

Technical Information

Lowenstein Jensen Medium Base w/o Starch

Product Code: DM 2542

Application: - Lowenstein Jensen Medium w/o Starch is recommended for susceptibility testing of Mycobacteria with addition of antitubercular drugs.

Composition**

Ingredients	Gms / Litre
L-Asparagine	3.600
Potassium dihydrogen phosphate	2.400
Magnesium sulphate	0.240
Magnesium citrate	0.600
Malachite green	0.400

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Lowenstein Jensen Medium Base w/o starch is used for resistance testing by WHO. Lowenstein- Jensen (L-J) Medium without potato starch with drugs incorporated before inspissation is the modification of the International Union Against Tuberculosis (IUAT) (5, 6, 7). The original LJ medium was developed by Lowenstein (1) and modified by Jensen (2) and Gruft (3, 4) with addition of two antimicrobial agents. Malachite green inhibits the growth of the majority of contaminants that survived the decontamination procedures for the specimen, thus encouraging earliest possible growth of Mycobacteria. 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 a pH indicator. Formation of blue zone indicates a decrease in pH by gram-positive contaminants (e.g. Streptococci) and yellow zones indicate dye destruction by gram-negative bacilli. Proteolytic contaminants cause localized or complete digestion of medium. Hardy et al (14) 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 (9-13).

Methodology

Suspend 7.24 grams of dehydrated powder media in 600 ml distilled water containing 12 ml glycerol (for bovine bacteria or other glycerophobic organisms, addition of glycerol is not desirable). Mix thoroughly & 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. Add and mix egg emulsion base gently to obtain uniform mixture. Distribute 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.

Quality Control

Appearance

Greenish blue to peacock blue homogeneous free flowing powder



Dehydrated Culture Media
Bases / Media Supplements

Colour and Clarity

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

Cultural Response

DM2542: Cultural characteristics observed in presence of 5-10% Carbon dioxide (CO₂), with added egg emulsion base, after an incubation at 35-37°C for 2-4 weeks.

Organism

Colony characteristics

<i>Mycobacterium avium</i> ATCC 25291	smooth, nonpigmented colonies
<i>Mycobacterium gordonae</i> ATCC 14470	smooth, yellow orange colonies
<i>Mycobacterium kansasii</i> ATCC 12478	photochromogenic, smooth to rough wrinkled,
<i>Mycobacterium smegmatis</i> ATCC 14468	creamy white colonies granular, rough,
<i>M. tuberculosis</i> H37RV ATCC 25618	warty, dry friable colonies

Storage and Shelf Life

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

Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

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10. Kent P. T and Kubica G. P., 1985, Public Health Mycobacteriology: A Guide to the level III Laboratory, USDHHS, Centers for Disease Control, Atlanta, Ga.
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13. Isenberg, (Ed.), 1992, Clinical Microbiology Procedures Handbook, Vol. I, ASM, Washington, D. C.
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