Technical Information

Lowenstein Jensen Medium (L.J. Medium)

Product Code: DM 1162

Application: - Lowenstein Jensen Medium (L. J. Medium) is used for the isolation and cultivation of *Mycobacterium* species.

Composition**

Ingredients	Gms / Litre
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

Principle & 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, LowensteinJensen Medium used 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]. Penicillin and Nalidixic acid (MS2053) along with malachite green prevents growth of the majority of contaminants surviving decontamination of the specimen while encouraging earliest possible growth of *Mycobacteria*. RNA (MS2053) 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
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 for isolation decontamination of the microorganism [1, 9-11].

Methodology

Suspend 37.24 grams of powder media in 600 ml distilled water containing 12 ml glycerol (for bovine bacteria or other glycerophobic organism's addition 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 egg emulsion base and Gruft Mycobacterial Supplement (MS2053) (if desired) 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

Physical Appearance
Greenish blue to peacock blue homogeneous
Gelling
free flowing powder
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.

Culture Response / Characteristic

DM1162: Cultural characteristics observed in presence of 5-10% Carbon dioxide, with added egg emulsion base, after an incubation at 35-37ºC for 2-4 weeks.

Organism	Growth	Growth with Gruft Supplement (MS2053)	Colony Characteristic
Mycobacterium avium ATCC 25291	luxuriant	good-luxuriant	smooth, nonpigmented colonies
Mycobacterium gordonae ATCC 14470	luxuriant	good-luxuriant	smooth, yellow, orange colonies
Mycobacterium kansasii ATCC 12478	luxuriant	good-luxuriant	photochromogenic, smooth to rough
Mycobacterium smegmatis ATCC 14468	luxuriant	good-luxuriant	wrinkled,creamy white colonies
M. tuberculosis H37RV ATCC 25618	luxuriant	good-luxuriant	granular, rough, warty, dry friable colonies

Storage and Shelf Life

Dried media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

1. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.
2. Lowenstein E., 1931, Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig., 120:127.
3. Jensen K. A., 1932, Zentralb. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. I Orig., 125:222.
4. Gruft, 1971, Health Lab. Sci., 8:79.
5. Gruft, 1963, Am. Rev. Respir. Dis., 88:412.
6. Boisvert H., 1960, Ann. Inst. Pasteur, 99:600.
7. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
8. Boisvert H., 1960, Ann. Inst. Pasteur, 99:600.
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.
9. Forbes B. A., Sahm A. S. and Weissfeld D. F., Bailey & Scotts Diagnostic Microbiology, 10th Ed., 1998, Mosby, Inc., St. Louis, Mo.
10. Cernoch P., Enns R., Saubolle M. and Wallace R., 1994, Cumitech, 16A, Laboratory Diagnosis of the Mycobacterioses coord , Ed., Weissfeld , ASM, Washington, D. C.
11. Isenberg, (Ed.), 1992, Clinical Microbiology Procedures Handbook, Vol. I, ASM, Washington, D. C.

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
- The product conforms solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at **CDH** is true and accurate.
- **Central Drug House Pvt. Ltd.** reserves the right to make changes to specifications and information related to the products at any time.
- Products are not intended for human or animal diagnostic or therapeutic use but for laboratory, research or further manufacturing of diagnostic reagents extra.
- Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.
- Do not use the products if it fails to meet specificatons for identity and performens parameters.

Replace Date 06-Mar.-2019