

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

MM162

Intended Use:

Recommended in accordance with IP for isolation and cultivation of *Mycobacterium* species.

Composition**

Ingredients	Gms / 600ml
Part A	-
L-Asparagine	3.600
Potassium hydrogen phosphate (K ₂ HPO ₄)	2.400
Magnesium sulphate (MgSO ₄)	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 purified/distilled water containing 12 ml glycerol. Heat if necessary to dissolve the medium completely. Sterilize by heating at 121°C for 25 minutes. Suspend 0.4 grams of Part B in 20 ml sterile purified/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 collected aseptically. To this add Part A and Part B solution aseptically. Distribute 5 ml aliquot into 25 ml McCartney bottles and screw the cap tightly. Lay the bottles horizontally, coagulate and inspissate the medium in an inspissator or hot air oven at 85°C for 60 minutes. Note: After addition of egg yolk emulsion, pH may shift to alkaline side, hence likely to decolorize the medium.

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 Lowensteins 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(7). 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 (8). 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 (9) recommended each specimen to be inoculated and incubated in triplicate,

- To identify saprophytes at room temperature (25°C).
- 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 (1,7,10,11,12).

Type of specimen

Isolate

Specimen Collection and Handling

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. This medium is general purpose medium and may not support the growth of fastidious organisms.
2. Certain gram-positive contaminants (e.g. Streptococci) and gram-negative bacilli may grow on the medium.
3. Certain Saprophytes may also grow on the medium.
4. 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 of Part A

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

Appearance of Part B

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% CO₂, with added egg emulsion base, after an incubation at 35-37°C for 4-5 weeks.

Organism	Growth	Colony Characteristic
<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 H37RV</i> ATCC 25618	Good-luxuriant	granular, rough, warty, dry friable 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 (10,11,3,14).

Reference

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