Principle of identification and differentiation of Human Pathogenic Bacteria:

A. Direct Examination and Techniques:

Direct examination includes Gram staining method. According to this method pathogenic bacterium can be classified into:

1. **Gram Positive Bacteria:**
   - **A. Gram Positive Cocci bacteria**
     1. **Streptococci and Enterococci appear in chain and pair.**
     2. **Staphylococci and Micrococci appear in irregular cluster and tetrads.**
   - **B. Gram Positive Bacilli bacteria**
     1. **Spore Forming Bacteria:**
        a. Aerobic Bacteria; like *Bacillus sp*.
        b. Anaerobic Bacteria; like *Clostridium sp*.
     2. **Non – Spore Forming Bacteria.**
        Corynebacterium diphtheriae.
2. **Gram Negative Bacteria:**
   - **A. Gram Negative Cocci bacteria** Like *Niesseria* spp.,
   - **B. Gram Negative Bacilli bacteria** Like *E. coli, Klebsiella* spp, *Proteus* sp, *Salmonella* spp,
   - **C. Gram Negative Coccobacilli bacteria** Like *Haemophillus influenzae*

B. Cultural Identification and Distinguishing of Human Pathogenic Bacteria:

Isolation of pathogenic bacteria frequently requires specialized media. Nonselective (non-inhibitory) media permit the growth of many microorganisms. Selective media contain inhibitory substances that permit the isolation of specific types of microorganisms. Differential Media that include ingredients, such as chemical indicators and differentiates between different organisms growing on the same plate.

Microbiological media which used in the differentiation of pathogenic bacteria are:

1. **Nutrient agar:**

- It is a simple medium that may be used in general purposes, (i.e. supports most non-fastidious bacteria, maintenance of strains) as well as a base for other specialized media.

2. Blood agar:

- Enriched media (Nutrient agar with 5% sheep blood).
- Cultivation of fastidious and non-fastidious bacteria. (Gram-positive and Gram- negative bacteria).
- Differential – especially for distinguishing between Streptococci groups. Identify hemolysis - Some bacteria secrete enzymes (hemolysins) that lyse red blood cells to produce either:

  Beta hemolysis: Enzymes lyse the blood cells completely, producing a clear area around the colony.

  Alpha hemolysis: Incomplete hemolysis produces a greenish discoloration around the colony

  Gamma hemolysis: No effect on the red cells.

3. MacConkey agar:

- Selective and differential medium.
- Selective media for Gram negative bacteria because contain bile salt and crystal violet which inhibit most Gram positive bacteria.
- Differentiate, use for differentiation between lactose fermenting (causes the neutral red indicator to make pink colonies) and non-lactose fermenting enteric bacteria (Pale colonies).

4. Eosin methylene blue agar:

- Selective and differential medium.
- Selective media for Gram negative bacteria because contain bile salt and two dyes eosin and methylene blue which inhibit most Gram positive bacteria
- Differentiate- Use for differentiation between lactose fermenting and non-lactose fermenting enteric bacteria. Bacteria that ferment the lactose produce acid which turns the colonies dark purple. In addition, certain lactose-fermenting produce flat, dark colonies with a green metallic sheen (*E. coli*). Other produce larger, mucoid colonies, often purple only in their center. Lactose non-fermenters are either colorless or amber.

5. Salmonella – Shigella agar:

- SS agar is a selective and differential medium used for isolation and differentiation of *Salmonella* and *Shigella* from clinical specimens.
- The selective agents are bile salts, and brilliant green dye, which inhibit Gram-positive organisms.
- The medium contains only lactose and thus differentiates organisms on the basis of lactose fermentation. The formation of acid on fermentation of lactose causes the neutral red indicator to make red colonies. Non lactose fermenting organisms are clear on the medium.
- SS agar contains sodium thiosulfate and ferric ammonium citrate allows the differentiation of organisms that produce H2S. Lactose fermenters, such as *E. coli*, have colonies which are pink with a precipitate, *Shigella* appears transparent or amber, and *Salmonella* appears transparent or amber with black centers.

6. Kligler Iron Agar:

- The fermentations of lactose and glucose, used to differentiate species of enterobacteria, result in acidification which makes phenol red (pH indicator) turn yellow.
- Microorganisms not fermenting lactose (*Salmonella* or *Shigella*) initially product a yellow slant due to the acidification resulting from glucose present in small quantities. When the glucose has been exhausted in the aerobic portion of the slant, the reaction becomes basic by oxidation of the acids produced, resulting in the phenomenon of a red color on the surface of the medium. This color does not appear in depth in the butt, where the color remains yellow.
- Bacteria fermenting lactose and glucose make the slant and the butt turn yellow because of the production of large quantities of acid. This is sufficient to maintain an acid pH on the surface.
- Microorganisms which ferment neither of these two sugars do not change the color of the medium.
- The production of H_2S is revealed in the base of the medium by the appearance of black iron sulfide, due to the reduction of thiosulfate in the presence of ferric citrate.
- The production of gas (H_2, CO_2) resulting from sugar fermentations is shown by the appearance of gas bubbles or by a fragmentation of the agar.

7. Mannitol salt agar:

- Selective and differential medium.
- Selective media for *Staphylococcus* sp. because contain 7.5% NaCl which inhibit most bacteria.
- Differentiate- Use for differentiation between pathogenic and non-pathogenic types. MSA contains the sugar mannitol and the pH indicator phenol red. Generally pathogenic staphylococci are able to ferment the mannitol, lowering the pH, and thereby turning the indicator yellow. The non-pathogenic staphylococci do not ferment mannitol and the medium remains pink.

C. Physiological Identification:

Colony and cellular morphology may permit preliminary identification. Growth characteristics under various conditions, utilization of carbohydrates and other substrates, enzymatic activity, immunoassays, and genetic probes are also used.