Virtual University of Pakistan

(Bt102)

(Microbiology)

Midterms Past Papers

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Good luck for exams

Midterm paper pattern

Mcqs = 20

SQs (2marks) = 2

SQs(3marks) = 2

LQs (5marks) = 2

Total marks = 40

Short Questions marks = 2

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.

Name two basic dyes?

In basic dye the color is in the positive ion. e.g
1-Crystal violet 2-Methylene 3-blue Malachite green 4-Safranin

3. **Differentiate direct count and viable count?**

4. **Direct microscopic count:** This method uses a hemocytometer which takes a specific volume of sample and the number of microbes can directly be counted under a light microscope. The number of microbes in one ml of original sample may be determined by multiplying with appropriate number. No incubation is required, so this method is quicker and it also gives a total count (viable as well as dead microbes). There is an auto version of this method where the cells are counted electronically. The name of the instrument is Coulter counter.

5. **Define pilli and also 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
- on bacterium to another:
- Conjugation (Sex pili)
- Also function in twitching Motility
- Gliding Motility is also the function of the pili.

6. **Respiration of glucose?**

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?

○ 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.

8. what is buffer?

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

9. What is resolution?

○ 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.
○ Light microscope resolving power is 0.2 μm.

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

○ n = refractive index, 1.5 for immersion oil ○ 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. 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.

11. 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.

12. What is function of 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.

13. Stages of Germination spore.3

• 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.

14. Give the characteristic of microaerophlis.(2)

15. Differentiate between differential and simple staining.(2)

• Simple Staining: only one stain is used.

- Methylene Blue staining

• Differential Staining: A couple of stains used.

- Gram Staining

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).

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

19. Write down type of bacteria?

20. **what is agar ? what are the characteristics**

Agar is 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 85^{0}C and solidifies at 43 ^{0}C Agar could be used to solidify medium in Petri plates or slants.

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.*

25. 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. Write down three advantages of staining?

• Most organisms appear colorless when seen under a microscope.

• Staining emphasizes certain structures of the organisms.
• Staining is just coloring with a dye.
• Staining increases visibility of microbes because staining increases contrast.
• Shape, size and arrangements of the organisms can be readily seen.
• Purity or contamination of a culture could be determined.
• Differentiation and classification of microbes is possible. For example, microbes can be categorized into Gram positive or Gram negative groups.
• Structures such as flagella, capsule and spores etc. of bacteria can be detected with staining.

31. How bacteria got nitrogen??

- By decomposing proteins
- From NH_4^+ ions
- From Nitrates
- Use Gaseous N_2

 These are nitrogen fixing bacteria.

32. 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

33. 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.

34. **Sporulation 2**

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

35. What is meant by Virulence Morbidity Morbidity rate Mortality Mortality rate Pathogenicity.

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

36. **What is passive transport? (2)**

Movement of substances with 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.

Short questions marks 3

1. **What is culture medium, culture and inoculums?**
   - This term is used to define growth of microbes. In other words, 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 organism
2. **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.
3. **write the name of five layers of endospore**
   - **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
4. **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
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   - 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.
5. **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. **What is function of 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.

9. **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..

10. **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

12. **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.*

13. **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. **What is fimbriae, write it 's location and funtion**

 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 Fimbriae help bacteria attach to surfaces before they can secrete biofilm Fimbriae also help attach to epithelial cells *Neisseria gonorrhoeae* E.coli O157: Diahrrea

15. **Magnetosomes**

• 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.

16. **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.

17. **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.

18. **Medical microbiology**

19. **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 on bacterium to another:
Conjugation (Sex pili)
 Also function in twitching Motility
 Gliding Motility is also the function of the pili.

20. **Stages of growth curve 3**

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.

21. 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

22. What are the physical and chemical methods for making Smear on the slide

• 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.

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

26. 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.

27. Write characteristics of integral proteins.

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.

Long Questions (5 marks)

1. **Function of cell membrane(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 as can be seen in the accompanying diagram.
Functions:
• 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.

2. **Types of microbes based on oxygen requirement (5 marks)**

 Oxygen is needed for aerobic respiration.
 Use of O2 means more ATP
 NADH and FADH2 during Krebs cycle oxidized via Electron Transport Chain which generates 36 or 38 ATP molecules in the organisms that use O2 as the final electron acceptor.
 Based on the use and concentration of Oxygen, microbes can be classified into five groups:
o **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.
o **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.
o **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

3. **write names of 5 kingdom system (5)**

In 1969, five kingdom classification was proposed by Robert Whittaker as under:
Monera: Bacteria
•**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.

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

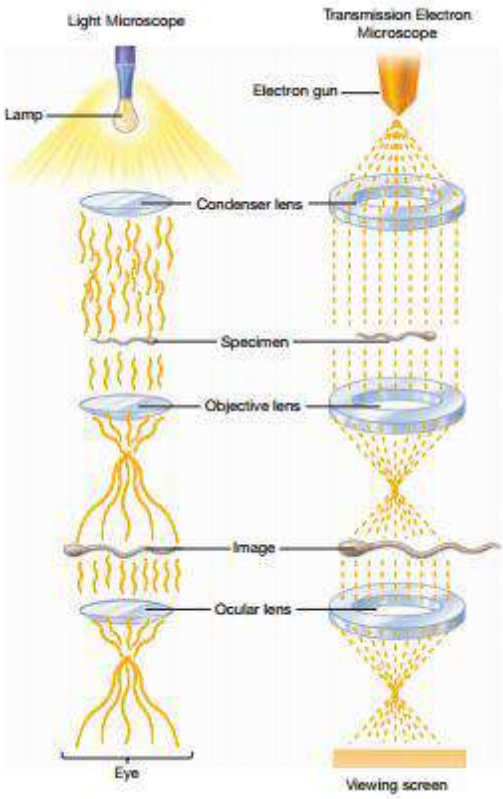
Characteristics of metachromatic granules5 • Also called volutin, they stain red with certain dyes such as methylene blue. That is why they are called metachromatic (stain in different color as methylene blue gives blue color but the color on these granules is red).

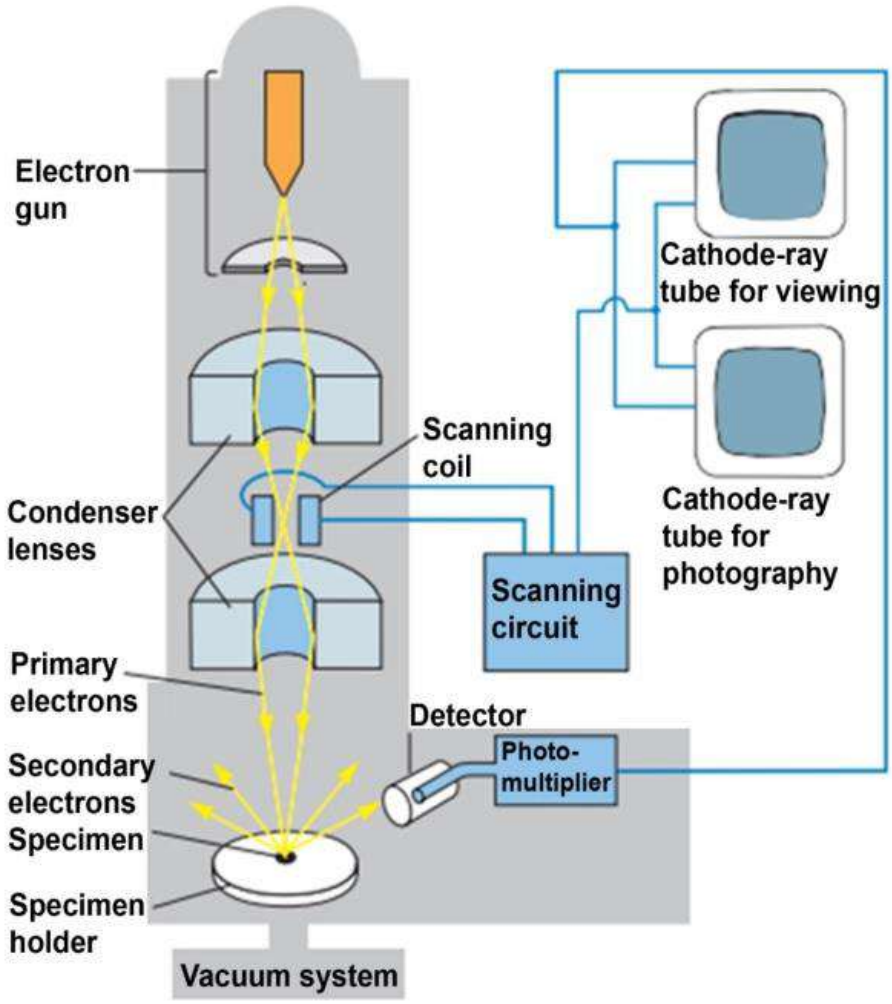
• Large inclusions • These inclusions contain inorganic phosphates.

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.**


Light Microscope
Transmission Electron Microscope
Lamp
Electron gun
Condenser lens
Specimen
Objective lens
Image
Ocular lens
Eye
Viewing screen



6. 5 layers of Endospore.5 marks

• **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.

7. Explain characteristics of aerophiles.

8. **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$_o$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 ○ 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.

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:
○ Ionizing and non-ionizing radiation
○ 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:

- **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.
  - **Silver nitrate (1%):** Commonly used as a disinfectant.
  - **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.
  - **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.

10. 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:
o **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.

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.
- 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.