

Isolation and Identification of *Salmonella* and *Shigella***Characteristics of *Salmonella*:**

1. Gram-negative bacteria.
2. Rod-shaped.
3. Non-spore forming, motile with the use of their peritrichous flagella.
4. They produce hydrogen sulfide (H₂S).
5. Most of these bacteria do not ferment lactose.
6. They may cause salmonellosis infection when *Salmonella*-containing foods and drinks are consumed.
7. *Salmonella* belongs to the Enterobacteriaceae family.
8. They are not usually found in the human gut unless there is an infection.
9. Among all the members of Enterobacteriaceae family, *Salmonella* is characterized by the following:
 - a. Motile
 - b. Non-capsule forming
 - c. Non-lactose fermenter when it is grown on MacConkey agar or Deoxycholate Citrate agar (DCA). Therefore, it appears as yellow or colorless (pale) colonies with or without dark (black) centers since these bacteria may produce H₂S.

10. Both *Salmonella* and *Shigella* are Gram-negative, facultative anaerobes, non-spore forming, and non-lactose fermenter bacteria which produce pale colonies on the above media. However, these two bacterial genera could be distinguished as follows:

Basis of Comparison	<i>Salmonella</i>	<i>Shigella</i>
Shape	Rod-shaped	Slender bacilli
Motility	Motile with peritrichous flagella	Non-motile
H ₂ S Production	Produces H ₂ S	Doesn't produce H ₂ S
Growth on Differential Media	Colonies appear with black centers	Colonies do not appear with black centers
Toxic Production	Produces enterotoxin	Produces heat labile toxin referred to as Shiga toxin
Causes	Mainly causes Salmonellosis	Mainly causes Shigellosis

There are several methods for the isolation of both *Salmonella* and *Shigella*:

1. Concentration of bacteria:

A- Swab technique: The swab is made from a light cotton cloth as strip, 4.5 cm in length. The strip is wrapped on a cord, then the swab is placed directly in the water sample and left for 3-5 days. After that, the swab is placed in a plastic cooled bag with ice and taken to the laboratory within a period not more than 6 hours. Samples are inoculated on an enrichment medium.

B- Membrane filtrations technique: Several liters of water are filtered through membranous filters (0.45micron diameter), under sterile conditions. Filters are placed on an enrichment medium.

2. Growth in enrichment media:

Enrichment media are used for this method which is very important because pathogens usually are present in few numbers. Many of these media allow the growth of pathogenic bacteria and inhibit non-pathogens. These media are:

- A- Selenite broth: This medium allows the growth of *Salmonella* and inhibits the growth of coliforms.
- B- Tetrathionate broth: The growth of *Salmonella* on this medium could be improved either by adding brilliant green dye which prevents the growth of non-pathogenic bacteria or by adding L-cystine that induce *Salmonella* growth.
- C- Gram-negative broth: This medium is suitable for the growth of both *Salmonella* and *Shigella*. Furthermore, it inhibits coliforms and fecal streptococci. Incubation period of this medium should not exceed more than 24 hrs, since long incubation allows the growth of *Proteus* and *Pseudomonas*.

3. Growth in selective media:

A- Differential media: These media differentiate between lactose fermenter and non-lactose fermenter Gram-negative bacteria. The media are also selective for the growth of Gram-negative and suppress Gram-positive bacteria. Inhibitory substances available in these media are eosin and methylene blue dyes in EMB agar, while they are bile salt and crystal violet in MacConkey agar.

B- Selective media:

1. Brilliant green agar: Selective medium for *Salmonella*.
2. Bismuth sulfite agar: Selective medium for *Salmonella typhi*.

3-Xylose-Lysine-Deoxycholate agar (XLD): Selective medium for *Salmonella* and *Shigella*.

4- *Salmonella Shigella* agar (S-S agar): Selective medium for *Salmonella* and *Shigella*. It contains high concentrations of sodium citrate and bile salts in addition to brilliant green that all inhibit Gram-positive and some of Gram-negative bacteria including coliforms. It also contains sodium thiosulphate as a source of sulfur to detect H₂S production as black precipitate.

4- Biochemical tests.

5- Serological tests.