Peptone Iron Agar

Intended Use

Peptone Iron Agar is used for detecting hydrogen sulfide production by microorganisms.

Summary and Explanation

Levine and co-workers^{1,2} described a medium containing proteose peptone and ferric citrate for detection of hydrogen sulfide production by coliform bacteria. They demonstrated that such a medium served to differentiate strains that were Voges-Proskauer negative, methyl-red positive and citrate positive from other members of the *Enterobacteriaceae*.

User Quality Control

Identity Specifications Difco™ Peptone Iron Agar

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Dehydrated Appearance:	Light beige, free-flowing, homogeneous.		
Solution:	3.6% solution, soluble in purified water upon boiling. Solution is light amber, very slightly to slightly opalescent.		
Prepared Appearance:	Light amber, slightly opalescent.		
Reaction of 3.6% Solution at 25°C:	рН 6.7 ± 0.2		

Cultural Response Difco[™] Peptone Iron Agar

Prepare the medium per label directions. Stab inoculate tubes with fresh cultures and incubate at 35 ± 2 °C for 18-48 hours.

ORGANISM	ATCC™	RECOVERY	H ₂ S PRODUCTION
Enterobacter aerogenes	13048	Good	_
Escherichia coli	25922	Good	_
Proteus vulgaris	6380	Good	+
Salmonella enterica subsp. enterica serotype Enteritidis	13076	Good	+



Levine reported that ferric citrate was a much more sensitive indicator of hydrogen sulfide production than lead acetate, producing a medium that gave definite reactions within 12 hours. Peptone Iron Agar is a modification of Levine's original formula in which peptone has been included with proteose peptone and the more soluble ferric ammonium citrate is used in place of ferric citrate.

Tittsler and Sandholzer³ compared Peptone Iron Agar with lead acetate agar for the detection of hydrogen sulfide and found that Peptone Iron Agar had the advantage of giving earlier reactions and clearer results.

Principles of the Procedure

Peptones are the nitrogen sources in Peptone Iron Agar. Ferric ammonium citrate and sodium thiosulfate are used to detect H_2S production. Sodium glycerophosphate is a buffering compound. Agar is the solidifying agent.

Formula

Difco[™] Peptone Iron Agar

Approximate Formula* Per Liter		
Peptone	15.0	g
Proteose Peptone	5.0	g
Ferric Ammonium Citrate	0.5	g
Sodium Glycerophosphate	1.0	g
Sodium Thiosulfate		g
Agar	15.0	q
*Adjusted and/or supplemented as required to meet performance criteria.		5

Directions for Preparation from Dehydrated Product

- 1. Suspend 36 g of the powder in 1 L of purified water. Mix thoroughly.
- 2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
- 3. Autoclave at 121°C for 15 minutes.
- 4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

- 1. Obtain a pure culture of a test organism. Pick the center of a single colony with an inoculating needle.
- Inoculate a tube of Peptone Iron Agar by the stab method. Stab the needle to within 1/4 to 1/2 inch of the bottom. Withdraw the needle following the initial line of inoculation.
- 3. Incubate tubes at $35 \pm 2^{\circ}$ C for 18-48 hours.
- 4. Read tubes for growth and hydrogen sulfide production.

Expected Results

Any blackening of the medium along the line of inoculation or throughout the butt indicates hydrogen sulfide production.

For a complete discussion of the identification of coliform bacteria, refer to appropriate references.⁴⁻⁶



References

- Levine, Vaughn, Epstein and Anderson. 1932. Proc. Soc. Exp. Biol. Med. 29:1022.
 Levine, Epstein and Vaughn. 1934. Am. J. Public Health 24:505.
 Tittsler and Sandholzer. 1937. Am. J. Public Health 27:1240.
 Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
 Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
 Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco[™] Peptone Iron Agar

Cat. No. 289100 Dehydrated - 500 g

