TCBS Agar

Typical colonial morphology on TCBS Agar are as follows

Microorganisms	Characteristics
Vibrio cholera	Flat yellow colonies, 2-3 mm in diameter
Vibrio alginolyticus	Large yellow colonies
Vibrio fluvialis, Vibrio vulnificus	Yellow or translucent colonies
Vibrio parahaemolyticus	Colorless colonies with a green center
Pseudomonas, Aeromonas	Blue colonies
Enterobacteria or others	Tiny transparent colonies

Composition of TCBS Agar

Ingredients per liter of deionized water

Ingredients	Gms / Litre	Ingredients	Gms / Litre
Sucrose	20.0 gm	Oxbile (Oxgall)	5.0 gm
Dipeptone	10.0 gm	**Sodium Cholate**	3.0 gm
Sodium Citrate	10.0 gm	**Ferric Citrate**	1.0 gm
Sodium Thiosulfate	10.0 gm	**Bromothymol Blue**	0.04 gm
Sodium Chloride	10.0 gm	**Thymol Blue**	0.04 gm
Yeast Extract	5.0 gm	**Agar**	15.0 gm

Principle of TCBS Agar:

TCBS Agar is used for the selective isolation of ***Vibrio cholerae*** and other enteropathogenic vibrios. **Thiosulfate** and **sodium** citrate, as well as the alkalinity of the medium, considerably inhibit the growth of **Enterobacteria**. **Ox bile** and **sodium cholate** slow the growth of **enterococci** and **inhibit** the development of **Gram-positive** bacteria. The **acidification** of the medium resulting from the fermentation of sucrose by Vibrio makes **bromthymol blue** turn yellow. **Bromthymol Blue** and **Thymol Blue** are pH indicators. Using thiosulfate as a sulfur source, the production of **hydrogen sulfide** is visualized in the presence of **ferric citrate**. **Yeast extract** and **peptone** provides the **nitrogen**, **vitamins**, and **amino** acids in TCBS Agar. Sodium chloride provide optimum growth and metabolic activity of halophilic Vibrio spp. Agar is a Solidifying agent.

- **Do not autoclave.**

Salmonella -Shigella Agar

Composition of Salmonella Shigella Agar

Ingredients	Gms / Litre	Ingredients	Gms / Litre
Beef Extract	5.00	Sodium Thiosulfate	8.50
Enzymatic Digest of Casein	**2.50**	**Ferric Citrate**	**1.00**
Enzymatic Digest of Animal Tissue	**2.50**	**Brilliant Green**	**0.00033**
Lactose	**10.00**	**Neutral Red**	**0.025**
Bile Salts	**8.50**	**Agar**	**13.50**
Sodium Citrate	**8.50**	**…….**	**……**

- **Do not autoclave.**

Principle of Salmonella Shigella Agar:

The inclusion of **Bile Salts**, **Sodium Citrate** and **Brilliant Green** serve to **inhibit gram-positive**, **coliform organisms** and **inhibit swarming *Proteus spp***., while allowing ***Salmonella spp***. to grow. **Beef Extract, Enzymatic** Digest of Casein, and Enzymatic Digest of Animal Tissue provide sources of nitrogen, carbon, and vitamins required for organism growth. **Lactose** is the **carbohydrate** present in **Salmonella Shigella Agar**. **Thiosulfate** and **Ferric Citrate** permit detection of **hydrogen sulfide** by the production of colonies with black centers. Neutral red turns red in the presence of an acidic pH, thus showing fermentation has occurred.

- **Salmonella will not ferment lactose, but produce hydrogen sulfide (H2S) gas. The resulting bacterial colonies will appear colorless with black centers.**
- **Shigella do not ferment lactose or produce hydrogen sulfide gas, so the resulting colonies will be colorless.**
- **Coliform bacteria such as E. coli will ferment the lactose in the media, resulting in bacterial growth with a pink color. They do not produce any hydrogen sulfide.**
- **Enterobacter and Klebsiella appears larger than E. coli, mucoid, pale, opaque cream to pink.**

Quality Control on Salmonella Shigella Agar:

Positive:

***Salmonella enteriditis* ATCC 13076= Colorless colonies with black center**

***Salmonella typhi* ATCC 6539 = Colorless colonies with black center**

***Salmonella typhimurium* ATCC 14028= Colorless colonies with black center**

***Shigella flexneri* ATCC 12022 = Colorless colonies**

MacConkey agar (MAC)

Composition of MacConkey Agar

Ingredients	Gms / Litre	Ingredients	Gms / Litre
Peptone (Pancreatic digest of gelatin)	**17 gm**	**Crystal Violet**	**0.001 g**
Proteose peptone (meat and casein)	**3 gm**	**Agar**	**13.5 gm**
Lactose monohydrate	**10 gm**	**Distilled Water**	**Add to make 1 Liter**
Bile salts	**1.5 gm**		
Sodium chloride	**5 gm**		
Neutral red	**0.03 gm**		

Principle of MacConkey Agar:

MacConkey agar is used for the isolation of **gram-negative** enteric bacteria and the differentiation of **lactose fermenting** from lactose **non-fermenting gram-negative** bacteria. Pancreatic digest of gelatin and peptones (meat and casein) provide the essential nutrients, vitamins and nitrogenous factors required for growth of microorganisms. Lactose monohydrate is the fermentable source of carbohydrate. The selective action of this medium is attributed to **crystal violet** and **bile salts**, which are **inhibitory** to most species of **gram-positive** bacteria. **Sodium chloride maintains the osmotic balance in the medium**. Neutral red is a pH indicator that turns red at a pH below 6.8 and is colorless at any pH greater than 6.8. Agar is theis the solidifying agent.

Result Interpretation on MacConkey Agar:

- Lactose fermenting strains grow as red or pink and may be surrounded by a zone of acid precipitated bile. The red colour is due to production of acid from lactose, absorption of neutral red and a subsequent colour change of the dye when the pH of medium falls below 6.8.
- Lactose non-fermenting strains, such as Shigella and Salmonella are colourless and transparent and typically do not alter appearance of the medium. Yersinia enterocolitica may appear as small, non-lactose fermenting colonies after incubation at room temperature.

Organism	Colour	Remarks
Escherichia coli	red/pink	non-mucoid
Aerobacter aerogenes	pink	mucoid
Enterococcus species	red	minute, round
Staphylococcus species	pale pink	opaque
Pseudomonas aeruginosa	green-brown	fluorescent growth