

## Lowenstein Jensen Medium (L.J. Medium), Granulated

GM162

Lowenstein Jensen Medium (L. J. Medium), granulated is used for the isolation and cultivation of *Mycobacterium* species.

### Composition\*\*

Ingredients	Gms / 600 ml
L-Asparagine	3.600
Monopotassium phosphate	2.400
Magnesium sulphate	0.240
Magnesium citrate	0.600
Potato starch, soluble	30.000
Malachite green	0.400

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

### Directions

Suspend 37.24 grams in 600 ml distilled water containing 12 ml glycerol (for bovine bacteria or other glycerophobic organisms additions of glycerol is not desirable). Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Meanwhile prepare 1000 ml of whole egg emulsion collected aseptically. Aseptically add and mix 1000 ml egg emulsion to sterile medium base. Mix gently to obtain uniform mixture. Distribute 8ml in sterile screw capped tubes. Arrange tubes in a slanted position. Coagulate and inspissate the medium in an inspissator water bath or autoclave at 85°C for 45 minutes. If desired selectivity may be obtained by addition of 0.05 ml of reconstituted Gruft Mycobacterial Supplement (FD053) onto each slant.

### 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).

Penicillin and Nalidixic acid (FD053) along with malachite green prevents growth of the majority of contaminants surviving decontamination of the specimen while encouraging earliest possible growth of *Mycobacteria*. RNA (FD053) acts as stimulant and help to increase the isolation rate of *Mycobacteria*. 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 so as

- 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, 9-11).

### Quality Control

#### Appearance

Greenish blue to peacock blue coloured granular medium

### 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% Carbon dioxide, with added egg emulsion base, after an incubation at 35-37°C for 2-4 weeks.

Organism	Growth	Growth with Gruft Supplement (FD053)	Colony Characteristic
<b>Cultural Response</b>			
<i>Mycobacterium avium</i> ATCC 25291	luxuriant	good-luxuriant	smooth, non-pigmented colonies
<i>Mycobacterium gordonae</i> ATCC 14470	luxuriant	good-luxuriant	smooth, yellow, orange colonies
<i>Mycobacterium kansasii</i> ATCC 12478	luxuriant	good-luxuriant	photochromogenic, smooth to rough
<i>Mycobacterium smegmatis</i> ATCC 14468	luxuriant	good-luxuriant	wrinkled, creamy white colonies
<i>M. tuberculosis</i> H37RV ATCC 25618	luxuriant	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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