Glucose Cysteine HiVeg™ Agar Base w/ Thiamine

MV433

Intended Use:

With addition of blood or haemoglobin or hemin, it is recommended for cultivation and enumeration of *Francisella tularensis*.

Composition**

Ingredients	g / L
HiVeg™ peptone No. 1	3.000
Soya peptone	10.000
Sodium chloride	5.000
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 sterile 4-5% defibrinated 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).

Glucose Cysteine HiVeg™ Agar Base w/ Thiamine is prepared by using vegetable peptones in place of animal based peptones which make the media free of BSE/TSE risks. HiVeg™ peptone No. 1 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

Water samples

Specimen Collection and Handling:

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

Warning and Precautions :

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. *F. tularensis* is highly virulent and laboratory infections can be acquired through aerosols or droplets, hence 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 the expiry period when stored at recommended temperature.

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 aqueous solution at 25°C. pH : 6.9±0.2

pH

6.70-7.10

Cultural Response

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

Organism	Growth
Francisella tularensis ATCC 29684	luxuriant

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Reference

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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. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater,23rd ed., APHA, Washington, D.C.
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