PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Urea Broth for AFB Procedure

Department of Microbiology

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1.0 Purpose and Principle

HPLC identification of *Mycobacterium* isolates frequently cannot separate *M. scrofulaceum* from MAI. Distinguishing between the two is important for subsequent susceptibility testing and for guiding patient therapy. Urea Broth for AFB is a liquid medium that may be used to differentiate *Mycobacterium* species based on their ability to hydrolyze urea. *M. scrofulaceum* can be differentiated from MAI based on its ability to hydrolyze urea overnight. While some strains of MAI can hydrolyze urea, they require more than 24 hours to produce a color change in the broth.

2.0 Scope

This procedure is classified under CLIA as Highly Complex. It should be carried out by technical personnel familiarized and trained to perform testing and interpret test results. Testing includes but is not limited to: basic troubleshooting, QC checks, and technical proficiency in accordance with the department SOP.

3.0 Safety - Personal Protective Equipment

Performance of this procedure may expose testing personnel to biohazardous material. All cultures must be handled as potentially infectious material as outlined in the Providence Sacred Heart Microbiology Safety Guidelines.

To perform this procedure, you must use:

- Laboratory Coat
- N95 Respirator
- Biological safety cabinet

Disinfectant following procedure:

- Bleach dilution sprayers can be used for on demand disinfectant.

4.0 Isolate Requirements

Isolates that produce an identification of MAIS complex (*M. avium/M. intracellulare*/*M. scrofulaceum*) by HPLC should be tested for overnight urease activity. Growth can be harvested from an actively growing culture on agar-based media such as 7H11.

5.0 Materials

5.1 Equipment

- Aerobic (non-CO_2) incubator at 33-37°C

5.2 Consumables

- Sterile disposable loops or swabs

5.3 Media

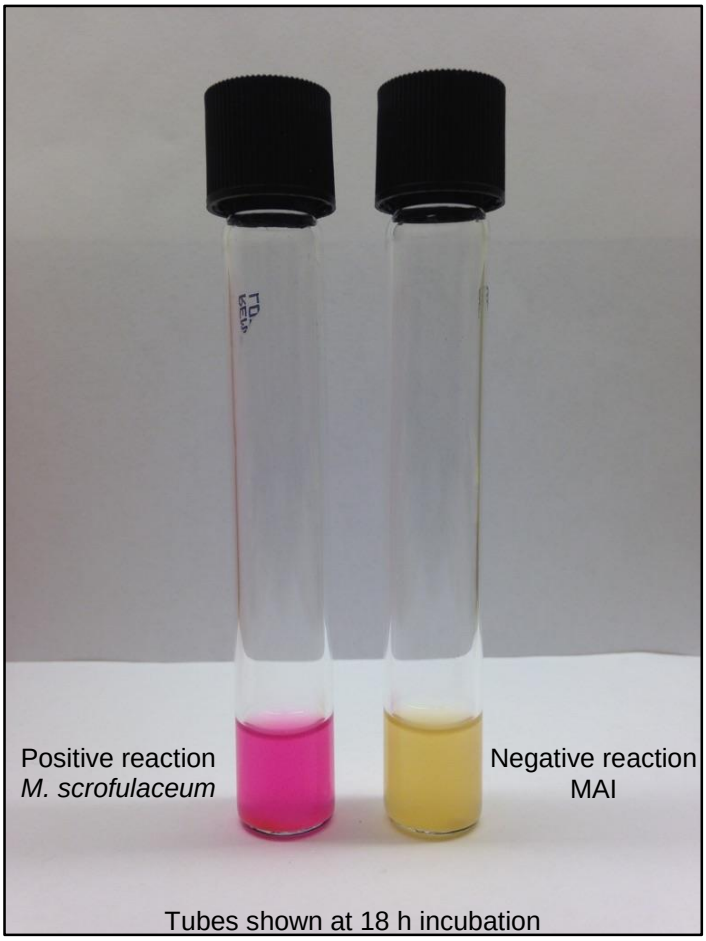
- 7H11 agar for culture
- Urea Broth for AFB (Remel item R065222)

6.0 Procedure

1. Perform culture manipulation and broth inoculation within a biosafety cabinet.
2. Harvest growth from a young, actively growing culture of the test isolate using a sterile loop or swab.
3. Vigorously mix the inoculum against the bottom and sides of the tube. The broth should be visibly turbid after inoculation.
4. Incubate tubes aerobically (not in CO_2) at 33-37°C overnight.
5. On the following day, observe the broth for a pink-red color. Do not observe tubes beyond 1 day of incubation.

7.0 Interpretation & Reporting of Results

M. scrofulaceum will produce a strongly-positive test within 18-24 h. This is indicated by the development of a dark pink to red color. Faint or light pink color reactions are not characteristic for *M. scrofulaceum.* Verify that the broth is visibly turbid, indicating that sufficient inoculum was used. If necessary, repeat the test using a heavier inoculum. Consult Rounds if weakly positive reactions are encountered. MAI isolates do not produce a positive reaction within 24 h and the broth will remain yellow.



Tubes shown at 18 h incubation

After the urea broth test has been performed, the report can be updated from *Mycobacterium* other than *Mycobacterium tuberculosis* to either *Mycobacterium avium-intracellulare* complex or *Mycobacterium scrofulaceum,* based in the test results.

8.0 Quality Control

Positive and negative control tubes should be inoculated each day of testing patient isolates.

Control Strain	**Results**
M. scrofulaceum 2013 CAP E08	Positive
M. avium ATCC 25291	Negative

9.0 Test Verification

A total of 31 isolates were used to verify the performance of the Remel Urea Broth for AFB. This included 30 clinical strains of MAI previously characterized by DNA hybridization with the AccuProbe® *Mycobacterium avium* Complex Culture Identification test. All 30 (100%) of the MAI

isolates remained negative after 24 h incubation of the urea broth. No clinical isolates of *M. scrofulaceum* were available for testing, and none were available for purchase from ARUP Laboratories. A *M. scrofulaceum* isolate from a CAP survey in 2013 (E08) was used. This isolate produced a strong positive reaction in less than 18 h (pictured above). While the number of *M. scrofulaceum* isolates in this verification study were limited to one, it did produce the expected result and verified the ability of the broth to detect the urease activity of this species.

10.0 References

1. Package insert: Remel Urea Broth for AFB IFU 65222, Revised August 31, 2009.
2. Garcia L, Isenberg H. *Clinical Microbiology Procedures Handbook*. Washington, DC: ASM Press; 2010.
3. Winn W, Koneman E. *Koneman's Color Atlas And Textbook Of Diagnostic Microbiology*. Philadelphia: Lippincott Williams & Wilkins; 2006.

11.0 Document Control History

Microbiology Director Approval: Dr. Ann Robinson 09/03/2015

Microbiology Supervisor Reviews: Jason Ammons 09/04/2015

Revisions & Updates: