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Rapid Detection of *Mycobacterium tuberculosis* in Contaminated BACTEC 12B Broth Cultures by Testing with Amplified Mycobacterium Tuberculosis Direct Test

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Contamination of broth cultures of acid-fast bacilli (AFB) by bacterial species other than *Mycobacterium* species frequently occurs. Many of these contaminated cultures require redecontamination and reincubation before the appropriate tests can be performed for identification, significantly affecting the turnaround time for reporting culture results. In this study, the Amplified Mycobacterium Tuberculosis Direct Test (MTD; Gen-Probe) was performed to detect the *Mycobacterium tuberculosis* complex (MTBC) in 125 BACTEC 12B broth cultures with positive growth indices. Among these, 41 grew non-AFB bacteria only, and all 41 were negative by the MTD. The remaining 84 bottles contained contaminated cultures that grew both AFB and other bacteria or yeasts. Repeat decontamination and reincubation of these specimens required a mean time of 13 days (range, 3 to 40 days). The MTD results were positive for 10 samples, 9 of which were MTBC culture positive and 1 of which grew *Myobacterium celatum*, a species known to cross-react in the MTD. All cultures growing other mycobacterial species were negative by the MTD. The results of this study demonstrate that the MTD is both sensitive and specific in detecting MTBC in contaminated broth cultures and that, when used selectively, the MTD can potentially rule in or out a diagnosis of MTBC as much as 12 days earlier than using nonamplified DNA probe testing alone can.

Tuberculosis remains a major cause of morbidity and mortality worldwide. Rapid and accurate detection of the *Mycobacterium tuberculosis* complex (MTBC) is a key aspect of effective tuberculosis treatment and control. Although the recently introduced nucleic acid amplification tests provide an opportunity for the early diagnosis of disease, routine culture of acid-fast bacilli (AFB) is still necessary for its higher sensitivity and ability to identify mycobacterial species other than MTBC and for the recovery of isolates for antimicrobial susceptibility testing. For the majority of specimens for AFB culture, modern culture techniques make it possible to meet the 21-day target turnaround time for detection and identification of MTBC as recommended by the Centers for Disease Control and Prevention (22). However, despite standardized techniques for decontamination and concentration and for the addition of antibiotics, contamination of broth cultures by bacteria other than AFB still occurs in 2 to greater than 10% of cultures (8, 14, 15, 18, 23, 24, 26). Many of these contaminated cultures grow AFB as well as other contaminating organisms, thus requiring a redecontamination step and reincubation before the appropriate tests (such as chromatographic or biochemical methods) can be performed for organism identification. The turnaround time for reporting culture results is thus significantly affected in this group of cultures. Although the use of DNA probes for detection and identification may be helpful in some of these contaminated cultures, such use is not specified in the manufacturer's instructions or discussed in published literature. Furthermore, since both the AFB and the contaminating bacteria in the bottle contribute to the growth index, it is difficult to estimate if and ensure that there is a sufficient amount of AFB for DNA probe testing. The enhanced Amplified Mycobacterium Tuberculosis Direct Test (MTD; Gen-Probe Inc., San Diego, Calif.) utilizes transcription-mediated amplification to detect MTBC rRNA and has been approved for detection of MTBC directly in respiratory specimens. The performance of the MTD directly on specimens has been extensively evaluated and reported (3, 5, 7, 9, 19, 27; X. Zheng, M. Pang, R. Watase, E. Ayling, K. Hirata, and T. Reppun, Abstr. 100th Gen. Meet. Am. Soc. Microbiol., abstr. C-3, p. 126, 2000). Studies have also found that the MTD (1, 2) and other nucleic acid amplification tests (11, 16, 17, 20, 25) can be used for early detection of MTBC in AFB broth cultures. However, the use of the MTD on contaminated broths has not been reported. In the present study, the MTD was performed to detect MTBC in 125 contaminated BACTEC 12B broth cultures to investigate if the MTD can shorten the time to MTBC identification in these types of cultures.

Specimen processing and organism identification. A total of 2,450 respiratory specimens were processed. Specimen processing, culture, sample redecontamination, and DNA probe testing and the MTD were performed at the Microbiology Laboratory, Diagnostic Laboratory Services, The Queen's Health Systems, Honolulu, Hawaii. Mycobacterial species other than MTBC and *Myobacterium avium-Mycobacterium intracellulare* complex were identified in the reference laboratory of Focus Technologies using a combination of high-performance liquid chromatography and conventional biochemical tests. Each

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specimen was mixed with Mucosol (Alpha-Tec Systems, Inc., Vancouver, Wash.) in a 1:1 ratio and left to stand for 15 min at room temperature. Specimens were then centrifuged at 3,000 × *g* for 20 min, and an equal volume of sodium hydroxide was added to the sediment to make a final 2% concentration of sodium hydroxide. After 15 min, phosphate buffer was added and specimens were concentrated as described above. The sediment was used to prepare a smear for staining with auramine-rhodamine and to inoculate a BACTEC 12B bottle (Becton Dickinson, Sparks, Md) and Middlebrook 7H10 solid medium. Cultures were incubated for 6 weeks. BACTEC 12B bottles were monitored for growth using the BACTEC 460TB instrument according to the manufacturer's instructions. Growth indices (GI) were measured twice a week for the first 2 weeks and once a week for the next 4 weeks. When the GI reached >10, the bottle was read daily until the GI was ≥100. Aliquots of broth were aseptically removed for (i) smears stained by the carbol-fuchsin method, (ii) identification by DNA probe testing or other methods, and (iii) inoculation of a 5% sheep blood agar plate for purity checks. Isolates of MTBC and *M. avium* complex were identified by nucleic acid hybridization using DNA probes (AccuProbe; Gen-Probe Inc.).

Redecontamination and enhanced MTD. The contaminated broth cultures were those in 12B bottles with elevated GI values containing non-AFB bacteria that grow on a sheep blood agar plate or are present on the smear, with or without the growth of AFB in the culture. To redecontaminate, contaminated broths were treated with sodium hydroxide (2.5% final concentration) and centrifuged. An aliquot of broth (0.5 ml) was removed before redecontamination for testing by the MTD. The MTD was performed according to the manufacturer's instructions (MTD product insert, Gen-Probe Inc.).

Comparison of MTD and culture results. At the microbiology laboratory of Diagnostic Laboratory Services, The Queen's Health Systems, the overall broth culture contamination rate is 5%. Contaminating organisms include gram-positive cocci in clusters (33%), coryneforms (27%), gram-negative rods (13%), and mixed organism types and yeasts (27%). A total of 125 broth samples contaminated with bacteria or yeasts (from 115 patients) were collected for testing by the MTD. Among these, 41 samples grew non-AFB contaminating bacteria only and were negative by the MTD. The remaining 84 contaminated cultures grew AFB as well as contaminating organisms. Redecontamination of these specimens required a mean time of 13 days (range, 3 to 40 days from the time the broth culture grew AFB to the time MTBC probe results were available). AFB and organisms recovered from the 84 contaminated cultures included 9 MTBC, 34 *M. avium-M. intracellulare* complex, 17 *Mycobacterium chelonae* species group, 17 *Mycobacterium fortuitum* species group, 4 *Mycobacterium gordonae*, 1 *Mycobacterium scrofulaceum*, 1 *Mycobacterium celatum*, 1 *Nocardia asteroides* complex (the complex includes *N. asteroides* sensu stricto, *Nocardia farcinica*, and *Nocardia nova*), and 1 *Tsukamurella* species. The MTD results for these broth specimens were positive for 10 samples, 9 of which were MTBC culture positive and 1 of which grew *M. celatum*, a species known to cross-react in the MTD (21; MTD product insert, Gen-Probe Inc.). All cultures growing other mycobacterial species were negative by the MTD (Table 1). These results demonstrate that the presence of contaminating organisms did not interfere with MTBC detection in broth cultures by the MTD.

TABLE 1. Comparison of MTD results with that of final identification of MTBC by culture

Culture result	No. of isolates with MTD result:	
	Positive	Negative
Positive	9	0
Negative	1[a]	115

[a] This isolate was identified as *M. celatum*.

The MTD has been reported to cross-react with *Mycobacterium terrae*-like organisms and *M. celatum* type 1 (21; MTD product insert, Gen-Probe Inc.). *M. celatum*, rarely seen in clinical specimens, has recently been found to be an important pathogen in both immunocompetent and immunocompromised patients (4, 21). Somoskövi et al. reported that performing the MTBC AccuProbe test with a selection temperature between 60 and 61°C, rather than 60 ± 1°C, helped to differentiate *M. celatum* from MTBC (21). Additionally, attention should be paid to colonial morphology and care should be taken to routinely include this organism as a negative control. The *M. celatum* isolate described in this report was identified using high-performance liquid chromatography and was not further typed.

Information regarding the interference of normal respiratory flora with the MTD is very limited in the published literature. According to the manufacturer's data, the MTD can detect MTBC rRNA at a concentration equivalent to 5 CFU per test and does not react with 150 species of microorganisms (including respiratory flora) at the level of 5×10^7 organisms per reaction (MTD product insert, Gen-Probe Inc.). In a study of the analytical sensitivity and specificity of the assay, the MTD was performed on 54 *Mycobacterium* species and on 124 species of bacteria and yeasts, including those frequently found in respiratory specimens (12). All non-MTBC species were negative by the MTD. One report suggests that *Mycobacterium kansasii* and *M. avium* may sometimes cause low-level positive MTD results (13). More recently, Desmond and Loretz reported using the MTD to detect MTBC in BACTEC 12B broth cultures from 239 smear-positive specimens (10). Of only two specimens from previous MTBC culture-positive patients that were now MTD positive but culture negative, one was found to be overgrown with nonmycobacterial contaminants and most likely contained MTBC and the second grew a non-MTBC mycobacterial species (NCP 201) that may have either grown over the MTBC in the specimen or cross-reacted with the MTBC probe used in the MTD procedure. All positive results were obtained only with the tuberculosis patients in the study (10). Overall, the specificity of the test as reported is very high, at over 99% in most studies (1–3, 5, 7; Zheng et al., Abstr. 100th Gen. Meet. Am. Soc. Microbiol.).

Although the MTD does not replace the AFB smear and culture, it can enhance rapid establishment of a diagnosis when used directly on clinical specimens (5, 6). However, for various reasons (ie., degree of suspicion, physician preference, and cost), most requests regarding specimens received in the laboratory are for AFB smear and culture only.

The AFB broth culture contamination rates differ among

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laboratories and depend on factors such as specimen handling, specimen delivery time, processing method, and culture medium used (8, 14, 15, 18, 23, 24, 26). To further identify an organism present in the broth, redecontamination and reincubation (sometimes more than once) are usually required to eliminate non-AFB organisms present in the broth. In our study, this process took an average of an extra 13 days before an identification test could be performed. This time requirement can significantly decrease a laboratory's ability to meet the Centers for Disease Control and Prevention-recommended 21-day target turnaround time for detection and identification of MTBC. Data from the present study have shown that utilization of the MTD on these contaminated 12B broths improved the turnaround time required to rule in or out a laboratory diagnosis of MTBC infection. The more rapid test results may be especially helpful in the medical management of hospitalized patients.

In summary, the results of this study demonstrate that the MTD is both a sensitive and specific method of detecting MTBC in contaminated BACTEC 12B broth cultures. Although redecontamination is still required for recovery of an isolate for subsequent antimicrobial susceptibility testing, MTBC can potentially be ruled in or out as much as 12 days earlier by the MTD than by using nonamplified DNA probe testing alone.

REFERENCES

1. **Bergmann, J. S., and G. L. Woods.** 1999. Enhanced Amplified Mycobacterium Tuberculosis Direct Test for detection of *Mycobacterium tuberculosis* complex in positive BACTEC 12B broth cultures of respiratory specimens. J. Clin. Microbiol. **37:**2099–2101.
2. **Bergmann, J. S., and G. L. Woods.** 1999. Enhanced Amplified Mycobacterium Tuberculosis Direct Test for detection of *M. tuberculosis* complex in positive ESP II broth cultures of nonrespiratory specimens. Diagn. Microbiol. Infect. Dis. **35:**245–248.
3. **Bergmann, J. S., G. Yuoh, G. Fish, and G. L. Woods.** 1999. Clinical evaluation of the enhanced Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test for rapid diagnosis of tuberculosis in prison inmates. J. Clin. Microbiol. **37:**1419–1425.
4. **Bux-Gewehr, I., H. P. Hagen, S. Rüsch-Gerdes, and G. E. Feurle.** 1998. Fatal pulmonary infection with *Mycobacterium celatum* in an apparently immunocompetent patient. J. Clin. Microbiol. **36:**587–588.
5. **Catanzaro, A., S. Perry, J. E. Clarridge, S. Dunbar, S. Goodnight-White, P. A. Lo-Bue, C. Peter, G. E. Pfyffer, M. F. Sierra, R. Weber, G. Woods, G. Mathews, V. Jonas, K. Smith, and P. Della-Latta.** 2000. The role of clinical suspicion in evaluating a new diagnostic test for active tuberculosis. JAMA **283:**639–645.
6. **Centers for Disease Control and Prevention.** 2000. Update: nucleic acid amplification tests for tuberculosis. Morb. Mortal. Wkly. Rep. **49:**593–594.
7. **Chedore, P., and F. B. Jamieson.** 1999. Routine use of the Gen-Probe MTD2 amplification test for detection of *Mycobacterium tuberculosis* in clinical specimens in a large public health mycobacteriology laboratory. Diagn. Microbiol. Infect. Dis. **35:**185–191.
8. **Cornfield, D. B., K. G. Beavis, J. A. Greene, M. Bojak, and J. Bondi.** 1997. Mycobacterial growth and bacterial contamination in the Mycobacteria Growth Indicator Tube and BACTEC 460 culture systems. J. Clin. Microbiol. **35:**2068–2071.
9. **Della-Latta, P., and S. Whittier.** 1998. Comprehensive evaluation of performance, laboratory application, and clinical usefulness of two direct amplification technologies for the detection of *Mycobacterium tuberculosis* complex. Am. J. Clin. Pathol. 110:301–310.
10. **Desmond, E. P., and K. Loretz.** 2001. Use of the Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test for early detection of *Mycobacterium tuberculosis* in BACTEC 12B medium. J. Clin. Microbiol. **39:**1993–1995.
11. **Hernandez, A., J. S. Bergmann, and G. L. Woods.** 1997. AMPLICOR MTB polymerase chain reaction test for identification of *Mycobacterium tuberculosis* in positive ESP II broth cultures. Diagn. Microbiol. Infect. Dis. **27:**17–20.
12. **Jonas, V., M. J. Alden, J. I. Curry, K. Kamisango, C. A. Knott, R. Lankford, J. M. Wolfe, and D. F. Moore.** 1993. Detection and identification of *Mycobacterium tuberculosis* directly from sputum sediments by amplification of rRNA. J. Clin. Microbiol. **31:**2410–2416.
13. **Jorgensen, J. H., J. R. Salinas, R. Paxson, K. Magnon, J. E. Patterson, and T. F. Patterson.** 1999. False-positive Gen-Probe Direct *Mycobacterium tuberculosis* amplification test results for patients with pulmonary *M. kansasii* and *M. avium* infections. J. Clin. Microbiol. **37:**175–178.
14. **Kanchana, M. V., D. Cheke, I. Natyshak, B. Connor, A. Warner, and T. Martin.** 2000. Evaluation of the BACTEC MGIT 960 system for the recovery of mycobacteria. Diagn. Microbiol. Infect. Dis. **37:**31–36.
15. **Katila, M.-L., P. Katila, and R. Erkinjuntti-Pekkanen.** 2000. Accelerated detection and identification of mycobacteria with MGIT 960 and COBAS AMPLICOR systems. J. Clin. Microbiol. **38:**960–964.
16. **Ninet, B., P. Rohner, C. Metral, and R. Auckenthaler.** 1999. Assessment of use of the COBAS AMPLICOR System with BACTEC 12B cultures for rapid detection of frequently identified mycobacteria. J. Clin. Microbiol. **37:**782–784.
17. **Pfyffer, G. E., C. Cieslak, H.-M. Welscher, P. Kissling, and S. Rüsch-Gerdes.** 1997. Rapid detection of mycobacteria in clinical specimens by using the automated BACTEC 9000 MB system and comparison with radiometric and solid-culture systems. J. Clin. Microbiol. **35:**2229–2234.
18. **Pfyffer, G. E., H.-M. Welscher, P. Kissling, C. Cieslak, M. J. Casal, J. Gutierrez, and S. Rüsch-Gerdes.** 1997. Comparison of the Mycobacteria Growth Indicator Tube (MGIT) with radiometric and solid culture for recovery of acid-fast bacilli. J. Clin. Microbiol. **35:**364–368.
19. **Scarparo, C., P. Piccoli, A. Rigon, G. Ruggiero, M. Scagnelli, and C. Piersimoni.** 2000. Comparison of enhanced *Mycobacterium tuberculosis* Amplified Direct Test with COBAS AMPLICOR *Mycobacterium tuberculosis* Assay for direct detection of *Mycobacterium tuberculosis* complex in respiratory and extrapulmonary specimens. J. Clin. Microbiol. **38:**1559–1562.
20. **Smith, M. B., J. S Bergmann, and G. L. Woods.** 1997. Detection of *Mycobacterium tuberculosis* in BACTEC 12B broth cultures by the Roche Amplicor PCR Assay. J. Clin. Microbiol. **35:**900–902.
21. **Somoskövi, A., J. E. Hotaling, M. Fitzgerald, V. Jonas, D. Stasik, L. M. Parsons, and M. Salfinger.** 2000. False-positive results for *Mycobacterium celatum* with the AccuProbe *Mycobacterium tuberculosis* complex assay. J. Clin. Microbiol. **38:**2743–2745.
22. **Tenover, F. C., J. T. Crawford, R. E. Huebner, L. J. Geiter, C. R. Horsburgh, Jr., and R. C. Good.** 1993. The resurgence of tuberculosis: is your laboratory ready? J. Clin. Microbiol. **31:**767–770.
23. **Tortoli, E., P. Cichero, M. G. Chirillo, M. R. Gismondo, L. Bono, G. Gesu, M. T. Simonetti, G. Volpe, G. Nardi, and P. Marone.** 1998. Multicenter comparison of ESP Culture System II with BACTEC 460TB and with Lowenstein-Jensen medium for recovery of mycobacteria from different clinical specimens, including blood. J. Clin. Microbiol. **36:**1378–1381.
24. **Tortoli, E., P. Cichero, C. Piersimoni, M. T. Simonetti, G. Gesu, and D. Nista.** 1999. Use of BACTEC MGIT 960 for recovery of mycobacteria from clinical specimens: multicenter study. J. Clin. Microbiol. **37:**3578–3582.
25. **Tortoli, E., F. Lavinia, and M. T. Simonetti.** 1998. Early detection of *Mycobacterium tuberculosis* in BACTEC cultures by ligase chain reaction. J. Clin. Microbiol. **36:**2791–2792.
26. **Zanetti, S., F. Ardito, L. Sechi, M. Sanguinetti, P. Molicotti, G. Delogu, M. P. Pinna, A. Nacci, and G. Fadda.** 1997. Evaluation of a nonradiometric system (BACTEC 9000 MB) for detection of mycobacteria in human clinical samples. J. Clin. Microbiol. **35:**2072–2075.
27. **Zheng, X., and G. D. Roberts.** 1999. Diagnosis and susceptibility testing, p. 57–64. *In* D. Schlossberg (ed.), Tuberculosis and nontuberculosis mycobacterial infections. W. B. Saunders Company, Philadelphia, Pa.

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