Journal of Clinical Microbiology

Increased Sensitivity of the BACTEC 460 Mycobacterial Radiometric Broth Culture System Does Not Decrease the Number of Respiratory Specimens Required for a Definitive Diagnosis of Pulmonary Tuberculosis

Jeff D. Harvell, W. Keith Hadley, and Valerie L. Ng 2000. Increased Sensitivity of the BACTEC 460 Mycobacterial Radiometric Broth Culture System Does Not Decrease the Number of Respiratory Specimens Required for a Definitive Diagnosis of Pulmonary Tuberculosis. J. Clin. Microbiol. 38(10):3608-3611.

JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2000, p. 3608–3611 Vol. 38, No. 10
0095-1137/00/$04.00+0

Increased Sensitivity of the BACTEC 460 Mycobacterial Radiometric Broth Culture System Does Not Decrease the Number of Respiratory Specimens Required for a Definitive Diagnosis of Pulmonary Tuberculosis

JEFF D. HARVELL,† W. KEITH HADLEY, AND VALERIE L. NG*

Department of Laboratory Medicine, School of Medicine, University of California, San Francisco, and Microbiology Division, Clinical Laboratories, San Francisco General Hospital, San Francisco, California

Received 19 June 2000/Returned for modification 26 June 2000/Accepted 18 July 2000

The BACTEC 460 radiometric mycobacterial broth culture system has consistently demonstrated faster and increased recovery of *Mycobacterium tuberculosis* from respiratory specimens of patients with pulmonary tuberculosis than conventional culture methods. We thus questioned whether three sputa were still necessary to definitively diagnose pulmonary tuberculosis if the BACTEC radiometric culture system were in use. We performed a retrospective analysis of 430 sequential respiratory specimens submitted from 143 patients and from which *M. tuberculosis* had been recovered by in vitro culture and simultaneously assessed the diagnostic yield of acid-fast smear in this same cohort. *M. tuberculosis* was recovered from the first specimen for 117 (82%) of the 143 patients, from the second for 14 patients (10%; cumulative rate, 92%), and from the third for 12 patients (8%; cumulative rate, 100%). With the exception of those for bronchial brushings, recovery rates of *M. tuberculosis* were comparable for all respiratory specimen types (expectorated sputum, induced sputum, tracheal aspirates, bronchoalveolar lavage fluids). Only 46 (32%) of these 143 patients had acid-fast bacilli detected in smears; acid-fast bacilli were detected in the first submitted specimen for 44 patients (96%) and in the second for the remaining 2 patients (4%; cumulative rate, 100%). Culture- or smear-positive rates for sequential specimens obtained from AIDS patients were comparable to those for non-AIDS patients. Overall, the diagnostic culture yield of sequentially submitted specimens was not different from previously published studies in which the BACTEC radiometric culture system had not been used. Despite the documented enhanced ability of the BACTEC 460 radiometric mycobacterial culture system to recover *M. tuberculosis* more often and faster than conventional methods, three sequential respiratory specimens (regardless of type) were still necessary to definitively diagnose pulmonary tuberculosis.

Pulmonary tuberculosis is usually considered to be definitively diagnosed when *Mycobacterium tuberculosis* is recovered from in vitro culture of respiratory specimens. Previous studies have demonstrated incrementally higher recovery rates of *M. tuberculosis* with each successive sputum specimen submitted (3, 9–11). It has been common cost-effective clinical practice, however, to limit the laboratory evaluation for tuberculosis by submitting only three expectorated sputa obtained on three successive days for in vitro culture and acid-fast smear microscopy.

The automated BACTEC 460 radiometric mycobacterial broth culture system was introduced into clinical practice in the 1980s and was in use at 37% of hospital-based clinical mycobacteriology laboratories surveyed in 1995 (19). Numerous comparative studies demonstrated that the BACTEC system, when used as the primary mycobacterial culture system (in conjunction with conventional solid media culture), detected mycobacterial growth sooner and more often than conventional methods (2, 6, 8, 13, 15–17, 20). The labor savings, increased sensitivity, and faster turnaround time of mycobacterial cultures using the BACTEC system led us to implement it as our primary mycobacterial broth culture system in 1992.

The increased sensitivity that the BACTEC system added to conventional methods for the in vitro recovery of *M. tuberculosis* led us to question whether a definitive laboratory diagnosis of tuberculosis could be established with fewer sputum specimens than the customary three. We also wanted to determine if fewer specimens were necessary to establish a definitive diagnosis of pulmonary tuberculosis in individuals infected with the human immunodeficiency virus (HIV), given that a 100% diagnostic yield (for both smear and culture) from just two respiratory specimens has been previously demonstrated (although it was not explicitly stated if a BACTEC radiometric culture system was used) (5). Finally, we wanted to compare the diagnostic yields of induced versus expectorated sputa for the diagnosis of pulmonary tuberculosis in our setting. To address these questions, we performed a retrospective analysis of culture results from sequential specimens obtained from 143 patients from which *M. tuberculosis* had been recovered during 1994 to 1996, a period during which the BACTEC system was firmly established within our mycobacteriology laboratory. We also evaluated the diagnostic yield of acid-fast smear microscopy in this same cohort.

(This work was presented in part at the 37th Annual Meeting of the Interscience Conference on Antimicrobial Agents and Chemotherapy, Toronto, Canada, 1997.)

* Corresponding author. Mailing address: Clinical Laboratories 2M24, San Francisco General Hospital, 1001 Potrero Ave., San Francisco, CA 94110. Phone: (415) 206-8588. Fax: (415) 206-3045. E-mail: ng@pangloss.ucsf.edu.

† Present address: Department of Pathology, School of Medicine, Stanford University, Stanford, Calif.

TABLE 1. Summary of respiratory specimens submitted for culture from the 143 patients from whose respiratory specimens *M. tuberculosis* was recovered

Specimen type	No. (%)
Expectorated sputum	167 (38.8)
Induced sputum	195 (45.3)
Tracheal aspirate	55 (12.8)
BAL fluid	11 (2.6)
Bronchial brushing	2 (0.5)
Total	430 (100)

TABLE 3. Recovery of *M. tuberculosis* from various types of respiratory specimens

Specimen type	Number (%) of specimens from which *M. tuberculosis* was recovered
Expectorated sputum (n = 167)	130 (78)
Induced sputum (n = 195)	163 (84)
Tracheal aspirate (n = 55)	47 (85)
BAL fluid (n = 11)	8 (73)
Bronchial brushing (n = 2)	0

MATERIALS AND METHODS

Study design. A total of 143 patients were identified between 1994 and 1996 from whom *M. tuberculosis* had been recovered from culture of respiratory specimens. Laboratory records were reviewed to document the sequence of and results for all specimens that had been submitted for each of the patients. The "look back" period for additional specimens submitted from the same patient encompassed 2 months before and after and the date of the first positive culture.

Patients with AIDS were identified if this diagnosis had been noted on laboratory documents (typically on requisitions accompanying specimens). Confidentiality laws in the state of California precluded us from identifying HIV-infected individuals who had not yet received a diagnosis of AIDS.

Laboratory procedures. Mycobacterial cultures were performed by digesting and decontaminating respiratory specimens with *N*-acetyl-L-cysteine-NaOH, concentrating by centrifugation, and resuspending the sediment in 1.0 ml of 0.067 M phosphate buffer, pH 6.8, as previously described (7, 21). A 0.5-ml aliquot of the resuspended sediment was inoculated into a radiometric BACTEC 12B vial (Becton Dickinson Laboratory, Cockeysville, Md.), 2 drops were plated onto Middlebrook 7H11 agar, and cultures were incubated for a maximum of 8 weeks. A positive culture result was defined as recovery of *M. tuberculosis* from either the BACTEC 12B and/or 7H11 culture. Once one of the two cultures yielded *M. tuberculosis*, the remaining negative paired culture was not further evaluated. Nucleic acid probe hybridization (AccuProbe; Gen-Probe, San Diego, Calif.) was used to identify *M. tuberculosis*. Smears were prepared from the concentrated sediment, stained with auramine-rhodamine, and examined using fluorescence microscopy. Kinyoun staining was performed as necessary to confirm positive auramine-rhodamine smears.

Statistical analysis. Fisher's exact test (two tailed probability) was performed as previously described (1).

RESULTS

A total of 430 respiratory specimens were submitted from the 143 patients. The mean and median number of specimens submitted per patient was three (range, 1 to 10). For 100 (70%) patients three or fewer specimens were submitted, and for 43 (30%) patients more than three specimens were submitted. A variety of respiratory specimens were submitted (Table 1). Of these various specimens, 50 patients submitted expectorated sputa only, 44 patients submitted induced sputa only, 8 patients submitted tracheal aspirates only and one patient submitted bronchoalveolar lavage (BAL) fluid only. The remaining 40 patients submitted more than one type of specimen among the first three specimens—26 submitted both induced and expectorated sputa, 6 submitted both induced sputa and BAL fluids, 2 submitted both tracheal aspirates and induced sputa, 4 submitted both tracheal aspirates and expectorated sputa, 1 submitted both tracheal aspirate and BAL fluid, and 1 submitted both expectorated sputa and BAL fluid.

The overall cumulative culture- and smear-positive rates for all sequentially submitted respiratory specimens in this study are shown in Table 2.

The overall culture-positive rate for all respiratory specimens is shown in Table 3. The overall culture-positive rates for all respiratory specimens except expectorated sputa were comparable to that of expectorated sputa.

A direct comparison of *M. tuberculosis* recovery rates from BACTEC 12B broth versus 7H11 agar was not performed as part of this study.

Acid-fast smear microscopy results. Although all 143 patients had *M. tuberculosis* cultured from one or more specimens, only 46 (32%) of the 143 patients had acid-fast bacilli detected in one or more concentrated smear preparations (Table 2). Within this group, there was complete concordance between detection of acid-fast bacilli and recovery of *M. tuberculosis* in in vitro culture.

Culture results for expectorated sputa versus induced sputa. Fifty patients submitted expectorated sputa only, and 44 patients submitted induced sputa only. The cumulative culture-positive rates for the two groups were identical (Table 4).

Diagnostic yield of specimens obtained from AIDS patients. Twenty-three (16%) of the 143 patients had a prior diagnosis of AIDS. Seventy-eight specimens had been submitted from these 23 AIDS patients, with a mean number of 3.4, a median of 4, and a range of 1 to 8 specimens submitted per patient. With the exception of seven AIDS patients for whom expectorated sputa (four patients) or induced sputa (three patients) only had been submitted, the remaining 16 AIDS patients had a variety of respiratory specimens submitted for laboratory evaluation. Specimens obtained from AIDS patients comprised 26 (16%) of the 167 expectorated sputa, 33 (17%) of the 195 induced sputa, 12 (22%) of the 55 tracheal aspirates, and six (55%) of the BAL fluids.

Ten (43%) of the 23 AIDS patients with pulmonary tuberculosis had acid-fast bacilli detected in smear preparations of their respiratory specimens, and all 10 had acid-fast bacilli detected on their first submitted specimen (Table 5). *M. tuberculosis* was recovered from culture of the first submitted spec-

TABLE 2. Cumulative culture- and smear-positive rates for sequentially submitted specimens for the 143 patients from whom *M. tuberculosis* was recovered in in vitro culture

Sequential specimen no.	Cumulative no. (%) of patients with *M. tuberculosis* recovered from culture (n = 143)	Cumulative no. (%) of smears with acid-fast bacilli detected (n = 46)
1	117 (82)	44 (96)
2	131 (92)	46 (100)
3	143 (100)	

TABLE 4. Cumulative culture-positive rates for sequential expectorated versus induced sputa

Sequential specimen no.	Cumulative no. (%) of patients with *M. tuberculosis* recovered from culture of expectorated sputa (n = 50)	Cumulative no. (%) of patients with *M. tuberculosis* recovered from culture of induced sputa (n = 44)
1	41 (82)	37 (84)
2	46 (92)	42 (95)
3	50 (100)	44 (100)

TABLE 5. Cumulative culture- and smear-positive rates for sequential specimens from AIDS versus non-AIDS patients

Sequential specimen no.	AIDS patients		Non-AIDS patients	
	Cumulative no. (%) of patients with *M. tuberculosis* recovered from culture (n = 23)	Cumulative no. (%) of smears with acid-fast bacilli detected (n = 10)	Cumulative no. (%) of patients with *M. tuberculosis* recovered from culture (n = 120)	Cumulative no. (%) of smears with acid-fast bacilli detected (n = 36)
1	22 (96)	10 (100)[b]	95 (80)	34 (94)[b]
2	23 (100)[a]		108 (90)[a]	36 (100)[b]
3			120 (100)[a]	

[a] $P = 0.08$; calculated using Fisher's exact test to compare the proportion of patients (AIDS vesus non-AIDS) for whom more than one specimen was required to establish a diagnosis of pulmonary tuberculosis.
[b] $P = 1$; calculated using Fisher's exact test to compare the proportion of patients (AIDS versus non-AIDS) for whom more than one specimen was required to detect acid-fast bacteria.

imen for 22 (96%) of these 23 patients and from the remaining patient from the second submitted specimen (Table 5). The diagnostic yield of either culture or smear from sequential specimens obtained from AIDS patients was not statistically significantly different than that for non-AIDS patients (Table 5).

Nondiagnostic specimens. The 43 patients (including 11 AIDS patients) from whom more than three specimens were submitted had a total of 210 nondiagnostic specimens submitted, of which 74 were the fourth or greater sequential specimen. These 74 nondiagnostic specimens represented 17% of the 430 total specimens in this study.

DISCUSSION

Despite the well-documented increased sensitivity for the BACTEC 460 radiometric mycobacterial broth culture system for recovery of *M. tuberculosis*, our study failed to demonstrate that this increased laboratory sensitivity could be extrapolated to a clinical recommendation that fewer respiratory specimens would be necessary for a definitive diagnosis of tuberculosis. In fact, the incremental diagnostic yield of culture for sequential specimens in our study was of similar magnitude to those in previously published studies conducted either with (14) or without (3, 4, 10) a BACTEC radiometric culture system (Table 6).

It had been questioned locally whether induced sputum has a higher diagnostic yield than expectorated sputum for respiratory tuberculosis. Our results failed to demonstrate a difference in diagnostic yield between these two specimen types, and they confirm those of Merrick and colleagues, who previously demonstrated comparable diagnostic culture yields for expectorated and induced sputa (12).

Our study did not demonstrate a higher diagnostic yield for specimens obtained from AIDS patients compared with non-AIDS patients. In contrast, Finch and colleagues were able to recover *M. tuberculosis* from culture of the first sputum (either expectorated or induced) for 20 (100%) of 20 HIV type 1-infected individuals (5) and detected acid-fast bacilli in the first sputum of 11 (79%) and in the second sputum of the remaining 3 (21%; cumulative rate, 100%) of their 14 smear-positive HIV-infected patients (5). Our differing results may have been related to a bias unknowingly introduced into our study by our inability to identify all HIV-infected patients who had not yet received a diagnosis of AIDS. An alternative explanation, however, may be related to the extraordinary diagnostic yield Finch and colleagues achieved in general—they recovered *M. tuberculosis* from culture of the first specimen from 142 (99%) of 143 non-HIV-infected patients (5), a much higher diagnostic yield from the first specimen than achieved by us or others (Table 6).

Seventy-four (17%) of the 430 specimens in this analysis represented the fourth or greater sequential specimen for individual patients. None of these 74 specimens yielded diagnostic results. Despite the observations that in vitro recovery of *M. tuberculosis* is proportional to the total number of specimens submitted (3, 9, 10), the minimal increase in recovery from the fourth or greater sequential specimen has led to the recommendation that only three sputum specimens are necessary for a definitive yet cost-effective diagnosis of pulmonary tuberculosis (4, 5, 14). Given our observation of fairly comparable isolation rates of *M. tuberculosis* from different types of respi-

TABLE 6. Comparison of previously published studies with this study of the cumulative diagnostic yield for recovery of *M. tuberculosis* from in vitro culture

Sequential specimen no.	Cumulative no. (%) of patients from whom *M. tuberculosis* was recovered from in vitro culture of sequential respiratory specimens in the study indicated[c]				
	Blair et al.[a] (1968–1973) (n = 445)	Levy et al.[a] (1986) (n = 106)	Cascina et al.[a] (1989–1998) (n = 84)	Nelson et al.[b] (1986–1996) (n = 120)	This study[b] (1994–1996) (n = 143)
1	333 (74.8)	89 (84.0)	64 (76.2)	80 (66.7)	117 (81.8)
2	372 (83.6)	99 (93.4)	77 (91.7)	113 (94.2)	131 (91.6)
3	396 (89.0)	101 (95.3)	84 (100)	120 (100)	143 (100)
4	405 (91.1)	103 (97.2)	ND[d]	ND	ND
5	416 (93.5)	104 (98.1)	ND	ND	ND
6	422 (94.9)	105 (99.1)	ND	ND	ND
7–17	445 (100)	106 (100)	ND	ND	ND

[a] Study conducted without the BACTEC radiometric broth culture system.
[b] Study conducted with the BACTEC radiometric broth culture system.
[c] The year(s) during which the studies were conducted is given in parentheses.
[d] ND, not done.

TABLE 7. Comparison of previously published studies with this study of the cumulative diagnostic yields for detection of acid-fast bacilli in smears from sequential specimens

Sequential specimen no.	Cumulative no. (%) of patients who had acid-fast bacilli detected in sequential respiratory specimens in the study indicated[b]				
	Blair et al. (1968–1973) (n = 270[a])	Levy et al. (1986) (n = 106)	Cascina et al. (1989–1998) (n = 84)	Nelson et al. (1986–1996) (n = 120)	This study (1994–1996) (n = 143)
1	128 (47.4)	58 (54.7)	46 (54.8)	41 (34.2)	44 (30.8)
2	143 (53.0)	65 (61.3)	51 (60.7)	52 (43.3)	46 (32.2)
3	157 (58.2)	69 (65.1)	56 (66.7)	56 (46.7)	46 (32.2)
4	160 (59.3)	71 (67.0)	ND[c]	ND	ND
5	163 (60.4)	ND	ND	ND	ND
6	167 (61.9)	ND	ND	ND	ND
7–17	181 (67.0)	ND	ND	ND	ND

[a] Includes all mycobacterial isolates (i.e., *M. tuberculosis*, *M. kansasii*, *M. avium* complex, etc.).
[b] The year(s) during which the studies were conducted is given in parentheses.
[c] ND, not done.

ratory specimens, we further recommend that only three sequential respiratory specimens—regardless of type—are necessary for a definitive and cost-effective laboratory diagnosis of pulmonary tuberculosis.

Our overall diagnostic yield for acid-fast smear microscopy for sequential specimens was comparable to that of previous studies (Table 7). Of note and for all patients from whose respiratory specimens *M. tuberculosis* was recovered in in vitro culture, there was complete concordance between detection of acid-fast bacilli and recovery of *M. tuberculosis* in culture. Our high diagnostic culture yield from smear-positive specimens also supports the recommendation that laboratory evaluation of only two smear-positive specimens is necessary to definitively diagnose pulmonary tuberculosis (5, 18).

ACKNOWLEDGMENTS

We gratefully acknowledge the expert technical advice and helpful suggestions of David Yajko, Marguerite Roemer, Ed Desmond, and Phil Hopewell.

REFERENCES

1. **Agresti, A.** 1992. A survey of exact inference for contingency tables. Stat. Sci. **7:**131–153.
2. **Anargyros, P., D. S. J. Astill, and I. S. Sim.** 1990. Comparison of improved BACTEC and Lowenstein-Jensen media for culture of mycobacteria from clinical specimens. J. Clin. Microbiol. **28:**1288–1291.
3. **Blair, E. B., G. L. Brown, and A. H. Tull.** 1976. Computer files and analyses of laboratory data from tuberculosis patients. II. Analyses of six years' data on sputum specimens. Am. Rev. Respir. Dis. **113:**427–432.
4. **Cascina, A., A. Fietta, and L. Casali.** 2000. Is a large number of sputum specimens necessary for the bacteriological diagnosis of tuberculosis? J. Clin. Microbiol. **38:**466.
5. **Finch, D., and C. D. Beaty.** 1997. The utility of a single sputum specimen in the diagnosis of tuberculosis: comparison between HIV-infected and non-HIV-infected patients. Chest **111:**1174–1179.
6. **Isenberg, H. D., R. F. D'Amato, L. Heifets, P. R. Murray, M. Scardamaglia, M. C. Jacobs, P. Alperstein, and A. Niles.** 1991. Collaborative feasibility study of a biphasic system. J. Clin. Microbiol. **29:**1719–1722.
7. **Kent, P. T., and G. P. Kubica.** 1985. Public health mycobacteriology: a guide for the level III laboratory. U.S. Department of Health and Human Services, Centers for Disease Control, Atlanta, Ga.
8. **Kirihara, J. M., S. L. Hillier, and M. B. Coyle.** 1985. Improved detection times for *Mycobacterium avium* complex and *Mycobacterium tuberculosis* with the BACTEC radiometric system. J. Clin. Microbiol. **22:**841–845.
9. **Krasnow, I., and L. G. Wayne.** 1969. Comparison of methods for tuberculosis bacteriology. Appl. Microbiol. **18:**915–917.
10. **Levy, H., C. Feldman, H. Sacho, H. van der Meulen, J. Kallenbach, and H. Koornhof.** 1989. A reevaluation of sputum microscopy and culture in the diagnosis of pulmonary tuberculosis. Chest **95:**1193–1197.
11. **Lipsky, B. A., J. Gates, and F. C. Tenover.** 1984. Factors affecting the clinical value of microscopy for acid-fast bacilli. Rev. Infect. Dis. **6:**214–222.
12. **Merrick, S. T., K. A. Sepkowitz, J. Walsh, L. Damson, P. McKinley, and J. L. Jacobs.** 1997. Comparison of induced versus expectorated sputum for diagnosis of pulmonary tuberculosis by acid-fast smear. Am. J. Infect. Control **25:**463–466.
13. **Morgan, M. A., C. D. Horstmeier, D. R. DeYoung, and G. D. Roberts.** 1983. Comparison of a radiometric method (BACTEC) and conventional culture media for recovery of mycobacteria from smear-negative specimens. J. Clin. Microbiol. **18:**384–388.
14. **Nelson, S. M., M. A. Deike, and C. P. Cartwright.** 1998. Value of examining multiple sputum specimens in the diagnosis of pulmonary tuberculosis. J. Clin. Microbiol. **36:**467–469.
15. **Park, C. H., D. L. Hixon, C. B. Ferguson, S. L. Hall, C. C. Risheim, and C. B. Cook.** 1984. Rapid recovery of mycobacteria from clinical specimens using automated radiometric technic. Am. J. Clin. Pathol. **81:**341–345.
16. **Roberts, G. D., N. L. Goodman, L. Heifets, H. W. Larsh, T. H. Lindner, J. K. McClatchy, M. R. McGinnis, S. H. Siddiqi, and P. Wright.** 1983. Evaluation of the BACTEC radiometric method for recovery of mycobacteria and drug susceptibility testing of *Mycobacterium tuberculosis* from acid-fast smear-positive specimens. J. Clin. Microbiol. **18:**689–696.
17. **Stager, C. E., J. P. Libonati, S. H. Siddiqi, J. R. Davis, N. M. Hooper, J. F. Baker, and M. E. Carter.** 1991. Role of solid media when used in conjunction with the BACTEC system for mycobacterial isolation and identification. J. Clin. Microbiol. **29:**154–157.
18. **Stone, B. L., W. J. Burman, M. V. Hildred, E. A. Jarboe, R. R. Reves, and M. L. Wilson.** 1997. The diagnostic yield of acid-fast-bacillus smear-positive sputum specimens. J. Clin. Microbiol. **35:**1030–1031.
19. **Tokars, J. L., J. R. Rudnick, K. Kroc, L. Manangan, G. Pugliese, R. E. Huebner, J. Chan, and W. R. Jarvis.** 1996. U.S. hospital mycobacteriology laboratories: status and comparison with state public health department laboratories. J. Clin. Microbiol. **34:**680–685.
20. **Wilson, M. L., B. L. Stone, M. V. Hildred, and R. R. Reves.** 1995. Comparison of recovery rates for mycobacteria from BACTEC 12B vials, Middlebrook 7H11-selective 7H11 biplates, and Lowenstein-Jensen slants in a public health mycobacteriology laboratory. J. Clin. Microbiol. **33:**2516–2518.
21. **Yajko, D. M., P. S. Nassos, C. A. Sanders, J. J. Madej, and W. K. Hadley.** 1994. High predictive value of the acid-fast smear for Mycobacterium tuberculosis despite the high prevalence of Mycobacterium avium complex in respiratory specimens. Clin. Infect. Dis. **19:**334–336.