

Comparison of MGIT and Myco/F Lytic Liquid-Based Blood Culture Systems for Recovery of *Mycobacterium tuberculosis* from Pleural Fluid

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The specificities and sensitivities of the Bactec mycobacterial growth indicator tube (MGIT) system for the recovery of *Mycobacterium tuberculosis* from pleural fluid are not statistically different than those of the Myco/F lytic liquid culture system. The time to positivity is shorter in the MGIT system (12.7 versus 20.7 days, respectively; $P = 0.007$). The Myco/F lytic culture system may be an alternative to the MGIT system for diagnosing pleural tuberculosis.

Tuberculosis (TB) is the most frequent cause of exudative pleural effusions in areas of high TB incidence. Studies have shown that *Mycobacterium tuberculosis* is the causative agent in up to 44% of HIV-seronegative people hospitalized with a pleural effusion (1–3), and the percentage is higher in HIV-seropositive people (4). Pleural TB is a paucibacillary disease. The pathogenesis of a tuberculous pleural effusion is likely due to a delayed hypersensitivity reaction to *M. tuberculosis* proteins (for a review, see reference 5) and not to a large burden of organisms. The scarcity of organisms makes it difficult to isolate *M. tuberculosis* from pleural fluid samples, leading to low rates of culture confirmation.

Rich culture media are generally more sensitive in detecting *M. tuberculosis* in sputum and other clinical samples (6). Few studies have compared different liquid media and examined their potential role in combination with solid media for the diagnosis of tuberculous pleurisy. In this study, we compared the Bactec 9120 Myco/F lytic blood culture system (Myco/F lytic) to the Bactec mycobacterial growth indicator tube (MGIT) 960 system (Becton Dickinson, Sparks, MD) (with each liquid system used in conjunction with locally prepared Middlebrook 7H11 solid medium) with respect to time to positivity (TTP), sensitivity, specificity, and percent culture yield of *M. tuberculosis* isolates from pleural fluid.

The Myco/F lytic and MGIT systems perform continuous monitoring. The MGIT system incubates the samples at 37°C, and the Myco/F lytic system incubates at 35°C. The Myco/F lytic culture is supplemented with Middlebrook 7H9 and brain heart infusion broth, which is a nonselective culture medium intended to be an adjunct to aerobic blood culture medium to improve the recovery of mycobacteria, yeast, and fungi from blood or for the culture of sterile body fluids when yeast or fungi are suspected. The MGIT culture contains modified Middlebrook 7H9 broth supplemented with polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin (PANTA) and oleic acid-albumin-dextrose-catalase (OADC) (a mycobacteria growth supplement). In these two systems, samples are determined to be negative if there is no detectable growth after 42 days of incubation. The list price of the BD Bactec 9120 system, which holds 120 bottles, is approximately \$20,000. The list prices of the MGIT 960 system (holds 960 vials) and the MGIT 320 system (holds 320 vials) are approximately \$49,000 and \$29,000, respectively. The list unit cost

per bottle of the Myco/F lytic medium is \$7.50, and the unit price per 7-ml MGIT and supplement is \$11.17.

The subjects of this study had new pleural effusions, with TB as the suspected etiology. The study took place in Uganda, a high-burden country for TB, with an TB incidence in 2012 of 179 cases per 100,000 population (7). This is a subset of a larger study, details of which were published previously (4, 8). The goals of the larger study are to analyze host immune responses against *M. tuberculosis*, with a particular focus on patients with TB pleurisy, to gain insight into the balance of pleural protective and pathogenic responses. For this substudy, patients were enrolled from September 2011 until October 2012. Adults aged 18 years and older with signs and symptoms consistent with TB but without a history of TB treatment and who had moderate-to-large pleural effusions were screened for enrollment at the National Tuberculosis Treatment Center, Mulago Hospital (Kampala, Uganda). The study protocol was approved by the Ugandan National AIDS Research Subcommittee and the Institutional Review Board for Human Investigations at Case Medical Center (Cleveland, OH). All the study participants gave written informed consent for HIV testing and study participation and received pre- and post-HIV test counseling. Patients with pleural TB underwent diagnostic thoracentesis, and up to 30 ml of pleural fluid was collected. Closed pleural biopsy specimens were also obtained from a subset of patients using the Abrams needle method (4, 8). The

Received 25 September 2014 Returned for modification 18 November 2014

Accepted 25 January 2015

Accepted manuscript posted online 4 February 2015

Citation Harausz E, Lusiba JK, Nsereko M, Johnson JL, Toossi Z, Ogwang S, Boom WH, Joloba ML, Tuberculosis Research Unit (TBRU). 2015. Comparison of MGIT and Myco/F lytic liquid-based blood culture systems for recovery of *Mycobacterium tuberculosis* from pleural fluid. *J Clin Microbiol* 53:1391–1394. doi:10.1128/JCM.02742-14.

Editor: B. A. Forbes

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doi:10.1128/JCM.02742-14

TABLE 1 Culture results for *Mycobacterium tuberculosis* and contamination rates for the different samples

Sample type	Positive culture (no. [%])	Negative culture (no. [%])	Contaminated (no. [%])
Pleural fluid			
Middlebrook 7H11 solid medium	15 (29)	36 (71)	2 (4)
MGIT	20 (39)	31 (61)	6 (12)
Myco/F lytic culture	22 (43)	29 (57)	10 (19)
Pleural biopsy ^a	26 (79)	7 (21)	0
Sputum ^b	7 (32)	15 (68)	1 (5)

^a The pleural biopsy was cultured in Bactec liquid culture and Lowenstein-Jensen medium.

^b The sputum was cultured on Middlebrook 7H11 solid medium and/or in an MGIT culture.

pleural fluid samples were divided, with 4 ml inoculated at bedside (4), into a Myco/F lytic culture vial, transported to the laboratory, and incubated in the Myco/F lytic system. The remaining pleural fluid was collected in a sterile 50-ml Falcon tube, transported to the laboratory, and spun at 3,000 × g, and the pellet was resuspended in a phosphate buffer solution. Five hundred microliters was inoculated into a 7-ml MGIT and incubated in the MGIT system. Two hundred microliters was inoculated onto 7H11 solid medium. The specimen was also stained with auramine fluorescent stain and examined for acid-fast bacilli (AFB). Pleural tissue was aseptically ground in a disposable tissue grinder and homogenized with 2.5 ml of sterile normal saline. One milliliter was inoculated into a liquid culture (Bactec), and 200 μl was inoculated onto a solid (Lowenstein-Jensen [LJ]) medium.

Two spontaneous expectorated sputum samples were collected from every patient who could produce a sample, and they were digested and decontaminated (at room temperature for 15 min) by the *N*-acetyl-L-cysteine-NaOH method to a final NaOH concentration of 1.5%. The samples were pelleted by centrifuging at 3,000 × g and resuspended in phosphate buffer. Two hundred microliters was inoculated onto 7H11 solid medium, and 500 μl was inoculated into MGITs as described above.

When a culture became positive, it was flagged, and the TTP was recorded. LJ tubes and 7H11 plates were incubated at 37°C at CO₂ concentrations of 5% to 10% and examined weekly for growth for a maximum of 42 days. All positive cultures were confirmed by Ziehl-Neelsen (ZN) staining. In all ZN-positive samples, *M. tuberculosis* complex was distinguished from nontuberculous mycobacteria with the Capilia assay (Tauns Laboratories, Numazu, Japan [9]). Bacterial contamination was ruled out by inoculating and incubating the sample on a blood agar plate.

Data were analyzed using SPSS Statistics version 21 (IBM). Comparisons of proportions between groups were done using McNemar's test or Fisher's exact test. A comparison of normally distributed means was done using an independent samples *t* test. A *P* value of <0.05 was used to reject the null hypothesis in all analyses.

In this substudy, 51 subjects who had pleural fluid cultures submitted to the Myco/F lytic system, the MGIT system, and 7H11 solid medium, were analyzed. The median age of the subjects was 28 years (interquartile range, 11), and 80% were male. Twenty-four subjects (47%) were HIV seropositive with a median CD4

TABLE 2 Comparison of *Mycobacterium tuberculosis* recovery in pleural fluid for the MGIT/Middlebrook 7H11 solid medium combination versus that of the Myco/F lytic/Middlebrook 7H11 solid medium combination^a

Myco/F lytic and solid medium	MGIT and solid medium		
	No. (%) positive	No. (%) negative	Total no. (%) of isolates
Positive	20 (39)	6 (12)	26 (51)
Negative	3 (6)	22 (43)	25 (49)
Total	23 (45)	28 (55)	51 (100)

^a Percentages are given as a proportion of total isolates (*n* = 51).

count of 223/mm³. Twenty-three (45%) of the subjects had sputum mycobacterial cultures done using the MGIT system and/or solid media, and 33 (65%) had pleural biopsy cultures. Two subjects (4%), both of whom were HIV seropositive, had pleural fluid samples that were AFB smear positive.

For analysis, a case of culture-confirmed pleural TB was defined as a patient with a culture positive for *M. tuberculosis* from any source except for the pleural fluid liquid and solid culture combination under investigation. Therefore, the reference standard for the pleural fluid MGIT/solid medium combination was a positive culture in the Myco/F lytic pleural fluid culture or in a sputum (in either a solid medium or an MGIT) or pleural biopsy specimen. The reference standard for the pleural fluid Myco/F lytic/solid medium combination was an *M. tuberculosis*-positive culture in the MGIT pleural fluid culture or in a sputum (in either a solid medium or an MGIT) or a pleural biopsy specimen.

The TTP was significantly shorter in the MGIT culture versus that in the Myco/F lytic liquid culture (12.7 days versus 20.7 days [sample sizes of 19 and 21], respectively; *P* = 0.007). Few studies have looked exclusively at pleural fluid, but the TTP for the MGIT culture in our study was similar to that for other studies that examined a variety of AFB-negative samples (10–13). However, the shorter TTP for the MGIT culture versus that for the Myco/F lytic culture may also have been due to the processing of the samples and not just to the culture method. The inoculum of *M. tuberculosis* may be lower in the 4 ml of pleural fluid directly inoculated into the Myco/F lytic culture versus that of the centrifuged concentrated pellet prepared from the pleural fluid that was resuspended and inoculated into the MGIT culture.

M. tuberculosis culture results for the various samples are presented in Table 1. The sensitivities and specificities for the MGIT/7H11 solid medium combination and for the Myco/F lytic/7H11 solid medium combination did not differ. The sensitivities were 60% and 69% and the specificities were 88% and 93% for the MGIT and Myco/F lytic cultures, respectively. Although not statistically significant, each liquid/solid culture combination was *M. tuberculosis* positive in pleural fluid cultures that were negative in the other liquid/solid culture combinations. The Myco/F lytic/solid culture was *M. tuberculosis* positive in 6 (or 12% of the samples submitted) cultures that were negative in the MGIT/solid culture combination. The MGIT/solid culture was *M. tuberculosis* positive in 3 cultures (or 6% of the total samples submitted) that were negative in the Myco/F lytic/solid culture combination (see Table 2). Thus, while the sensitivities and specificities of these two culture methods did not differ, those methods that use different media did provide different culture results for individual samples.

This may, in part, be due to subtle differences in *M. tuberculosis* strains that allow growth and recovery in different media.

Overall, the Myco/F lytic/solid culture combination was positive for *M. tuberculosis* in 51% (26/51) of the cultures submitted. The MGIT/solid culture combination was positive for *M. tuberculosis* in 45% (23/51) of the cultures submitted (Table 2). It is difficult to compare culture yields of this study to those of other studies, as frequently pleural fluid cultures are combined with other culture results, various volumes are inoculated into the medium, the reference standard is difficult to define, and the pretest probability of TB in the populations studied varies. Not surprisingly, MGIT culture yields in studies that examined the culture yields from a variety of respiratory and nonrespiratory AFB-negative samples varied from 16% to 76% of the submitted samples (13–15). In a 2011 paper from South Africa, in 25 subjects with an undiagnosed pleural effusion but with high clinical suspicion for TB, the pleural fluid culture sensitivity of the MGIT system was 45% and the specificity was 100% (16). In a 2011 study from India, 102 pleural fluid samples from clinically suspected cases of pleural TB had a culture positivity rate of 46% in the Bactec liquid culture system (17).

The 7H11 solid medium contributed to the *M. tuberculosis* culture yield. The solid medium grew 4 positive cultures (8% of all pleural fluid samples submitted) that the Myco/F lytic liquid broth used alone would have missed, and it grew 3 (6% of all pleural fluid samples submitted) cultures that the MGIT culture alone would have missed.

Culture contamination rates between the MGIT liquid culture and Myco/F lytic liquid culture (12% versus 19%, respectively) were not statistically significantly different (see Table 1). There was only one instance in which a sample was contaminated in both of the systems. The contamination rate for the MGIT system is similar to that in other studies using MGIT; although there have been no other studies evaluating the Myco/F lytic system in this manner, the contamination rate of 19% is somewhat higher than that of other liquid culture media (10–13, 18).

None of these statistical measures, culture yields, or TTP were significantly different when HIV-seropositive and HIV-seronegative subjects were compared.

Our data demonstrate that the diagnostic performance of pleural fluid culture using the Myco/F lytic liquid culture, combined with 7H11 solid medium, is not statistically different from that of the MGIT culture, when it is also combined with 7H11 solid medium, with respect to sensitivity, specificity, and contamination rates. However, pleural fluid cultures became positive in the MGIT cultures more rapidly (roughly 1 week earlier) than cultures done using the Myco/F lytic system.

Our data suggest that the Myco/F lytic culture may be a suitable liquid culture replacement for the MGIT culture if a site did not have MGIT available. This may be particularly significant at some sites considering the list price for the Bactec 9120 system is about \$8,000 less expensive than the MGIT 320 system and \$28,000 less expensive than the MGIT 960 system, although the MGIT systems do hold 2.5 to 8 times as many samples as the Bactec 9120 system.

Some *M. tuberculosis*-positive pleural fluid cultures that grew in the Myco/F lytic system were negative in the MGIT/solid medium combination and vice versa (12% and 6% of total submitted pleural fluid cultures, respectively). This raises the possibility that culturing the pleural fluid in a second liquid culture may improve the overall diagnostic yield.

ACKNOWLEDGMENTS

We acknowledge the staff of the National Tuberculosis Treatment Centre at Mulago Hospital, Alphonse Okwera and the Ugandan National Tuberculosis and Leprosy Program and the Uganda Tuberculosis Investigation Bacteriological Unit, Wandegaya, and the study medical officers, nurses, laboratory and data personnel, and Joy Baseke, Sarah Zalwango, and Brenda Okwera for their contributions to this study. This study would not have been possible without the generous participation of the Ugandan patients and families.

This work was supported by the Tuberculosis Research Unit (TBRU), established with Federal funds from the United States National Institutes of Allergy and Infectious Diseases and the United States National Institutes of Health and Human Services (contract NO1-AI-95383). We have received no outside funding that poses a potential conflict of interest.

We have no conflicts of interest to report.

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