



## Lowenstein Jensen Medium (L.J. Medium) (Twin pack)

MM162

For isolation and cultivation of *Mycobacterium* species.

### Composition\*\*

Ingredients	Gms / Litre
Part A	-
L-Asparagine	3.600
Potassium hydrogen phosphate (K <sub>2</sub> HPO <sub>4</sub> )	2.400
Magnesium sulphate (MgSO <sub>4</sub> )	0.240
Magnesium citrate	0.600
Part B	-
Malachite green	0.400

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 6.84 grams of Part A in 600 ml distilled water containing glycerol. Sterilize by heating at 121°C for 25 minutes. Suspend 0.4 grams of Part B in 20 ml sterile distilled water under aseptic precautions, allowing the dye to dissolve by incubating for 1 to 2 hours at 37°C. Shake the solution before use. Meanwhile prepare 1000 ml of egg emulsion solution collected aseptically. To this add Part A and Part B solution aseptically. Distribute 5 ml aliquot into 25 ml McCartney bottles and screw the caps tightly. Lay the bottles horizontally. Coagulate and inspissate the medium in an inspissator or hot air oven at 85°C for 60 minutes.

### Principle And Interpretation

Solid media used for isolation and cultivation of Mycobacteria are either egg-based or agar-based. Egg-based media contain whole eggs or egg yolk, potato flour, salts and glycerol and are solidified by inspissation. Of the egg-based media, Lowenstein Jensen Medium is most commonly used (1). L.J. Medium was originally formulated by Lowenstein, containing congo red and malachite green dyes (2). Jensen (3) modified Lowenstein's medium by altering the citrate and phosphate contents, eliminating the congo red dye and by increasing the malachite green concentration. Gruft (4, 5) further modified L. J. Medium with the addition of two antimicrobics to increase selectivity. This medium supports the growth of a wide variety of Mycobacteria and can also be used for niacin testing (6). This medium is recommended by Indian Pharmacopoeia (12).

Malachite green prevents growth of the majority of contaminants surviving decontamination of the specimen. Do not add glycerol to the medium if bovine or other glycerophobic strains are to be cultured (7). Malachite green serves as an inhibitor and also as pH indicator. Formation of blue zone indicates a decrease in pH by gram-positive contaminants (e.g. *Streptococci*) and yellow zones of dye destruction by gram-negative bacilli. Proteolytic contaminants cause localized or complete digestion of medium. Hardy et al (8) recommended each specimen to be inoculated and incubated in triplicate (>,<)

a. To identify saprophytes at room temperature (25°C).

b. To identify presence or absence of pigmentation by photochromogenes and scotochromogenes at 35°C alternately in light and dark as per the type of organism.

Routinely cultivation is carried out aerobically at 35°C.

Refer appropriate references for standard test procedures of decontamination and isolation (1, 9-11).

### Quality Control

#### Appearance of Part A

Part A : White to off-white homogeneous free flowing powder

#### Appearance of Part B

Please refer disclaimer Overleaf.

Part B : Greenish blue homogeneous free flowing crystals

### Colour and Clarity of prepared medium

The mixture of sterile basal medium and whole egg emulsion, when inspissated, coagulates to yield pale bluish green coloured, opaque smooth slants

### Cultural response

Cultural characteristics observed in presence of 5-10% CO<sub>2</sub>, with added egg emulsion base, after an incubation at 35-37°C for 2-4 weeks.

### Cultural Response

Organism	Growth	Colony Characteristic
<b>Cultural response</b>		
<i>Mycobacterium avium</i> ATCC 25291	Good-luxuriant	smooth, non-pigmented colonies
<i>Mycobacterium gordonae</i> ATCC 14470	Good-luxuriant	smooth, yellow, orange colonies
<i>Mycobacterium kansasii</i> ATCC 12478	Good-luxuriant	photochromogenic, smooth to rough
<i>Mycobacterium smegmatis</i> ATCC 14468	Good-luxuriant	wrinkled, creamy white colonies
<i>M. tuberculosis</i> H37RV ATCC 25618	Good-luxuriant	granular, rough, warty, dry friable colonies

### Storage and Shelf Life

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

### Reference

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