TRIPLE SUGAR IRON AGAR (7162)

Intended Use

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

Product Summary and Explanation

In 1911, Russell described the use of two sugars in a medium to differentiate Gram-negative organisms of intestinal origin.[1] Lead or iron salts were added to Russell's medium to detect the presence of hydrogen sulfide. Kligler added lead acetate to Russell Double Sugar Agar, resulting in a medium capable of differentiating typhoid, paratyphoid, and dysentery.[2,3] A modification of this medium was developed, Kligler Iron Agar, using Phenol Red as an indicator and iron salts to detect hydrogen sulfide production. In 1940, Sulkin and Willett described a triple sugar ferrous sulfate medium for use in identification of enteric organisms.[4] Triple Sugar Iron Agar is essentially the formula originally described by Sulkin and Willett.[4]

Triple Sugar Iron Agar is recommended for differentiation of enteric, Gram-negative bacilli from dairy samples and food products.[5-7]

Principles of the Procedure

Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, and Yeast Enriched Peptone provide the nitrogen, carbon, and vitamins required for organism growth. Triple Sugar Iron Agar contains three carbohydrates, Dextrose, Lactose and Sucrose. When the carbohydrates are fermented, acid production is detected by the Phenol Red pH indicator. Sodium Thiosulfate is reduced to hydrogen sulfide, and hydrogen sulfide reacts with an iron salt yielding the typical black iron sulfide. Ferric Ammonium Citrate is the hydrogen sulfide (H_2S) indicator. Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Casein	5 g
Enzymatic Digest of Animal Tissue	5 g
Yeast Enriched Peptone	10 g
Dextrose	1 g
Lactose	10 g
Sucrose	10 g
Ferric Ammonium Citrate	0.2 g
Sodium Chloride	5 g
Sodium Thiosulfate	0.3 g
Phenol Red	0.025 g
Agar	13.5 g

Final pH: 7.3 ± 0.2 at 25°C

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

Precautions

1. For Laboratory Use Only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 60 g of the medium in one liter of purified water.
3. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Dispense into tubes and autoclave at 121°C for 15 minutes.
4. After autoclaving, allow medium to solidify in a slanted position.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light pink-beige to beige.

Prepared Appearance: Prepared medium is reddish-orange and trace to slightly hazy.

Expected Cultural Response: Cultural response in Triple Sugar Iron Agar at 35 ± 2°C after 18 – 24 hours of incubation.

Microorganism	Approx. Inoculum (CFU)	Expected Results				
		Recovery	Slant	Butt	Gas	H_2S
Escherichia coli ATCC® 25922	Heavy	growth	A	A	+	-
Proteus mirabilis ATCC® 12453	Heavy	growth	K	A	-	+
Pseudomonas aeruginosa ATCC® 27853	Heavy	growth	K	K	-	-
Salmonella typhimurium ATCC® 14028	Heavy	growth	K	A	+/-	+
Shigella flexneri ATCC® 12022	Heavy	growth	K	A	-	-

The organisms listed are the minimum that should be used for quality control testing.
KEY: A, acid, K, alkaline, +, positive, -, negative, +/-, usually negative, positive, growth

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.

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.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

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 the Procedure

1. Padron and Dockstader[8] 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.[9]
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.[9]
4. It is recommended to streak only half way up the prepared slant to avoid reversion of sugar to an alkaline reaction (pink/red).

Packaging

Triple Sugar Iron Agar	**Code No.**	**7162A**	**500 g**
		7162B	**2 kg**
		7162C	**10 kg**

References

1. **Russell, F. F.** 1911. The isolation of typhoid bacilli from urine and feces with the description of a new double sugar tube medium. J. Med. Res. **25**:217.
2. **Kligler, I. J.** 1917. A simple medium for the differentiation of members of the typhoid-paratyphoid group. Am. J. Public Health **7**:1042-1044.
3. **Kligler, I. J.** 1918. Modifications of culture media used in the isolation and differentiation of typhoid, dysentery, and allied bacilli. J. Exp. Med. **28**:319-322.
4. **Sulkin, S. E., and J. C. Willett.** 1940. A triple sugar-ferrous sulfate medium for use in identification of enteric organisms. J. Lab. Clin. Med. **25**:649-653.
5. **P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
6. **Marshall, R. T. (ed.).** Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
7. **Bacteriological Analytical Manual,** 8th ed. AOAC International, Gaithersburg, M.D.
8. **Padron, A. P. and W. B. Dockstader**. 1972. Selective medium for hydrogen sulfide production. Appl. Microbiol. **23**:1107.
9. **MacFaddin, J. F.** Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.

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