

Technical Data

Glucose Cysteine Agar Base w/ Thiamine Intended Use:

M433

With added blood or haemoglobin or hemin, it is used for cultivation and enumeration of Francisella tularensis.

Composition**

Ingredients	g/L
HM peptone#	3.000
Soya peptone	10.000
Sodium chloride	5.000
L-Cysteine hydrochloride	1.000
Dextrose (Glucose)	25.000
Thiamine	0.0005
Agar	14.000
Final pH (at 25°C)	6.9 ± 0.2

^{**}Formula adjusted, standardized to suit performance parameters

Directions

Suspend 58.0 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. Cool to 45-50°C and aseptically add sterile packed erythrocytes at a final concentration of 2% or 4-5% defibrinated sheep/rabbit blood. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Francisella tularensis, a gram-negative aerobic bacillus, is the etiological agent of tularemia, which is primarily a disease of wild animals that is perpetuated in nature by ectoparasites, contaminated environment, cannibalism and acute or chronic carriers. Biting and blood sucking insects serve as vectors (1). Francisella (formerly known as Pasteurella) cannot be cultured on ordinary medium but require a complex medium containing blood or tissue extracts, thiamine and cysteine (2,3). Glucose Cysteine Agar Base w/ Thiamine when supplemented with blood / haemoglobin is recommended for cultivation and enumeration of F.tularensis (Pasteurella tularensis)(4).

HM peptone and soya peptone provide essential growth nutrients. Dextrose serves as an easily metabolisable carbohydrate source while sodium chloride maintains the osmotic balance. Thiamine and cysteine hydrochloride serves as growth factor promoters required for culturing *Pasteurella*. Minute droplet like colonies develops in 48 hours.

Type of specimen

Clinical samples - swabs or scrapings of skin lesions, pharyngeal swabs, sputum specimens, or gastric aspirates, depending on the form of illness; Water samples

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(7) After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions:

In Vitro diagnostic use. For professional 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:

F. tularensis is highly virulent and laboratory infections can be acquired through aerosols or droplets, hence clinical specimens must be handled with extreme caution and suspected specimens of containing F.tularensis should be handled following Biological Safety Level-2 (BSL-2) procedures.

Performance and Evaluation

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

[#] Equivalent to Meat peptone

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Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.4% Agar gel.

Colour and Clarity of prepared medium

Basal medium: Amber coloured, clear to slightly opalescent gel forms.

On addition of 4-5% sterile defibrinated sheep/rabbit blood:cherry red coloured opaque gel forms in Petri plates.

Reaction

Reaction of 5.8% w/v agueous solution at 25°C. pH : 6.9 ± 0.2

pН

6.70-7.10

Cultural Response

Cultural characteristics observed with added 4-5% defibrinated sheep blood after an incubation at $35-37^{\circ}$ C for 48-72 hours in presence of 10% CO₂

Organism

Growth

Francisella tularensis ATCCluxuriant 29684

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°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. 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 (5,6).

Reference

1.Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.

2.Collee J. G., Marmion B. P., Fraser A. G., and Simmons A., (Eds.), Mackie and McCartney Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone, New York.

3.Manual of Diagnostic Tests and Vaccine for Terrestrial Animals, 2004, 5th Edi, OIE World Organization for Animal Health.

4.Atlas R. M., 2004, Handbook of Microbiological Media, Lawrence C. Parks, (Ed.), 3rd Edition, CRC Press, pg. no 717.

5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

6.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.

7.Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

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HiMedia Laboratories Pvt. Limited, Plot No.C-40, Road No.21Y, MIDC, Wagle Industrial Area, Thane (W) -400604, MS, India



In vitro diagnostic medical device



Storage temperature



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package is damaged

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