LOWENSTEIN-JENSEN (L-J) MEDIA

PRODUCT:

Tube Media:[a]

Lowenstein -Jensen Medium, item no. T3300, T6840
Lowenstein -Jensen Medium, Deep, item no. T6845
Lowenstein -Jensen Medium, 5% Sodium Chloride, item no. T6847
Lowenstein -Jensen Medium, Mycobacterium Selective, item no. T6850
Lowenstein -Jensen Medium, Gruft Modification, item no. T6842

[a]see catalog for ordering options

PURPOSE:

Lowenstein-Jensen (L-J) media are used in qualitative procedures primarily for the isolation and propagation of *Mycobacterium* species.

PRINCIPLE:

Lowenstein-Jensen Medium was first developed by Lowenstein[11,12] using congo red or malachite green dye to inhibit unwanted bacteria. Jensen[8] later modified the citrate, phosphate, and dye contents of the medium. The Lowenstein-Jensen formula is a glycerated egg-base medium. These ingredients provide fatty acids and protein essential for the metabolism of mycobacteria. The glycerol is a source of carbon and energy that are favorable to the growth of the human-type tubercle bacillus at the expense of the bovine type. Asparagine and ribonucleic acid (RNA) are added to provide a source of nitrogen and a growth stimulant. The coagulation of the egg albumin during the inspissation process provides a solid medium for inoculation purposes.

Specimens cultured for mycobacteria often contain a mixture of contaminating microorganisms necessitating the use of selective antibiotics in the media used for isolation purposes. Nalidixic acid inhibits most of the gram-negative bacteria. Lincomycin inhibits gram-positive bacteria. Cycloheximide suppresses saprophytic fungi. L-J Medium Deep is used for semi-quantitative catalase testing.[15] L-J Medium, 5% Sodium Chloride differentiates certain group IV mycobacteria from other species.[10]

Studies have shown this medium is satisfactory for growth of pathogenic mycobacteria, particularly *Mycobacterium tuberculosis,*[2,6,7] and for their primary isolation.[9,14] *Nocardia* species are also able to grow on this medium.[5]

FORMULAS:

Approximate, per 600 ml deionized filtered water.

(1) Lowenstein-Jensen Medium:

Asparagine	3.60 g
Monopotassium Phosphate	2.50
Magnesium Citrate	0.60
Magnesium Sulfate	0.24
Potato Flour	30.00
Malachite Green	0.40
Eggs (fresh, whole)	1000.00 ml
Glycerol	12.00

Final pH 6.9 ± 0.3 at 25°C

(2) Lowenstein-Jensen Medium With 5% Sodium Chloride:
Same as (1) above except it also contains 80.0 g of Sodium Chloride.

(3) Lowenstein-Jensen Medium, Mycobacterium Selective:
Same as (1) above except it also contains 0.64 g of Cycloheximide, 3.2 mg of Lincomycin, and 56.0 mg of Nalidixic Acid.

(4) Lowenstein-Jensen Medium, Gruft Modification:
Same as (1) above except it also contains 56.0 mg of Nalidixic Acid and 80.0 mcg of Ribonucleic Acid.

PRECAUTIONS:*

For in vitro diagnostic use. Observe approved biohazard precautions. Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. Prevent aerosols. Perform all manipulations in a biological safety hood.

Storage: Upon receipt, store at 2-8°C in the dark. The media should not be used if there are signs of contamination, deterioration (shrinking, cracking or discoloration), or if the expiration date has passed. After storage in the upright position, tubes of coagulated egg media may appear to have a large amount of water at the bottom of the slants. The fluid will be reabsorbed into the medium if the tubes are placed on their sides, allowing the fluid to cover the slant surface.

Limitations: Malachite green is extremely photosensitive. Protect L-J media from all sources of light.

Selective media often inhibit, to some extent, specific strains of organisms for which they are designed to isolate.

Mycobacterium bovis will not grow on glycerol-containing L-J media.

L-J media may range in color from a pale green to a dark blue-green. Media that has turned yellow should not be used as it will interfere with the interpretation of the pigmentation of the mycobacteria.

PROCEDURE:

Specimen Collection: Specimens must be collected in clean, sterile containers and sent directly to the laboratory for immediate processing. For more detailed information, appropriate references should be consulted. [9,10]

Method of Use: The procedures used for processing mycobacteria specimens are those recommended by the Centers for Disease Control (CDC).[14] N-Acetyl-L-Cysteine with a Sodium Hydroxide/Sodium Citrate solution is recommended as an effective digesting and decontaminating agent to use when preparing specimens for the isolation of mycobacteria. Other acceptable methods may be used. For further information refer to Sputum Digestant Reagents, Data #700. Inoculate the medium with the sediment of the centrifuged specimen. Following inoculation, the tubes are laid on their sides, slightly elevated, with caps loose, and incubated in an atmosphere containing 5-10% carbon dioxide at 35°C. Cultures should be read within 5-7 days after incubation and once a week thereafter. Retighten caps after 5-7 days. The incubation time required for full growth of most mycobacteria is at least eight weeks.

Interpretation: In general, examine and record each type of colony morphology, pigment, and growth rate. Definitive identification requires biochemical testing.

Materials Required but Not Supplied: Standard microbiological supplies such as loops, burner, staining reagents, slides, microscope, incubator, centrifuge, and biological safety hood are not provided.

QUALITY CONTROL:*

Microorganism Used (ATCC#):	Expected Results:
Mycobacterium tuberculosis (25177)	Growth; no growth on 5% NaCl
Mycobacterium kansasii Group I (12478)	Growth; no growth on 5% NaCl
Mycobacterium scrofulaceum Group II (19981)	Growth; no growth on 5% NaCl
Mycobacterium intracellulare Group III (13950)	Growth; no growth on 5% NaCl
Mycobacterium fortuitum Group IV (6841)	Growth
Escherichia coli (25922), on selective media only	Inhibition, partial to complete
Staphylococcus aureus (25923), on selective media only	Inhibition

User Quality Control: Check for signs of contamination and deterioration.

BIBLIOGRAPHY:

1. Bailey, W. R., and E. G. Scott, *Diagnostic Microbiology*, 4th ed., C. V. Mosby, St. Louis, 1974.
2. Cummings, M. M., *Am. H. Clin. Pathol.*, 21:684, 1951.
3. Gruft, H., *J. Bacteriol.*, 90:829, 1965.
4. Hardy, A. V., et al., *Am. J. Publ. Health*, 48:754, 1958.
5. Hosty, T J., et al., *J. Clin. Med.*, 58:107, 1961.
6. Hughes, E. E., et al., *J. Clin. Pathol.*, 24:621, 1954.
7. Jeffries, M. B., et al., *H. Rev. Resp. Dis.*, 81:259, 1960.
8. Jensen, F., *Zentralbl. Bakteriol. Parasetenkd. Infektionskr.,* Abt. 1 Orig., 125:222, 1932.
9. Kubica, G. P., and W. E. Dye, USDHEW PHS Publication No. 1547, U.S. Government Printing Office, Washington, D. C., 1967.
10. Lennette, E. H., et al., *Manual of Clinical Microbiology*, 4th ed., American Society for Microbiology, Washington, D. C., 1985.
11. Lowenstein, E., *Ann. Inst. Pasteur.*, 50:161, 1933.
12. Lowenstein, E., *Zentralbl. Bakteriol. Parasetenkd. Infektionskr.*, 120:127, 1931.
13. Petran, E. L., and H. D. Vera, *Health Lab. Sci.*, 8:225, 1971.
14. Vestal, A. L., *Procedures for the Isolation and Identification of Mycobacterium*, DHEW Publication No. CDC 79-8230, CDC, Atlanta, 1978.
15. Wayne, L. G., *Am. Rev. Respir. Dis.*, 86:651, 1962.

*For more detailed information, consult appropriate references and/or details in the preface of the PML Technical Manual.

PML MICROBIOLOGICALS, INC.
Data #465

Revision Date: January 2001