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

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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

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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.

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

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expectorated sputum</td>
<td>167 (38.8)</td>
</tr>
<tr>
<td>Induced sputum</td>
<td>195 (45.3)</td>
</tr>
<tr>
<td>Tracheal aspirate</td>
<td>55 (12.8)</td>
</tr>
<tr>
<td>BAL fluid</td>
<td>11 (2.6)</td>
</tr>
<tr>
<td>Bronchial brushing</td>
<td>2 (0.5)</td>
</tr>
<tr>
<td>Total</td>
<td>430 (100)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sequential specimen no.</th>
<th>Cumulative no. (%) of patients with M. tuberculosis recovered from culture (n = 143)</th>
<th>Cumulative no. (%) of smear with acid-fast bacilli detected (n = 46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>117 (82)</td>
<td>44 (96)</td>
</tr>
<tr>
<td>2</td>
<td>131 (92)</td>
<td>46 (100)</td>
</tr>
<tr>
<td>3</td>
<td>143 (100)</td>
<td></td>
</tr>
</tbody>
</table>

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

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

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

<table>
<thead>
<tr>
<th>Sequential specimen no.</th>
<th>Cumulative no. (%) of patients with M. tuberculosis recovered from culture of expectorated sputa (n = 50)</th>
<th>Cumulative no. (%) of patients with M. tuberculosis recovered from culture of induced sputa (n = 44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>41 (82)</td>
<td>37 (84)</td>
</tr>
<tr>
<td>2</td>
<td>46 (92)</td>
<td>42 (95)</td>
</tr>
<tr>
<td>3</td>
<td>50 (100)</td>
<td>44 (100)</td>
</tr>
</tbody>
</table>
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 5).

Seventy-four (17%) of the 430 specimens in this analysis represented the fourth or greater sequential specimen. 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, 4, 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 5. Cumulative culture- and smear-positive rates for sequential specimens from AIDS versus non-AIDS patients

<table>
<thead>
<tr>
<th>Sequential specimen no.</th>
<th>AIDS patients</th>
<th>Non-AIDS patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cumulative no. (%) of patients with M. tuberculosis recovered from culture (n = 23)</td>
<td>Cumulative no. (%) of patients with M. tuberculosis recovered from culture (n = 120)</td>
</tr>
<tr>
<td></td>
<td>Cumulative no. (%) of smears with acid-fast bacilli detected (n = 10)</td>
<td>Cumulative no. (%) of smears with acid-fast bacilli detected (n = 36)</td>
</tr>
<tr>
<td>1</td>
<td>22 (96)</td>
<td>95 (80)</td>
</tr>
<tr>
<td></td>
<td>23 (100)*</td>
<td>108 (90)*</td>
</tr>
<tr>
<td>2</td>
<td>10 (100)*</td>
<td>120 (100)*</td>
</tr>
<tr>
<td>3</td>
<td>10 (100)*</td>
<td>34 (94)*</td>
</tr>
<tr>
<td></td>
<td>36 (100)*</td>
<td>56 (100)*</td>
</tr>
</tbody>
</table>

|                         | Cumulative no. (%) of smear-positive culture results for sequential specimens (n = 5) |
|                         | Cumulative no. (%) of smear-positive culture results for sequential specimens (n = 120) |
|                         | Cumulative no. (%) of smear-positive culture results for sequential specimens (n = 36) |

### 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).

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

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>333 (74.8)</td>
<td>89 (84.0)</td>
<td>64 (76.2)</td>
<td>80 (66.7)</td>
<td>117 (81.8)</td>
</tr>
<tr>
<td>2</td>
<td>372 (83.6)</td>
<td>99 (93.4)</td>
<td>77 (91.7)</td>
<td>113 (94.2)</td>
<td>131 (91.6)</td>
</tr>
<tr>
<td>3</td>
<td>396 (89.0)</td>
<td>101 (95.3)</td>
<td>84 (100)</td>
<td>120 (100)</td>
<td>143 (100)</td>
</tr>
<tr>
<td>4</td>
<td>405 (91.1)</td>
<td>103 (97.2)</td>
<td>NDa</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>416 (93.5)</td>
<td>104 (98.1)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>422 (94.9)</td>
<td>105 (99.1)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>7–17</td>
<td>445 (100)</td>
<td>106 (100)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

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.
ratory specimens, we further recommend that only three se-
quently respiratory specimens—regardless of type—are nec-
necessary for a definitive and cost-effective laboratory diagnosis of
diagnostic 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 defini-
tively diagnose pulmonary tuberculosis (5, 18).

**ACKNOWLEDGMENTS**

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Phil Hopewell.

**REFERENCES**