ARENA
BioScien

Block 5, Street 9 inside The Ismailia free zone. Ismailia- Egypt
Post Code-41511
Tel.-Fax: +202 21813500/ +202 21813600
Mob.: +2 01211550941/ +2 01119225860
E-mail: sales@arenabioscientific.com
Website: www.arenabioscien.com

Lowenstein Jensen Medium Base (L.J. Medium)

Recommended for isolation and cultivation of Mycobacterium species.

REF: BS.1/LJ01.100.0100	100 Gram	REF: BS.1/LJ01.250.0250	250 Gram
REF: BS.1/LJ01.500.0500	500 Gram		

CLINICAL SIGNIFICANCE

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 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).

METHOD PRINCIPLE

Penicillin and Nalidixic acid along with malachite green prevents growth of the majority of contaminants surviving decontamination of the specimen while encouraging earliest possible growth of Mycobacteria. RNA 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,

1. To identify saprophytes at room temperature (25°C).
2. 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.

MEDIA COMPOSITION

Item	Formula per 600 ml of medium
- L-Asparagine	3.600 gm
- Potassium dihydrogen phosphate	2.400 gm
- Magnesium sulphate	0.240 gm
- Magnesium citrate	0.600 gm
- Potato starch, soluble	30.00 gm
- Malachite green	0.400 gm

PRECAUTIONS AND WARNINGS

Media to be handled by entitled and professionally educated person. Do not ingest or inhale.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the L.J. Medium material safety data sheet.

STORAGE AND STABILITY

BioScien L.J. Medium should be stored between 10-30°C in a firmly 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 avoid lump development due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in a 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.

PREPARATION

Suspend 37.24 grams in 600 ml purified / 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. Cool to 45-50°C. Meanwhile prepare 1000 ml of whole egg emulsion collected aseptically. Aseptically add and mix egg emulsion base and Gruft Mycobacterial Supplement (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.

Deterioration

The color of ***BioScien*** L.J. Medium is Greenish blue to peacock blue homogeneous free flowing powder. The mixture of sterile basal medium and whole egg emulsion, when inspissated, coagulates to yield pale bluish green coloured, opaque smooth slants. If there are any physical changes for powder or signs of deterioration (shrinking, cracking, or discoloration), and contaminations for hydrated media, discard the medium.

SPECIMEN

Clinical samples : Sputum.

EQUIPMENT REQUIRED NOT PROVIDED

- Screw capped Tubes
- Sterile Test tubes
- Incubator
- Autoclave

PERFORMANCE CHARACTERISTICS

Cultural characteristics observed in presence of 5-10% CO2, with added egg emulsion base, after an incubation at 35-37ºC for 2-4 weeks.

Oranism	Growth	Growth with Gruft Supplement	Colony Characteristic
Mycobacterium avium ATCC 25291	luxuriant	good-luxuriant	smooth, non-pigmented 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

QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control values are found outside the defined range, check the system performance. If control still out of range please contact ***BioScien*** technical support.

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. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
8. A.V. Hardy, et al, Am. J. Publ. Hlth. 48(1), 754 (1958)
9. Isenberg, (Ed.), Clinical Microbiology Procedures Handbook 2nd Edition
10. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
11. Cernoch P., Enns R., Saubolle M. and Wallace R., 1994, Cumitech, 16A, Laboratory Diagnosis of the Mycobacterioses coord , Ed., Weissfeld , ASM, Washington, D. C.
12. Forbes B. A., Sahm A. S. and Weissfeld D. F., Bailey & Scotts Diagnostic Microbiology, 10th Ed., 1998, Mosby, Inc., St. Louis, Mo.

SYMBOLS IN PRODUCT LABELLING			
IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		