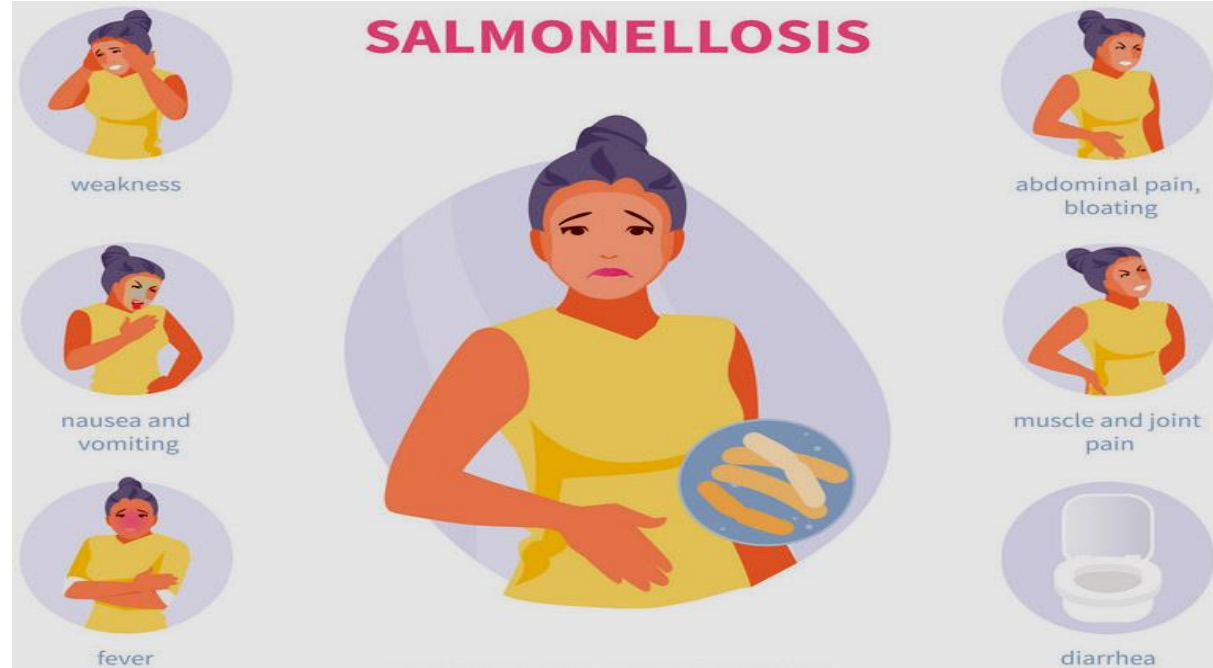


# **Enumeration Techniques of *Salmonella and Shigella***

✓ *Salmonella* cause diarrhoeal illness in people.

✓ **Salmonellosis:**



✓ Contaminated food, mainly of animal origin, is mode of transmission.

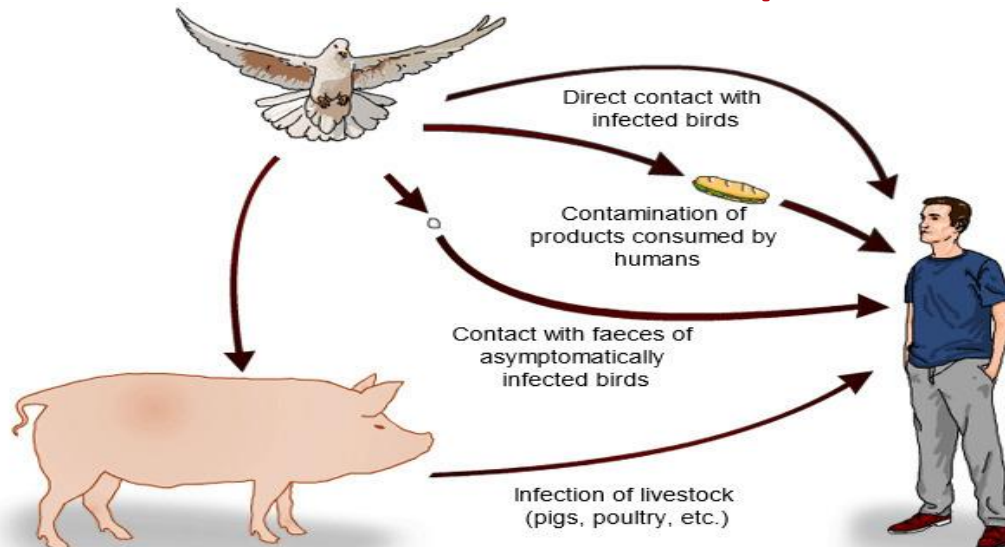
✓ Typhoid fever and enteritis.

✓ Outbreak of *Shigella sonnei* gastroenteritis reported.

✓ Fresh pasteurized milk cheese as the vehicle of infection.

# Transmission and Source of Infection of *Salmonella*

- *Salmonella* infections are zoonotic; they can be transmitted by humans to animals and vice versa.
- Infection via food is also possible.
- Transmitted to humans by eating foods contaminated with animal faeces.
- Contaminated foods usually of animal origin (beef, poultry, milk, or eggs), but any food including vegetables, may become contaminated.
- Cooking kills *Salmonella*.
- Food become contaminated by hands of an infected food handler who don't wash hands with soap.



# Isolation and Enumeration Principles of *Salmonella* and *Shigella*

Many methods used for examining these foods employ step of pre-enrichment, selective enrichment, differential and selective plating for isolation and identification of selected isolates.

**Pre-enrichment :** Twenty-five (25) g or ml of sample is added to 225 ml of buffered peptone water and incubated at 37°C for 24 h.

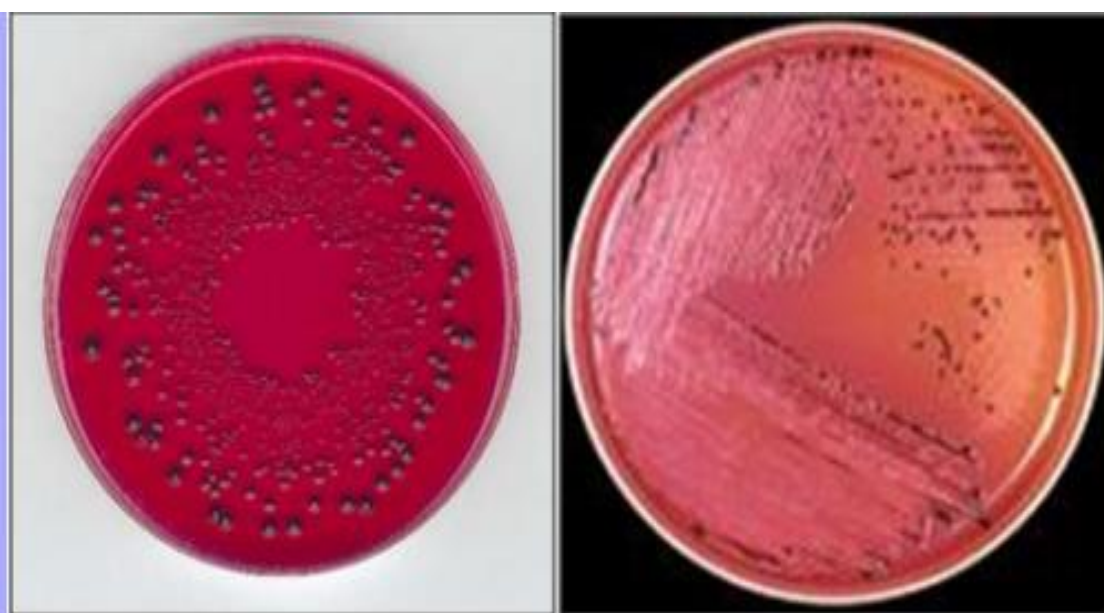
**Selective enrichment:** Transfer one ml portion from pre-enrichment step to each 10 ml of selenite eosine broth and tetrathionate broth and incubated at 37°C for overnight. Contents of both tubes mixed and loopful was streaked on xylose lysine deoxycholate agar (XLDA), bismuth sulphite agar (BSA) plate and Hektoen enteric agar (HEA). Plates were incubated at 37°C for 24 h and continued up to 72 h.

# Typical *Salmonella* and *Shigella* colonies on different selective media

## Xylose lysine deoxycholate (XLDA) agar:

- Developed to improve recovery of *Salmonella* and *Shigella* species.
- Lactose, sucrose, and xylose are fermentable carbohydrates present and phenol red is used as pH indicator.
- Bacteria that ferment none of these sugars, e.g., *Shigella*, appear as red, translucent colonies.
- Yellow colonies indicate rapid fermentation of lactose and acid pH, by *E. coli*.
- Since *Salmonella* ferment xylose as readily as coliforms, a second differential mechanism, lysine decarboxylase, is utilized. Those organisms that ferment xylose as well as decarboxylate lysine exhaust xylose rapidly and lysine reaction causes a pH reversal to alkaline reaction similar to *Shigella*.

- Sodium thiosulfate and ferric ammonium citrate are indicators of H<sub>2</sub>S production only when alkaline conditions exist; *Salmonella* will form red colonies with black center in 24 h.
- Sodium deoxycholate added to inhibit Gram-positive growth and retard growth of coliforms.
- *Salmonella* may produce pink colonies with or without glossy black centres/appear as complete black colonies.

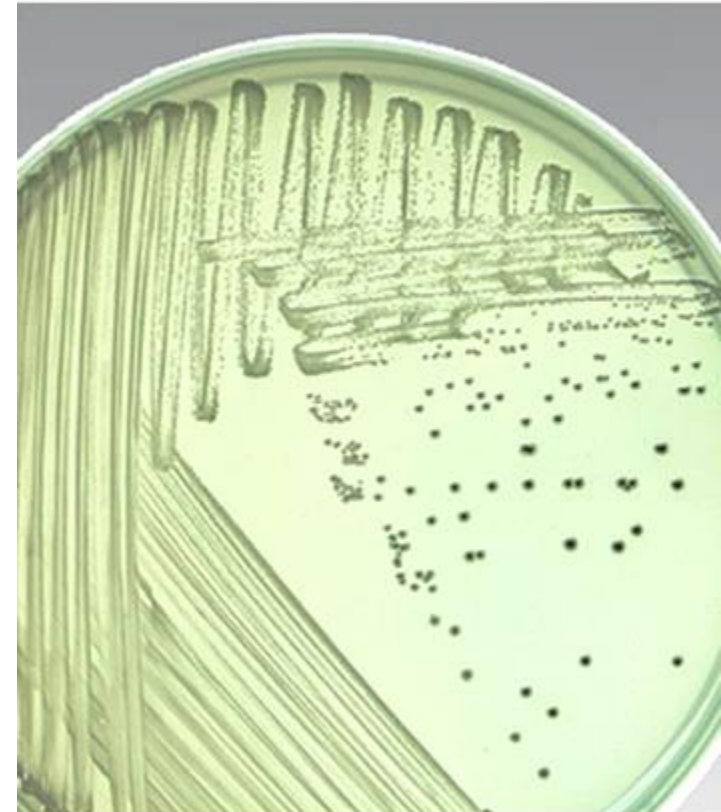


Pink and black color colony of *Salmonella* on XLD agar



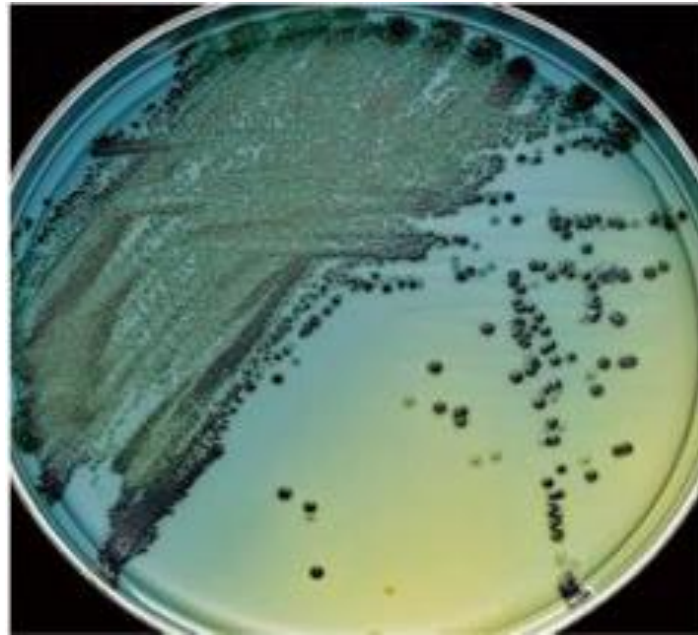
## Bismuth sulphite agar (BSA):

- Brown, grey, or black colonies; sometimes have metallic sheen.
- Surrounding medium is usually brown at first, but may turn black with increased incubation, producing halo effect.
- BSA is a modification of Wilson and Blair selective medium for isolation and preliminary identification of *Salmonella typhi* and other sp. from sewage, water supplies, food etc.
- Freshly precipitated bismuth sulphite acts together with brilliant green as selective agent by suppressing growth of coliforms, whilst permitting growth of Salmonellae.
- Sulphur compounds provide substrate for H<sub>2</sub>S production, whilst metallic salts in medium stain colony and surrounding medium black or brown in presence of hydrogen sulphide.

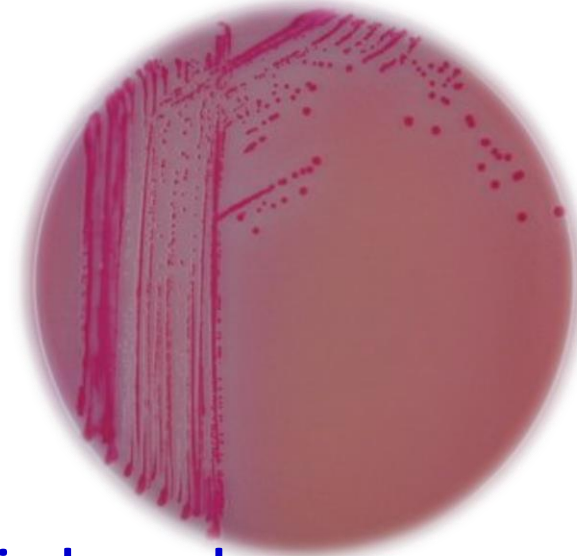


## Hektoen Enteric Agar (HEA):

- Increased content of peptone and three fermentable carbohydrates (lactose, sucrose, salicin) as sources of carbon and energy reduce inhibitory action of the bile salts on *Salmonella* and *Shigella* spp.
- Bromothymol blue and acid fushchin are pH indicators.
- Sodium thiosulphate provides sulphur and ferric ammonium citrate is indicator for H<sub>2</sub>S production.
- H<sub>2</sub>S positive colonies are blue-green to blue colonies with or without black center.







## **RAMBACH Agar (chromogenic medium):**

- Identify non-typhi *Salmonella* in foodstuffs and clinical samples.
- Nutritive substrates in RAMBACH Agar enable Enterobacteriaceae to multiply.
- Sodium deoxycolate inhibits the accompanying Gram-positive flora.
- RAMBACH Agar differentiates *Salmonella* by adding propylene glycol.
- Salmonellae form acid with propylene glycol, so that, in combination with pH indicator, colonies have characteristic red color.
- To differentiate coliforms from Salmonellae, medium contains chromogen indicating presence of  $\beta$ -galactosidase, a characteristic for coliforms.
- Coliform microorganisms grow as blue-green or blue-violet colonies.
- *Proteus*, *Pseudomonas*, *Shigella*, *S. typhi* and *S. paratyphi* grow as colorless to yellow colonies.

# Biochemical tests for detection of *Salmonella* and *Shigella*

Lysine-decarboxylase broth, phenol red dulcitol broth, TSI Agar slant, tryptone broth, potassium cyanide (KCN) broth, malonate broth, indole production test, phenol red sucrose broth, MRVP broth.

## Indole and H<sub>2</sub>S production:

1. Determine ability of organisms to degrade tryptophan to indole.
2. Tryptophan undergo oxidation by some bacteria possessing enzyme tryptophanase to indole.
3. Presence of indole is detected by adding Kovac's reagent composed of p-dimethyl amino benzaldehyde, butanol & hydrochloric acid. Indole is extracted from the medium into the reagent layer by the acidified butanol and forms complex with p-dimethyl amino benzaldehyde to give cherry red color.
4. Heavy metals are usually incorporated to detect H<sub>2</sub>S production.
5. Addition of 3-4 drops of Kovac's Indole reagent on surface after incubation indicated production of Indole from tryptophan.



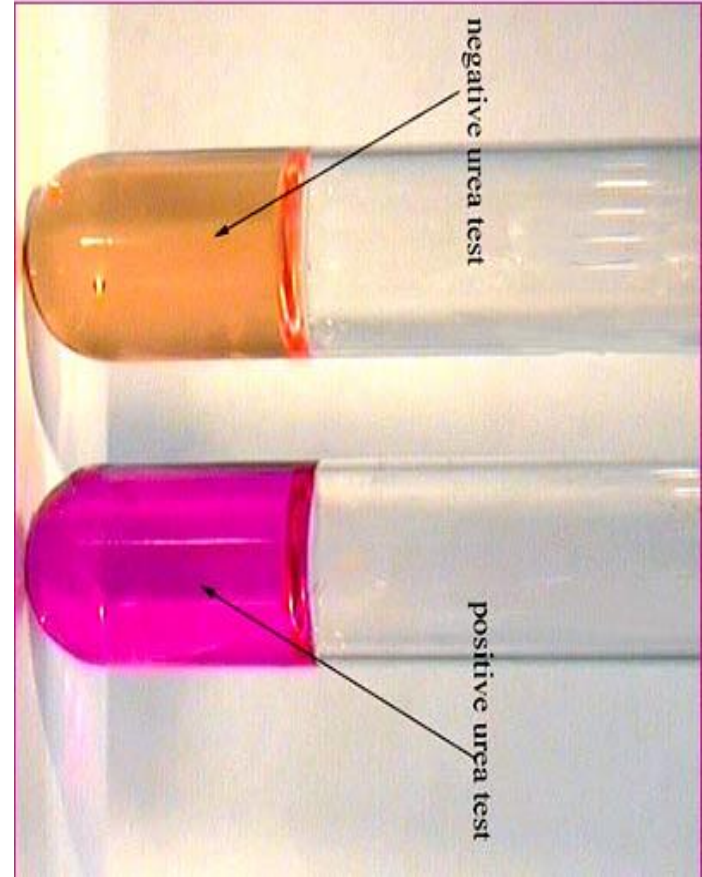
## Citrate utilization:

1. Streaking was done (SCA) from inoculum and incubated at 37°C for 24 h, color change from green to blue shows citrate utilization positive.
2. Determines ability of organism to use citrate as a source of carbon in absence of glucose & lactose this ability depends upon citrate permeases that facilitates its transport into cell.
3. Citrate is major intermediate of Kreb's cycle & produced by condensation of active acetyl CoA with oxaloacetic acid.
4. During its conversion to pyruvic acid, medium becomes alkaline as CO<sub>2</sub> generated combines with sodium and water to form Na<sub>2</sub>CO<sub>3</sub> whose presence is detected by the bromothymol blue indicator which undergoes color changes from green to deep blue.



## Urease test:

1. Depends upon ability of microorganism to degrade urea in presence of urease enzyme produced by bacteria and is indicated by phenol red indicator which undergoes color change from peach to pink.
2. Upon streaking on urease agar slant and on incubating it at  $37^{\circ}\text{C}$  for 24 h, color changes to pink indicate bacteria is urease positive.



## **Methyl red reduction (MR) Test):**

- 1. Determine the pathway to ferment glucose.**
- 2. Methyl red (MR) test determines whether or not large quantities of acid have been produced from mixed acid fermentation of glucose.**
- 3. End products of this pathway include lactic, acetic, formic and succinic acids.**
- 4. Voges-Proskauer (VP) test determines whether specific neutral metabolic intermediate, acetoin, has been produced instead of acid from glucose.**
- 5. Acetoin is last intermediate in butanediol pathway.**
- 6. pH indicator methyl red detects acidic end product by formation of red color.**
- 7. Tube of MR-VP broth is inoculated with suspected colony incubated at 37°C for 24 h.**
- 8. After incubation pour 2-3 drops of methyl red.**



## Triple sugar iron (TSI) Test:

1. Composed of three sugars viz. glucose, sucrose and lactose.
2. Ferrous sulphate and phenol act as indicator, employed for detection of fermentation of sugars, indicated by change in color due to production of organic acid.
3. Appearance of bubbles indicates production of gas from fermentation of sugar by an organism.
4.  $H_2S$  production is indicated by reduction of ferrous sulphate of medium to ferric sulphate (black precipitate).
5. TSI slant stabbed, streaked and incubated at  $37^\circ C$  for 24 h.
6. After incubation, sugar fermenting bacteria ferment sugars and may produce  $H_2S$ .



Reaction	Fermentation
Acid butt (yellow), alkaline Slant (red)	Glucose fermented
Acid throughout medium, butt and slant yellow	Lactose or Sucrose or both fermented
Gas bubbles in butt, medium sometimes split	Aerogenic culture
Blackening of the butt	Hydrogen sulfide
Alkaline slant and butt (medium entirely red)	None of the three sugars fermented

**Reactions observed on TSI slants**

Test or substrate	Result		<i>Sabmonella</i> species reaction	<i>Shigella</i> species reaction
	Positive	Negative		
Glucose (TSI)	Yellow Butt	Red Butt	+	-
Lysine decarboxylase (LIA)	Purple butt	Yellow butt	+	-
H <sub>2</sub> S (TSI)	Blackening	No blackening	+	-
Urease	Purple-red color	No color change	-	-
Phenol red dulcitol broth	Yellow color and/ or gas	No gas; no color change	+	-
KCN broth	Growth	No growth	-	+
Malonate broth	Blue color	No color change	-	+
Indole test	Violet color at surface	Yellow color at surface	-	±
Phenol red lactose broth	Yellow color and/ or gas	No gas; no color change	-	-
Phenol red sucrose broth	Yellow color and/ or gas	No gas; no color change	-	-
Voges-Proskauer test	Pink-to-red color	No color change	-	-
Methyl red test	Diffuse red color	Diffuse yellow color	+	+
Citrate utilization			+	+
P-phenyl Pyruvic acid			-	-

**Biochemical reactions of *Salmonella* and *Shigella***