PRODUCT SPECIFICATION SHEET

Lowenstein Jensen Medium Base / L.J. Medium (DM135)

Intended Use

Lowenstein Jensen Medium Base / L.J. Medium (DM135) is recommended for isolation and cultivation of *Mycobacterium* species.

Product Summary and Explanation

Of the egg-based media, LowensteinJensen 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) 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. Mycobacterial infections, particularly tuberculosis, are a worldwide health problem. Almost three million people worldwide die of uberculosis each year.(7) Non-tuberculous mycobacteria infections have also increased since 1985(8) At least 25 species of mycobacteria are associated with human disease and produce usually slowly developing, destructive granulomas that may undergo necrosis with ulceration or cavitation. The use of egg-based media for primary isolation of mycobacteria have two significant advantages. First, egg-based media support a wide variety of mycobacteria. Second, growth of mycobacteria on egg media can be used for niacin testing. Liquification of Lowenstein–Jensen Medium can occur if contaminated with proteolytic organisms. Lowenstein-Jensen Medium is a modification of Lowenstein Medium,(9) modified by Jensen(10) Jensen modified the medium by alternating the citrate and phosphate contents, eliminating congo red dye, and increasing malachite green concentration.(11) Lowenstein-Jensen Medium is commonly used in the clinical laboratory to isolate acid fast organisms from sterile and nonsterile sources.(12) Hughes and Dixon and Cuthbert(13-14) reported that the addition of pyruvic acid to egg-based media resulted in improved recovery of tubercle bacilli compared to recovery on egg-based media supplemented only with glycerol. Dixon and Cuthbert recommended using pyruvic acid-egg medium in addition to media supplemented with glycerol for optimum recovery of tubercle bacilli from clinical specimens. Additionally, the medium is available with the addition of 5% sodium chloride. Most rapid growers, the slowly growing *M. triviale* and some strains of *M. flavescens* grow on NaClncontaining media. The inability of *M. chelonae* subsp. *Chelonae* to grow helps differentiate it from other members of the *M. fortuitum* complex (e.g., *M. chelonae* subsp. *abscessus*). In the semi-quantitative catalase test, mycobacteria can be differentiated into groups, based upon catalase activity.

Principles of the Procedure

L-Asparagine and Potato Starch are sources of nitrogen and vitamins in Lowenstein–Jensen Medium. Monopotassium Phosphate and Magnesium Sulfate enhance organism growth and act as buffers. Glycerol and the Egg Suspension provide fatty acids and protein required for the metabolism of mycobacteria. The coagulation of the egg albumin during sterilization provides a solid medium for inoculation purposes. Sodium Citrate and Malachite Green are selective agents to prevent growth of most contaminants and allow early growth of mycobacteria. Penicillin and Nalidixic acid in the Gruft's Mycobacterial Supplement (MS049) along with malachite green prevents growth of the majority of contaminants surviving decontamination of the specimen while encouraging earliest possible growth of *Mycobacteria* . RNA in the Gruft's Mycobacterial Supplement (MS049) 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 .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 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.

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Formula / Liter

Ingredients	Gms / Liter
L-Asparagine	3.60
Monopotassium phosphate	2.40
Magnesium sulphate	0.24
Magnesium citrate	0.60
Potato starch, soluble	30.00
Malachite green	0.40
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.
3.

Directions

1. Suspend 37.24 grams of the medium in 600 ml of distilled water containing 12 ml glycerol (for bovine bacteria or other glycerophobic organisms additions of glycerol is not desirable).
2. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
3. Meanwhile prepare 1000 ml of whole egg emulsion collected aseptically.
4. Aseptically add and mix egg emulsion base and Gruft Mycobacterial Supplement (MS049) (if desired) gently to obtain uniform mixture.
5. 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 Specifications

Dehydrated Appearance	Greenish blue to peacock blue homogeneous free flowing powder
Prepared Medium	The mixture of sterile basal medium and whole egg emulsion, when inspissated, coagulates to yield pale bluish green coloured, opaque smooth slants
Reaction of 3.7% solution	--
Gel Strength	Firmness observed due to coagulation of egg emulsion

Expected 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.

Sr. No.	Organisms	Results to be achieved		
		Growth	Growth with Gruft Supplement (MS049)	Colony Characteristics
1.	*Mycobacterium avium ATCC 25291*	Luxuriant	Good-luxuriant	Smooth, non-pigmented Colonies
2.	*Mycobacterium gordonae ATCC 14470*	Luxuriant	Good-luxuriant	Smooth, yellow, Orange colonies
3.	*Mycobacterium kansasii ATCC 12478*	Luxuriant	Good-luxuriant	Photochromogenic, Smooth to rough

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4.	*Mycobacterium smegmatis ATCC 14468*	Luxuriant	Good-luxuriant	Wrinkled, creamy White colonies
5.	*M. tuberculosis H37RV ATCC 25618*	Luxuriant	Good-luxuriant	Granular, rough, Warty, dry friable colonies

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. The test procedures are those recommended by the Centers for Disease Control and Prevention (CDC) for primary isolation from specimens containing mycobacteria.6 N-Acetyl-Lcysteine-sodium hydroxide (NALC-NaOH) solution is recommended as a gentle but effective digesting and decontaminating agent.
2. These reagents are provided as Mycobacterial Specimen Digestion/Decontamination Kits. For detailed decontamination and culturing instructions, consult an appropriate reference.
3. Following inoculation, keep test containers shielded from light and place them in a suitable system providing an aerobic atmosphere enriched with carbon dioxide.
4. Incubate at 35 ± 2°C. Slanted and bottled media should be incubated in a horizontal plane until the inoculum is absorbed.
5. Tubes and bottles should have screw caps loose for the first 3 weeks to permit the circulation of carbon dioxide for the initiation of growth.
6. Thereafter, to prevent dehydration, tighten caps; loosened briefly once a week. Stand tubes upright if space is a problem.
7. NOTE: Cultures from skin lesions suspected to be *M. Marinum* or *M. ulcerans* should be incubated at 25-33°C for primary isolation; cultures suspected to contain *M. avium* or *M. Xenopi* exhibit optimum growth at 40-42°C.
8. Incubate a duplicate culture at 35-37°C. For LJ Medium with Iron, specimens must first be isolated in pure culture on an appropriate solid medium.
9. Inoculate LJ Medium with Iron with one drop of a barely turbid suspension of the culture to be tested. For the semi-quantitative catalase test, 1 mL of a 1:1 mixture of 10% polysorbate 80 and 30% hydrogen peroxide is added to each inoculated tube after 2 weeks of incubation.
10. The height of the column of bubbles is recorded after 5 minutes as <45 mm or >45 mm.

Results

1. Cultures should be read within 5-7 days after inoculation and once a week thereafter for up to 8 weeks.

Record Observations:

- Number of days required for colonies to become macroscopically visible. Rapid growers have mature colonies within 7days; slow growers require more than 7 days for mature colony forms.
- Pigment production

2. White, cream to buff - Nonchromogenic (NC)
3. Lemon, yellow, orange, red-Chromogenic (Ch)
4. Stained smears may show acid-fast bacilli, which are reported only as "acid-fast bacilli" unless definitive tests are performed.
5. Bottles may be examined by inverting the bottles on the stage of a dissecting microscope.
6. Read at 10-60× with transmitted light. Scan rapidly at 10-20× for the presence of colonies.
7. Higher magnification (30-60×) is helpful in observing colony morphology; i.e., serpentine cord-like colonies.
8. Examine LJ Medium with Iron for rusty-brown colonies with a tan discoloration in the surrounding medium, indicating uptake of the iron.
9. The presence or absence of growth in the tube of medium containing 5% NaCl aids in the differentiation of mycobacterial isolates. T

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10. he salt tolerance test is positive when numerous colonies appear on the control medium and more than 50 colonies grow on the medium containing 5% NaCl.
11. Colonies on the control medium, but no visible growth on the test medium after a total of 4 weeks of incubation constitutes a negative test.
12. In the semi-quantitate catalase test, mycobacteria fall into two groups with *M. tuberculosis* falling into the group producing a column of bubbles less than 45 mm in height.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Negative culture results do not rule-out active infection by mycobacteria.
2. Some factors that are responsible for unsuccessful cultures are:
   - The specimen was not representative of the infectious material; i.e., saliva instead of sputum.
   - The mycobacteria were destroyed during digestion and decontamination of the specimen.
   - Gross contamination interfered with the growth of the mycobacteria.
   - Proper aerobic conditions and increased CO2 tension were not provided during incubation.
3. Mycobacteria are strict aerobes and growth is stimulated by increased levels of CO2. Screw caps on tubes or bottles should be handled as directed for exchange of CO2.

Packaging

Product Name : Lowenstein Jensen Medium Base

Product Code : DM135

Available Pack sizes : 100gm / 500gm

References

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. Musser, J. M. 1995. Antimicrobial resistance in Mycobacteria: molecular genetic insights. Clinical Microbiology Reviews. 8:496- 514.
8. Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.). 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
9. Lowenstein, E. 1931. Die Zachtung der Tuberkelbazillen aus dem stramenden Blute. Zentralb. Bakteriol Parasitenkd. infektionskr. hyg. Abt. I orig., 120:127.
10. Jensen, K. A. 1932. Rinzuchtung und Typenbestimmung von Tuberkelbazillentamen. Zentralb. Bakteriol Parasitenkd. infektionskr. hyg. Agt. I Orig., 125:222.
11. MacFaddin, J. F. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.
12. Isenberg, H. D. (ed.). 1992. Clinical microbiology procedures handbook, vol. 1 American Society for Microbiology, Washington, D.C.
13. Hughes. 1966. J. Clin. Pathol. *19*:73.
14. Dixon and Cuthbert. 1967. Am. Rev. Respir. Dis. *96*:119.

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Further Information

For further information please contact your local MICROMASTER Representative.

MICROMASTER LABORATORIES PRIVATE LIMITED
Unit 38/39, Kalpataru Industrial Estate,
Off G.B. Road, Near 'R-Mall' , Thane (W) – 400607. M.S. INDIA.
Ph: +91-22-25895505, 4760, 4681. Cell: 9320126789.
Email: micromaster@micromasterlab.com
sales@micromasterlab.com

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Prepared By	Checked By	Approved By
01.01.2018	01.01.2018	01.01.2018
Microbiologist	**Head Quality Control**	**Head Quality Assurance**

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