Dubos Oleic HiVeg™ Agar Base

MV179

Intended Use:

Recommended for preparation of solid agar media for plate or tube cultures of *Mycobacteria.*

Composition**

Ingredients	g / L
HiVeg™ hydrolysate	0.500
L-Asparagine	1.000
Potassium dihydrogen phosphate	1.000
Disodium hydrogen phosphate	2.500
Ferric ammonium citrate	0.050
Magnesium sulphate	0.010
Calcium chloride anhydrous	0.0005
Zinc sulphate	0.0001
Copper sulphate	0.0001
Agar	15.000
Final pH (at 25°C)	6.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 4 grams in 180 ml of 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°. Aseptically add 20 ml sterile Oleic Albumin Supplement (FD020) and 5,000 to 10,000 units of Penicillin to sterile, cooled 180 ml medium. Mix thoroughly and distribute in sterile tubes or plates.

Principle And Interpretation

Mycobacterium tuberculosis, the causative agent of tuberculosis in man, is carried in airborne particles known as droplet nuclei that are generated when patients with pulmonary tuberculosis cough. Infections occur when a susceptible person inhales the droplet nuclei containing the bacterium (1). Mycobacteria are generally isolated on medium containing either coagulated egg as base or on media containing agar. Middlebrook and Dubos media contain agar whereas Lowenstein media contain egg. The advantage of using agar is that accompanying contaminating proteolytic organisms does not liquefy the medium. Agar medium are generally recommended for testing samples obtained from non-sterile sites (2). Agar containing media can be made selective by the addition of antibiotics since the media are solidified by addition of agar and not by inspissation as against egg containing media. Dubos and Middlebrook (3) recommended Dubos Oleic Broth Base for the primary isolation and subsequent cultivation of the tubercle bacilli. On comparative studies of various media, Dubos Oleic Agar Base was found to be superior to other media for the primary isolation of the bacterium (4,5). Dubos Oleic HiVeg™ Agar Base is prepared by using vegetable peptones in place of animal based peptones which make the media free of BSE/TSE risks. Dubos media contain tryptone and L-aspargine as sources of nitrogen. The phosphates (together with calcium chloride) buffer the media as well as serve as sources of phosphates. Magnesium sulphate, zinc sulphate, copper sulphate and ferric ammonium citrate provide trace metals and sulphates. Dubos Oleic Agar is prepared without glycerol or dextrose to avoid growth of commensals. Standard procedures for the isolation of Mycobacteria from test samples should be followed (5). The specimen should be appropriately decontaminated before culturing as per standard methods (1,2,6 and 7).

Type of specimen

Clinical samples : Sputum

Specimen Collection and Handling

Standard procedures for the isolation of Mycobacteria from test samples should be followed (5). The specimen should be appropriately decontaminated before culturing as per standard methods (1,2,6,7). 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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Proper aerobic conditions and increased CO_2 tension were not provided during incubation, it may lead to negative result.

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

Light yellow to brownish yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light amber coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 2.0% w/v aqueous solution at 25°C. pH : 6.6±0.2

pH

6.40-6.80

Cultural Response

Cultural characteristics observed in presence of 5-10% CO2, with added sterile Oleic Albumin Supplement(FD020) and 5,000-10,000 units of Penicillin at 35-37°C upto 7 days. Further growth may be observed for 2-4 weeks

Organism	Growth	Colony Morphology
Mycobacterium avium ATCC 25291	luxuriant	smooth, thin, non-pigmented colonies
Mycobacterium gordonae ATCC 14470	luxuriant	smooth, yellow to orange colonies which are occasionally rough
Mycobacterium kansasii ATCC 12478	luxuriant	photochromogenic with flat,smooth/ somewhat granular surface slightly undulating margins
M. tuberculosis H37 Rv (25618)	luxuriant	flat, rough, dry and usually non-pigmented
Mycobacterium smegmatis ATCC 14468	luxuriant	rough or smooth, white dome shaped colonies.

Storage and Shelf Life

Store below 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 (4,5).

Reference

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4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. 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.
6. Kent and Kubica, 1985, Public Health Mycobacteriology : A Guide For the Level III Laboratory, USDHHS, Center for Disease Control, Atlanta.
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Please check reference

Revision : 03/2024