Technical Information

Cystine Heart Agar Base

Product Code: DM 1172

Application: - Cystine Heart Agar when enriched with haemoglobin is recommended for the cultivation of Francisella tularensis. Without enrichment it supports excellent growth of gram-negative cocci and other pathogenic organisms.

Composition**

Ingredients	Gms / Litre
Beef heart, infusion from	500.000
Proteose peptone	10.000
Dextrose	10.000
Sodium chloride	5.000
L-Cystine	1.000
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Francisella tularensis the cause of tularaemia, a plague-like disease of rodents and other small organisms was first described in humans in 1907 [(1)]. The organisms are strict aerobes; fresh isolates fail to culture on ordinary medium but require a complex medium containing blood, or tissue extracts and cystine for their growth. Several media formulations were tried to isolate this microorganism. Blood Dextrose Cystine Agar, described by Francis [(2)] was found to be satisfactory for cultivating *F. tularensis.* Addition of 0.05% cystine and 1% dextrose to Heart Infusion Agar can also be employed for cultivation of F. tularensis! [(3)]. Subsequently haemoglobin was added to Cystine Heart Agar Base to develop a satisfactory cultivation medium for *F. tularensis* [(4)]. This medium is also known as Cystine Glucose Blood Agar and is the most suitable medium for isolating *F. tularensis* [(2)]. Hemoglobin provides additional nutrients and growth factors. This medium also supports growth of gram-negative cocci and other pathogenic microorganisms without additional enrichment. Cystine Heart Agar Base can be supplemented with Rabbit blood and antimicrobial agents [(5)].

This medium is a nutritionally rich medium, which may also be used for cultivating many other organisms generally difficult to grow.

Beef heart infusion and proteose peptone are sources of carbon, nitrogen, vitamins and minerals. Dextrose is an energy source. L-Cystine is the source of amino acid. Sodium chloride provides the essential ions. Overgrowth due to contaminating organisms can be reduced by incorporating 100-500 units penicillin per ml into the medium [(1)].

F. tularensis is a Biosafety Level 2 pathogen that can be transmitted by aerosols or by penetration of unbroken skin [(5)]. Use of PPE like gowns, gloves and masks is recommended for people handling suspected infectious material [(6)].

Methodology

Suspend 51 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. When to be enriched with haemoglobin (2%), suspend 10.2 grams of medium in 100 ml distilled water. Sterilize as above. Cool medium to 50°C and aseptically add 100 ml of 2% sterile haemoglobin solution. Mix well and pour into sterile Petri plates.

Quality Control

Physical Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Basal medium :Amber coloured clear to slightly opalescent gel After addition of 2% haemoglobin solution: Chocolate brown coloured opaque gel forms in Petri plates

Reaction

Reaction of 5.1% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH range 6.60-7.00

Cultural Response/ characteristices

DM 1172: Cultural characteristics observed with added 2% Haemoglobin after an incubation at 35-37°C for 40-48 hours.

Organism	Growth
Francisella tularensis ATCC 29684	luxuriant
Neisseria meningitidis ATCC 13090	luxuriant
Streptococcus pneumoniae ATCC 6303	luxuriant
Streptococcus pyogenes ATCC 19615	luxuriant

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

1. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Tenover F. C., Yolken R. H., (Ed.), 1999, 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, 1999, Biosafety in Microbiological and Biomedical Laboratories, 4th Ed., HHS Publication.

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