Dubos Broth Base

M067

Intended Use:

Recommended for preparation of liquid medium for rapid cultivation of pure cultures of *Mycobacterium tuberculosis* and related microorganisms.

Composition**

Ingredients	g / L
Tryptone	0.500
L-Asparagine	2.000
Polysorbate 80 (Tween 80)	0.200
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
Final pH (at 25°C)	6.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 1.3 grams in 180 ml purified/distilled water containing 10 ml glycerol. Heat if necessary 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 20 ml sterile bovine albumin V or sterile serum or one vial of sterile Albumin Glucose Supplement (FD201) to each 180 ml of broth base.

Principle And Interpretation

Tuberculosis remains a major global public health problem worldwide. *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). Dubos Broth is formulated as per Dubos, et al (2), and is a modification of the medium originally formulated by Dubos and Davis (3) and Dubos and Middlebrook (4).

Dubos media contain tryptone and L-aspargine as sources of nitrogen. Polysorbate 80, an oleic acid ester also acts as a surfactant. It therefore supplies the essential fatty acids for the replication of Mycobacteria and also increases the growth by dispersing the bacilli. The phosphates (together with calcium chloride) buffers 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. Bovine albumin binds the free fatty acids, which may be toxic to Mycobacteria. Albumin is heat treated to inactivate the lipase, which may release fatty acids from Polysorbate 80 incorporated in the medium.

Dubos Broth Base enriched with serum will generally initiate growth from smaller inocula and yield more luxuriant growth than the basal medium enriched with albumin V. Growth is generally more granular with the serum enrichment, while it is more diffused with albumin enrichment. Maximum care should be taken while handling Mycobacterial cultures, as they are highly infectious.

Type of specimen

Clinical samples : Sputum

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use only. 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

1. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
2. Proteolytic contaminants cause localized or complete digestion of medium.

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 beige homogeneous free flowing powder

Colour and Clarity of prepared medium

Light yellow coloured, clear solution without any precipitate

Reaction

Reaction of 0.65% w/v aqueous solution with 1% glycerol at 25°C. pH : 6.6±0.2

pH

6.40-6.80

Cultural Response

Cultural characteristics observed with added Albumin Glucose Supplement (FD201) or sterile bovine albumin V or sterile serum after an incubation at 35-37°C for 2-6 weeks with 5-10% CO_2.

Organism	Growth
Mycobacterium avium ATCC 25291	luxuriant
Mycobacterium gordonae ATCC 14470	luxuriant
Mycobacterium kansasii ATCC 12478	luxuriant
Mycobacterium smegmatis ATCC 14468	luxuriant
M. tuberculosis H37 Rv ATCC 25618	luxuriant

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 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. Dubos R. J., Fenner F. and Pierce C. H., 1950, Am. Rev. Tuberc., 61:6 6.
3. Dubos R. J. and Davis B.D., 1946, J. Exp. Med., 83:409.
4. Dubos R. J., and Middlebrook G., 1947, Am. Rev. Tuberc., 56:334
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.

Revision :03/2024

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

CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
www.cepartner4u.eu

IVD ***In vitro*** **diagnostic medical device**

10°C – 30°C **Storage temperature**

CE Marking

Do not use if package is damaged