Dubos Broth Base

M067

Dubos Broth Base with added supplements is recommended for the preparation of a liquid medium for the rapid cultivation of pure cultures of *Mycobacterium tuberculosis* and related microorganisms.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	0.500
L-Asparagine	2.000
Polysorbate 80	0.200
Monopotassium phosphate	1.000
Disodium phosphate	2.500
Ferric ammonium citrate	0.050
Magnesium sulphate	0.010
Calcium chloride	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 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 50°C and aseptically add 20 ml sterile bovine albumin V or sterile serum or 1 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 Casein enzymic hydrolysate 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.

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
M067: 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% CO2.

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 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

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

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