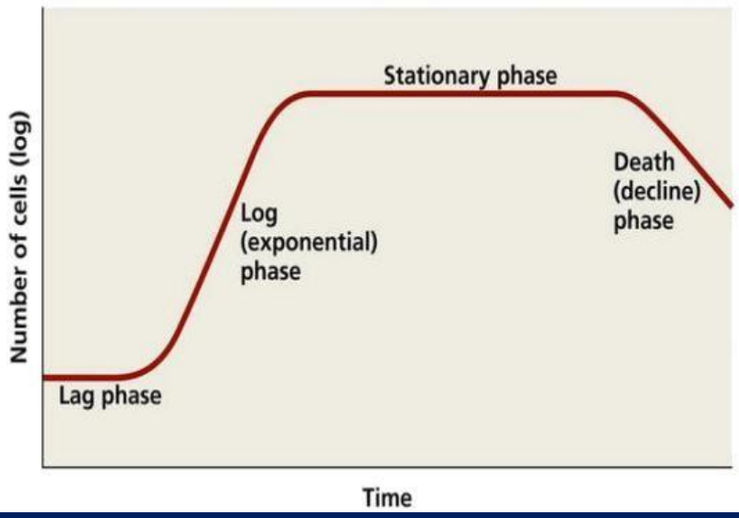
## 14. Enlist three chemical that effect bacteria 03

1 : Penicillin

2 : Aminoglycosides

3 : Ofloxacin.

## 15. Growth curve of bacteria 03




## 16. Mircoaerophiles ?

**Microaerophiles:**

– Aerobes but O2 is required in low amounts.

– Produce O2 toxic compounds if exposed to more O2

Listeria, Vibrio

## 17. Sporulation 2

Formation of spores is called sporulation or sporogenesis.

## 20. Prokaryote and eukaryotes difference 3

**Prokaryotes and Eukaryotes**

• Prokaryotes and eukaryotes are chemically similar.

– All made up of nucleic acids, protein, lipids, and carbohydrates.

– All metabolically similar.

**Prokaryotes**

• DNA is not enclosed in a nuclear membrane.

• Chromosome: mostly Single, circular DNA not associated with histones

• No membrane enclosed organelles

• Cell wall has peptidoglycan, a complex carbohydrate.

• Divide by binary fission.

**Eukaryotes**

➢ DNA is enclosed in a membrane-bound nucleus.

➢ DNA is found in multiple chromosomes.

➢ Chromosomes are linear, thread-like structures

## 21. endospore .

**Endospores**

➢ Specialized resting cells

➢ Highly durable, dehydrated forms of bacteria

➢ Formed inside a bacterium

➢ Survive extremes of temperature

## 25. Method of microbial control in detail .(5)

• Terms used for microbial controls and their meaning

**Terminology**

➢ Sepsis: Microbial contamination

➢ Asepsis: Absence of significant contamination

➢ Aseptic surgery techniques prevent microbial contamination of wounds

➢ Sterilization: Removing all microbial life including spores

➢ Disinfection: Removing pathogens significantly

➢ Antisepsis: Removing pathogens from living tissue

➢ Degerming: Removing microbes from a limited area (injection site)

➢ Sanitization: Lowering microbial counts on eating utensils

➢ Biocide/germicide: Kills microbes

➢ Bacteriostasis: Inhibiting, not killing, microbes

➢ Chemotherapy: killing microbes within the host tissue

## 24. What is blood agar ?

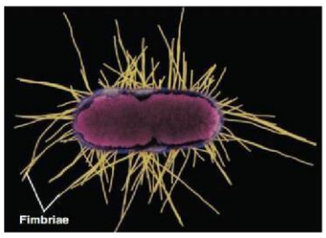
**Blood Agar**

• Nutrient agar + blood
• Enriched and differential
• Used for fastidious organisms
• Differential: hemolysin
– β-hemolysis: clear zone
Strep pyogenese
– α-hemolysis: greenish halo

## 25. Functions of cell membrane.?

**Functions of Cell Membrane**

• Selective permeable barrier
• Passive and Active Transport
• Respiration
• Photosynthesis
• Lipid synthesis
• Cell wall parts
• Receptors




## 26.Difference b/w prokaryotes and eukaryotes ? vip L-5

| | Prokaryotes | Eukaryotes |
|---|---|---|
| **Type of Cell** | Always unicellular | Unicellular and multi-cellular |
| **Cell size** | Ranges in size from 0.2 µm – 2.0 µm in diameter | Size ranges from 10 µm – 100 µm in diameter |
| **Cell wall** | Usually present; chemically complex in nature | When present, chemically simple in nature |
| **Nucleus** | Absent. Instead, they have a nucleoid region in the cell | Present |
| **Ribosomes** | Present. Smaller in size and spherical in shape | Present. Comparatively larger in size and linear in shape |
| **DNA arrangement** | Circular | Linear |
| **Mitochondria** | Absent | Present |
| **Cytoplasm** | Present, but cell organelles absent | Present, cell organelles present |
| **Endoplasmic reticulum** | Absent | Present |
| **Plasmids** | Present | Very rarely found in eukaryotes |
| **Ribosome** | Small ribosomes | Large ribosomes |
| **Lysosome** | Lysosomes and centrosomes are absent | Lysosomes and centrosomes are present |
| **Cell division** | Through binary fission | Through mitosis |

| | | |
|---|---|---|
| **Flagella** | The flagella are smaller in size | The flagella are larger in size |
| **Reproduction** | Asexual | Both asexual and sexual |
| **Example** | Bacteria and Archaea | Plant and Animal cell |

## 27. Acid fast staining ?

Acid Fast Staining

➢Differential stain
➢1882, Paul Ehrlich observation on Mycobacterium
➢Not easily stained
➢Once stained do not loose the stain even with acid alcohol
➢Mycobacterium have mycolic acid (waxy)
➢Makes cell wall impermeable to most stains
➢Ziehl Neelsen method is commonly used
➢Make a smear
➢Carbolfuchsin on slides
➢Heat the slide for 5-10 min
➢Wash with water and then with acid alcohol for 1 min.
➢Counterstain with methylene blue for 1 min.
➢See under the microscope.

Acid fast bacteria have mycolic acid that can be stained by heating the organisms with the stain.

## 28. Metachromatic granules?

➢ Also called volutin
➢Large inclusions
➢Stained red with methylene blue
➢Contain inorganic phosphates
➢Characteristics of *Corynebacterium diphtheriae*

## 29. Definition of the culture media ?

### Culture Media

➢Culture medium: A nutrient material for growth ➢ Some grow easily, other do not grow at all.
➢Inoculum: Microbes introduced into a culture medium that initiate growth
• Culture: Microbes that grow or multiply in a culture medium

## 30. why 70percent alcohol best instead of100percent ?

**Alcohols**

• Ethanol, isopropanol
Denature proteins, dissolve lipids Require water 70% is more effective than 100%

## 31. Integral proteins

### Integral proteins

– Imbedded in the membrane
– Insoluble in water
– Amphipathic in nature
• Some act as receptors

## 32. Pili and its function?

**Pili** :

Pili are hair-like structures in bacterial walls that allow bacterial cells to adhere to other surfaces throughout their environment.

**Function** :

The primary function of pili are to attach a bacterial cell to specific surfaces or to other cells.

## 34. Types of bacteria on basis of oxygen requirement?

### *1.* Obligate aerobes

Bacteria/organism that requires oxygen to live are called obligate aerobes oxygen must

### 2. Facultative anaerobes

The such-type organism can grow in the presence or absence of oxygen but greater growth in the presence of oxygen. E.coil

### 3. Obligate anaerobes

Such Microorganisms are unable to use oxygen for growth. Growth is restricted in the presence of oxygen (cannot tolerate oxygen), only grow under anaerobic. These bacteria obtain oxygen atoms from water.

**Example**: Clostridium bacteria that cause tetanus and botulism.

### 4. Aerotolerant anaerobes

Aerotolerant anaerobes do not require oxygen for grow but tolerate oxygen fairly well
**Example** lactic acid-producing lactobacillus

### 5. Microaerophiles

These are aerobic required (required oxygen but in lower concentration) but grow only in oxygen concentration lower than those in air.

## 35. Spontaneous generation ?

**Spontaneous Generation**

• Aristotle: Simpler invertebrate could arise by spontaneous generation
Francesco Redi challenged

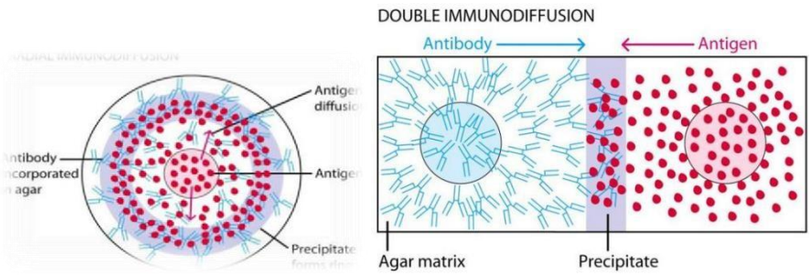
## 36. Microbe indentification? 5

**Microbes identified by various methods**
Serological Methods for microbial identification

**Serological Testing**

• Antigen antibody reactions
Antibody Production by Lymphocytes
• Precipitation test
• Soluble antigen binds with antibodies
• Bacterial Identification III
• Precipitation test
• Soluble antigen binds with antibodies

**Radial Immunodiffusion Test**




Precipitation is with soluble antigen **Serological Testing**
• Some antigen antibody reactions are not visibleto the eyes.
• Such reactions are visualized indirectly using various probes.
• Probes include enzymes, radioisotopes etc.
• Enzymes or radioisotopes are conjugated to antibodies
• Presence of enzymes detected by incubating with substrate that forms colored compounds.
• ELISA used for antigen or antibody detection in a samp **Western blotting**
• Bacterial Identification V

## 46. Layers of endospore 5

**Endospores**

➢ Specialized resting cells
➢ Highly durable, dehydrated forms of bacteria
➢ Formed inside a bacterium
➢ Survive extremes of temperature
• Lack of water Radiation
• Toxic environment
• Mostly formed by Gram positive bacteria –
Clostridium
– Bacillus
• One gram negative: *Coxiella burnetii* also has it.
• Formation of spores is called sporulation or sporogenesis.
Exosporium: A thin delicate covering
Thick, composed of several protein layers
– Resistant to chemicals
– Contains enzymes germination
**Cortex**: Has peptidoglycan
Spore cell wall or core wall: Surrounds the protoplast or spore core **Spore core:**

## 47. Photoautotroph 3 marks

**Photoautotrophs**

➢ Use light as a source of energy
➢ CO2 as a source of Carbon
➢ Hydrogen atoms of water are used to reduce CO2

**Photoautotrophs**

Oxygen is given off (Oxygenic)
• Photosynthetic bacteria
• Algae
• Green plants
✓ Green bacteria: Non-oxygenic
✓ Do not use hydrogen from water
✓ Use hydrogen from H2S or H2 gas to reduce CO2
✓ Need total anaerobic conditions
✓ Use light as a source of energy
Use organic compounds as Carbon source
– Alcohol
– Fatty acids – Carbohydrates etc.
• They are anoxygenic.
– *Rhodopseudomonas*

**Chemoautotrophs**

• Use electrons from inorganic compounds as energy source
1. H2S
2. Sulfur
3. Ammonia
4. H2
• CO2 as carbon source – *Thiobacillus*
• Energy source and carbon source are usually the same organic compound – Glucose
• Typically, use hydrogen atoms of organic compounds as electron source – Disease causing microbes

**Summary**

• Anaerobic respiration
– O2 is not electron acceptor
• Lipids and proteins can enter at various points in the glycolysis and Krebs • cycle Microbes classified on the basis of nutritional requirements

## 48. Bacteria kinds on the basis of oxygen ? 5 marks

**On the basis of oxygen requirements, bacteria can be divided into the following different categories:**

**1 : Aerobes**

Aerobes grow in ambient air, which contains 21% oxygen and a small amount of (0.03%) carbon dioxide. Aerobes obtain some of their energy from glycolysis but they get most of the energy through aerobic respiration (via the Krebs cycle and oxidative phosphorylation). Aerobes require molecular oxygen as a terminal electron acceptor so cannot grow in its absence. **For example**., *bacillus cereus.*

**2 : Obligate aerobes**

They have an absolute requirement of free oxygen in order to grow.

**For example**, Pseudomonas aeruginosa, Mycobacterium tuberculosis.

**Anaerobes 3 :** :

Anaerobes can not grow in the presence of oxygen, oxygen is toxic for them. Anaerobes do not use free O2 as their final electron acceptor instead they use inorganic oxygen-containing molecules such as nitrate ($NO_3$-), nitrite ($NO_2$−), and sulfate ($SO_4^2$-), in a process called anaerobic respiration.

**4 : Obligate anaerobes**

Obligates anaerobes are killed by free oxygen. These bacteria grow only under the condition of high reducing intensity. Clostridium perfringens, Clostridium botulinum etc.

**5 : Facultative anaerobes**

Facultative anaerobes are versatile organisms, capable of growth under both aerobic and anaerobic conditions. They use oxygen if it is available but can function without it. When oxygen is present, they **preferentially use oxygen** as a terminal electron acceptor and carry on aerobic metabolism, but they shift to anaerobic metabolism when oxygen is absent.

e.g., ***Enterobacteriaceae*** Aerotolerant family, ***Staphylococcus aureus***, etc.

**6 : Aerotolerant anaerobes**

Are anaerobic bacteria that are not killed by exposure to oxygen. anaerobes **can survive in the presence of oxygen but do not use it** in their metabolism. *Lactobacillus*,

**for example**, always captures energy by fermentation, regardless of whether the environment contains oxygen.

## 7: Capnophiles

Capnophilic (or carbon dioxide–loving) bacteria require increased concentration of carbon dioxide (5% – 10%) and approximately 15% oxygen. This condition can be achieved by a candle jar (3% carbon dioxide) or carbon dioxide incubator, jar, or bags.

**Examples** of capnophilic bacteria include Haemophilus influenzae, Neisseria gonorrhoeae etc.

## Microaerophiles

Microaerophiles (mipkro-aer`o-filz) are those groups of bacteria that can grow best under reduced oxygen (5% to 10%) and increased carbon dioxide (8% to 10%) concentrations. Higher oxygen tensions may be inhibitory to them.

In a nutrient broth, they grow below the surface of the medium in a culture tube at the level where oxygen availability matches their needs. This environment can be obtained in specially designed jars or bags. Microaerophiles such as Campylobacter are also capnophiles. They thrive under conditions of low oxygen and high carbon dioxide concentration.

**Examples** of microaerophiles are Campylobacter jejuni, Helicobacter pylori, etc.

## 49. Protocol urease test 3 marks

The urease test identifies those organisms that are capable of hydrolyzing urea to produce ammonia and carbon dioxide. It is primarily used to distinguish urease-positive Proteeae from other Enterobacteriaceae. Two media types are commonly used to detect urease activity.

Christensen's urea agar is used to detect urease activity in a variety of microorganisms. Stuart's urea broth is used primarily for the differentiation of Proteus species. For Christensen's urea agar, urease production is indicated by a bright pink (fuchsia) color on the slant that may extend into the butt after 1-6 hours of incubation. The culture medium will remain a yellowish color if the organism is urease negative. For Stuart's urea broth, urease production is indicated by a bright pink (fuchsia) color throughout the broth.

## 50. What is acid-fast staining method?

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

## 51. Complex medium and basal medium ?

Serum-free media (SFM) are often categorized as either basic/basal or complex. Basal mediums are those without supplements. Once growth supplements are added to a **basal medium**, it becomes a **complex medium**.

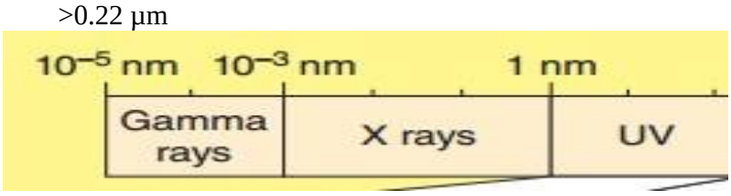
## 52. Ionizing Radiation ?

### Ionizing Radiation

- Mechanical removal includes Filtration
  1. Air
  2. Liquid

### Filtration

- HEPA removes microbes >0.3 µm
- Membrane filtration removes microbes >0.22 µm




## 53. Medical microbiology? 2

### Medical Microbiology

- ➢ Deals with diseases of humans and animals
- ➢ Identifications of disease causing agents
- ➢ Control and elimination of microbes
- ➢ Tracking down new pathogens

## 54. Steps of glucose respiration. 3

### Respiration of Glucose

- Occurs in three stages:
  – Glycolysis
  – The Krebs cycle
  – Electron transport chain
- Most of ATP is generated in the last stage.

### Glycolysis

- Glycolysis is the oxidation of glucose to pyruvic acid with the production of some ATP and energy-containing NADH.

### The Krebs Cycle

- Oxidation of acetyl CoA to carbon dioxide, with the production of some ATP and energycontaining NADH and FADH2

### Electron Transport Chain

- NADH and FADH2 are oxidized through a series of electron carriers to produce ATP

## 55. Magnetosomes 5

### Magnetosomes

- Inclusions of iron oxide
- Surrounded by invaginations of plasma membrane
- Present in G negative bacteria – Act like magnet
  – Decompose H2O2

### Summary

- ➢ Cytoplasm
- ➢ Bacterial chromosome
- ➢ Plasmid
- ➢ 70S Ribosomes
- ➢ Inclusion bodies Metachromatic granules
- Gas vacuoles

➢ Magnetosomes

## 56. What is coenzyme? (3 marks)

### Coenzymes

- Act as electron carriers
- Receive electrons from nutrients
- Donate electrons to other molecules in subsequent reactions

**Examples of coenzymes:**

- $NAD^+$
- $NADP^+$
- FMN and FAD

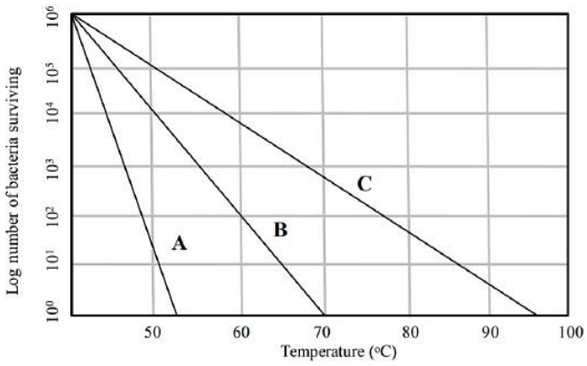
## 57. What is agar?

Agar, or agar-agar, is a jelly-like substance consisting of polysaccharides obtained from the cell walls of some species of red algae, primarily from ogonori and "tengusa".

## 58. What is thermal death point and (Time) rate? (2 marks)

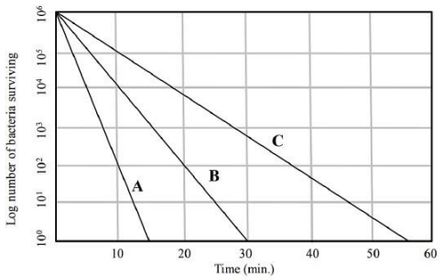
### Thermal Death Point (TDP)

The TDP is the lowest temperature that is required to kill a population of microbes when applied for a specific time.




### Thermal Death Time (TDT)

The TDT is the minimum time it takes to kill a population of microbes at a specific temperature.




## 59. In Dark field microscopy which specimens are used? (2 marks) .

Very thin histological sections can be used if unstained or if only certain components are stained, as in silver stains. Biological fluids from animals and plants, cell cultures, microbes, foods, fibers, crystals, colloids, and sub-microscopic particles are all suitable for dark field microscopy. Preparations of autoradiography and gold labeling are also suitable.

## 60. spoilage of cheese and pickeles

Nisin and natamycin prevent spoilage of cheese

### Effect of pH

- Very few grow below pH 4

– Pickles and Many Cheeses not spoiled

- Acid is produced by bacteria during fermentation

## 61. Name of four membrane bound organalles ?

- Mitochondria
- Lysosomes
- endoplasmic reticulum
- Golgi apparatus

## 62. Sepsis , Antisepsis Sanitization ?

**Sepsis:** Microbial contamination

**Antisepsis**: Removing pathogens from living tissue

**Sanitization**: Lowering microbial counts on eating utensils

## 64. Classify bacteria on basis of temperature ?

**Classification of bacteria on the basis of Temperature**

Bacteria can be classified into the following major types on the basis of their temperatures response as indicated below:

**1.Psychrophiles:**

- Bacteria that can grow at 0°C or below but the optimum temperature of growth is 15 °C or below and maximum temperature is 20°C are called psychrophiles
- Psychrophiles have polyunsaturated fatty acids in their cell membrane which gives fluid nature to the cell membrane even at lower temperature.
- Examples: Vibrio psychroerythrus, vibrio marinus, Polaromonas vaculata, Psychroflexus.

2. **Psychrotrops (facultative psychrophiles):**

 Those bacteria that can grow even at 0°C but optimum temperature for growth is (20-30)°C.

3. **Mesophiles:**

- Those bacteria that can grow best between (25-40)o C but optimum temperature for growth is 37C
- Most of the human pathogens are mesophilic in nature.
- Examples: E. coli, Salmonella, Klebsiella, Staphylococci.

4. **Thermophiles:**

- Those bacteria that can best grow above 45C.
- Thermophiles capable of growing in mesophilic range are called facultative thermophiles.
- True thermophiles are called as Stenothermophiles, they are obligate thermophiles,
- Thermophils contains saturated fattyacids in their cell membrane so their cell membrane does not become too fluid even at higher temperature.
- Examples: Streptococcus thermophiles, Bacillus stearothermophilus, Thermus aquaticus.

5. **Hypethermophiles:**

- Those bacteria that have optimum temperature of growth above 80C.
- Mostly Archeobacteria are hyperthermophiles.
- Monolayer cell membrane of Archeobacteria is more resistant to heat and they adopt to grow in higher remperature.
- Examples: Thermodesulfobacterium, Aquifex, Pyrolobus fumari, Thermotoga. 

## 65. Active and passive transport ?

**Passive Movement**

➢ Movement with the concentration gradient

➢ No energy expenditure

➢ Substances move from area of high concentration to low concentration.

**Active Movement**

• Movement against the concentration gradient

• From low concentration to high concentration

• Requires energy (ATP)

# FINAL TERM

## 1. Karyogamy plasmogamy and meiosis? 2MARKS (PPT 58)

**Plasmogamy**: A haploid nucleus of a donor cell (+) penetrates the cytoplasm of a recipient cell (-)
Both cells are haploid (one set of chromosomes).
**Karyogamy**: The (+) and (-) nuclei fuse to form a diploid zygote nucleus.
**Meiosis:** The diploid nucleus gives rise to haploid nuclei (sexual spores).

## 2. Advantage of Dry Heat relatively? 2 MARKS

**Advantages of dry heat sterilization** include: it is inexpensive, it does not pollute or cause toxic fumes, and it does not corrode or rust metal objects.
**Disadvantages** include: it is relatively slow and many objects cannot withstand the high temperatures.

## 3. How Microorganism cause damaged the body?

Infection with a **pathogen** does not necessarily **lead** to disease. Infection occurs when viruses, **bacteria**, or other **microbes** enter your **body** and begin to multiply. ... Pathogenic **microbes** challenge the immune system in many ways. Viruses make us sick by killing cells or disrupting cell function.

## 4. Microbial Identification?

**Microbial identification** can be **defined** as "**microbial** characterization by a limited spectrum of tests pre-chosen and appropriate to the problem being studied"
**Methods:** Classical or Conventional Method, Serological testing, Nucleic acid based testing.

## 5. Write two method? Viral Replication and Its mechanism? Stages of Viral Replication?

Viral replication involves six steps:
**1)** attachment,
**2)** penetration,
**3)** uncoating,
**4)** replication,
**5)** assembly,
**6)** release.
... During uncoating, replication, and assembly, the viral DNA or RNA incorporates itself into the host cell's genetic material and induces it to replicate the viral genome.

## 6. Immune and Its Types? Define Imunity and Its types? Innate and adaptive immunity? (10)

Immunity is the balanced state of multicellular organisms having adequate biological defenses to fight infection, disease, or other unwanted biological invasion, while having adequate tolerance to avoid allergy, and autoimmune diseases.
**Innate Immunity**
Plants and animals have what is called innate immunity. Innate immunity is the first line of defense against pathogens. It involves several cell types, proteins, and even an organ. The organ involved is your skin. Yes, skin is part of the first line of defense. It protects you and prevents pathogens from getting inside your body.
**Adaptive immunity** is an important part of the immune system. It is protection from an infectious disease agent that is mediated by B- and T- lymphocytes following exposure to specific antigen, and characterized by immunological memory.

## 7. Economies use of fungi?

They play an important **role** in medicine yielding antibiotics, in agriculture by maintaining the fertility of the soil and causing crop and fruit diseases, forming basis of many industries and as important means of food.

## 8. Gram Staining Procedure?

Crystal violet is added. – Primary stain • Crystal violet is washed off. • Iodine is added to enhance binding. – Mordant. • The slide is washed off with alcohol. – Decolorizing agent • Gram positive bacteria retain crystal violet • Gram negative bacteria appear colorless. • Crystal violet and iodine make a complex in the cytoplasm • Peptidoglycan layer is thicker in Gram positive bacteria and CV-I is retained. • Alcohol is rinsed off and stained with safranin. – Counterstain • The smear is washed again. • Blotted dry and examined microscopically.

## 9. Difference Simple and Differential Media?

Selective and differential media are used to isolate or identify particular organisms. Selective media allow certain types of organisms to grow, and inhibit the growth of other organisms. ... Differential media are used to differentiate closely related organisms or groups of organisms.

## 10. Horizontal and Vertical Gene Transfer? 3

**Vertical gene transfer** – Parents to offsprings
**Horizontal** – From cells to cells within the same generation – From bacteria to bacteria.

## 11. Difference LB Broth and LB Agar?

**LB Broth:** **broth** (**LB**) is a nutritionally rich **medium** primarily used for the growth of bacteria. Its creator, Giuseppe Bertani, intended **LB** to stand for lysogeny **broth**, but **LB** has also come to be commonly referred to as **Luria broth**, Lennox **broth**, or**Luria**-Bertani **medium**.

**LB Agar:**
Luria broth (**LB**) is a nutrient-rich media commonly used to culture bacteria in the lab. The addition of **agar** to **LB** results in the formation of a gel that bacteria can grow on, as they are unable to digest the **agar** but can gather nutrition from the **LB**within.

## 12. Chemical used in Gram staining?

Both gram-positive and gram-negative cells have peptidoglycan in their cell walls, so initially, all bacteria stain violet. Gram's iodine (iodine and potassium iodide) is applied as a mordant or fixative. Gram-positive cells form a crystal violet-iodine complex. Alcohol or acetone is used to decolorize the cells.

## 13. Viruses, Virion, and Virusoid and Prion?

**Viruses**: Acellular organisms – Proteins and nucleic acids
**Viroids**: composed only of circular ssRNA (potato spindle tuber viroid)
**Virusoids**: Contain circular ssRNA, need helper viruses for replication and encapsidation; also called satellite viruses **Prions**: Infectious proteins

## 14. Uses of UV in Lab?

- Transilluminators. **Used** for the visualization of molecular samples, transilluminators are indispensable tools for researchers at life-sciences **laboratories**. ...
- **UV** Crosslinkers. ...
- Fluorescence Analysis Viewing Cabinets and Work Stations. ...
- **Ultraviolet** Lamps. ...
- Digital Radiometers/Photometers

## 15. Epidemiology and Its type?

Monitoring diseases in a population • Detection of outbreaks and developing epidemics • Implementation of appropriate control measures • Surveillance studies.
**There are four most common types of epidemiological studies:**

- Cohort Study.
- Case Control Study.
- Occupational Epidemiological Study.
- Cross-Sectional Study.
- Epidemiologists use statistical methods to determine whether the differences they see are real or due to chance fluctuations.

## 16. Streaking Plates method?

**Streak plate** technique is used for the isolation into pure culture of the organisms (mostly bacteria), from mixed population. The inoculum is streaked over the agar surface in such a way that it “thins out” the bacteria. Some individual bacterial cells are separated and well spaced from each other.

## 17. Process of bacterial identification?

The **identification** of **bacteria** is a careful and systematic **process** that uses many different techniques to narrow down the types of **bacteria** that are present in an unknown **bacterial** culture. It produces benefits for many aspects of the research of microorganisms and helps physicians correctly treat patients.

## 18. Physical Method of Sterlization?

Heat **Method of Sterilization**
This is the most common **method of sterilization**. The heat used kills the microbes in the substance. ... In heat**sterilization** process, the longer the exposure to heat the better is the **sterilization** at a given temperature.

## 19. Disadvantage of Chlorine Sterilization?

disadvantages as well; First of all high concentration of this agent corrodes metals and also damages cloths. Secondly the strength of this solution decreases with time so whenever you want to use it always prepare its fresh solution.

## 20. Advantage of dry heat Sterilization?

**Advantages** of dry heat **sterilization** include: it is inexpensive, it does not pollute or cause toxic fumes, and it does not corrode or rust metal objects.**Disadvantages** include: it is relatively slow and many objects cannot withstand the high temperatures.

## 21. Virulence

The extent of pathogenicity is called virulence of a pathogen.

## 22. Name the three subgroups of spiral bacteria

These are curved shaped bacteria. They are further divided into 3 more subgroups.

a. Vibrio: curved rods
b. Spirillum: Helical but rigid
c. Spirochete: Helical but flexible

Spirochetes move by axial filaments which are enclosed by the outer membrane

## 23. flagellum and function (2

Flagellum: Long filamentous structure that propels bacteria It helps the bacteria in movement.

## 24. Name the Types of Bacteria on the basis of Oxygen Requirement

a. Obligate Aerobes: Do not grow without oxygen. It is necessary.
b. Facultative Anaerobes: Oxygen is necessary but start become anaerobes in its absence and can grow without of $O_2$.
c. Obligate Anaerobes: Cannot grow in the presence of oxygen.
d. Aerotolerant aerobes: Do not need $O_2$, also do not bother its presence
e. Microaerophiles: Aerobes but need small amount of oxygen. Cannot grow if available in large amount.

## 25. conjugation, transformation and transduction (3)

### Transformation

Naked DNA in solution is transferred from one bacterium to another during this process.

### Conjugation

Genes transfer process can be mediated by plasmids. Plasmids are extra chromosomal circular DNA fragments that replicate independently of bacterial chromosome. Conjugation requires direct cell to cell contact and cells have to be opposite mating types.

### Transduction

Transferring of a gene from a bacterium to another bacterium via a virus is called transduction. o When a bacteriophage infects a bacterium such as E. coli, it replicates inside E. coli and also produces a protein coat (which is called a capsid) in which viral DNA is packed before the virus is released.

## 26. how disease can be transmitted (5)

Direct Transmission: person to person physical contact is a direct contact. Influenza spread is a typical example.

Indirect Transmission: A nonliving object is involved. Objects in the use of a patient such as handkerchief, utensils, pillow and bedding are called fomites. These can transmit organisms to susceptible individuals.

Droplet Transmission: Mucus droplets are created when you sneeze for example. These droplets carry organisms that may infect another individual who comes in contact with the droplets. One sneeze may produce 20,000 droplets. Influenza can be spread by this route.

Vehicle Transmission: Transmission of disease agents by a medium, such as water, food, or air. Other media include blood and other body fluids, drugs, and intravenous fluids. 

What are Vectors? A vector is a vehicle (animate or inanimate) that carries a pathogen from one host to another.

Arthropods are vectors for many diseases. There are two ways these arthropods can transmit organisms to susceptible hosts:

Mechanical Transmission: In this type of transmission, organisms stick to the body parts of insects and get transmitted passively from one location to another. Biological Transmission: Microbes multiply in the insect and is transmitted actively by insect bites from one individual to another

## 27. Any two microbial identification method (5)

Classical or Conventional Method

Serological testing

Nucleic acid based testing … plz See detail on 49 to 54 lessons on handouts

## 28. epidemiology and methods (5)

The study of where and when diseases occur and how they are transmitted in a population

Descriptive Epidemiology: (recording the data about disease). It includes location and time of cases of disease, gender, health, age of patients etc. (Basically, frequency and distribution of risk factors in population are recorded). Prevalence and incidence of disease are typical examples. Such studies can be retrospective (after they occur) or prospective (before they occur). So, in summary, in descriptive epidemiology, information about an already existing disease is recorded.

Analytical Epidemiology: It relates to determining the cause of the disease. Analysis of data, mode of transmission of diseases and means to prevent diseases also come in this discipline. Such studies can be done with case control methods in which factors that have preceded the disease are determined. A group of people who have the disease is compared with another group of people in the same location who are free of the disease. A Cohort method tracks two groups forward from exposure to outcome. Cohort study compares the experience of a group exposed to the factor with another group not exposed to the factor. An association between exposure and its outcome is determined which makes it easy to see the risk factor involved. In other words, cohort studies begin with a group of people (a cohort) free of disease. The people in the cohort are grouped by whether or not they are exposed to a potential cause of disease. The whole cohort is followed over time to see if the development of new cases of the disease (or other outcome) differs between the groups with and without exposure.

Experimental Epidemiology: A hypothesis relating to a disease is tested in this approach. For example, smoking cause cancer can be tested by designing an experiment in mice in which they are exposed to certain levels of smoke over time and then the outcome is observed. Another example will be testing of a drug for prevention of a disease.

## 29. Magnetostome (5)

Inclusions of iron oxide

Surrounded by invaginations of plasma membrane

Present in G negative bacteria

Act like a magnet. Bacteria can stick to iron containing rocks for nutrition.

Decompose $H_2O_2$ which is toxic for cells.

## 30. Define immunity, difference between innate and adaptive immunity in Detail (10)

Immunity: Ability of the body to protect itself from harmful effects of diseases whereas Susceptibility is the lack of immunity which provides the pathogens easy establishment to cause the diseases Types of Immunity

### Innate (Natural):

Present at birth: One is born with these defenses, so it is present without any exposure to the microbes. That is the reason, it is non-specific.

Rapid to respond: Because it is already there, it interacts with the microbes as soon as they enter the body.

Nonspecific: It means that it does not differentiate if it is an E. coli or a Staph or Salmonella. Body defense system against these entire pathogens acts in a similar fashion. In other words, the same macrophage can phagocytize E. coli, Staph or Salmonella at the same time (if these pathogens are present there).

Does Not Have Memory. Do not increase in responses to the repeated exposure to the same agent. Therefore, it has no memory.

First line of defense: Physical barriers (skin, mucus membrane) provide the first line of defense as they prevent the entry of the organisms by providing a physical barrier which if broken will let the microbes go inside.

Second line of defense: Phagocytes and their secretions provide a second line of barriers for microbes. So, if microbes succeed in breaking the first barrier, macrophages, neutro-phils and such other cells phagocytize these microbes and kill them before these can establish infection in the body.

### Adaptive (Acquired):

It is the third line of defense. It is acquired by experience and specific to an organism.

Specific: It means that antibodies made against E. coli will destroy E. coli only, and will not do any harm to a Staph for example.

Have memory: It means if antibodies have once been made against E. coli, this exposure will be remembered by the body, and if a second encounter is made with the same type of E. coli again, the body would mount a heightened immune response against E. coli because it has memory cells developed in the body which upon second stimulation proliferate quickly and mount a greater defensive response.

Increases with repeated exposure: Because of memory, body's immune response increase with every repeated exposure. It has a limit also!

Slower to respond: For the first time exposure, the body's response is slower and takes a long time compared with the innate immunity which is immediate.

Adaptive Immune Response can further be divided into:

Humoral immune response (antibody response by B cells or lymphocytes) and Cellular immune response (T cells are made which kills microbial harboring cells)

## 31. cell membrane function

a. Selective permeable barrier
b. Passive and Active Transport
c. Respiration in microbes (bacteria)
d. Photosynthesis in microbes
e. Lipid synthesis
f. Cell wall parts are transported by a molecule called bactoprenol which is present in the plasma membrane.
g. PM has many receptors in it with which various ligands can bind and initiate signals for gene expression etc.

## 32. Glycoclyx, its composition and function

It is the outermost layer of the cell wall, viscous and gelatinous in nature that surrounds the cells. It is composed of polysaccharide and polypeptide or both. If glycocalyx is organized and firmly attached to the cell, it is called a capsule. – Plays important role in virulence (degree of pathogenicity) – Can also be a part of the vaccine against the bacteria to which it belongs.

Glycocalyx is also part of biofilms that bacteria make to attach to surfaces. Bacteria secrete extracellular polymeric substance (EPS) Biofilm protects cells within it.

It also facilitates communication amongst cells

If glycocalyx is loosely attached to the cell, it is called slime.

## 33. Differentiate between vertical and horizontal gene transfer and name three horizontal gene transfers

Vertical Gene Transfer:
This is a normal way of transferring genes from parents to offspring. This happens when a cell divides. Each daughter cell receives exactly what its parent cell has.

Horizontal Gene Transfer:
When genes are transferred from cells to cells within the same species, the process is called horizontal gene transfer. This can happen between cells of the same species, or across different species of organisms. Horizontal gene transfer involves a donor and a recipient cell. The recipient cell then incorporates received DNA into its own genome and this genome becomes a recombinant molecule or recombinant DNA. The cell that has this recombinant DNA in it is called a recombinant cell. Three processes are known by which genes can be transferred horizontally from one cell to the other, and they include:

1. Transformation
2. Conjugation
3. Transduction

## 34. three types of passive movement

Diffusion Facilitated Difussion Osmosis

## 35. Economic benefits of fungi

Saccharomyces are used for alcoholic beverages under anaerobic conditions and if incubated aerobically, it is used in bread making because it metabolizes glucose to produce $CO_2$ (some ethanol is also produced but gets evaporated during baking) which expands the dough causing it to rise.

This yeast is also extensively used for molecular biology work and vaccine production.

Vitamin C is obtained from Aspergillus niger.

Cellulase from Trichoderma
Antibiotics from many fungi and anticancer Taxol from Taxomyces are some other examples of useful fungal products.

Entomophaga (kills gypsy moth), hence is used as a biological pest control.

Mushrooms can be cooked and eaten as a source of proteins for humans.

## 36. Pili and its functions

These are hair-like structure composed of pilin, usually one to ten in number. Longer than fimbriae
Used for attachment to:

Host cells

Bacteria

Used for DNA transfer from one bacterium to another during Conjugation (Sex pili)

Also function in twitching Motility

Gliding Motility is also the function of the pili.

## 37. physical nature of media 2

- Solid Media
- Liquid Media

## 38. germination of spore, 5

Exosporium: A thin delicate outermost covering of the spore

Spore coat: 2 nd layer underneath the exosporium. It is thick and composed of several protein layers. Resistant to chemicals

It contains enzymes for germination. Germination of spores into vegetative form occurs when environment becomes favorable for their growth. Cortex: It is the 3rd layer from outside in. It has peptidoglycan in it.

Spore cell wall or core wall: Surrounds the protoplast or spore core Spore core: Contains nucleoid and ribosomes

## 39. Give two advantages of solid media in bactrial culturing.2

Solidification of the medium is done for purifying organisms from each other as solid medium provides surface for individual colonies to grow well separated from each other.

Secondly, the solid media are used to study the colony characteristics.

## 40. Types of microscopy 5

- Dark field microscopy
- Phase contrast microscopy
- Fluorescent microscopy
- Transmission electron microscopy
- Scanning electron microscope

## 41. Writes stages of respiration of glucose 3

Glycolysis: Oxidation of glucose to pyruvic acid

Krebs cycle: Oxidation of acetyl CoA to CO2

Electron Transport Chain: Coenzymes that carry electrons from Krebs cycle or glycolysis are oxidized to create ATP.

## 42. Differentiate between horizontal and vertical gene transfer.

- **Vertical Gene Transfer** o This is a normal way of transferring genes from parents to offspring. This happens when a cell divides. Each daughter cell receives exactly what its parent cell has.

- **Horizontal Gene Transfer**

 When genes are transferred from cells to cells within the same species, the process is called horizontal gene transfer. This can happen between cells of the same species, or across different species of organisms. Horizontal gene transfer involves a donor and a recipient cell. The recipient cell then incorporates received DNA into its own genome and this genome becomes a recombinant molecule or recombinant DNA. The cell that has this recombinant DNA in it is called a recombinant cell. Three processes are known by which genes can be transferred horizontally from one cell to the other, and they include:

1. Transformation 2. Conjugation 3. Transduction

## 43. Define buffers.

- Buffers are used to maintain desired pH.
- Bacteria grown in lab produce acids. o Growth inhibited by acids. o Peptones and amino acids are used as buffers. o Phosphates are also used for buffers.

## 44. Define immunity, innate immunity and adaptive immunity.

- **Immunity: Ability of the body to protect itself from harmful effects of diseases.**
- **Innate (Natural):**

- Present at birth: One is born with these defenses, so it is present without any exposure to the microbes. That is the reason, it is non-specific. o Rapid to respond: Because it is already there, it interacts with the microbes as soon as they enter the body. o Nonspecific: It means that it does not differentiate if it is an E. coli or a Staph or Salmonella. Body defense system against these entire pathogens act in a similar fashion. In other words, the same macrophage can phagocytize E. coli, Staph or Salmonella at the same time (if these pathogens are present there). o Does not increase in response with repeated exposures to the same agent. Therefore, it has no memory.

- **First line of defense:** Physical barriers (skin, mucus membrane) provide the first line of defense as they prevent the entry of the organisms by providing a physical barrier which if broken will let the microbes go inside. o **Second line of defense:** Phagocytic cells and their secretions provide a second line of barriers for microbes. So, if microbes succeed in breaching or breaking the first barrier, macrophages, neutrophils and

such other cells phagocytize these microbes and kill them before these can establish infection in the body.
**Adaptive Immunity:**
The **adaptive immune** system, also known as the acquired **immune** system or, more rarely, as the specific **immune** system, is a subsystem of the overall **immune**system that is composed of highly specialized, systemic cells and processes that eliminate pathogens or prevent their growth.
- **Adaptive (Acquired):** It is the third line of defense. Acquired by experience, specific to an organism.

## 45. What is epidemiology? Describe its tyes.

o The study of where and when diseases occur and how they are transmitted in a population. There are three basic types of investigations relating to epidemiology:

**Descriptive Epidemiology**

**Analytical Epidemiology**
**Experimental Epidemiology**:

## 46. Enlist microbial identification methods.

Classical or Conventional Method
Serological Methods
Nucleic Acid based Methods

## 47. Write economical uses of fungi.

Saccharomyces are used for alcoholic beverages under anaerobic conditions for alcohol production (fermentatively). However, if Saccharomyces are incubated aerobically (for bread making), they metabolize glucose to produce $CO_2$ (some ethanol is also produced but gets evaporated during baking) which expands the dough causing it to rise. This yeast is also extensively used for molecular biology work and vaccine production. Vitamin C is obtained from *Aspergillus niger*; cellulase from Trichoderma, antibiotics from many fungi and anticancer Taxol from Taxomyces are some other examples of useful fungal products. Entomophaga (kills gypsy moth), hence is used as a biological pest control. Mushrooms can be cooked and eaten as a source of proteins for humans.

## 48. What is sporulation?

• Formation of spores takes place within a vegetative cell and the process is called sporulation or sporogenesis. It is initiated when nutrients become unavailable.

## 49. What is flagellum? Write its functions.

**Flagellum:** Long filamentous structure that propels bacteria

## 50. What is meant by gene transfer? Name the types.

**Vertical Gene Transfer**
**Horizontal Gene Transfer**

**1.** Transformation 2. Conjugation 3. Transduction

## 51. Describe classification system proposed by Robert H Whittaker.

In 1969, five kingdom classification was proposed by Robert Whittaker as under:

•Plantae: plants

•Animalia: Animals

•Fungi: Yeasts, molds and mushrooms

•Protista: These are unicellular eukaryotes. Organisms that do not fit into any other category are placed in Protista. They are larger than prokaryotes. They include algae, protozoa, slime molds and water molds.

•Prokaryotae or Monera: Bacteria

## 52. What is taxonomic hierarchy?

All organisms can be grouped into a series of subdivisions that make up the taxonomic hierarchy.

Specie:

A bacterial **species** represents —a monophyletic and genomically coherent cluster of individual organisms that show a high degree of overall similarity with respect to many independent characteristics, and is diagnosable by a discriminative phenotypic property‖

Genus

Order

Family

Group

Class

Phylum

Kingdom

Domain

## 53. What is western blotting?

**Western Blotting:** We can use this technique for the detection of antigen in the serum. Microbial proteins can be separated on SDS-PAGE by electrophoresis and the presence of these proteins can be detected by enzyme-tagged antibodies specific to those proteins. A color band will be seen where the specific protein (antigen) is present on the gel. Please remember that proteins in the gel are first transferred to a paper strip before they could be detected by specific antibodies

## 54. Enlist types of passive transport.

Simple Diffusion

Facilitated Diffusion

Osmosis

## 55. Name types of spiral bacteria.

These are curved shaped bacteria. They are further divided into 3 more subgroups.

**Vibrio:** curved rods

**Spirillum:** Helical but **rigid**
**Spirochete:** Helical but **flexible**
 Spirochetes move by axial filaments which are enclosed by the outer membrane.

## 56. Define passive movement.

**Passive Movement**

 Movement of substances with the concentration gradient. This means that substances will move from higher concentration of a substance to lower concentration. Gradient provides the force for movements of molecules and this happens with no energy expenditures.

## 57. What is function of lysozymes?

A lysosome is a membrane-bound organelle found in nearly all animal cells. They are spherical vesicles that contain hydrolytic enzymes that can break down many kinds of biomolecules. Lysosomes are organelles that contain digestive enzymes. They digest excess or worn out organelles, food particles, and engulfed viruses or bacteria.

## 58. Name types of culture medium on the basis of function.

a. Differential medium
b. Selective medium
c. Enriched medium
d. Basal medium
e. Reducing medium
f. Selective medium
g. General purpose medium

## 59. Write functions of cell wall, flagella and ribosomes.

**Cell wall** gives support and protection against plasmolysis.

**Flagella** help bacteria in movement.

**Ribosomes** are organelles that assist in protein synthesis during translation.

## 60. What are types of microscopes?

a. Light microscope
b. Electron microscope
c. Scanning electron microscope
d. Transmission electron microscope
e. Dark field microscope

f. Bright field microscope
g. Fluorescent microscope

## 61. What do you know about types of techoic acid?

G-positive cell wall contains teichoic acid which may be divided into wall teichoic (remains within the cell wall) acid and lipoteichoic acid (is inserted into the cell membrane).

 Teichoic acid is antigenic in nature and helps the cell wall by providing rigidity.

## 62. Write characteristics of integral proteins.

i. Integral proteins act as channels or carriers in facilitated diffusion. Integral proteins are called transporters or permeases ii. No energy is required during transportation of substances through these integral proteins.

iii. Two kinds of transporters are known: i. Nonspecific transporters 1. Ions ii. specific transporters

1. Change in shape 2. Sugars, vitamins

## 63. What are benefits of solid medium?

Solidification of the medium is done for purifying organisms from each other as solid medium provides surface for individual colonies to grow well separated from each other. A well isolated colony is assumed to be the progeny of a single cell. Secondly, the solid media are used to study the colony characteristics.

## 64. Define refractive index.

Refractive Index: Ability of a medium to bend the light
o Light rays move in a straight line through a single medium o Light rays bend when the pass from one medium to another

## 65. Define medical microbiology.

**Medical microbiology** is a branch of **medical** science concerned with the prevention, diagnosis and treatment of infectious diseases. In addition, this field of science studies various **clinical** applications of microbes for the improvement of health.

## 66. What is resolution?

Resolution is the ability of the lenses to distinguish between two closely lying objects as separate.

$$d = \frac{0.5\lambda}{n \sin \theta}$$

o Light microscope resolving power is 0.2 μm.

## 67. Write three differences between prokaryotes and eukaryotes.

**Prokaryotes**

| |
|---|
| • DNA is not enclosed in a nuclear membrane. |
| • Chromosome: mostly Single, circular |
| • DNA not associated with histones |
| • No membrane enclosed organelles |
| • Cell wall has peptidoglycan, a complex |
| carbohydrate. |
| • Divide by binary fission. |

**Eukaryotes**

| |
|---|
| • DNA is enclosed in a membrane-bound nucleus. |
| • DNA is found in multiple chromosomes. |
| • Chromosomes are linear, thread-like structures. |
| • DNA is associated with histones. |
| • Cell wall, if present, is chemically simple.<br>Cellulose or Chitin |
| • They have membrane enclosed organelles.<br>Mitochondria chloroplasts Endoplasmic reticulum Golgi apparatus Lysosomes etc |

## 68. Define virulence. Virulence:

The extent of pathogenicity

## 69. Write methods for

1. Plate Counts

a. Pour Plate Method:
b. Spread Plate Method:
2. Filtration:
3. The most Probable Number Method:
4. Direct Microscopic Count

## 70. Write steps of germination of spores.

• Spore germinates when it finds a conducive environment for its growth. Germination has 3 phases or stages:

**Activation:** It prepares the spore for germination.

Heat can activate the spore when appropriate moisture and nutrients are present in the envir onment.

It becomes metabolically active.

**Germination:** Spore starts swelling and loosing its coats etc.

**Outgrowth**: New components are made.

## 71. Define culture, media and inoculum.

Microbial growth in the lab is called a **culture**.

A nutrient material that supports the growth of microbes in the lab is called a **culture medium**.

Microbes introduced into a culture medium that initiate growth of organisms are known as **inoculum.**

## 72. Define antiseptics.

Compounds that are used to remove pathogens from living tissue are known as antiseptics.

## 73. Define phosphorylation.

The addition of an inorganic phosphate group to a chemical compound is called phosphorylation. e.g conversion of ADP into ATP in living tissues.

## 74. Explain phases of growth curve.

In a batch culture, organisms go through various phases of growth. If we follow this growth with respect to time, and make a graph of this growth pattern, it will give us what we call as a growth curve. There are four distinct phases of this curve. **1-Lag phase 2- Log phase 3- stationary phase 4- Death phase.**

## 75. What is high frequency of combination?

In some cells that carry $F_+$, $F_+$ (Fertility factor) gets incorporated in the chromosomal DNA which converts $F_+$ cells to high frequency of recombination cells (Hfr cells). When conjugation occurs between an Hfr cell and an $F_-$ cell, the Hfr cell's chromosome (with its integrated F factor) replicates, and a parental strand of the chromosome is transferred to the recipient cell. Since this transfer starts from the middle of F factor gene, and most of the time, this transfer is not complete; the whole chromosome of Hfr cell is not transferred. However, some genes can be transferred during this process. When these genes become integrated into the genomic DNA of the recipient cells, the recipient cells acquires new versions of genes that were not part of its genome previously. It may be noted that $F_-$ cells remains $F_-$ negative cell because F factor is not transferred completely. This whole process is illustrated in the accompanying diagram.These newly acquired genes can be mapped easily with respect to the time they get transferred to $F_-$ cells.

## 76. Differentiate between clone and strain?

**Clone**: Population of cells derived from a single cell that are genetically identical.

**Strain:** A genetic variant of a clone is called a strain.

## 77. What is zoonosis?

**Zoonosis:** Primarily an animal infection, but can be transmitted to humans. It can assume epidemic proportions in humans and is difficult to control or eradicate.

## 78. What are interferons? Give their function.

**Interferons** are antiviral proteins, produced by macrophages and lymphocytes in response to viral infections. Fibroblasts can also secrete it. These proteins interfere with viral replication. Thereare two types of interferons:

**Species specific:** This means that interferon produced by humans cannot be used in dogs or cats or

vice versa.

**Not viral specific:** This means that interferons produced in response to virus A can also be effective against virus B.

**Long Questions 5 & 10 marks.**

## 79. Explain characteristics of aerophiles.

## 80. Describe conjugation, transduction and transformation. Horizontal gene 10

**transfer:**

When genes are transferred from cells to cells within the same species, the process is called horizontal gene transfer. This can happen between cells of the same species, or across different species of organisms. Horizontal gene transfer involves a donor and a recipient cell. The recipient cell then incorporates received DNA into its own genome and this genome becomes a recombinant molecule or recombinant DNA. The cell that has this recombinant DNA in it is called a recombinant cell. Three processes are known by which genes can be transferred horizontally from one cell to the other, and they include:

1. Transformation 2. Conjugation 3. Transduction

**1. Transformation** o Naked DNA in solution is transferred from one bacterium to another during this process. Frederick Griffith observed this process in 1928 when he was working with *Strep pneumonia* in his lab. This bacterium has two strains: one is capsulated and is called a Smooth strain (which is pathogenic) and the other is Rough strain (non-pathogenic). o When live encapsulated bacteria are injected into a mouse, the mouse gets killed by the microbe. When rough strain is injected, the mouse is not killed. Moreover, when heat-killed encapsulated bacteria are injected, it also does not kill the mouse. However, when heat-killed strain is mixed with rough live strain, and a mouse is injected with this mixture, the mouse dies. If you isolate the bacterium from these dead mice, the bacteria resemble with encapsulated strain of *Strep pneumonia.* The interpretation of this experiment led to the mechanism that rough live bacteria take up DNA from the heat-killed encapsulated strain and becomes pathogenic by acquiring the ability to form a capsule. Very few bacteria have this property of accepting foreign DNA and most of it is degraded (only small fragments of DNA get incorporated in the genome of the recipient. The cells that can take up DNA are called competent cells. We can create such microbes in the lab that can accept a given DNA during gene transfers that we undertake in our DNA work. The cells that take up the DNA and incorporate this in their genome successfully are called transformed cells.

2. **Conjugation** o Genes transfer process can be mediated by plasmids. Plasmids are extra-chromosomal circular DNA fragments that replicate independently of bacterial chromosome. In other words, a given bacterial cell can have multiple copies of plasmid with the same cell. Plasmids often have genes that are not strictly needed by the microbe for its growth. However, the genes on the plasmids provide some advantages to the bacterium. For example, antibiotic resistant genes offer a mechanism for the bacterium to resist the presence of that antibiotic. In other words, bacterium that has a plasmid with an antibiotic gene on it can still multiply in the presence of that antibiotic.

- Conjugation requires direct cell to cell contact and cells have to be opposite mating types. Plasmids in G –ive cells have genes that code for sex pili because pili are needed to make a bridge between the two mating type cells. Since there is no pilus present in G +ve bacteria, they secrete sticky surface molecules instead which create contact between the two cells. The pilus along with two mating cells is shown in the diagram below: o These plasmids are transmissible from one bacterium to another during conjugation.

- Plasmid is replicated and a single copy of the plasmid is transferred to the recipient bacterium through the pilus.
- A complementary copy is then made in both the cells to make the DNA of the plasmid double stranded molecule.
- In E. coli, fertility factor (F factor) plasmid was the first plasmid observed to be transferred from one organism to the other, hence those bacteria that have this plasmid are called $F_+$ cells to differentiate from those that do not have one ($F^-$). However, once $F^-$ cells acquire $F_+$ plasmid, they also become $F_+$.

**3. Transduction** o Transferring of a gene from a bacterium to another bacterium via a virus is called transduction. o When a bacteriophage infects a bacterium such as E. coli, it replicates inside E. coli and also produces a protein coat (which is called a capsid) in which viral DNA is packed before the virus is released. What happens is that when phages cause lysis of infected cells, bacterial chromosomal DNA also gets fragmented and such fragments can sometimes be packed into the viral capsids much like the viral DNA. Now, the capsid carrying the bacterial gene or genes in that fragment can infect another E. coli and can inject its DNA (bacterial DNA fragment) into it resulting in the transfer of genes from one bacterium to another.

## 85. Explain methods of microbial control in detail.

These methods can be broadly classified into four groups: Physical, Mechanical, Chemical and Biological

## Physical methods of microbial control

**Heat:** It is the cheapest sources of all and easily available to control microbial growth. There are a few heat related concepts that we must appreciate. Heat denatures enzymes.

 **Thermal Death Point:** It is the lowest temperature at which all cells in a culture are killed in 10 min.

It will be a specific temperature for a specific species of organism.

 **Thermal Death Time:** Time (minimum) during which all cells in a culture are killed at a given temperature. This will vary from temperature to temperature for the same organism. Obviously, higher temperatures will take less time to kill the organisms than low temperatures.

 Methods that use heat can fu rther be classified into **dry and moist** heat methods.

Heat kills by oxidation

Form of dry heat include:

1. Dry heat (hot-air oven),
2. Flaming (Platinum loop sterilization in the flame of Bunsen burner),
3. Incineration (burning to ashes).

 Similarly, moist heat can also be grouped into autoclaving (15psi), boiling at 100 $^{o}$C, and Tyndallization (100$^{o}$C for 3 consecutive days to sterilize sugar solution that can be degraded by autoclaving) and pasteurization (usually at low temperature than 100$^{o}$C).

**Pasteurization:** This technique typically employs low heat for killing pathogenic and food spoiling bacteria in milk. However, it does not kill thermoduric organisms. Thermoduric organisms are not pathogenic though. Products other than milk, such as ice cream, yogurt, and beer, all have their own pasteurization times and temperatures, which often differ considerably. Dairy industry tests phosphatase to determine if pasteurization of dairy product has occurred effectively because phosphatase present in raw milk gets inactivated with effective pasteurization temperatures. The following three equivalent heat treatments can be given to achieve pasteurization of milk:

- 63°C for 30 min
- o High-temperature short-time: 72°C for 15 sec
- Ultra-high-temperature: 140°C for 4 sec

**Other Physical Methods**

Low temperature inhibits microbial growth by slowing down chemical reactions.

**Refrigeration:** Materials stored at 2-8°C can last for a day or two.

**Deep-freezing:** Long term storage of materials is possible at minus temperatures in the deep freezers.

**Lyophilization**: Freeze drying is another method for long term storage of food and other materials.

**High pressure** deshapes and denatures proteins: This technique is used for preserving juices and wines.
**Desiccation** prevents metabolism: This method is very old and still in use today. Food stored in high concentrations of salt or sugar solution can keep for long.

**Osmotic pressure** causes plasmolysis. High salt concentrations for example can take the water content of the cells out leaving them starved of water.

**Filtration:** It is also a physical method. It can be used to filter air (using high efficiency particulate air filter) or liquid medium using membrane filters.

**Radiation:** o Ionizing and non-ionizing radiation o Ionizing Radiation: High energy waves that destroys microbes.

- Ionization radiations include gamma rays, X rays, or high-energy electron beams. The ionizing radiation possesses a wavelength shorter than that of nonionizing radiation, less than about 1 nm. All these short wavelengths cause ionization of water generating high reactive hydroxyl radicals which are damaging to the cells as they bind to DNA and results in mutations. Medical plastic supplies, medicines and meat products can be sterilized by radiation.

 Non -ionizing Radiation: wavelengths longer than 1nm fall into non-ionizing radiation. The best example is UV light. UV light causes mutations in the DNA by dimerization of thymidine bases. These *thymine dimers* inhibit correct replication of the DNA during reproduction of the cell. The most useful wavelength is 260 nm. This wavelength is specifically absorbed by DNA. UV radiation is used to control organisms in the air mostly. However, surfaces are also sterilized by UV radiation. UV light cannot penetrate deep into the fluids, so only surface bacteria are killed.

**ETHYLENE OXIDE FOR MICROBIAL CONTROL**

o **Ethylene Oxide:** Many heat-sensitive items such as disposable plastic, petri dishes and syringes, heart-lung machine components, sutures, and catheters are sterilized by ethylene oxide. Ethylene oxide is a strong alkylating agent that kills by reacting with functional groups of DNA and proteins to block replication and enzymatic activity. It rapidly penetrates packing materials, even plastic wraps. EtO is explosive, supplied in a 10% to 20% concentration mixed with either $CO_2$ or dichlorodifluoromethane. A clean object can be sterilized if treated for 5 to 8 hours at 38°C or 3 to 4 hours at 54°C when the relative humidity is maintained at 40 to 50% and the EtO concentration at 700 mg/L.

**CHEMICAL METHODS FOR MICROBIAL CONTROL**

Chemicals are used to control microbial growth. Factors that influence the efficacy of a chemical disinfectant include the concentration of the disinfectant, presence of organic matter in the environment (matrix) where the disinfectant is being used, pH of the environment (disinfectants are more effective at acidic pH), and time of exposure to the disinfectant.

## 82. How infectious diseases are transmitted? Explain in detail.

1) how infectious disease can be transmitted explain it briefly transformission occur to contact ,vehicle n vector.(10)

**Direct Transmission:** person to person physical contact is a direct contact. Influenza spread is a typical example.

**Indirect Transmission:** A nonliving object is involved. Objects in the use of a patient such as handkerchief, utensils, pillow and bedding are called fomites. These can transmit organisms to susceptible individuals.

**Droplet Transmission:** Mucus droplets are created when you sneeze for example.

These droplets carry organisms that may infect another individual who comes in contact with the droplets. One sneeze may produce 20,000 droplets. Influenza can be spread by this route.

**Vehicle Transmission:** Transmission of disease agents by a medium, such as water, food, or air. Other media include blood and other body fluids, drugs, and intravenous fluids.

**Vectors** o A **vector** is a vehicle (animate or inanimate) that carries a pathogen from one host to another. o Arthropods are vectors for many diseases. There are two ways these arthropods can transmit organisms to susceptible hosts:

**Mechanical Transmission**: In this type of transmission, organisms stick to the body parts of insects and get transmitted passively from one location to another.
**Biological Transmission**: Microbes multiply in the insect and is transmitted actively by insect bites from one individual to another.

**Nosocomial Infections** o Hospital acquired infections are called nosocomial infections.

## 83. Explain salient features of viruses, virions and viroids.

**VIROIDS**

These are infectious agents that consist only of RNA and found in plants only. No capsids, no envelopes, just RNA. They are covalently closed circular ssRNAs about 250 nucleotides long. The circular RNA normally exists as a rodlike shape due to intrastrand base pairing, which forms doublestranded regions with single-stranded loops. They do not encode any gene products, so they are replicated by cellular DNA dependent RNA polymerase. A plant infected with such viroids may not show any signs of illness. However, in other plants, they may cause severe disease. They cause the disease by RNA silencing process.

**VIRUSOIDS** o They are similar to viroids; however, they encode one or two gene products and need a helper virus to infect the host cells. The helper virus supplies gene products and other materials needed by the virusoid for completion of its replication cycle. Hepatitis D virusoid is a typical example which uses Hepatitis B virus as a helper.

**PRIONS**

o These are proteinaceous infectious particles that cause neurodegenerative diseases such as scrapie in sheep, bovine spongiform encephlopthy, and Kuru in humans. These are abnormal form of cellular proteins. How these proteins accumulate in the cell is not clear, although, some genetic components are known to be involved.

## 84. How fungi can be identified by biochemical testing?

Yeast if grown on solid medium grows like bacteria. In other words, colonies of yeast look like bacterial colonies but are bigger in size.

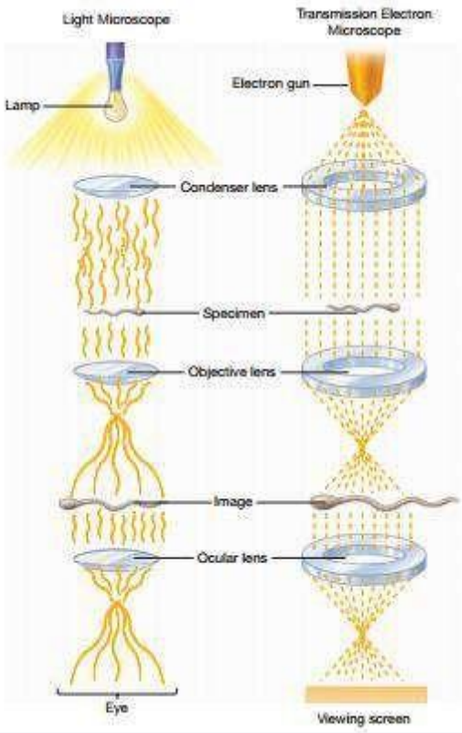
 Yeasts can grow as facultative anaerobes. Saccharomyces if given oxygen to grow, it converts carbohydrates aerobically and produces $CO_2$ and water; however, if oxygen is denied, carbohydrates get converted fermentatively into ethanol and $CO_2$.

## 85. IDENTIFICATION OF SELECTED FUNGI

 Filamentous fungi are mainly identified by morphology, and not by biochemical testing. Fungi are cultured using a slide (slide culture) on top of Sabouraud's agar. Spores are placed on top of the medium or embedded in the

medium. A block of the medium can be used to grow fungi or a glass coverslip can be used to grow spores into hyphae and mycelium and spore arrangements can be studied under the microscope by staining with lactophenol cotton blue. Slides stained with lactophenol cotton blue can also be seen under the microscope.Stained slides can show specific arrangements of spores, their sizes and other morphological features as seen in the figure for Aspergillus.

## 86. Label the diagram of Electron microscope with numbers mentioned.




## 87. Write functions of cell membrane of bacteria.

- Selective permeable barrier
- Passive and Active Transport
- Respiration in microbes (bacteria)
- Photosynthesis in microbes
- Lipid synthesis
- Cell wall parts are transported by a molecule called bactoprenol which is present in the plasma membrane.
- PM has many receptors in it with which various ligands can bind and initiate signals for gene expression etc.

## 88. Write names of 5 types of bacteria with respect to temperature. 

Organisms are basically classified into three groups based on the temp requirements:

1. **Psychrophiles:** These are further divided into strict psychrophiles and psychrotrophs:

- **Psychrotrophs:** Cold loving: 15 $^0$C
- **Psychrotrophs:** Optimum temp is 20-30 $^0$C. Food spoilage bacteria that can spoil food during refrigeration.

2. **Mesophiles:** 25 – 40 $^0$C: These are the ones that cause diseases in animals and humans.

o Moderate temp loving organisms o Optimum: 37 $^0$C as this is the body temperature of humans and animals.

3. **Thermophiles:** 50 – 60 $^0$C

- Heat loving o Important in organic compost piles o These are further divided into hyperthermophiles that grow optimally at 80 $^0$C. They live in hot springs. Every group of these organisms has a:
- Minimum growth temp o Optimum growth temp. o Maximum growth temp.

## 89. Write characteristics of metachromatic granules.

**Metachromatic Granules**

• Also called volutin, they stain red with certain dyes such as methylene blue. That is why they are called metachromatic (stain in different color as methylene blue gives blue color but the color on these granules is red). • Large inclusions • These inclusions contain inorganic phosphates. Inorganic phosphates are used up in ATP synthesis. • Characteristics of *Corynebacterium diphtheria: This bacterium can be identified by the presence of these granules in it.*

## 90. Explain five kingdom classification systems.

**In 1969, five kingdom classification was proposed by Robert Whittaker as under:**
**•Plantae: plants**

**•Animalia: Animals**

**•Fungi: Yeasts, molds and mushrooms**

**•Protista: These are unicellular eukaryotes. Organisms that do not fit into any other category are placed in Protista. They are larger than prokaryotes. They include algae, protozoa, slime molds and water molds.**

**Prokaryotae or Monera: Bacteria and Archea**

## 91. Explain layers of endospore.

**Endospore and its various parts/structures**

• **Exosporium:** A thin delicate outermost covering of the spore

• **Spore coat:** 2nd layer underneath the exosporium. It is thick and composed of several protein layers.

Resistant to chemicals

It contains enzymes for germination. Germination of spores into vegetative form occurs when environment becomes favorable for their growth.

• **Cortex:** It is the 3rd layer from outside in. It has peptidoglycan in it.

• **Spore cell wall or core wall:** Surrounds the protoplast or spore core

• **Spore core:** Contains nucleoid and ribosomes

## 92. Enlist types of bacteria on the basis of oxygen.

Based on the use and concentration of Oxygen, microbes can be classified into five groups:

**Obligate Aerobes**: Oxygen must be present for their growth. These organisms do not grow if $O_2$ is not present in the medium. If you test these organisms in a tube containing nutrient agar, the growth will remain confined to the surface only (see the diagram for the concept). Mycobacterium tuberculosis is an example of such an organism.

**Facultative Anaerobes:** These organisms prefer aerobic respiration; however, if oxygen is not available, they can use anaerobic respiration or fermentative modes for generating ATPs. These organisms will be present throughout the tube, however, more growth will be seen on the top (close to the surface) because these organisms prefer using oxygen if it is available.

**Obligate Anaerobes:** Although, these organisms do not use oxygen, they cannot grow in the presence of oxygen. The reason is that oxygen creates toxic compounds which can kill these cells. Normally, oxygen toxic compounds are eliminated by the cells, however, strict anaerobe do not have a system to get rid of these toxic compounds when they are made in the cells. So using or not using oxygen is one thing and growing in the presence of oxygen (and not utilizing it) is another. The growth will remain confined to the bottom which has little oxygen. o **Aerotolerant Aerobes:** These organisms do not use oxygen and also are not bothered by the presence of oxygen. In other words, they have a better system to dispose of toxic oxygen compounds. They will grow evenly throughout the tube of medium

## 93. Name five types of microscopy . 5 marks

**Dark field microscopy**

- This microscope makes use of a special condenser that provides illumination from the sides only as depicted in the diagram below:
- This special arrangement of the condenser throws light rays at an oblique angle so that if the specimen is not present on the slide, there will be no image formed. However, if the the organisms are present on the glass slide, those organisms will reflect the light into the objective lens and we will see the cells as bright objects in a dark background much like we see stars in the sky at night.
- The beauty of this microscope is that one does not have to stain the organisms to see them under the microscope.

**Phase contrast microscopy**

This instrument is built on the concept of phases of light waves. The light ray that passes through an organism gets retarded about ¼ of its wavelengths than the light that passes outside the organism (through the glass slide only). To further retard the retarded light ray, a phase plate is inserted into the body of the microscope (above the objective lens) which is thicker in the center than the ends. This arrangement causes the retarded light to further slow down about ¼ of its wavelength, which when recombines with the un-retarded light creates a contrast which makes the cells/organisms visible.

- This method for visualizing organisms also does not involve staining.

**Fluorescent microscopy** o This microscope is equipped with UV light that provides the source of light for illumination. However, this light source does not pass through the slide, it instead falls over the slide (having specimen) and creates fluorescence.

- Fluorochromes are substances that absorb short wavelength of light and emit longer wavelength (visible light spectrum)
- Fluorochromes are attached to antibodies that are specific to organisms. So, if an organism is present in the specimen, we can use specific antibodies to determine the presence of those organisms by use of fluorochrome tagged antibodies. See the accompanying diagrams for details. Look for UV lamp (Mercury Lamp).

**Transmission electron microscope**

- Electron microscope (EM) makes use of electron beam as an alternative to light rays. Since electron beam has much shorter wavelength, resolution of EM is 0.1nm compared with light microscope which is 200nm.
- Second difference of EM with light microscope is that focusing of electron beam is done not by glass lenses but by electromagnets that acts like lenses.
- The third difference in EM is that the image of the specimen is formed on a fluorescent screen like a TV screen.

**Scanning electron microscope**
This is a modified version of electron microscope. Electron beam is allowed to fall on to the specimen and the image is formed from the reflected electrons that are collected and assembled into an image by the detector. It gives a three dimensional picture.

## 94. Explain ELISA.

There are some interactions (Antigens and Antibodies) that are not directly evident. Interactions may have taken place, but there is no visible direct clue if the interaction has taken place or not. For such interactions, we use indirect methods. Say, there is big body of smoke visible to you from a kilometer ahead of you. What is your guess? Yes, you guessed it right. There is a big fire there. Did you see the fire? Probably not. You guessed on the basis of smoke that there is a fire there. This is called indirect way of interpretations. A beautiful example of such a test is ELISA where we do not know if the interaction has taken place or not. However, we can see it indirectly. We will see that in the following paragraph which discusses ELISA.

**Enzyme-Linked Immunosorbent Assay:** This test is done by coating the ELISA plastic plates either with the antigen or the antibodies. Suppose we have Brucella antigen coated ELISA plates that can detect antibodies from the serum of a Brucella infected animal. You take the serum and put into the wells of ELISA plate. You allow time for antigen and antibody to interact and then wash these wells with some buffer to remove unbound antibodies. These bound antibodies are not visible at this step. So, how do we see this interaction? Well, we use another antibody that has been made against the antibody bound to the antigen. This special antibody has been tagged with an enzyme. After allowing time for interaction of this 2nd antibody with the 1st antibody, 2nd antibody is also given a washing to remove unbound antibody molecules. Again, we cannot say if interaction has taken place or not. Here comes an indirect way of detecting this interaction. Remember that the 2nd antibody has a molecule of an enzyme attached to it. Now, if we provide substrate to the enzyme in the wells, enzyme will convert the substrate into a color compound visible to the eyes. The color change can be measured by a spectrophotometer as well which gives a quantitative data

## 95. Explain types of algae.

- **Phaeophyta (Kelp):** Commonly known as brown algae, brownish in color, contains cellulose and alginic acid cell walls, are multicellular and contain chlorophyll *a* and *c*, xanthophylls. These store carbohydrates and are used for algin which is a thickener in ice creams. Can grow upto 20 cm in one day.
- **Rhodophyta:** Red Alage, these are reddish in color and contain cellulose in their cell walls. Most are multicellular and contain chlorophyll a and d, and phycobiliproteins. They store glucose polymer and used for agar and carrageenan (both are used as thickeners of foods).
- **Chlorophyta:** Green Algae: have all the features of red algae except that chlorophyll is of a and b types only. These algae are believed to have given rise to terrestrial plants.
- **Bacillariophyta:** Cell wall is composed of pectin and silica. They are unicellular and possess chlorophyll a and c, and carotene xanthophylls. These algae store oil and produce domoic acid with which human can be intoxicated by eating contaminated mussels.

Diatoms: Come in beautiful colors and shapes.

- **Dinoflagellata:** These are unicellular algae collectively called as plankton (free floating organisms). These cells have cellulose in their cytoplasmic membrane which gives the membrane rigidity. They have chlorophyll *a* and *c*, and carotene. These algae store starch in them. Dinoflagellates in the genus Alexandrium produce neurotoxins (called saxitoxins) which can cause paralytic shellfish poisoning in humans when they consume contaminated mussels and clams. Blooms of Alexndrium gives ocean a deep red color commonly known as red tide.

- **Oomycota (Water molds):** They are basically decomposers of dead matter. Superficially, these resemble with fungi; however, these are motile with flagella and their cell wall is composed of cellulose not chitin. Their spores are called zoospores.
- Terrestrial members of water molds are also plant parasite. *Phytophthora infestans* was responsible for Irish potato blight during mid-1800s.

  *P. ramorum* causes sudden oak death

## 1. Define and type of algae. 5 marks

### Define :

The definition of algae is a single or multi-cellular organism that has no roots, stems or leaves and is often found in water

These are simple eukaryotic cells. Some are unicellular, others are multicellular (thallus); however, they lack tissues such as roots, stem and leaves typically seen in plants. Algae absorb nutrients from water through their surfaces and are mostly photoautotrophs; however, a few are chemoheterotrophs. They are responsible for 80% atmospheric O2 on the face of the earth.

### Types of algae:

1. **Phaeophyta (Kelp):** Commonly known as brown algae, brownish in color, contains cellulose and alginic acid cell walls, are multicellular and contain chlorophyll *a* and *c*, xanthophylls. These store carbohydrates and are used for algin which is a thickener in ice creams. Can grow upto 20 cm in one day.

2. **Rhodophyta:** Red Alage, these are reddish in color and contain cellulose in their cell walls. Most are multicellular and contain chlorophyll a and d, and phycobiliproteins. They store glucose polymer and used for agar and carrageenan (both are used as thickeners of foods).

3. **Chlorophyta:** Green Algae: have all the features of red algae except that chlorophyll is of a and b types only. These algae are believed to have given rise to terrestrial plants.

4. **Bacillariophyta:** Cell wall is composed of pectin and silica. They are unicellular and possess chlorophyll a and c, and carotene xanthophylls. These algae store oil and produce domoic acid with which human can be intoxicated by eating contaminated mussels.
5. **Diatoms**: Come in beautiful colors and shapes.

6. **Dinoflagellata:** These are unicellular algae collectively called as plankton (free floating organisms). These cells have cellulose in their cytoplasmic membrane which gives the membrane rigidity. They have chlorophyll *a* and *c*, and carotene. These algae store starch in them. Dinoflagellates in the genus Alexandrium produce neurotoxins (called saxitoxins) which can cause paralytic shellfish poisoning in humans when they consume contaminated mussels and clams. Blooms of Alexndrium gives ocean a deep red color commonly known as red tide.

7. **Oomycota (Water molds):** They are basically decomposers of dead matter. Superficially, these resemble with fungi; however, these are motile with flagella and their cell wall is composed of cellulose not chitin. Their spores are called zoospores. Terrestrial members of water molds are also plant parasite. *Phytophthora infestans* was responsible for Irish potato blight during mid-1800s. *P. ramorum* causes sudden oak death

## 2. HYPERSENSITIVITY AND ITS TYPE 5 marks

### What is hypersensitivity?

It is a normal Immune response but in a damaging way. In other words, immune system is acting in a way for which it has made to. So when an antigen gets into the body, immune system recognizes it as foreign and start making antibodies against the antigen. However, the consequences are bad and damaging to the body. It may be called an abnormal antigenic response. Allergy is another name for hypersensitivity. Those antigens that act in a bad way are called allergens **Types of hypersensitivity:**

Hypersensitivities can be grouped into 4 types based on the types of antibodies or cells involved.

1. **Type I (Anaphylactic):** Also called anaphylaxis or immediate hypersensitivity. This is IgE mediated.
2. **Type II (Cytotoxic):** IgG and IgM mediated as these antibodies attach to cells and destroy them by activating complement.
3. **Type III (Immune complex mediated):** Immune complexes are formed by IgM or IgG antibodies and these complexes lodge in the capillary beds and cause damage by complement activation.

4. **Type IV (delayed type cell-mediated):** CD4+ helper cells along with macrophages are involved.

**3. Define antigen and epitope**

1. **Antigen**: A foreign substance that provokes immunity is called an antigen.
   The definition of antigen is a harmful substance which enters the body which causes the body to make antibodies as a response to fight off disease.
   More specifically called as an immunogen. So immunogen and antigen although slightly different but used interchangeably. o Proteins are best antigen, though glycoproteins and lipoproteins can also act as antigens. Carbohydrates and lipid alone are poor antigens. o Antigens are foreign to the body. o **Degradability**: Inert molecules are not antigenic.
2. **Epitopes**: Specific regions on an antigen. In other words, any antigen has multiple regions each one of those act as a small antigen. These are called epitopes.These epitopes are also called as antigenic determinants. o Most antigens have MW >10,000 D. Molecules that are less than 10,000 D are generally poor antigen.

   A small molecular region of an antigen that binds to a particular antibody or antigen receptor on a T cell; an antigenic determinant. A single antigen can have multiple epitopes

## 4. Describe two transported system in the blood and why it is necessary 10 marks

**Cardiovascular** and **lymphatics** are the two transport systems of the body by which cells of the immune system patrol the whole body.

Cardiovascular system consists of the heart and associated blood vessels (arteries and veins) in which blood circulates

lymphatics are channels that start from tissue spaces and collect tissue fluid (called lymph) from tissue spaces and bring them back to the heart. Along these lymphatic channels, there are strategically placed lymph nodes which filter lymph for microbes much like blood is filtered in the spleen. Lymph nodes contain aggregations of B and T cells along with macrophages and dendritic cells which interact with each other inducing adaptive immune responses.

**Why Cardiovascular** and **lymphatics are needed?**

Well, in unicellular organisms, nutrients can be absorbed directly from the environment in which a unicellular organism is living. However, a multicellular organism does not have this easy access to the nutrients. So, nutrients must surround the cells in a multicellular organism. These nutrients are provided by cardiovascular system which pumps blood to all organs. Remember that only fluid part of blood leaves blood vessels. RBCs never leave blood vessels. Sometimes proteins also leak from these blood vessels; however, once they leak from the blood vessels, they never can enter back into blood vessels because of their bigger size. If proteins remain in the tissue spaces, they exert osmotic pressure which attracts water into these space resulting in edema. However, nature has provided another mechanism in the form of lymphatic channels which start from tissue spaces and can drain tissue fluid and proteins back to the heart. This is the role of blood vessels and lympahtics that they ensure

circulation of nutrients throughout the body without causing any edema or abnormality. B cells and T cells also keep circulating between these two channels (cardiovascular and lymphatics). Unfortunately, microbes can also use these two systems to spread themselves in the body. That is the reason, spleen and lymph nodes are placed in these channels by the nature to deal with these microbes that find their ways into blood and lymph. Lymphatic System consists of the following: o Bone marrow o Lymph nodes o Spleen o Tonsils o Thymus o Mucosa associated o Bursa of Fabricius in birds

## 5. Disk diffusion method

A standardized inoculum of the organisms is spread on agar plate. Filter paper disks soaked with anti bacterials are then placed on the lawn of the organism. Plates are incubated at 37oC and results are recorded. Drugs diffuse from the paper disks radially and if the organisms are sensitive to the drug, a clear area with no growth around the disks develops. The size of the zone directly relates to the degree of sensitivity of the drug. This zone is compared to a standard table for recording the results of sensitivity test. Results are reported as sensitive or resistant or intermediate (between the two extremes).

## 7. Define immunity and difference between innate immunity and adaptive immunity. 10 marks

**What is immunity?**

**Immunity**: Ability of the body to protect itself from harmful effects of diseases.

**Susceptibility**: lack of immunity which leads to easy establishment of disease.

**Types of immunity**

### Innate (Natural):

**Present at birth:** One is born with these defenses, so it is present without any exposure to the microbes. That is the reason, it is non-specific.

**Rapid to respond:** Because it is already there, it interacts with the microbes as soon as they enter the body.

**Nonspecific:** It means that it does not differentiate if it is an E. coli or a Staph or Salmonella. Body defense system against these entire pathogens act in a similar fashion. In other words, the same macrophage can phagocytize E. coli, Staph or Salmonella at the same time (if these pathogens are present there).

Does not increase in response with repeated exposures to the same agent. Therefore, it has no memory.

**First line of defense**: Physical barriers (skin, mucus membrane) provide the first line of defense as they prevent the entry of the organisms by providing a physical barrier which if broken will let the microbes go inside.

**Second line of defense:** Phagocytic cells and their secretions provide a second line of barriers for microbes. So, if microbes succeed in breaching or breaking the first barrier, macrophages, neutrophils and such other cells phagocytize these microbes and kill them before these can establish infection in the body.

**Adaptive Immunity**: This will be studied in detail in a separate lecture soon.

**Adaptive (Acquired):** It is the third line of defense. Acquired by experience, specific to an organism.

**Specific**: It means that antibodies made against E. coli will destroy E. coli only, and will not do any harm to a Staph for example.

**Have memory**: It means if antibodies have once been made against E. coli, this exposure will be remembered by the body, and if a second encounter is made with the same type of E. coli again, the body would mount a heightened immune response against E. coli because it has memory cells developed in the body which upon second stimulation proliferate quickly and mount a greater defensive response.

O Because of memory, body's immune response increase with every repeated exposure. It has a limit also!

**Slower to respond**: For the first time exposure, the body's response is slower and takes a long time compared with the innate immunity which is immediate.

**Adaptive Immune Response can further be divided into:**

**Humoral immune response** (antibody response by B cells or lymphocytes)

**Cellular immune response** (T cells are made which kills microbial harboring cells).

## 8. Agglutination and Neutralization 2 marks

**Agglutination**: Antibodies can agglutinate antigens which are then cleared as a group by macrophages

**Neutralization**: Toxins bind with antibodies and they become incapable of binding to their target receptors thus the toxins become harmless.

## 9. What is plaque 2 marks

Plaques are equivalent to bacterial colonies. Basically a plaque is an area where the virus infection has lysed the cells. These plaques are formed on cultured cells in a culture dish. A monolayer of the cells is infected with the virus to be enumerated and then the culture is overlaid with the semisolid agar so that infected and lysed cells do not move away from the site in the dish. Plaques can be counted easily much like bacterial colonies

## 10. Difference between capsule and slime

**Capsule**: If glycocalyx is organized and firmly attached to the cell, it is called a capsule.

– Plays important role in virulence (degree of pathogenicity)

– Can also be a part of the vaccine against the bacteria to which it belongs.

– Can be demonstrated by negative staining technique.

**Slime**: If glycocalyx is loosely attached to the cell, it is called slime.

## 11. PCR method 3 marks

PCR goes through 3 step cycles for about 30 to 35 times. Each cycle consists of a denaturing stage (about 95oC), primer annealing (50 to 56oC) and extending stage (72oC). A primer is a fragment of DNA which is specific to the signature sequence. Two primers span the signature sequence actually. At the end of 30 or so such cycles, enough DNA accumulates that one can visualize on an agarose gel easily.

## 12. Type of T cell

T helper cells

TH1 (CD4+)

TH2 (CD4+)

T cytotoxic cells (CD8+)

## 13. Inhibits growth cell wall drug name

Penicillins and cephalosporins are the major antibiotics that **inhibit** bacterial **cell wall** synthesis. They are called beta-lactams because of the unusual 4-member ring that is common to all their members.

## 14. Microbiota factor and distribution composition

**Nutrients**: Availability of various nutrients determines the type of organisms present in a system. Similarly, physical and chemical factors also influence the type of microbiota. Other factors include pH, O2, and CO2. Host defenses, level of stress and age of an individual also matters for allowing microbiota of an individual.

## 15. Capsule function 2 marks

The capsule impairs phagocytosis and help the organisms evade the immune system; however, antibodies can help cells of the immune system in phagocytosis of such encapsulated organisms. Only a few bacteria produce capsule which include Strep pneumonia, Klbesiella pneumonia.

## 16. Viral replication stages

Viral multiplication goes through various stages in the cell. These include viral attachment, entry into the cell by pinocytosis or fusion with the plasma membrane, uncoating of the virus, biosynthesis of capsids and nucleic acids, maturation (assembly of the virus) and final release of the virus from the cell.

## 17. Economic benefits of fungi 3 marks

Saccharomyces are used for alcoholic beverages under anaerobic conditions for alcohol production (fermentatively). However, if Saccharomyces are incubated aerobically (for bread making), they metabolize glucose to produce CO2 (some ethanol is also produced but gets evaporated during baking) which expands the dough causing it to rise. This yeast is also extensively used for molecular biology work and vaccine production. Vitamin C is obtained from Aspergillus niger; cellulase from Trichoderma, antibiotics from many fungi and anticancer Taxol from Taxomyces are some other examples of useful fungal products. Entomophaga (kills gypsy moth), hence is used as a biological pest control. Mushrooms can be cooked and eaten as a source of proteins for humans.

## 18. Types of symbiosis 3 marks

Interaction of microbiota with the host leads to a relationship called symbiosis. There are a few outcomes of this symbiosis which we must know. The two participants of this relationship are microbiota and the host. At least one of these participants is benefited and sometimes both can be benefited from this mutual relationship. Different names or terms have been given to these mutual relationships as under:

**Mutualism**: both are benefited

**Parasitism**: one is harmed.

Commensalism: One is benefited, other is unharmed.

## 19. Inflammation and write its consequence 5 marks

Damage to body tissues triggers a local defensive response which is called inflammation. It causes redness, pain, swelling, heat, and loss of function of that organ

**During inflammation, various mediators of inflammation are released, some of which include:**

Histamine

**Kinins**: Almost all cells.

Prostaglandins by damaged cells

Leukotrienes by mast cells

Cytokines are released (TNF-alpha), and they cause release of acute phase proteins from the liver

## 20. Cause and diagnose of anaphylaxis

The patient also experiences difficulty in breathing. It is an emergency as it may kill the person. Anaphylaxis may be local or systemic.

- Shock from drug reactions, venoms, and common allergens causing asthma are some examples of type I hypersensitivity.

**Hypersensitivity can be diagnosed** by demonstration of IgE by intradermal injections of allergens one by one. If specific IgE are present in the serum, a reaction will be visible on the skin locally in the form of a wheel of inflammation. Moreover, IgE levels can be measured by ELISA. Measurement of IgE is more practical as nature of allergens is not known most of the time.

- Rx: Epinephrine & antihistaminic drugs provide a means of treatment.

## 21. Two name of bacteria which is capsulated

Bacillus anthracic, streptococcus pneumoniae and Klebsiella are examples of capsulated organisms.

## 22. What is Epidemiology and its types

The study of where and when diseases occur and how they are transmitted in a population.

There are three basic types of investigations relating to epidemiology

1. Descriptive Epidemiology
2. Analytical Epidemiology
3. Experimental Epidemiology:

## 24. Capsomere

Capsid is made up of protein subunits called capsomeres that are arranged in 3 possible ways to give a helical, or icosahedral or complex symmetry to the viruses.

- Capsomeres: Subunit called protomers aggregate to form capsomeres.

## 25. Character of antigen

Antigen: A foreign substance that provokes immunity is called an antigen.

More specifically called as an immunogen. So immunogen and antigen although slightly different but used interchangeably.

Proteins are best antigen, though glycoproteins and lipoproteins can also act as antigens. Carbohydrates and lipid alone are poor antigens.

Antigens are foreign to the body.

Degradability: Inert molecules are not antigenic.

## 26. Algae structure

Body of multicellular alga such as seaweed is called a thallus which consists of branched holdfasts (anchor alga to rock) stemlike hollow stipes and leaflike blades. There is no vascular tissue in these algae. Also, the stipe is not lignified or woody, so it does not provide support to the weed. Surrounding water provides the support for the thallus. Some algae have a gas filled body inside them which keeps them floating in the water. This gas filled structure is called pneumatocyst or float

## 27. Acid fast stanning 5 marks

Some bacteria such as Mycobacteria have a waxy material in their cell wall.

Specific name for this waxy substance is mycolic acid

**Mycobacterium tuberculosis**: Causative agent for tuberculosis

**M. leprae**: Causative agent for leprosy

- **Principle of Acid-fast staining**: Mycobacteria are lipophilic, not easy to stain

But once stained, they are resistant to acidic alcohol decolorization process.

- **Overall Procedure for Acid-fast staining**:

**Carbol fuchsin:** It is a primary stain Heat is used to enhance penetration of carbol fuchsin.

Acid alcohol is a decolorizer.

Methylene blue is a counterstain.

Please note, Mycobacteria are gram positive, however, they look red in acidfast staining.

## 28. Capsule staining 10 marks

**Capsule**: If glycocalyx is organized and firmly attached to the cell, it is called a capsule.

– Plays important role in virulence (degree of pathogenicity)

– Can also be a part of the vaccine against the bacteria to which it belongs.

– Can be demonstrated by negative staining technique.

– Capsulated organism make glistening colonies as seen in the accompanying diagram below:

- Bacillus anthracic, streptococcus pneumoniae and Klebsiella are examples of capsulated organisms.
- Thickness of the capsule depends upon the culture conditions.

Capsules are mostly are water soluble. Also remember that capsular organisms usually make the broth viscous and stringy

**Capsule staining procedure**

1. Prepare a thick smear in a loopful of congo red (1%) stain
2. Fixation in acid alcohol for 15 sec
3. Wash with dH2O
4. Cover it with acid fuchsin for 1 min.
5. Wash with water
6. A bacterium stains red, capsule remains colorless and is seen in the dark blue background as seen in the accompanying diagram.

## 29. Define vaccine and its types 10 marks

# What is vaccine:

A product that creates immunity against an infection is called a vaccine. It could be the whole organism or a part of the organism.

The observation that people or animals once recover from an infection do not get sick easily to that organism again led to the discovery of vaccines and vaccination.

# Types of vaccine:

**Live Attenuated Vaccines**: These are weakened organisms so that they do not harm the body, but divide or replicate in the same way as pathogenic version of the organism does in the body. So, when a live virus or bacteria multiplies in the body, it stimulates the immune system to create an adaptive immune response which is protective against the disease for which vaccine was given.

The benefits of live vaccines are a better immunity because live microbes mimic actual infection (although they do not cause harm to the body). Live vaccines induce both humoral and cellular immune responses together, so live vaccines are a better choice if available. Because live microbes multiply in the body, this replication results in the increase of antigenic amount needed for stimulation of the immune system. Also, this antigen remains for a long time in the body, continuous presence of antigen continuously keeps the immune system active for longer time resulting in long term immunity.

**Inactivated Killed Vaccines**: Sometimes a non-pathogenic version of the microbe is not available, and to make a vaccine in this situation, the organism is killed with formalin or phenol and then used as a killed inactivated vaccine. Because organisms in such a vaccine do not replicate in the body, frequent boosters are required to achieve a good level of immunity (antibodies). Also, remember that this is a killed vaccine, so classical cellular immunity is not created with killed vaccines.

**Toxoids**: Some diseases are caused by bacterial toxins, and not by bacteria. In such cases, toxin is modified (inactivated) and used as a vaccine. Such a vaccine is known as a toxoid. Tetanus toxoid is a good example.

**Conjugated Vaccines:** Sometimes special parts of organisms are required for generating protective immunity. However, those substances are poor antigens if used alone. In order to enhance immunity, such poor antigens are attached to proteins, and such vaccines are called conjugated vaccines. Haemophilus influenzae type b is a good example.

**DNA vaccines:** We now know that sometimes only certain proteins are required for generating a good immune response. In such cases, the genes responsible for those proteins can act as vaccines. DNA coding for such a protein is injected into cells, and that DNA gets transcribed by the cells of the body into the required protein and that protein induces the antibody production (because that protein is foreign to the body). West Nile Virus is a good example of DNA vaccine

**Subunit vaccines:** Also called recombinant vaccines, such vaccines are composed of a portion of viral or bacterial proteins that can induce immunity. In other words, this is another version of DNA vaccines. In subunit vaccines, antigenic epitopes are included in the genome which is inserted into an expression vector that makes those subunit proteins. These subunit proteins are collected and used as vaccines to stimulate the immune system. Again, these act like a killed vaccine. There is another mechanism being explored in these days for recombinant vaccines. A subunit vaccine for Foot and Mouth disease virus is available.

Such genes are being incorporated in plants now that are acting like an expression vector. Animals can eat those plants and subunit vaccines can induce immune response through M cells of the intestine. Success is limited though so far.

## 30. Describe features of fungi 5 marks

These are simple eukaryotic cells. Some are unicellular, others are multicellular (thallus); however, they lack tissues such as roots, stem and leaves typically seen in plants. Algae absorb nutrients from water through their surfaces and are mostly photoautotrophs; however, a few are chemoheterotrophs. They are responsible for 80% atmospheric O2 on the face of the earth. Microscopic exam is needed to identify unicellular and filamentous algae. However, multicellular algae that are commonly known as seaweeds are macroscopic in nature and can be identified morphologically without the help of a microscope. Four groups of such algae

include blue-green algae, green algae, brown algae and red algae. These algae are located in the sea at various locations and absorb light of various wavelengths, hence red algae are located r from the surface and can use blue light from the sun as blue light is of shorter wavelength and can penetrate deep in the sea. Please see the accompanying figure (above) for a better understanding of how wavelength relates with various algae. Also remember, blue-green algae need magnification in order to be correctly identified, although they are not microscopic.

## 31. Treatment of hypersensitivity ii 3 marks

This type of hypersensitivity can be diagnosed by history and symptoms. Glomerulonephritis and cellulitis is very common. Deposition of immune complexes in the kidneys can be diagnosed by presence of IgG in kidney cells by using anti-IgG antibodies.

## 32. What are antibiotics 3 marks

antibiotics are used to kill organisms in disease states, some antibiotics are not very effective for this purpose; however, these antibiotics could be used in food to prevent food spoilage. Nisin and natamycin prevent spoilage of cheese

## 33. Define virion and viroid 2 marks

A **virion** is an infectious particle composed of nucleic acid that is surrounded by a protein coat (capsid). Viruses are classified based on their nucleic acid and protein coat. Viruses have an extracellular and an intracellular state. Outside of a cell, in the extracellular state, a virus is called a virion.

**VIROIDS:** They are composed only of circular single stranded (ss) RNA.

## 34. Write entry point of microorganism 2 marks

**Mucus membranes:** These include linings of digestive tract, respiratory tract, urogenital system, and conjunctiva

**Skin**: Surface area wise, skin is the largest organ of the body. Unbroken skin is impermeable to organisms

**Parenteral Route:** Injury to the skin or mucous membrane leads to easy access for the organisms in the body. Any cut received on the skin for example becomes a portal of entry

## 35. Role of thin clonal selection 2 marks

According to this theory, B cells are generated in the bone marrow randomly without seeing the antigen. When such a B cell encounters its specific antigen in the body, it starts proliferating into a clone which ultimately becomes plasma cells and start secreting antibodies. A beautiful example to understand clonal selection theory is like going to a shoe store and select a shoe size that fits you very well. Note, that the shoe that you selected was made randomly without any regard to your foot size.

## 35. Antigen presenting cell 3 marks

These cells are able to present antigens to B cells for making antibodies by B cells.

There are three cells that fall into this category: Dendritic cells, macrophages, and B cells

## 36. How antibodies provide defense against microbes 5 marks

## 37. Reverse transcription 2 marks

A reverse transcriptase is an enzyme used to generate complementary DNA from an RNA template, a process termed reverse transcription

## 38. Bactericidal and bacteriostatic drug 2 marks

**Bactericidal**: Those antibiotics that kill and lyse the cells are called bactericidal.

**Bacteriostatic**: Such antibiotics stop the growth of the organisms, they do not kill or lyse the cells. Cells of the immune system then clear them from the body

## 39. Define plasmogamy karyogamy and meiosis 3 marks

**1.Plasmogamy**: A haploid nucleus of a donor cell (+) penetrates the cytoplasm of a recipient cell (-). Both cells are haploid (one set of chromosomes)

2. **Karyogamy** The (+) and (-) nuclei fuse to form a diploid zygote nucleus.

3. **Meiosis**: The diploid nucleus gives rise to haploid nuclei (sexual spores).

## 40. Mechanism of hypersensitivity iv 5 marks

It is called as delayed type because its bad effect becomes visible 2-3 days after exposure to the antigen. This is the only hypersensitivity that involves cells rather than antibodies. Robert Koch discovered this when he

injected tuberculin in patients. Contact dermatitis is the most common example of this kind of hypersensitivity. This is basically an interaction between an antigen, T cells and macrophages. Transplant rejection is another good example of delayed type IV hypersensitivity. Reaction to hair dyes is another example. Poison Ivy is another example in which contact with this plant initiate type IV hypersensitivity response. Chemicals from poison ivy interact with skin proteins and change them into foreign-looking protein molecules. Body's immune system mounts an immune response against these proteins resulting in a damaging type of intense inflammatory response

## 41. Cytokines 2 marks

**Cytokines** are released as a result of defense cell stimulation by PAMPs and TLRs interactions. Cytokines recruits more immune cells and also stimulate adaptive immune response

## 42. Note on infectious disease transmission with vector vehicle and contacts?

**Direct Transmission**: person to person physical contact is a direct contact. Influenza spread is a typical example.

**Vehicle Transmission**: Transmission of disease agents by a medium, such as water, food, or air. Other media include blood and other body fluids, drugs, and intravenous fluids.

**A vector is a vehicle** (animate or inanimate) that carries a pathogen from one host to another. o Arthropods are vectors for many diseases. There are two ways these arthropods can transmit organisms to susceptible hosts:

**Mechanical Transmission**: In this type of transmission, organisms stick to the body parts of insects and get transmitted passively from one location to another.

**Biological Transmission:** Microbes multiply in the insect and is transmitted actively by insect bites from one individual to another.

## 43. Incidence and Prevalence 2 marks

**Incidence**: # of new cases in a given time period in a population

**Prevalence**: New + existing cases

## 44. PRIMARY AND SECONDARY IMMUNE RESPONSE 5 marks

When a person is exposed to an antigen (a microbe for example), the antigen is presented to B cells which start making antibodies. Initially, there are no detectable antibodies in the serum. However, after 3-5 days, IgM antibodies are secreted and then 7-10 days after, the same B cell starts making IgG. The level of IgG rises to a level and then it starts declining. This is a typical response of an antigen exposure and is called a primary immune response. It is interesting to note that a second exposure with the same antigen results in a heightened response in terms of very high levels of antibodies that are achieved in a very short time (within 2-7 days). This is called a secondary immune response. It is also called a memory or anamnestic response because memory cells are involved that ensure a quick response to the second exposure by the same antigen (to which the immune system has already been exposed). The accompanying figure illustrates this concept (next page). Blue line in the figure depicts a primary and a secondary response.

Adaptive Immune Response can be acquired naturally or artificially. Each of these categories can further be divided either into active or passive type.

**Naturally Acquired Active Immunity**: This type of adaptive immunity develops in a person who gets infected with a microbe but then recovers from that illness making antibodies.

**Naturally Acquired Passive Immunity:** A typical example of this type of immunity is transfer of antibodies from colostrum or milk of a mother to the offspring.

**Artificially Acquired Active Immunity:** When a person receives a vaccine against a microbe and the body makes antibodies in response to that vaccine, it is called artificial acquired active immunity because body makes these antibodies actively by itself.

**Artificially Acquired Passive Immunity:** When premade antibodies are given to a person in order to protect a person from an illness or a snake bite, this constitutes an artificially acquired passive immunity type. The following figure illustrates these four types.

## 45. Vegetative Hyphae and Aerial hyphae 2 marks

**Vegetative Hyphae:** Hyphae that are embedded in the medium and are used to obtain food are called vegetative hyphae.

**Aerial or Reproductive Hyphae:** The portion of hyphae that is concerned with reproduction. This portion is projected into the air. They bear reproductive spores.

## 46. Signature sequence and primer

**signature sequences**

There are many tests that involve nucleic acid sequence detection for the presence of microbes in a clinical sample. Please remember, as antibodies are specific to the organisms, DNA or RNA sequences must be

uniquely specific for the microbe that we are going to detect. Such sequences that are specific to an organism are called **signature sequences**

Primer

A primer is a fragment of DNA which is specific to the signature sequence

Feature of yeast cells

## 47. Active and passive transport

**active Movement**

Movement against the concentration gradient

From low concentration to high concentration of a substance

Requires energy (ATP)

Substances that are actively transported into the cells undergo either no change or they are chemically modified to keep them there in the cell. Remember, active transport brings substances inside the cell against the gradient; hence molecules brought in can go out. That is why these molecules may be modified to keep them inside the cells. In other words, this modification avoids passive diffusion of those molecules from the cells outwards.

Passive Movement

Movement of substances with the concentration gradient. This means that substances will move from higher concentration of a substance to lower concentration. Gradient provides the force for movements of molecules and this happens with no energy expenditures.

## What are the 2 benefits algae get from oceans 2 marks

Algae are important for aquatic life. They are at the bottom of the food chain because they fix CO2 into organic molecules (Carbs) which could be used by other organisms.

They produce 80% of molecular oxygen on earth.

Algal blooms which result from fluctuation of temperature sometimes may cause problems for nature but that happens occasionally.

Algal blooms tell us about the status of pollution in the environment. Planktonic organisms (dead and decaying) are responsible for oil production. Petroleum and natural gas are the residues (hydrocarbons) of diatoms and other planktonic organisms.

## Define lichens 2 marks

Lichens are a combination of green alga (or a cyanobacterium) and a fungus. Green alga or cyanobacterium is also called a phycobiont and the fungus is called a mycobiont in this relationship. Both partners have a symbiotic relationship with each other

## Hypersensitivity type 1 3 marks

It involves IgE and it occurs within 30 minutes of exposure to the allergen. Antigens called allergens preferentially induce IgE production instead of IgG against the antigens. These IgEs get bound to mast cells in the tissues and a second exposure to the same allergen activates mast cells to secrete histamine and other mediators of inflammation. Shock from drug reactions, venoms, and common allergens causing asthma are some examples of type I hypersensitivity.

Hypersensitivity can be diagnosed by demonstration of IgE by intradermal injections of allergens one by one. If specific
IgE are present in the serum, a reaction will be visible on the skin locally in the form of a wheel of inflammation

## Transmission of disease 5 marks

**Direct Transmission**: person to person physical contact is a direct contact. Influenza spread is a typical example.

**Indirect Transmission:** A nonliving object is involved. Objects in the use of a patient such as handkerchief, utensils, pillow and bedding are called fomites. These can transmit organisms to susceptible individuals.

**Droplet Transmission:** Mucus droplets are created when you sneeze for example. These droplets carry

organisms that may infect another individual who comes in contact with the droplets. One sneeze may produce 20,000 droplets. Influenza can be spread by this route.

**Vehicle Transmission:** Transmission of disease agents by a medium, such as water, food, or air. Other media include blood and other body fluids, drugs, and intravenous fluids.

## Hypersensitivity type3 5 marks

This hypersensitivity involves soluble antigen circulating in the serum and antibodies (IgG or sometimes IgM). Complement gets activated when these complexes get deposited in capillary beds as we see in the kidneys for example. A typical example will be the use of antivenom (antibodies) that was prepared in horses but given to a human to save the life. What happens is that horse antibodies will be recognized by human body as a foreign protein and human immune system will generate antibodies against this. This type of hypersensitivity can be diagnosed by history and symptoms. Glomerulonephritis and cellulitis is very common. Deposition of immune complexes in the kidneys can be diagnosed by presence of IgG in kidney cells by using anti-IgG antibodies.

BEST OF LUCK

BEST OF LUCK