Dubos Oleic Agar Base

M179

Dubos Oleic Agar Base is used for isolation and susceptibility testing of M. *tuberculosis* .

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	0.500
L-Asparagine	1.000
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
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 distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. 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 media contain casein enzymic hydrolysate and L-aspargine as sources of nitrogen. *Mycobacterium* . 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).

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
M179: 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
Cultural Response		
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 30°C in tightly closed container and prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference
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5. Byham, 1950, Am. J. Clin. Pathol., 20:678
6. Kent and Kubica, 1985, Public Health Mycobacteriology : A Guide For the Level III Laboratory, USDHHS, Center for Disease Control, Atlanta c.a.
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