B1116I	TRIPLE SUGAR IRON AGAR
Formula	
Ingredients:	gms/lit.
Peptic digest of animal tissue	20.00
Yeast extract	3.00
Meat Extract B#	3.00
Lactose	10.00
Sucrose	10.00
Dextrose	1.00
Sodiumchloride	5.00
Ferric citrate	0.30
Sodium thiosulphate	0.30
Phenol red	0.024
Agar	12.00
#-Equivalent to Beef extract	
Final pH(at25°C): 7.4±0.2	

Directions:

Suspend 64.62 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Mix well and distribute in to 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. Note: For better results, the medium can be sterilized by autoclaving at 10 lbs pressure (115°C) for 15 minutes

Principle:

Triple Sugar Iron Agar was originally proposed by Sulkin and Willett and modified by Hajna for identifying *Enterobacteriaceae*. This medium complies with the recommendation of APHA, for the examination of meat and food products, for the examination of milk and dairy products and for microbial limit test for confirming the presence of *Salmonella* and in the identification of gram-negative bacilli. ISO Committee has recommended a slight modification in the original medium for the identification of *Salmonella*. Peptone, yeast extract and meat extract provide nitrogenous compounds, sulphur, trace elements and vitamin B complex etc. Sodium chloride maintains osmotic equilibrium. Lactose, sucrose and dextrose(glucose) are the fermentable carbohydrates. Sodium thiosulphate and ferrous ions make H2S indicator system. Phenol red is the pH indicator. Organisms that ferment glucose produce a variety of acids, turning the colour of the medium from red to yellow. More number of acids are liberated in butt (fermentation) than in the slant (respiration). Growing bacteria also form alkaline products from the oxidative decarboxylation of peptone and these alkaline products neutralize the large amounts of acid present in the butt. Thus, the appearance of an alkaline (red) slant and an acid (yellow) butt after incubation indicates that the organism is a glucose fermenter but is unable to ferment lactose and/or sucrose. Bacteria that ferment lactose or sucrose (or both), in addition to glucose, produce large amounts of acid enables no reversion of pH in that region and thus bacteria exhibit an acid slant and acid butt. Gas production (CO2) is detected by the presence of cracks or bubbles in the medium, when the accumulated gas escapes. Thiosulphate is reduced to hydrogen sulphide by several species of bacteria and H2S combines with ferric ions of ferric salts to produce the insoluble black precipitate of ferrous sulphide. Reduction of thiosulphate proceeds only in an acid environment and blackening usually occurs in the butt of the tube. Triple Sugar Iron Agar should be used in

parallel with Urea Agar/Broth to distinguish between *Salmonella* and *Proteus* species. The reactions can be summarized as follows:
Alkaline slant / acid butt-only glucose fermented
Acid slant / acid butt-glucose and sucrose fermented or glucose and lactose fermented or all the three sugars, glucose, lactose and sucrose fermented.
Bubbles or cracks present-gas production, Black precipitate present-H2S gas production

QC Tests–(I)Dehydrated Medium						
	Colour:	Light yellow to 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	+	-
	Escherichia coli (8739)	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.					
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 forH_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:	Recommended for identification of members of *Enterobacteriaceae* especially *Salmonella* species. The composition and performance criteria of this medium are as per the specifications laid down in ISO 1993, Draft ISO DIS 6579-1:2017				
Storage:	Dehydrated medium-below30°C Prepared medium–Between 2 to 8°C.				
Packing:	500gm. bottle				
Product profile:	Reconstitution	Quantity on Preparation (500g)	pH (25°C)	Supplement	Sterilization
B048	64.62g/l	7.73L	7.4±0.2	Nil	121^{0}C/15 min.

Disclaimer:

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