LOWENSTEIN-JENSEN (L-J) MEDIUM w/ 5% NaCl

INTENDED USE
Remel Lowenstein-Jensen (L-J) Medium w/ 5% NaCl is a solid medium recommended for use in qualitative procedures to detect sodium chloride tolerance which aids in the differentiation of *Mycobacterium* species.

SUMMARY AND EXPLANATION
In 1931, Lowenstein formulated a medium for cultivation of mycobacteria containing 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 congo red dye, and increasing the malachite green concentration.[2] The Lowenstein-Jensen formulation, with the addition of 5% sodium chloride, differentiates slowly growing mycobacteria from rapidly growing mycobacteria on the basis of salt tolerance.

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. Sodium chloride (5%) in the medium affects the osmotic/electrokinetic equilibria, thereby permitting differentiation of mycobacteria on the basis of salt tolerance.[3] Most rapid growers and the slower growing *Mycobacterium triviale* will grow on the salt-containing medium.[4,5] Some strains of *Mycobacterium flavescens* may also grow on L-J Medium w/ 5% NaCl. The inability of *Mycobacterium chelonae* subsp. *chelonae* to grow on this medium distinguishes it from other members of the *Mycobacterium fortuitum* complex.[6]

REAGENTS (CLASSICAL FORMULA)*

Sodium Chloride	80.0	g	Magnesium Sulfate	0.24	g
Potato Flour	30.0	g	Whole Eggs	1000.0	ml
Asparagine	3.6	g	Glycerol	12.0	ml
Monopotassium Phosphate	2.4	g	Malachite Green	0.4	ml
Magnesium Citrate	0.6	g	Demineralized Water	600.0	ml

*Adjusted as required to meet performance standards.

PROCEDURE
Note: Follow established laboratory guidelines and safety procedures when processing acid-fast cultures.[7]

1. For each test isolate, set up one tube of L-J Medium w/ 5% NaCl and one tube of sodium chloride-free L-J Medium (REF R08502) as a control.
2. Prepare a light suspension of the test isolate from a pure culture.
3. Transfer 0.1 ml of the suspension into each L-J tube.
4. Incubate both tubes in 5-10% CO_2 at 35-37°C with caps loosened for up to 4 weeks.
5. Examine weekly for growth. The test isolate must grow in the control tube before a negative report can be made.

INTERPRETATION OF THE TEST
Positive Test - Growth on both the L-J Medium w/ 5% NaCl slant and the control slant
Negative Test - Growth only on the control slant

QUALITY CONTROL
All lot numbers of Lowenstein-Jensen Medium w/ 5% NaCl have been tested using the following quality control organisms and have been found to be acceptable. 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
Mycobacterium flavescens ATCC® 14474	5-10% CO_2, 2 weeks @ 35-37°C	Positive
Mycobacterium kansasii ATCC® 12478	5-10% CO_2, 2 weeks @ 35-37°C	Negative

LIMITATIONS
1. Media containing malachite green, such as L-J Medium, is photosensitive and should not be exposed to light during storage.[3]
2. Mycobacteria require moisture for growth. L-J tubes should be tightly sealed prior to inoculation to avoid dehydration of media.[3]

BIBLIOGRAPHY

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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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