Lab – 1 Diagnosis of pathogenic bacteria

Pathogenic bacteria: are bacteria that can cause infection. Although most bacteria are harmless or often beneficial, some are pathogenic. One of the bacterial diseases with the highest disease burden is tuberculosis, caused by the bacterium *Mycobacterium tuberculosis*, which kills about 2 million people a year, mostly in sub-Saharan Africa. Pathogenic bacteria contribute to other globally important diseases, such as pneumonia, which can be caused by bacteria such as *Streptococcus* and *Pseudomonas*, and foodborne illnesses, which can be caused by bacteria such as *Shigella*, *Campylobacter*, and *Salmonella*. Pathogenic bacteria also cause infections such as tetanus, typhoid fever, diphtheria, syphilis, and leprosy. Pathogenic bacteria are also the cause of high infant mortality rates in developing countries

What distinguishes a pathogen from a non-pathogen?

The bodies are host to many microbes, most of which do not cause disease and many of which are beneficial. Occasionally, however, microbes do cause infection and disease. Pathogens such as Staphylococcus, Vibrio cholera and Mycobacterium tuberculosis differ from normal non-pathogenic microbes in that they cause damage to the host. This damage allows the pathogen to colonize novel sites, antagonizes the host immune response, and facilitates spread of the pathogen. Pathogens inflict damage on their hosts by secreting toxins that act on host cell membranes or translocate across the cell membrane and usurp normal cellular functions.

Diagnostic tests

Microbial culture

Microbiological culture is the primary method used for isolating infectious disease 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.**

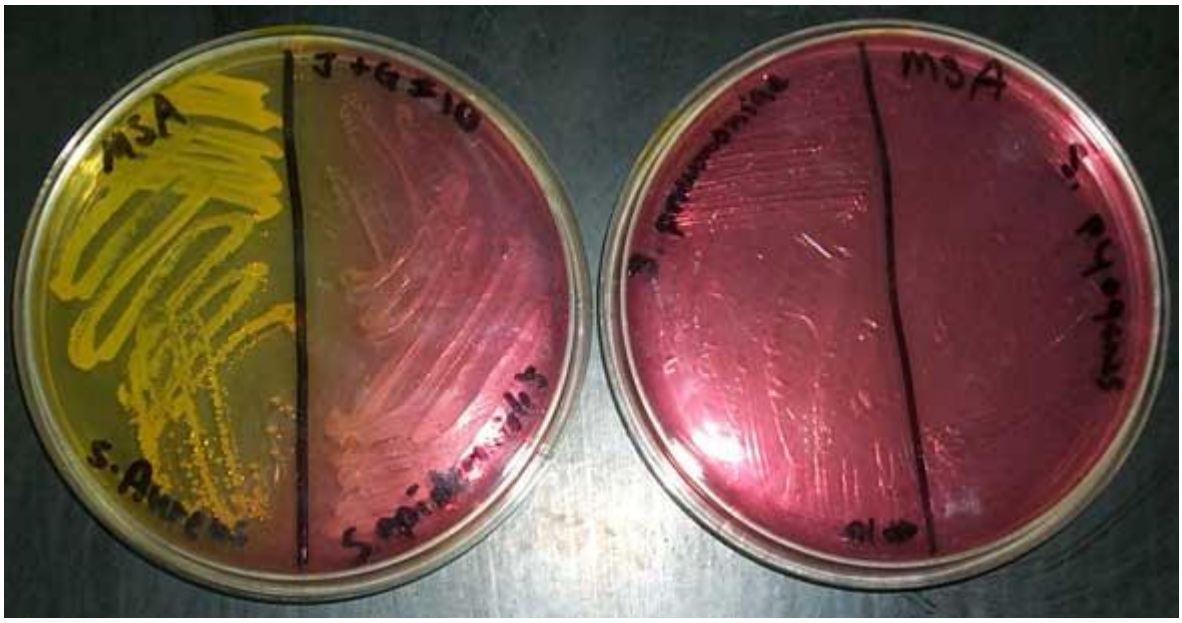
Media culture used to identify Gram Positive Bacteria and Gram Negative Bacteria

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 .

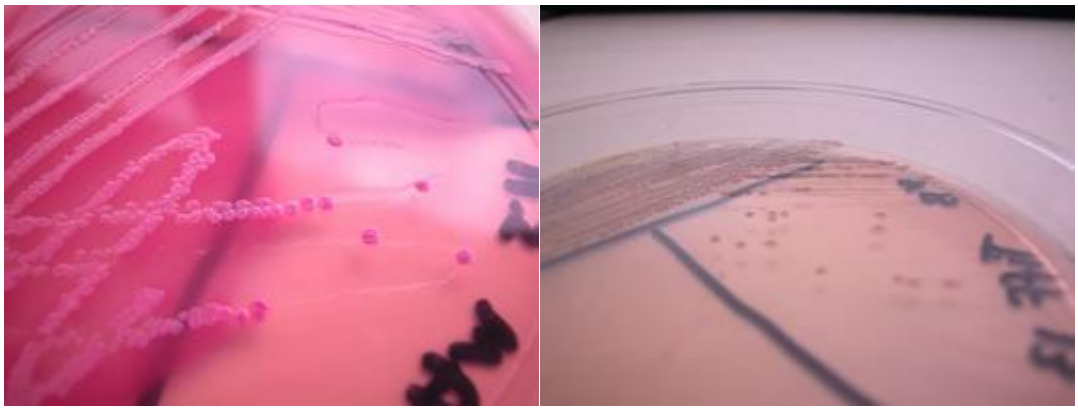
The differential ingredient in MSA is the sugar Mannitol. Organisms capable of using Mannitol as a food source will produce acidic byproducts of fermentation that will lower the pH of the media. The acidity of the media will cause the pH indicator, phenol red, to turn yellow. *Staphylococcus aureus* 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.

Microscopy:

Culture techniques will often use a microscopic examination to help in the identification of the microbe. Instruments such as compound light microscopes can be used to assess critical aspects of the organism. This can be performed immediately after the sample is taken from the patient and is used in conjunction with biochemical staining techniques, allowing for resolution of cellular features. Electron microscopes and fluorescence microscopes are also used for observing microbes in greater detail for research. There are different kinds of stains used to staining the bacterial cell for examples simple stain , Gram stain ,acid fast stain , spore stain , capsule stain , flagella stain ,nucleotide stain ……..etc.

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 Gram Positive Bacteria

1. **Catalase Test**
2. **Mannitol Salt Agar (MSA)**
3. **Blood Agar Plates (BAP)**
4. **Taxos P (optochin sensitivity testing)**
5. **Taxos A (bacitracin sensitivity testing)**
6. **CAMP Test**
7. **Bile Esculin Agar**
8. **Nitrate Broth**
9. **Starch hydrolysis test**
10. **Motility Agar**

Tests used to identify Gram Negative Bacteria

1. **Oxidase Test**
2. **Sugar (eg glucose) broth with Durham tubes**
3. **Methyl Red / Voges-Proskauer (MR/VP)**
4. **Kliger's Iron Agar (KIA)**
5. **Nitrate Broth**
6. **Motility Agar**
7. **MacConkey agar**
8. **Simmon's Citrate Agar**
9. **Urease test**
10. **Sulfur Indole Motility Media (SIM)**

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.[29] 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.