

LOWENSTEIN-JENSEN (L-J) MEDIUM w/ 2.5% Ferric Ammonium Citrate

INTENDED USE

Remel Lowenstein-Jensen (L-J) Medium w/ 2.5% Ferric Ammonium Citrate is a solid medium recommended for use in qualitative procedures to detect iron-uptake ability 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 Silcox developed a medium to differentiate mycobacteria on the basis of iron-uptake ability, by adding 2.5% ferric ammonium citrate to the L-J formulation.³

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.⁴ Ferric ammonium citrate (2.5%) is added to determine the ability of some *Mycobacterium* species to take up iron.⁵ *Mycobacterium fortuitum* and a few other rapid and slow growers can convert ferric ammonium citrate to iron oxide resulting in rusty-brown colonies and a tan discoloration in the medium.^{6,7}

REAGENTS (CLASSICAL FORMULA)*

Ferric Ammonium Citrate	40.0 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	Deminerlized 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.⁸

1. For each test isolate, set up one tube of L-J Medium w/ 2.5% Ferric Ammonium Citrate and one tube of iron citrate-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% CO₂ 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

	L-J Medium w/ 2.5% Ferric Ammonium Citrate	L-J Medium (control)
Positive Test -	Rusty-brown colonies, medium may be tan to rusty brown	Growth of colonies without rusty-brown pigment
Negative Test -	Growth of colonies without rusty-brown pigment	Growth of colonies without rusty-brown pigment

QUALITY CONTROL

All lot numbers of L-J Medium w/ 2.5% Ferric Ammonium Citrate 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

Mycobacterium fortuitum ATCC® 6841
Mycobacterium marinum ATCC® 927

INCUBATION

5-10% CO₂, 2 weeks @ 35-37°C
5-10% CO₂, 2 weeks @ 35-37°C

RESULTS

Positive
Negative

LIMITATIONS

1. Media containing malachite green, such as L-J Medium, is photosensitive and should not be exposed to light during storage.⁴
2. Mycobacteria require moisture for growth. L-J tubes should be kept tightly sealed prior to inoculation to avoid dehydration of media.⁴

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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