



20% FERRIC AMMONIUM CITRATE

INTENDED USE

Remel 20% Ferric Ammonium Citrate reagent is recommended for use in qualitative procedures for determining iron uptake in mycobacteria.

SUMMARY AND EXPLANATION

In 1931, Lowenstein formulated a medium for cultivation of mycobacteria containing congo red and malachite green dyes to inhibit certain bacterial species.¹ Jensen modified this medium by eliminating congo red dye, increasing malachite green, and altering the citrate and phosphate contents.² Lowenstein-Jensen medium is used with 20% Ferric Ammonium Citrate in the modified iron uptake test of Szabo and Vandra to identify certain mycobacteria, including *Mycobacterium fortuitum*.³⁻⁴

PRINCIPLE

The iron uptake test detects those mycobacteria capable of converting ferric ammonium citrate to an iron oxide which results in rusting that colors both the colonies and the medium.⁵ Most rapid growers, including *Mycobacterium fortuitum*, are usually positive while *Mycobacterium chelonae* is negative. Slow growers are usually not capable of accumulating iron oxides.

REAGENTS (CLASSICAL FORMULA)*

Ferric Ammonium Citrate (CAS 1185-57-5)..... 200.0 g
Demineralized Water (CAS 7732-18-5) 1000.0 ml

*Adjusted as required to meet performance standards.

PRECAUTIONS

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after their use. Directions should be read and followed carefully.

STORAGE

This product is ready for use and no further preparation is necessary. Store product in its original container at 2-8°C until used. Allow product to equilibrate to room temperature before use. Protect from light.

PRODUCT DETERIORATION

This product should not be used if (1) the color has changed, (2) the expiration date has passed, or (3) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT

Specimens should be collected and handled following recommended guidelines.⁶

MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, swab, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) Lowenstein-Jensen Medium (R08500), (7) Mycobacteriological safety equipment, (8) Pipette.

PROCEDURE

1. Inoculate two L-J slants for each patient sample with one drop of a barely turbid suspension of the test organism.

2. Incubate at 35-37°C in 5-10% CO₂ in a slanted position with caps loosened until colonies are grossly visible.
3. Add 20% Ferric Ammonium Citrate to one of the patient sample tubes, one drop of 20% Ferric Ammonium Citrate for each milliliter of L-J Medium. The other L-J slant serves as a negative color control.
4. Reincubate cultures at 28°C in 5-10% CO₂ and examine weekly for 3 weeks.

INTERPRETATION

- Positive Test - Colonies appear rusty brown
- Negative Test - Pigmentation of colonies remains the same as that observed on L-J Medium that did not receive 20% Ferric Ammonium Citrate Solution

QUALITY CONTROL

All lot numbers of 20% 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	INCUBATION	RESULTS
<i>Mycobacterium fortuitum</i> ATCC® 6841	CO ₂ , up to 3 weeks @ 28°C	Positive
<i>Mycobacterium kansasii</i> ATCC® 12478	CO ₂ , up to 3 weeks @ 28°C	Negative
<i>Mycobacterium tuberculosis</i> ATCC® 25177	CO ₂ , up to 3 weeks @ 28°C	Negative




BIBLIOGRAPHY

1. Lowenstein, E. 1931. Zentralb. Bakteriell. Parasitenkd. Infektionskr. Hyg. Abt. Orig. 120:127-129.
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3. Szabo, I. and E. Vandra. 1963. Acta. Microbiol. Acad. Sci. Hung. 10:215-223.
4. Wayne, L.G. and J.R. Dubek. 1968. Appl. Microbiol. 16:925-931
5. Kent, P.T. and G.P. Kubica. 1985. Public Health Mycobacteriology, A Guide for the Level III Laboratory. U.S. Dept. of H.H.S. and CDC, Atlanta, GA.
6. Garcia, L.S. 2010. Clinical Microbiology Procedures Handbook. 3rd ed. ASM Press, Washington D.C.

PACKAGING

REF R21216, 20% Ferric Ammonium Citrate25 ml/Btl

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

ATCC® is a registered trademark of American Type Culture Collection.
CAS (Chemical Abstracts Service Registry No.)

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