

Evaluation of the Use of Mitchison 7H11 Agar Plates in Conjunction with the BACTEC MGIT 960 Broth System for Recovery of Mycobacteria in a Public Health Laboratory.

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RESULTS

ABSTRACT

Background: Current guidelines recommend the use of both a liquid and a solid medium for the isolation of mycobacteria from clinical specimens. We conducted a four-month trial using Mitchison 7H11 selective agar plates (7H11) in conjunction with the BACTEC MGIT 960 automated broth system (MGIT) to determine the impact of using 7H11 on the recovery rate of *Mycobacterium* species in clinical specimens, time to detection (TTD), and contamination rates. We also looked at the cost-effectiveness of using 7H11. **Methods:** 594 specimens were processed using the standard N-acetyl-L-cysteine (NALC)-NaOH decontamination method, concentrated by centrifugation, and each inoculated into a MGIT broth tube and a 7H11 plate. Additionally, 188 specimens from sterile and/or surgical sites were concentrated (without decontamination) and inoculated into the two media types. **Results:** A total of 114 *Mycobacterium* isolates were detected from 782 clinical specimens, including 32 *M. tuberculosis* complex (MTBC) isolates and 82 non-tuberculous mycobacteria. The overall recovery rate for *Mycobacterium* species was higher in the MGIT (95.6%, 109/114) than the 7H11 plates (47.3%, 54/114). Recovery rates for MTBC were 100% (32/32) for the MGIT and 56% (18/32) for 7H11 plates. While there were 60 *Mycobacterium* species (including 14 MTBC) isolated in the MGIT only, there were only 4 *Mycobacterium* isolates (3 *M. avium* complex and 1 *M. gordonae*) that were isolated solely on the 7H11 plates. The average TTD was shorter for the MGIT (18 days) than the 7H11 plates (26 days); isolates detected on both media types were never detected first on the 7H11 plates. Moreover, the average TTD for MTBC was 18 days for the MGIT and 24 days for the 7H11 plates. Contamination rates for the MGIT were higher for the four month period (9.0%, 71/782) than for the 7H11 media (2%, 16/782). **Conclusion:** Although contamination rates were lower, 7H11 did not increase the recovery rate of mycobacteria from clinical specimens nor decrease TTD when used in combination with the MGIT system. Our lab would save \$1,532 in a four month period or approximately \$4500 per year by omitting 7H11 plates from our routine protocol.

INTRODUCTION

Effective tuberculosis (TB) control and progress toward TB elimination require rapid diagnostic testing. Although rapid molecular methods exist to detect MTBC directly from patient specimens, the definitive diagnosis of mycobacterial disease still relies on isolation and identification of mycobacteria from culture, the "gold standard" of detection. Actively growing cultures are also necessary for antimicrobial susceptibility testing. Clinical microbiology laboratories are challenged to culture mycobacteria as quickly as possible in a cost-effective manner. Current guidelines recommend the use of both liquid and solid media for the isolation of mycobacteria from clinical specimens. The CDC recommends use of liquid media to increase recovery of mycobacteria and decrease detection times. Solid media may maximize recovery and allow examination of colony morphology^{1,2}.

Our four-month study evaluated the contribution of selective solid media (Mitchison 7H11) when used in combination with the automated MGIT (modified 7H9 broth) system for recovery and identification of mycobacteria from clinical specimens. Our evaluation included comparison of MGIT and 7H11 using the following criteria: numbers and types of mycobacteria isolated, time to detection, contamination rates, and cost of media.

METHODS

Non-sterile patient specimens were decontaminated using the standard N-acetyl-L-cysteine (NALC)-NaOH method (1% final concentration of NAOH) and concentrated by centrifugation at 3300 X g for 15 minutes. Specimens from sterile and/or surgical sites were concentrated by centrifugation as above (without decontamination). Cell pellets were resuspended in 1 ml 0.067M phosphate buffer (pH 6.8) and 0.5 ml of the sediment suspension was inoculated into each 7 ml MGIT tube containing PANTA supplement. Three drops of sediment suspension were inoculated to each 7H11 plate, drops evenly spaced. MGIT tubes were incubated in the BACTEC MGIT 960 system at 35-38°C degrees for up to 42 days and automatically monitored for positivity. 7H11 plates were incubated at 35-37°C with 5-10% CO2 for 6 weeks, and inspected at least weekly for growth.

Growth from plates or broth was stained by the Kinyoun method. All positive cultures containing acid fast organisms were analyzed by HPLC for identification purposes. Non-acid fast growth in MGIT broth or on 7H11 was considered "contamination". Time to detection was calculated from the date the specimen was inoculated onto media until the date the BACTEC MGIT 960 instrument flagged the sample as positive for MGIT tubes, or the date on which there was evidence of growth by visual examination using a light microscope at 5-10X magnification for 7H11.

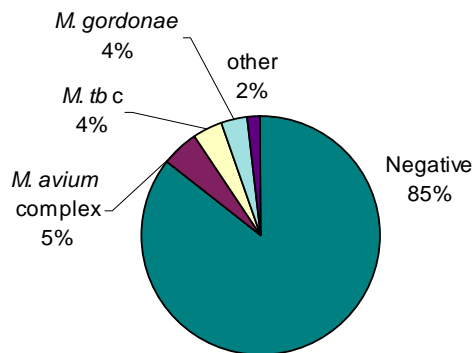


Figure 1. Isolations from 782 Clinical Specimens.

From 782 clinical specimens, there were 108 positive cultures (13.8% overall positivity rate) with 114 acid-fast organisms isolated including: 40 MAC (5%), 32 MTBC (4%), and 30 *M. gordonae* (4%). Other organisms (2%) included: 4 *Gordonia* species, 3 *M. fortuitum* group, 2 *M. abscessus*, 2 *Rhodococcus* species, and 1 *M. chelonae*.

Table 1. Isolations Based on Media Type.

MGIT Broth Only		MGIT Broth and 7H11 Plate		7H11 Plate Only	
Number of Isolations	Identification	Number of Isolations	Identification	Number of Isolations	Identification
14	MTBC	21	MAC	3	MAC
16	MAC	18	MTBC	1	<i>M. gordonae</i>
21	<i>M. gordonae</i>	8	<i>M. gordonae</i>		
4	<i>Gordonia</i> sp.	1	<i>M. fortuitum</i> group		
2	<i>M. fortuitum</i> group	1	<i>M. chelonae</i>		
2	<i>Rhodococcus</i> sp.	1	<i>M. abscessus</i>		
1	<i>M. abscessus</i>				
60 TOTAL		50 TOTAL		4 TOTAL	

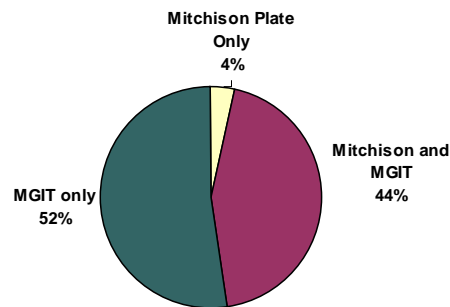


Figure 2. Percentage of Acid-Fast Organisms Isolated in Broth and Solid Media.

Of 114 acid-fast organisms identified (see Table 1), 50 isolates (44%) grew in both MGIT and 7H11 ("Mitchison"). 60 isolates (52%) grew only in the MGIT broth, and 4 isolates (4%) grew only on 7H11 plates. Overall recovery rate was 96% (110/114) for MGIT and 47.3% (54/114) for 7H11.

Table 2. Time to Detection in Broth and Solid Media.

Media	Average Time to Detection in Days for Cultures containing Acid-fast Organisms	Average Time to Detection in Days for <i>M. tuberculosis</i> complex
BACTEC MGIT 960 Broth System	18 (range 3-41 days)	18 (range 3-36 days)
Mitchison 7H11 Selective Plate	26 (range 3-42 days)	24 (range 3-42 days)

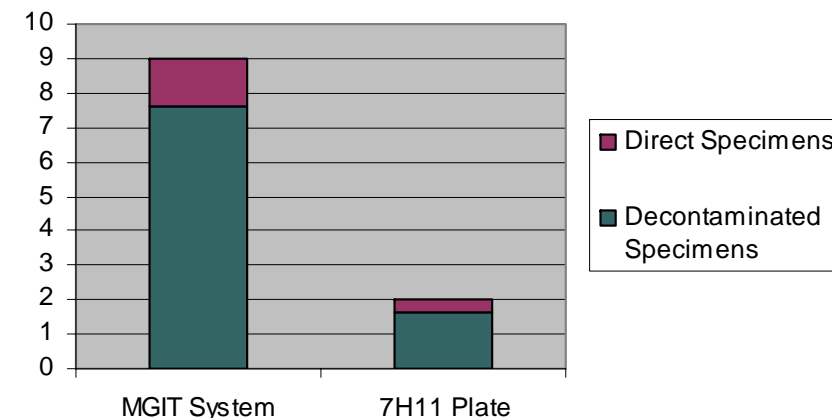


Figure 3. Contamination Rates in Broth and Solid Media.

Of 782 clinical specimens, 594 were decontaminated by the NALC-NaOH method and 188 sterile and/or surgical specimens were concentrated and inoculated directly onto media ("Direct Specimens"). In MGIT broth, 60/782 (7.6%) of decontaminated specimens and 11 (1.4%) of direct specimens were contaminated with non-acid fast growth. On 7H11 plates, 13/782 (1.66%) of decontaminated specimens and 3 (0.38%) of direct specimens were contaminated. Total contamination rates were 9.0% and 2.0% for MGIT and 7H11 respectively.

Table 3. Cost of Media.

Media	Cost	4-month period (782 specimens)	1 Year (approximately 2300 specimens)
Mitchison 7H11 selective plate (Remel, Lenexa, KS, USA)	\$1.96 each	\$1532	\$4508
BACTEC MGIT 7 ml tube plus PANTA growth supplement (Becton Dickinson & Co., Sparks, MD, USA)	\$5.30 each	\$4144	\$12,190
TOTAL		\$5676	\$16,698

CONCLUSIONS

- The overall recovery rate for *Mycobacterium* species and other acid-fast organisms was higher in the MGIT than the 7H11 plate.
- 100% of MTBC isolates were recovered in the MGIT while 56% were recovered on 7H11, with 14 MTBC isolates growing only in the MGIT broth.
- Average TTD was eight days shorter in the MGIT compared to 7H11.
- Overall contamination rates were higher in the MGIT (7.6%) than 7H11 (1.66%) for non-sterile specimens.
- Using 7H11 plates in conjunction with the MGIT broth resulted in additional media costs of \$1532 in four months or approximately \$4500 in one year. Significant labor costs were also incurred for inoculation and examination of plates.
- Omitting 7H11 plates from routine culture inoculations would not significantly reduce the number or type of acid fast organism isolated from clinical specimens in our laboratory.
- In this study, MGIT culture alone was sufficient to detect all MTBC isolates in a rapid and cost-effective manner.

REFERENCES

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- Tenover FC, et al. The resurgence of tuberculosis: Is your laboratory ready? *J. Clin Microbiol.* 1993. 31:767-770.