Triple Sugar Iron (TSI) Agar (NCM0144)

Intended Use

Triple Sugar Iron (TSI) Agar is used for the differentiation of microorganisms on the basis of dextrose, lactose, and sucrose fermentation and hydrogen sulfide production. Triple Sugar Iron (TSI) Agar is not intended for use in the diagnosis of disease or other conditions in humans.

Description

This is a modification of the Krumwiede and Kohn medium of 1917 which differentiates some of the *Enterobacteriaceae* on the basis of four reactions; fermentation of lactose, glucose and sucrose and H2S production. This medium should be used in conjunction with a urease test to eliminate *Proteus* spp. when screening for *Salmonella* spp.

Typical Formulation

Beef Extract	3.0 g/L
Yeast Extract	3.0 g/L
Peptone Mixture	20.0 g/L
Sodium Chloride	5.0 g/L
Lactose	10.0 g/L
Sucrose	10.0 g/L
Glucose	1.0 g/L
Ferric Citrate	0.3 g/L
Sodium Thiosulphate	0.3 g/L
Phenol Red	0.025 g/L
Agar	12.0 g/L

pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

Refer to SDS

Preparation

1. Suspend 65 grams of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Dispense into tubes.
4. Autoclave at 121°C for 15 minutes
5. Allow to set as a slope ensuring that the slant is over a butt approximately 3 cm deep.

Test Procedure

For specific procedures, refer to appropriate references using Triple Sugar Iron Agar.

Note: It is recommended to streak only half way up the prepared slant to avoid reversion of sugar to an alkaline reaction (pink/red) in the thin tip of the slant.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is red and clear with no precipitate.

Expected Cultural Response: Cultural response in Triple Sugar Iron Agar at 37 ± 1°C after 18 – 24 hours of incubation.

Effective Date: 4/10/2019

Revision: 0

Microorganism	Approx Inoculum	Expected Results				
		Recovery	Slant	Butt	Gas	H_2S
Escherichia coli ATCC® 25922	Heavy	Pos.	A	A	+	-
Proteus mirabilis ATCC® 12453	Heavy	Pos.	K	A	-	+
Salmonella typhimurium ATCC® 14028	Heavy	Pos.	K	A	+/-	+
Shigella flexneri ATCC® 12022	Heavy	Pos.	K	A	-	-
Shigella sonnei NCTC 8574	Heavy	Pos.	K	A	-	-

The organism listed is the minimum that should be used for quality control testing.
*KEY: A= acid, K= alkaline, + = positive, - = negative, Pos. for recovery = growth

Results
An alkaline slant-acid butt (red/yellow) indicates fermentation of dextrose only. An acid slant/acid butt (yellow/yellow) indicates fermentation of dextrose, lactose and/or sucrose. An alkaline slant-alkaline butt (red/red) indicates dextrose or lactose were not fermented (non-fermenter). Cracks, splits, or bubbles in medium indicate gas production. A black precipitate in butt indicates hydrogen sulfide production.

Expiration
Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of Procedure
1. Padron and Dockstader found not all H_2S positive *Salmonella* are positive on TSI.
2. Sucrose is added to TSI to eliminate some sucrose-fermenting non-lactose fermenters, such as Proteus and Citrobacter spp.
3. Do not use inoculating loop to inoculate a tube of Triple Sugar Iron Agar. While stabbing butt, mechanical splitting of medium occurs, causing a false positive result for gas production.
4. It is recommended to streak only half way up the prepared slant to avoid reversion of sugar to an alkaline reaction (pink/red).

Storage
Store dehydrated culture media at 2-30°C away from direct sunlight. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

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