Cystine Heart Agar

Intended Use

Cystine Heart Agar is used with hemoglobin for cultivating *Francisella tularensis* and without enrichment for cultivating gram-negative cocci and other microorganisms.

Summary and Explanation

Francisella tularensis was first described in humans in 1907.[1] Several media formulations were employed to isolate this microorganism. Initial formulations contained egg or serum and were difficult to prepare. Edward Francis,[2] who dedicated his career to the study of this organism, reported that blood dextrose cystine agar was a satisfactory medium for cultivating this fastidious pathogen. Shaw[3] added 0.05% cystine and 1% dextrose to Heart Infusion Agar for the cultivation of *F. tularensis*.

While experimenting with Francis' blood dextrose cystine agar, Rhamy[4] added hemoglobin to Cystine Heart Agar to develop a satisfactory medium for growth of *F. tularensis*.

Cystine Heart Agar, also known as Cystine Glucose Blood agar, is the historical medium of choice for isolating *F. tularensis*.[2]

Principles of the Procedure

Infusions from beef heart, peptone and L-cystine provide nitrogen, vitamins and amino acids in Cystine Heart Agar. Dextrose is a carbon source. Sodium chloride maintains the osmotic balance and agar is the solidifying agent.

Enrichment with 2% hemoglobin provides additional growth factors. Without enrichment, Cystine Heart Agar supports excellent growth of gram-negative cocci and other pathogenic microorganisms. Rabbit blood and antimicrobial agents can be added to this medium.[5]

Formula

Difco™ Cystine Heart Agar

Approximate Formula* Per Liter

Beef Heart, Infusion from 500 g	10.0	g
Proteose Peptone	10.0	g
Dextrose	10.0	g
Sodium Chloride	5.0	g
L-Cystine	1.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions

Francisella tularensis is a Biosafety Level 2 pathogen that can be transmitted by aerosols or by penetration of unbroken skin.[5] Wearing of gowns, gloves and masks is advocated for laboratory staff handling suspected infectious material.[6]

Directions for Preparation from Dehydrated Product

Enriched Medium

1. Suspend 10.2 g of the powder in 100 mL 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. Cool to 50-60°C.
4. Add 100 mL sterile 2% hemoglobin solution and mix well. Use:
   - Hemoglobin Solution 2%; or,
   - Prepare a 2% hemoglobin solution as follows: Place 2 g of hemoglobin powder in a dry flask. Add 100 mL of cold purified water while agitating vigorously. Continue intermittent agitation for 10-15 minutes until solution is complete. Autoclave at 121°C for 15 minutes. Cool to 50-60°C.
5. Dispense into sterile Petri dishes or tubes.
6. Test samples of the finished product for performance using stable, typical control cultures.

Unenriched Medium

1. Suspend 51 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.

User Quality Control

Identity Specifications

Difco™ Cystine Heart Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	5.1% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent, may have fine precipitate.
Prepared Appearance:	Plain – Light to medium amber, slightly opalescent, may have a fine precipitate. With Hemoglobin – Chocolate, opaque.
Reaction of 5.1% Solution at 25°C:	pH 6.8 ± 0.2

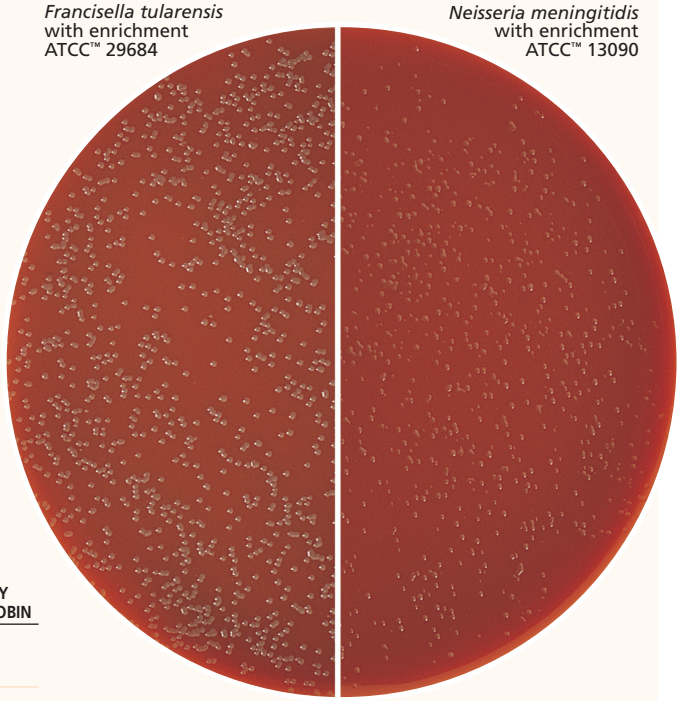
Cultural Response

Difco™ Cystine Heart Agar

Prepare the medium per label directions without and with hemoglobin. Incubate inoculated medium at 35 ± 2°C aerobically for 66-72 hours. Incubate *Neisseria meningitidis* under increased CO_2.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY W/O HEMOGLOBIN	RECOVERY W/HEMOGLOBIN
Francisella tularensis (BD 16223)*		10^2-10^3	N/A	Good
Neisseria meningitidis	13090	10^2-10^3	Good	Good
Staphylococcus aureus	25923	10^2-10^3	Good	Good
Streptococcus pneumoniae	6303	10^2-10^3	Good	Good

**Minimally, one strain of* F. tularensis *should be used for performance testing.* F. tularensis *ATCC 29684 can be substituted for BD Diagnostics strain 16223.*



Procedure

1. Inoculate and streak specimens as soon as possible. For a complete discussion on the inoculation and identification of *Francisella*, consult appropriate references.
2. Overgrowth by contaminating organisms can be reduced by incorporating 100-500 units penicillin per mL into the medium.[1]

Expected Results

Refer to appropriate references and procedures for results.

References

1. Wong and Shapiro. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
2. Francis. 1928. JAMA *91*:1155.
3. Shaw. 1930. Zentr. Bakt. I. Abt. Orig. *118*:216.
4. Rhamy. 1933. Am. J. Clin. Pathol. *3*:121.
5. Isenberg (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
6. U.S. Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. 2007. Biosafety in microbiological and biomedical laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington, D.C.

Availability

Difco™ Cystine Heart Agar

Cat. No. 247100 Dehydrated – 500 g

BBL™ Hemoglobin, Bovine, Freeze-Dried

Cat. No. 212392 Dehydrated – 500 g

BBL™ Hemoglobin Solution 2%

Cat. No. 211874 Bottle – 10 × 100 mL