FAQ's

1. **What is a Hemocytometer**

   A hemocytometer is a counting chamber. It is a thick glass slide containing a well in the central section. On the bottom of the well, a grid is etched containing squares of known area. Each square is 0.04 mm^2. A cover slip is placed over this well forming a chamber of known depth (0.1mm). Thus, the volume of liquid that each square can hold is 0.004 mm^3 ($0.04 \times 0.1 = 0.004mm^3$). The sample to be counted is placed in the well by placing a drop of the sample at the edge of the cover slip so that it runs into the well and over the grid. The hemocytometer is then viewed using a microscope. The number of microorganisms in several squares is counted, and the average number of micro-organisms is calculated. This number of microorganisms is then used to calculate the number of microorganisms in the original sample.

2. **What is wet mount method?**

   It is a method for observing microbial samples under microscope. Here a desired liquid sample is placed on the slide. After that, a cover slip is placed without forming any air bubbles. The fluid spreads out in a thin layer between coverslip and slide. The mount is now examined under the microscope at appropriate magnifications (e.g., 10x100 X). This method is commonly used to view microscopic organisms that grow on liquid media, especially when studying their movement and behavior.

3. **What is Dry mounts?**

   Microorganisms like bacteria being too small need their permanent preparations which are made by drying and fixing them on the clean slide with or without staining.

   For preparing a dry mount, a drop of distilled water with a small amount of culture is spread as a thin smear on a clean slide. The smear is allowed to dry and then 'fixed' by passing it through a flame two to three times with the smeared slide away from the flame. If desired, this dried and fixed culture may be stained and dried again for observation under the microscope.

4. **What is Hanging drop mount?**

It is used to observe the motility or germination or fission of microorganisms. In this method, a cavity slide, which has a circular concavity in the center, is used. The periphery of the concavity on the cavity slide is smeared with Vaseline. A drop of liquid microbial culture is placed in the center of the cover glass for liquid culture. If the culture is grown on solid media, it is mixed with a drop of distilled water before placing on the cover glass. The cover glass is inverted over the concavity so that the drop hangs freely and the edge of cover glass adheres tightly to the Vaseline coated periphery of the concavity. The microorganisms present in the hanging drop are now observed for their type of mobility under the microscope.

5. **What is Gram's staining**

Gram's staining is a differential staining involves the usage of 2 or more stains. It involves heating. It can differentiate various groups of bacteria, e.g. between Gram-positive and Gram-negative bacteria.

The reaction of bacteria to Gram's staining method is a consequence of differences in the chemical structure of the bacterial cell wall and is a key feature in their identification.

The basis of Gram's staining method is the ability of the cell to get stained with crystal violet to retain the colour when treated with a differentiating agent, usually alcohol (although professionals sometimes use acetone). They are further stained in the contrasting colour of a counter stain, usually pink/red. Bacteria that retain the violet/purple colour are called Gram-positive. Those that lose the violet/purple colour but take the pink/red are called Gram-negative

6. **List the advantages and disadvantages of membrane filtration**

Advantages	Disadvantages
Flexible sample volume range enabling the use of large sample volume and therefore increased sensitivity Water soluble impurities interfering with	Quality of membrane varies Solid particles and chemicals absorbed from samples to the membrane during filtration may

the growth of target organisms separated from the sample in the filtration step Quantitative result and good precision if the number of colonies grown adequate Further cultivation steps not always needed, which lowers the cost and time required for the analysis When confirmation is required isolation from well-separated colonies on membrane is easy	interfere with the growth of the target organism Not applicable to turbid samples Scoring of typical colonies not always easy Can be used for only samples with low microbial counts

7. **What is selective medium?**

   Selective medium: A selective medium is one that favors' the growth of a particular organism or group of organisms. It often suppresses the growth of others organisms. An enrichment culture uses a selective media component to encourage the growth of an organism present in low numbers. For example, mannitol salt agar is selective for Staphylococci because most other bacteria cannot grow in its high-salt environment. Another selective medium is brilliant green agar, a medium that inhibits Gram-positive bacteria while permitting Gram-negative organisms such as Salmonella species to grow

   .

8. **What is differential medium?**

   A differential medium allows colonies of a particular organism to be differentiated from others growing in the same culture. These media provide environments in which different bacteria can be distinguished from one another. For instance, violet red bile agar is used to distinguish coliform bacteria such as Escherichia coli from non-coliform organisms. The coliform bacteria appear as bright pink colonies in this media, while non-coli forms appear a light pink or clear.

9. Classify microorganisms on the basis of temperature?

Microorganism can be categorized based on the temperature

Mesophiles: Microorganism has optimal growth around 20 to 45 ℃.

Example: *Staphylococcus aureus, Salmonella,*

Thermophiles: Those microorganisms capable of growth within a range of about 40 to 80 ℃, with optima around 50 to 60 ℃ They are adapted to not only surviving but thriving at much higher temperature.

Example: *Hydrogneobaculum, Thiomonas, Acidimicrobium,*

Extreme thermophiles or Hyperthermophiles: These are microorganism which has optimum value and can tolerate temperature excess 100 ℃.

Example: *Thermococcus barophilus, Thermus aquaticus, Thermoccus itorali*

Psychrophiles: Psychrophiles occupy the other extreme of the temperature range; they can grow at 0 ℃, with optimal growth occurring at 15 ℃ or below.

Example: Arthrobacter spp. Psychrobacter spp. Halomonas spp,

Psychrotrophs: They can also grow at 0 ℃, have much higher temperature optimum (20-30 ℃).

10. Classify microorganisms on the basis of oxygen?

Various types of oxygen requirement by microbes.

a) Aerobes: Microorganism, which requires oxygen for growth.

Examples: Lactobacillus, Leuconostoc, Micrococcus, Bacillus, Rhodotorula, Molds Aspergillus, Penicillium, Mucor,

b) Anaerobes: They can survive in the absence of oxygen or grow in the absence of oxygen.

Examples: Bacteria (Alcaligenes, Clostridium spp)

c) Obligate anaerobes: Microorganism cannot tolerate oxygen at all requires culturing in special anaerobic chambers.

Example: *Clostridium botulinum, C. tetani, C. perfringens,*

d) Facultative anaerobes: They can grow like aerobes in the presence of oxygen, but have the added facility of being able to survive when conditions become anaerobic.

Example: *Staphylococcus spp, Listeria spp, Saccharomyces cervisiae.*

e) Aerotolerant anaerobes: They are basically anaerobic, not inhibited by oxygen, which they do not utilize it.

Example: Thiobacillus spp, Thiococcus spp.

f) Microaerophiles: They require oxygen, but are only able to tolerate low concentration i.e., 2-10%.

Example: Campylobacter spp, Helicobacter pylori,

11. What is aerobic plate count?

Aerobic Plate Count (APC) is used as an indicator of bacterial populations on a sample. It is also called the aerobic colony count, standard plate count, Mesophilic count or Total Plate Count.

The test is based on the assumption that each cell forms a visible colony when mixed with agar containing the appropriate nutrients. It is not a measure of the entire bacterial population, but it is a generic test for organisms that grow aerobically at mesophilic temperatures (25 to 40 °C). The count is expressed as colony forming unit (CFU)/gm or ml.

APC does not differentiate types of bacteria. APC can be used to gauge sanitary quality, organoleptic acceptability, adherence to good manufacturing practices, and to a lesser extent, as an indicator of safety. APC may also provide information regarding shelf life or impending organoleptic change in food.

12. What is standard plate count?

Standard Plate Count or Plate Loop Count (SPC or PLC) is the measure of the total number of aerobic bacteria in the milk. The most common causes of a high SPC could be unhygienic milking equipment, poor cooling, and poor udder preparations. Mastitic cows can be responsible for high counts. The regulatory limit for SPC is 100,000 bacteria/ml of milk.

13. What is dye reduction test?

Dye reduction test involve the use of redox dyes like methylene blue to determine the quality of milk.

Methylene blue is reduced and loses its color in the presence of actively growing bacteria. The time taken for the reduction of methylene blue is inversely proportional to the number of viable bacteria.

The shorter the methylene blue reduction time higher is the microbial count and poorer is the quality of the milk

14. What is a complex media?

Complex media are rich in nutrients, they contain water soluble extracts of plan or animal tissue (e.g. enzymatically digested animal proteins such as peptone and tryptone). Usually a sugar, often glucose is added to serve as the main carbon and energy source. The combination of extracts and sugar creates a medium which is rich in minerals and organic nutrients, but since the exact composition is not know, the medium is called complex.

15. What are the methods of measure microbial growth?

Direct cell counts, direct microscopic count, total cell count, viable count , membrane filter count method, spread count method, pour plate method.

16. What are types of microorganisms based temperature?

Mesophilies, thermophiles, extreme theromophiles or hyperthermophiles, psychrophiles: psychrotrophs:

17. What are types of microorganisms based oxygen requirements?

Aerobes, anaerobes, obligate anaerobes, facultative anaerobes, aerotolerant anaerobes: Microaerophiles.

18. What are types of microorganisms based cultivation mode?

Autotroph, heterotroph, phototroph, chemotroph, lithotroph, organotroph