U-083 Evaluation of a Rapid, Fluorescent Stain for the Detection of Mycobacteria in Clinical Specimens

Cindy Hendry, Kim Dionne, Karen Carroll, and Nicole Parrish

The Johns Hopkins Medical Institutions, Baltimore, Maryland

ABSTRACT

Due to the steady increase in Mycobacterial disease, rapid detection is essential for early diagnosis and treatment of infection. A common method used for screening clinical specimens suspected of containing mycobacteria is microscopic examination of stained smears for the presence of acid-fast bacilli (AFB). We compared 2 Auramine-O stains: the Remel TB Auramine-O stain (RAO) and the Rapid Modified Auramine-O stain from Scientific Device Laboratory (MAO). The RAO procedure required 8 steps using 3 stains and ~22 minutes for completion. The MAO procedure required 6 steps using 2 stains and ~2 minutes for completion. Testing included pooled specimens from the following digested / decontaminated sources: tissue, sputum, bronchial lavage, peritoneal fluid, and (undigested) cerebrospinal fluid. Each source was divided into separate aliquots and inoculated with a dilution series of *Mycobacterium gordonae* to reflect the burden of organism typically seen in clinical samples. 100 duplicate slide sets were prepared according to manufacturer's protocols and divided into sets 'A' (RAO-stained) and 'B' (MAO-stained). Slides were graded for quantity of organism, and brightness of both AFB and background debris. In comparing both methods, all slides were positive for AFB with no significant quantification difference in organism between stains. Approximately 40% of the MAO slides were brighter than their paired RAO counterpart. Overall, MAO-stained slides exhibited less background debris staining (4%) versus RAO stained slides (30%). MAO staining required significantly less time (~2 min) versus the RAO stain (~22 min).
Results of this study suggest that the MAO-stain has several favorable characteristics for use in a clinical laboratory setting: it is rapid, provides equivalent AFB quantitation as compared with the RAO stain, but with less non-specific background fluorescence. As such, the MAO stain has the potential to be more cost effective and efficient in presenting presumptive evidence of mycobacteria in clinical specimens.

INTRODUCTION

Rapid diagnosis of mycobacterial infections is essential for initiation of infection control practices when necessary and to provide appropriate antimicrobial therapy. A common method used for screening clinical specimens suspected of containing Mycobacteria is microscopic examination of stained smears for the presence of acid-fast bacilli (AFB). Effective time management can be a major factor contributing to the efficiency of mycobacteriology laboratories with high testing volumes. Thus, rapid staining methods for AFB are essential to provide the fastest turn-around-time for reporting results. Additionally, more rapid staining methods will provide for decreased technician time resulting in improvement of overall laboratory performance. In this study, we compared 2 Auramine-O stains: the Remel TB Auramine-O stain (RAO) and the Rapid Modified Auramine-O stain from Scientific Device Laboratory (MAO).

METHODS

RAO procedure: 8 steps, 3 stains, total time ~22 minutes.
MAO procedure: 6 steps, 2 stains, total time ~2 minutes.

Part I: Testing included pooled specimens from the following digested / decontaminated sources: tissue, sputum, bronchoalveolar lavage, peritoneal fluid, and (undigested) cerebrospinal fluid. Each source was divided into separate aliquots and inoculated with a dilution series of *Mycobacterium gordonae*, to reflect the burden of organism typically seen in clinical samples. In a blind study, 100 duplicate slide sets were prepared according to manufacturer's protocols and divided into sets 'A' (RAO-stained) and 'B' (MAO-stained). Slides were read and graded independently by multiple qualified technologists and the results compared. Results included both the quantity of organism observed (1+ to 4+) and the brightness (1+ to 4+) of both the AFB and background debris.

Part II: The same procedure as listed in Part I was repeated using additional species (2 strains each) of Mycobacteria including *M. tuberculosis, M. avium, M. kansasii, M. lentiflavum, M. abscessus, M. chelonae, M. scrofulaceum, M. neoaurum,* and *M. mucogenicum*. This comparative analysis between the two methods was performed in a blinded manner in which both species identification and dilution number were removed. Slides were graded as outlined above.

Table 1. RAO versus MAO by mycobacterial species: average AFB and debris quantitation between 5 microscopists in spiked sputum samples.

M. tuberculosis

Stain	AFB (average)	% agreement	Debris (average)	% agreement
RAO	4+	100%	3.2+	80%
MAO	4+	100%	1.2+	80%

M. avium

Stain	AFB (average)	% agreement	Debris (average)	% agreement
RAO	3.2+	80%	2.1+	60%
MAO	3+	100%	1+	100%

M. fortuitum

Stain	AFB (average)	% agreement	Debris (average)	% agreement
RAO	4+	100%	1.4+	80%
MAO	4+	100%	1.2+	80%

Table 2. RAO versus MAO by specimen type with *M. tuberculosis*: average AFB and debris quantitation between 5 microscopists.

RAO

Specimen Type	AFB	% agreement	Debris	% agreement
Tissue	4+	80%	2+	80%
Sputum	4+	80%	1+	60%
BAL	3.6+	60%	2+	80%
CSF	4+	100%	0	100%
Pleural Fluid	4+	100%	1+	80%

MAO

Specimen Type	AFB (average)	% agreement	Debris (average)	% agreement
Tissue	4+	92%	1.4+	80%
Sputum	3.8+	68%	1+	80%
BAL	3.6+	80%	1+	80%
CSF	4+	100%	0	100%
Pleural Fluid	4+	100%	1+	100%

Averages and % agreement between the 2 methods computed based on readings by 5 independent microscopists. Spiked sputum samples contained 5 X 10^6 to 1 X 10^7 CFU/mL. 80% agreement represents perfect concordance between 4/5 microscopists with only one discrepant result.

MTB with RAO

MAC with RAO

Debris with RAO

MTB with MAO

MAC with MAO

Debris with MAO

SUMMARY

MAO versus RAO: which stain?

- The MAO stain is more rapid than the RAO stain.
- The MAO stain provides equivalent AFB quantitation versus the RAO stain.
- The MAO stain shows less non-specific background fluorescence versus the RAO stain.
- The MAO stain decreases the technician time required for the procedure.
- The MAO stain decreases the turn-around-time in reporting results.
- Thus, the MAO stain has the potential to improve laboratory effectiveness.