BW048	**TRIPLE SUGAR IRON AGAR**

Formula

Ingredients :	**gms/lit.**
Peptic digest of animal tissue	20.00
Yeast extract	3.00
Meat extract	3.00
Lactose	10.00
Sucrose	10.00
Dextrose	1.00
Sodium chloride	5.00
Ferric sulphate heptahydrate	0.20
Sodium thiosulphate,pentahydrate	0.30
Ammonium iron(III) citrate	0.30
Phenol red	0.024
Agar	12.00

Final pH (at 25°C) : 7.4 ± 0.2

Directions :

Suspend 65 gms.in 1000 ml. distilled water. Heat to boiling to dissolve the medium completely. Mix well and distribute into test tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the medium to set in sloped form with a butt about 1 inch long.

Principle :

Beef extract, Yeast extract, Peptic digest of animal tissue and Casein enzyme hydrolysate provide nitrogen, vitamins, and minerals. Triple sugar iron agar contains three carbohydrates (dextrose, lactose and surcrose). When these carbohydrates are fermented, the resulting production of acid is detected by the phenol red indicator. The colour changes that result are yellow for acid production and red for alkalinization. Sodium thiosulfate is reduced to hydrogen sulfide. Hydrogen sulfide then reacts with an iron salt yielding the typical black iron sulfide. Sodium chloride maintains the osmotic balance of the medium. Agar is a solidifying agent.

QC Tests – (I)Dehydrated Medium	
Colour :	Light pink
Appearance :	Homogeneous Free Flowing powder
(II)Rehydrated medium	
PH (post autoclaving/heating) :	7.4 ± 0.2
Colour (post autoclaving/heating) :	Pinkish red
Clarity (post autoclaving/heating) :	Clear to slightly opalescent
(III)Q.C. Test Microbiological	

Cultural characteristics observed after 18 – 48 hrs.at 35- 37°C.

MICROORGANISM (ATCC)	GROWTH	SLANT	BUTT	GAS	H_2S
Citrobacter freundii (8090)	Luxuriant	A	A	+	+
Enterobacter aerogenes (13048)	Luxuriant	A	A	+	-
Escherichia coli (25922)	Luxuriant	A	A	+	-
Klebsiella pneumoniae (13883)	Luxuriant	A	A	+	-
Proteus vulgaris (13315)	Luxuriant	K	A	-	+
Salmonella paratyphi A	Luxuriant	K	A	+	-
Salmonella typhi (6539)	Luxuriant	K	A	-	+
Salmonella typhimurium (14028)	Luxuriant	K	A	+	+
Shigella flexneri (12022)	Luxuriant	K	A	-	-

Key : A = acidic, yellow K = alkaline, no change
+ = blackening (H_2S), positive reaction
- = no reaction.

Refer disclaimer Overleaf

Rev: December 2020

Precautions :	1. For Laboratory Use.				
	2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.				
Limitations :	1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.				
	2. Hydrogen sulfide production may be evident on Kligler Iron Agar but negative on Triple Sugar Iron Agar. Studies by Bulmash and Fulton showed that the utilization of sucrose could suppress the enzymatic mechanisms responsible for H_2S production. Padron and Dockstader found that not all H_2S – positive Salmonella are positive on TSI.				
	3. Sucrose is added to TSI to eliminate some sucrose – fermenting non – lactose fermenters such as Proteus and Citrobacter spp.				
	4. Further biochemical tests and serological typing must be performed for definite identification and confirmation of organisms.				
	5. Do not use an inoculating loop to inoculate a tube of Triple Sugar Iron Agar. While stabbing the butt, mechanical splitting of the medium occurs, causing a false positive result for gas production.				
	6. A pure culture is essential when inoculating Triple Sugar Iron Agar. If inoculated with a mixed culture, irregular observations may occur.				
	7. Tubes should be incubated with caps loosened. This allows a free exchange of air, which is necessary to enhance the alkaline condition on the slant.				
Use :	To differentiate enteric pathogens by ability to determine carbohydrate fermentation and hydrogen sulphide production.				
Storage :	Dehydrated medium- below 30°C Prepared medium– Between 2 to 8°C.				
Packing :	500 gm. bottle				
Product profile:	Reconstitution	Quantity on Preparation (500g)	pH (25°C)	Supplement	Sterilization
BW048	65g/l	7.692L	7.4 ± 0.2	NIL	121^0C /15 min.

Rev: December 2020