Microbial culture.

Microbiological culture is the primary method used for isolating infectious agents for study in the laboratory. Tissue or fluid samples are tested for the presence of a specific pathogen, which is determined by growth in a selective or differential medium.

The 3 main types of media used for testing are:

1. Solid culture: A solid surface is created using a mixture of nutrients, salts and agar. A single microbe on an agar plate can then grow into colonies (clones where cells are identical to each other) containing thousands of cells. These are primarily used to culture bacteria and fungi.
2. Liquid culture: Cells are grown inside a liquid media. Microbial growth is determined by the time taken for the liquid to form a colloidal suspension. This technique is used for diagnosing parasites and detecting mycobacteria.
3. Cell culture: Human or animal cell cultures are infected with the microbe of interest. These cultures are then observed to determine the effect the microbe has on the cells. This technique is used for identifying viruses.

Cultural media used to identify G+ ve Bacteria and G+ ve Bacteria

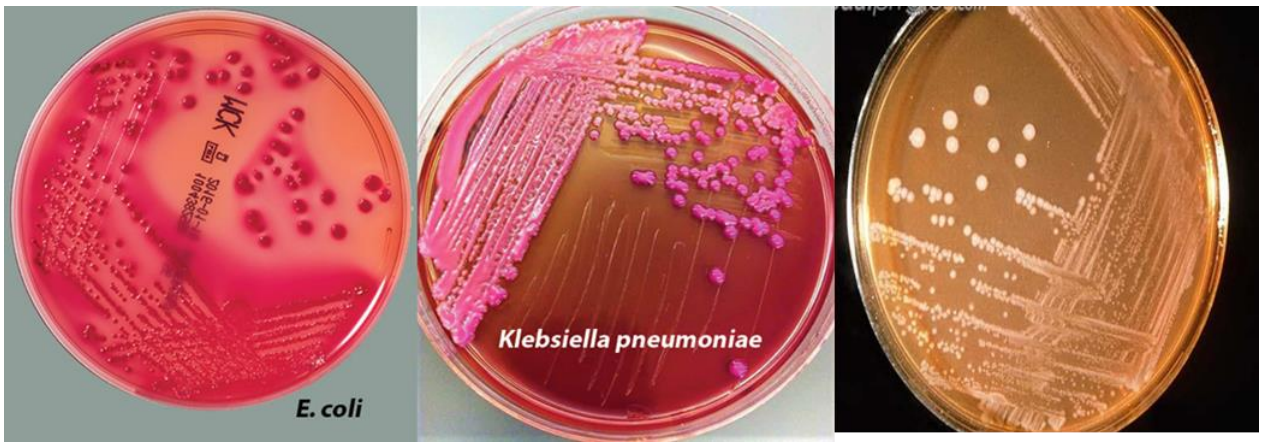
- **Mannitol Salt Agar (MSA)**

This type of medium is both selective and differential. The MSA will select for organisms such as *Staphylococcus* species which can live in areas of high salt concentration. This is in contrast to *Streptococcus* species, whose growth is selected against by this high salt agar.

The differential ingredient in MSA is the sugar Mannitol. Organisms capable of using Mannitol as a food source will produce acidic by products of fermentation that will lower the pH of the media. The acidity of the media will cause the pH indicator, phenol red, to turn yellow. *Staphylococcus aureus* is capable of fermenting Mannitol while *Staphylococcus epidermidis* is not.

- **MacConkey agar.**

This medium is both selective and differential. The selective ingredients are the bile salts and the dye, crystal violet which inhibit the growth of Gram-positive bacteria. The differential ingredient is lactose. Fermentation of this sugar results in an acidic pH and causes the pH indicator, neutral red, to turn a bright pinky-red color. Thus, organisms capable of lactose fermentation such as *Escherichia coli*, form bright pinky-red colonies MacConkey agar is commonly used to differentiate between the *Enterobacteriaceae*.



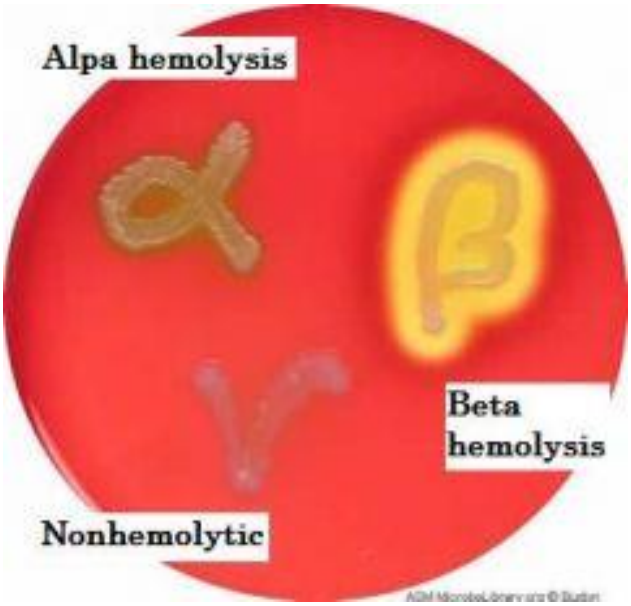
Organism on left is positive for lactose fermentation and that on the right is negative.

- **Blood Agar Plates (BAP)**

This is a differential medium. It is a rich, complex medium that contains 5% sheep red blood cells. BAP tests the ability of an organism to produce hemolysins, enzymes that damage/lyse red blood cells (erythrocytes).

The degree of hemolysis by these hemolysins is helpful in differentiating members of the genera *Staphylococcus*, *Streptococcus* and *Enterococcus*.

- Beta-hemolysis is complete hemolysis. It is characterized by a clear (transparent) zone surrounding the colonies. *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus agalactiae* are beta-hemolytic.
- Partial hemolysis is termed alpha-hemolysis. Colonies typically are surrounded by a green, opaque zone. *Streptococcus pneumoniae* and *Streptococcus mitis* are alpha-hemolytic.
- If no hemolysis occurs, this is termed gamma-hemolysis. There are no notable zones around the colonies. *Staphylococcus epidermidis* is gamma-hemolytic.



Biochemical tests.

Fast and relatively simple biochemical tests can be used to identify infectious agents. For bacterial identification, the use of metabolic or enzymatic characteristics are common due to their ability to ferment carbohydrates in patterns characteristic of their genus and species. Acids, alcohols and gases are usually detected in these tests when bacteria are grown in selective liquid or solid media, as mentioned above. In order to perform these tests en masse, automated machines are used. These machines perform multiple biochemical tests simultaneously, using cards with several wells containing different dehydrated

chemicals. The microbe of interest will react with each chemical in a specific way, aiding in its identification.

	Tests used to identify G+ve Bacteria.	Tests used to identify G -ve Bacteria.
1	Catalase Test.	Oxidase Test
2	Mannitol Salt Agar (MSA)	Sugar (e.g. glucose) broth with Durham tubes.
3	Blood Agar Plates (BAP)	Methyl Red / Voges-Proskauer (MR/VP)
4	Taxo P (optochin sensitivity testing)	Kligler Iron Agar (KIA)
5	Taxo A (bacitracin sensitivity testing)	Nitrate Broth
6	CAMP Test	Motility Agar
7	Bile Esculin Agar	MacConkey agar
8	Nitrate Broth	Simmon Citrate Agar
9	Starch hydrolysis test	Urease test
10	Motility Agar	Sulfur Indole Motility Media (SIM)
11	Coagulase Test	

Serological methods.

Are highly sensitive, specific and often extremely rapid laboratory tests used to identify different types of microorganisms. The tests are based upon the ability of an antibody to bind specifically to an antigen. The antigen (usually a protein or carbohydrate made by an infectious agent) is bound by the antibody, allowing this type of test to be used for organisms other than bacteria. This binding then sets off a chain of events that can be easily and definitively observed, depending on the test. More complex serological techniques are known as immunoassays. Using a similar basis as described above, immunoassays can detect or measure antigens from either infectious agents or the proteins generated by an infected host in response to the infection.

Polymerase chain reaction:

Polymerase chain reaction (PCR) assays are the most commonly used molecular

technique to detect and study microbes. As compared to other methods, sequencing and analysis is definitive, reliable, accurate, and fast. Today, quantitative PCR is the primary technique used, as this method provides faster data compared to a standard PCR assay. For instance, traditional PCR techniques require the use of gel electrophoresis to visualize amplified DNA molecules after the reaction has finished. Quantitative PCR does not require this, as the detection system uses fluorescence and probes to detect the DNA molecules as they are being amplified. In addition to this, quantitative PCR also removes the risk of contamination that can occur during standard PCR procedures (carrying over PCR product into subsequent PCRs). Another advantage of using PCR to detect and study microbes is that the DNA sequences of newly discovered infectious microbes or strains can be compared to those already listed in databases, which in turn helps to increase understanding of which organism is causing the infectious disease and thus what possible methods of treatment could be used. This technique is the current standard for detecting viral infections such as AIDS and hepatitis.