Cystine H Agar Base

M172

Intended use

Cystine H 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
HM infusion solids B#	10.000
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
-Equivalent to Beef heart infusion (solids)

Directions

Suspend 51 grams in 1000 ml purified/ distilled water. Heat to boiling 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.

Principle And Interpretation

Francisella tularensis is the cause of tularaemia, a plague-like disease of rodents and other small organisms. It was first described in humans in 1907 (4). The organisms are strict aerobes; fresh isolates cannot be cultured on ordinary medium but require a complex medium containing blood, or tissue extracts and cystine. Several media formulations were employed to isolate this microorganism. Blood Dextrose Cystine Agar, described by Francis (1) 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* (6). Subsequently haemoglobin was added to Cystine Heart Agar Base to develop a satisfactory cultivation medium for *F.tularensis* (5). This medium is also known as Cystine Glucose Blood Agar and is the most suitable medium for isolating *F.tularensis* (1). 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 (2).

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

HM infusion solids B 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 by contaminating organisms can be reduced by incorporating 100-500 units penicillin per ml into the medium (4).

F.tularensis is a Biosafety Level 2 pathogen that can be transmitted by aerosols or by penetration of unbroken skin (2). Wearing of gowns, gloves and masks is recommended for people handling suspected infectious material (7).

Type of specimen

Clinical samples - Blood

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,3).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Further biochemical and serological tests must be carried out for further identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

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

6.60-7.00

Cultural Response

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

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.
Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. Francis, 1928, JAMA, 91:1155.

2. Isenberg, (Ed.), 1992, Clinical Microbiology Procedures Handbook, Vol. 1. American Society for Microbiology, Washington, D.C.

3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

4. 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.

5. Rhamy, 1933, Am. J. Clin. Pathol., 3:121.

6. Shaw, 1930, Zentr. Bakt. I. Abt. Orig., 118:216.

7. 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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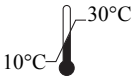
In vitro diagnostic medical device

CE Marking



Storage temperature

Do not use if package is damaged

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