LOWENSTEIN-JENSEN (L-J) MEDIUM, L-J GRUFT, and L-J MYCOBACTOCEL

INTENDED USE

Remel Lowenstein-Jensen (L-J) Medium, L-J Gruft, and L-J Mycobactocel are solid media recommended for use in qualitative procedures for the isolation and cultivation of *Mycobacterium* species.

SUMMARY AND EXPLANATION

In 1931, Lowenstein formulated a medium for the cultivation of mycobacteria using congo red and malachite green dyes to partially inhibit a number of bacterial species.[1] In 1932, Jensen modified the medium by altering the citrate and phosphate contents, eliminating the congo red dye, and increasing the malachite green concentration.[2] In comparison studies of media for recovery of pathogenic *Mycobacterium* spp., L-J Medium was found to be superior to other media tested, including ATS (American Trudeau Society) Medium.[3,4] In 1960, Boisvert successfully used L-J Medium for niacin testing with *M. tuberculosis*.[5] Gruft modified L-J Medium by adding ribonucleic acid as a growth stimulant and penicillin and nalidixic acid as selective inhibitors of contaminating bacteria.[6] Petran and Vera increased the selectivity of L-J Medium by incorporating cycloheximide, lincomycin, and nalidixic acid.[7]

PRINCIPLE

L-J Medium is a glycerated egg-potato medium. The glycerol and egg mixture provide fatty acids and protein required for the metabolism of mycobacteria. Malachite-green dye is added to inhibit microorganisms other than mycobacteria and as a pH indicator.[8] The incorporation of antibiotics into L-J Medium also serves to inhibit contaminating bacteria. Penicillin G and lincomycin inhibit gram-positive bacteria and nalidixic acid inhibits some gram-negative bacteria encountered in clinical specimens. Cycloheximide inhibits saprophytic molds and yeast. Ribonucleic acid increases the percentage of positive cultures over the standard L-J Medium.

REAGENTS (CLASSICAL FORMULA)*

L-J Medium:

Potato Flour	30.0	g	Malachite Green	0.4	g
Asparagine	3.6	g	Magnesium Sulfate	0.24	g
Monopotassium Phosphate	2.4	g	Whole Eggs	1000.0	ml
Magnesium Citrate	0.6	g	Glycerol	12.0	ml
			Demineralized Water	600.0	ml

Optional ingredients per liter of medium:

L-J Gruft:

Nalidixic Acid	56.0	mg
Ribonucleic Acid	80.0	µg
Penicillin G	80,000	U

L-J Mycobactocel:

Cycloheximide	0.64	g
Nalidixic Acid	56.0	mg
Lincomycin	3.2	mg

*Adjusted as required to meet performance standards.

PROCEDURE

Note: Follow established laboratory guidelines and safety procedures when processing specimens for acid-fast cultures. Inoculate media according to test procedures recommended by the Centers for Disease Control and Prevention (CDC) or consult appropriate references.[9-11]

1. After inoculation, allow media to stand at room temperature for several hours or until the inoculum dries or is absorbed.
2. Protect tubes from light and incubate at 35-37°C in an atmosphere of 5-10% CO_2. It is advisable to incubate media in a slanted position for at least one week to allow even distribution and absorption of the inoculum into the media. Caps should be loosened for the first week to permit the circulation of CO_2 to initiate growth. Thereafter, tighten caps to prevent dehydration of media.
3. Inoculate a second set of media to incubate at room temperature (25-30°C) for specimens obtained from skin or soft tissue in which *Mycobacterium marinum* or *Mycobacterium ulcerans* is suspected.[11]
4. Examine cultures within 5-7 days after incubation and once a week thereafter for a minimum of 8 weeks. Prolonged incubation for 10-12 weeks or more may be necessary in selected cases or if the original smear was positive and the culture remains negative at 8 weeks.
5. Observe cultures for growth rate, pigment production, and colony morphology.
6. Perform an acid-fast stain from the colony to confirm acid-fastness of isolate.
7. Initiate identification tests according to established laboratory procedures. Consult appropriate references for further instructions.[9-11]

QUALITY CONTROL

All lot numbers of L-J Medium, L-J Gruft, and L-J Mycobactocel have been tested using the following quality control organisms and have been found to be acceptable. This quality control testing conforms with or exceeds CLSI standards.[12] Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
L-J Medium:		
**Mycobacterium fortuitum* ATCC® 6841	CO_2, up to 21 days @ 35-37°C	Growth
**Mycobacterium intracellulare* ATCC® 13950	CO_2, up to 21 days @ 35-37°C	Growth
**Mycobacterium kansasii* ATCC® 12478	CO_2, up to 21 days @ 35-37°C	Growth
**Mycobacterium scrofulaceum* ATCC® 19981	CO_2, up to 21 days @ 35-37°C	Growth
**Mycobacterium tuberculosis* ATCC® 25177	CO_2, up to 21 days @ 35-37°C	Growth

(Continued on back)

L-J Gruft Medium:

**Mycobacterium fortuitum* ATCC® 6841	CO_2, up to 21 days @ 35-37°C	Growth
**Mycobacterium intracellulare* ATCC® 13950	CO_2, up to 21 days @ 35-37°C	Growth
**Mycobacterium kansasii* ATCC® 12478	CO_2, up to 21 days @ 35-37°C	Growth
**Mycobacterium tuberculosis* ATCC® 25177	CO_2, up to 21 days @ 35-37°C	Growth
**Mycobacterium scrofulaceum* ATCC® 19981	CO_2, up to 21 days @ 35-37°C	Growth
**Escherichia coli* ATCC® 25922	CO_2, up to 21 days @ 35-37°C	Inhibition (partial to complete)
Staphylococcus aureus ATCC® 25923	CO_2, up to 21 days @ 35-37°C	Inhibition (partial to complete)

L-J Mycobactocel Medium:

**Mycobacterium fortuitum* ATCC® 6841	CO_2, up to 21 days @ 35-37°C	Growth
**Mycobacterium intracellulare* ATCC® 13950	CO_2, up to 21 days @ 35-37°C	Growth
**Mycobacterium kansasii* ATCC® 12478	CO_2, up to 21 days @ 35-37°C	Growth
**Mycobacterium scrofulaceum* ATCC® 19981	CO_2, up to 21 days @ 35-37°C	Growth
**Mycobacterium tuberculosis* ATCC® 25177	CO_2, up to 21 days @ 35-37°C	Growth
Cryptococcus neoformans ATCC® 34877	CO_2, up to 21 days @ 35-37°C	Inhibition (partial to complete)
**Escherichia coli* ATCC® 25922	CO_2, up to 21 days @ 35-37°C	Inhibition (partial to complete)
Staphylococcus aureus ATCC® 25923	CO_2, up to 21 days @ 35-37°C	Inhibition (partial to complete)

*CLSI recommended organism

LIMITATIONS

1. Media containing malachite green, such as L-J Medium, is photosensitive and should not be exposed to light during storage.[8]
2. Mycobacteria require moisture for growth. L-J tubes should be kept tightly sealed prior to inoculation to avoid dehydration of media.[8]
3. Lowenstein-Jensen Medium may display some variation in the light-green color throughout the tube. This does not interfere with the growth of mycobacteria; however, color changes showing bright yellow or dark blue zones may indicate contamination.[8]
4. *Mycobacterium bovis* is inhibited by glycerol and will not grow on L-J Medium.[8]

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Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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