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Current Practices in Mycobacteriology: Results of a Survey of State Public Health Laboratories

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Fifty-six state and territorial public health laboratories were surveyed to determine whether currently available rapid methods for the identification and drug susceptibility testing of *Mycobacterium tuberculosis* were being performed. Forty (71%) laboratories use fluorochrome rather than conventional basic fuchsin stains for screening clinical specimens for acid-fast bacilli. Of the 55 laboratories that routinely culture for mycobacteria, 16 (29%) use the more rapid radiometric methods. Species identification of isolates is done by biochemical tests in 13 (23%) laboratories; 40 (72%) use nucleic acid probes, high-performance liquid chromatography, or the BACTEC *p*-nitro-α-acetylamino-β-hydroxypropiophenone (NAP) test (rapid tests); 3 laboratories do not perform species identification. Drug susceptibility testing is performed with solid media by 36 of 45 (80%) laboratories, while the more rapid radiometric methods are used by 9 (20%) laboratories. Compared with the laboratories that use conventional methods, laboratories that use rapid methods report results more quickly: for species identification, 43 days (conventional) versus 22 days (rapid); for drug susceptibility testing, 44 days (conventional) versus 31 days (rapid) from specimen processing. Rapid technologies for microscopy and species identification are being used by many, but not all, state and territorial public health laboratories; however, most laboratories do not use the more rapid radiometric methods for routine culture or drug susceptibility testing of mycobacteria. Implementation of such rapid technologies can shorten turnaround times for the laboratory diagnosis of tuberculosis and recognition of drug resistance.

Cases of tuberculosis in the United States increased 18% from 1985 through 1991. The Centers for Disease Control (CDC) estimates that during that period more than 39,000 excess cases of tuberculosis occurred in the United States (6). These are cases which would not have been expected had the downward trend experienced before 1985 continued. In addition to the increase in the number of cases, more cases that are caused by isolates that are highly resistant to one or more of the primary drugs used for treatment are being identified. This pattern of resistance adds significantly to the costs and duration of treatment while reducing the efficacy of therapy. To control this resurging health problem, it is imperative that cases of tuberculosis be identified and that patients with tuberculosis be placed on effective chemotherapy as quickly as possible.

Mycobacteriology laboratories play a pivotal role in the control of multidrug-resistant tuberculosis through the rapid detection, isolation, identification, and drug susceptibility testing of *Mycobacterium tuberculosis* in clinical specimens. To assess how widely the most rapid technologies were being used, CDC, in collaboration with the Association of State and Territorial Public Health Laboratory Directors, conducted a survey of state and territorial mycobacteriology laboratories. This report summarizes the results of that survey.

MATERIALS AND METHODS

In December 1991, questionnaires were mailed to the 54 public health laboratory directors representing the 50 states, the District of Columbia, Puerto Rico, the Virgin Islands, and Guam. Surveys were completed by laboratory personnel in 57 laboratories; responses from the two branches of the state laboratory in Alaska and the three branches of the state laboratory in Florida were included as independent laboratories because each laboratory used different methodologies. In one state, all mycobacteriology is done at a facility other than the state laboratory; therefore, that questionnaire was excluded from the analysis.

Information was requested on the methods used for acid-fast microscopy, routine culture, species identification, and drug susceptibility testing, as well as the manner and length of time needed to report results. The laboratories also were asked to provide the number of clinical specimens and referred mycobacterial isolates processed for identification, the number of isolates identified as *M. tuberculosis*, and the number of isolates tested for drug susceptibility during the 9-month interval from 1 January to 30 September 1991. Data from one laboratory which had only recently begun mycobacterial testing were excluded from analyses of the monthly volume of specimens processed.

When completed, the forms were returned to CDC, and the data were coded, entered, and then analyzed by using the Epi Info statistical software package (7).

RESULTS

Clinical specimens arrive at state laboratories an average of 2.8 days (range, 1 to 5 days) following collection. Only 46% of the laboratories surveyed reported receiving specimens within 2 days of collection, and only 6% reported receiving specimens within 1 day of collection (data not shown). The numbers of specimens processed and the strains identified and tested for drug susceptibility between 1 January and 30 September 1991 are given in Table 1. Thirty-six (65%) mycobacteriology laboratories process 500 or fewer clinical specimens per month, and 10 (18%) laboratories process more than 1,000 clinical specimens per month.

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TABLE 1. Specimens processed between 1 January and 30 September 1991 by 55 state and territorial laboratories[a]

Monthly vol	No. (%) of laboratories[b]
No. of specimens processed:	
≤100	9 (16)
101–500	27 (49)
501–1,000	9 (16)
>1,000	10 (18)
No. of specimens positive for *M. tuberculosis*[c]:	
≤10	20 (38)
11–50	17 (32)
51–100	12 (23)
>100	4 (7)
No. of *M. tuberculosis* isolates tested for drug susceptibility[d]:	
≤10	18 (40)
11–50	21 (47)
51–100	3 (7)
>100	3 (7)

[a] One survey was excluded because of the recent implementation of mycobacterial testing (see text).
[b] Percentages may not total 100 because of rounding.
[c] Two laboratories do not perform species identification.
[d] Ten laboratories do not perform drug susceptibility testing.

Sixteen laboratories (30%) identify more than 50 isolates per month as *M. tuberculosis*; 6 (13%) laboratories perform drug susceptibility testing on more than 50 isolates of *M. tuberculosis* per month (Table 1).

Microscopic examination of sputum for acid-fast bacilli (AFB) is performed in all of the laboratories surveyed. Forty of 56 (71%) laboratories use fluorochrome staining, while the remaining 16 (29%) use conventional stains, such as Ziehl-Neelsen or Kinyoun (Table 2).

TABLE 2. Procedures used in state and territorial laboratories for detection and identification of *M. tuberculosis*

Procedure	No. (%) of laboratories that use the procedure[a]	% of total specimens processed by the procedure
AFB screening stains		
Fluorochrome	40 (71)	77
Ziehl-Neelsen	12 (21)	13
Kinyoun	4 (7)	10
Primary culture medium		
Solid	39 (70)	68
Radiometric	1 (2)	1
Solid and radiometric	15 (27)	31
Do not do	1 (2)	0
Species identification tests		
Biochemical tests	13 (23)	18
Nucleic acid probes	12 (21)	25
BACTEC NAP	0 (0)	0
HPLC	1 (2)	1
More than one of the above[b]	27 (48)	56
Do not do	3 (5)	0

[a] Percentages may not total 100 because of rounding.
[b] Seventeen laboratories use biochemical tests and nucleic acid probes, five laboratories use BACTEC NAP with biochemical tests and/or nucleic acid probes and/or HPLC, and five laboratories use HPLC in conjunction with biochemical tests and/or nucleic acid probes.

TABLE 3. Methods used for drug susceptibility testing of *M. tuberculosis* isolates in 45 state and territorial laboratories

Test method	No. (%) of laboratories	% of specimens processed
Inoculum[a]		
Direct only	0 (0)	0
Indirect only	19 (42)	31
Both	26 (58)	69
Medium		
Solid	36 (80)	20
Radiometric	4 (9)	14
Both	5 (11)	66
Isolates tested		
All	21 (47)	53
As requested	4 (9)	6
Other[b]	20 (44)	41

[a] Direct testing is done from specimen concentrates; indirect testing is done from pure cultures of the organisms.
[b] Done by a predetermined schedule, such as one test per patient at defined intervals.

Ninety-eight percent (55 of 56) of the laboratories culture for mycobacteria; 39 (70%) laboratories use solid media such as Lowenstein-Jensen or Middlebrook agar, 1 laboratory uses radiometric methods alone, and 15 (27%) laboratories use solid media in conjunction with radiometric culture methods (Table 2). Two laboratories use radiometric methods for blood and specimens from other sterile sites and solid media for specimens from nonsterile sites, i.e., sputum or bronchial washings (data not shown). Thirty-two percent of the specimens processed for mycobacteria are cultured by radiometric techniques (Table 2). Of the 10 laboratories that process the greatest number of clinical specimens for mycobacteria, 3 currently use radiometric methods for routine isolation of the organisms.

Fifty-two (93%) of the laboratories surveyed identify the mycobacteria to the species or complex level; reference laboratories provide species identification for the remaining laboratories. Isolates are identified by standard biochemical tests (catalase, niacin production, and nitrate reduction) in 13 (23%) laboratories, while 12 (21%) laboratories use nucleic acid probes and one laboratory uses high-performance liquid chromatography (HPLC). The remaining 27 (48%) laboratories use a combination of biochemical tests, nucleic acid probes, HPLC, and the BACTEC *p*-nitro-α-acetyl-amino-β-hydroxypropiophenone (NAP) test. Laboratories that process 82% of the specimens identify isolates of *M. tuberculosis* (complex) by using either nucleic acid probes, HPLC, or the BACTEC NAP test (Table 2).

Among the 52 laboratories that culture and identify *M. tuberculosis* (complex), 45 (87%) perform drug susceptibility testing and 7 (13%) send isolates to reference laboratories for such testing. All 45 laboratories perform drug susceptibility tests on pure cultures of the organisms (indirect method); 26 of 45 (58%) laboratories also test directly from specimen concentrates (direct method) (Table 3). Four (9%) laboratories perform drug testing only if it is requested; the remainder of the laboratories test either all isolates or one isolate from each patient at defined intervals. Susceptibility tests are performed on solid medium (Middlebrook 7H10 or 7H11) in 41 (91%) laboratories, 5 of which also use a radiometric method. Only radiometric methods are used for susceptibility testing in four laboratories. Eighty percent of the isolates

TABLE 4. Method of reporting mycobacteriology results by state and territorial laboratories

Reporting method	No. (%) of laboratories reporting to:	
	Hospital, clinic, or physician	Tuberculosis control program
Written report only	33 (59)	37 (66)
Telephone only	3 (5)	6 (11)
Written and telephone report	20 (36)	12 (21)
Computer diskette	0 (0)	1 (2)

processed for mycobacteria are tested for drug susceptibility by radiometric methods (Table 3).

All 45 laboratories that test drug susceptibility use isoniazid, rifampin, and ethambutol. Additionally, streptomycin is used in 43 (96%) laboratories, *p*-aminosalicylic acid is used in 14 (31%) laboratories, pyrazinamide is used in 12 (27%) laboratories, and other drugs (ethionamide, kanamycin, ciprofloxacin, cycloserine, and capreomycin) are used in 26 (58%) laboratories.

Reporting of results to the hospital, clinic, physician, or state tuberculosis control program is performed primarily by written report; some laboratories also report by telephone (Table 4). In addition, one state communicates results to the tuberculosis control program through electronic or computer systems.

The time required to report test results is variable. Results of AFB smear microscopy take an average of 2 days (range, 1 to 30 days) from the time of specimen receipt; identification of *M. tuberculosis* in clinical specimens averages 31 days (range, 5 to 80 days). Drug susceptibility results are available in 42 days (range, 18 to 76 days).

Laboratories that use radiometric methods for culture and either nucleic acid probes, HPLC, or the BACTEC NAP test are able to report results of species identification in an average of 22 days, while those that culture on solid medium and perform standard biochemical tests report results of species identification in an average of 43 days (Table 5). Laboratories that use radiometric methods for culture or drug susceptibility testing are able to report results more quickly than those that use solid media (Table 6).

DISCUSSION

Since 1990, several nosocomial outbreaks of multidrug-resistant tuberculosis, involving transmission of *M. tuberculosis* to patients, health care workers, and employees of correctional institutions, have been reported to CDC (5, 8).

TABLE 5. Days needed to report results by laboratories that perform identification of *M. tuberculosis*

No. (%) of laboratories	% of isolates processed	Culture method	Identification method	Report time (days [mean + SD])
13 (25)	18	Solid medium	Biochemicals	43 ± 18
24 (45)	49	Solid medium	Rapid[a]	31 ± 8
16 (30)	33	Radiometric	Rapid	22 ± 9
53[b] (100)	100	All	All	31[c]

[a] Use of nucleic acid probes, HPLC, or the BACTEC NAP test.
[b] Three laboratories do not perform species identification of *M. tuberculosis*.
[c] Range, 5 to 80 days.

TABLE 6. Days needed to report results by laboratories that perform drug susceptibility testing of *M. tuberculosis*

No. (%) of laboratories	% of isolates processed	Culture/identification methods	Drug testing method	Report time (days [mean ± SD])
10 (22)	13	Solid/biochemical	Solid medium	44 ± 7
19 (42)	31	Solid/rapid[a]	Solid medium	48 ± 13
2 (4)	15	Solid/rapid	Radiometric	40 ± 8
7 (16)	8	Radiometric/rapid	Solid medium	36 ± 9
7 (16)	33	Radiometric/rapid	Radiometric	31 ± 11
45[b] (100)	100	All	All	42[c]

[a] Use of nucleic acid probes, HPLC, or the BACTEC NAP test.
[b] Eleven laboratories do not perform drug susceptibility testing of *M. tuberculosis*.
[c] Range, 18 to 76 days.

To date, more than 200 multidrug-resistant tuberculosis cases have been identified in connection with these outbreaks. Isolates from most cases are characterized by resistance to at least isoniazid and rifampin; many of the strains also show resistance to other drugs, such as ethambutol, streptomycin, ethionamide, kanamycin, and rifabutin. Laboratory delays in both the identification of *M. tuberculosis* and the recognition of drug resistance contributed to the nosocomial transmission of these multidrug-resistant organisms. Outbreaks such as these, along with the resurgence of drug-susceptible disease, underscore the need for rapid laboratory diagnosis and drug susceptibility testing.

Microscopy is the simplest and most rapid procedure currently available to detect the presence of AFB in clinical specimens. Both basic fuchsin (Ziehl-Neelsen and Kinyoun) and a fluorochrome dye (auramine O) are available for staining AFB. Fluorescing dyes offer several advantages over conventional basic fuchsin stains; smears stained with auramine O are easier to read; the yellow-fluorescing bacilli are easier to detect than the red fuchsin-stained organisms on the blue background that is present after Ziehl-Neelsen or Kinyoun staining. With basic fuchsin stains, at least 300 fields should be examined at ×800 to ×1,000 magnification before the smear is considered negative (14). Because auramine O and other fluorescent acid-fast stains are scanned at ×250 to ×450 magnification, a larger area of the smear can be examined in less time by the fluorescence technique (10). Although fluorescence microscopes are costly and training is required to obtain reliable results, use of fluorochrome acid-fast stains can reduce the time and effort required for acid-fast screening of clinical specimens. This is a true acid-fast staining technique, and results are as meaningful as those obtained with the Ziehl-Neelsen stain.

The 54 recognized species of *Mycobacterium* cannot be reliably differentiated by microscopy. In addition, 30 to 50% of patients with pulmonary tuberculosis have negative sputum smears (11, 16). For a definitive diagnosis of tuberculosis, the organism must be isolated on culture medium (1) and identified. Conventional culture methods involve the inoculation of solid media (Lowenstein-Jensen, Middlebrook 7H10 or 7H11) with incubation at 35 to 37°C in 10% CO_2–90% air; under such conditions, colonies of *M. tuberculosis* can be detected in 3 to 4 weeks. Use of radiometric techniques can shorten detection times significantly. BACTEC, the most widely used radiometric method, is based on the metabolism of ^{14}C-labeled palmitic acid in the liquid medium and the subsequent release of radiolabeled CO_2. By this

TABLE 7. Summary of proportion of state and territorial laboratories that use rapid methods

No. of specimens processed/mo	No. of laboratories that use the indicated method/total no. of laboratories in group					
	Fluorochrome stains	Radiometric culture	Rapid identification	Radiometric drugs	Telephone hospital	Telephone tuberculosis control program
1–100	7/10	1/10	5/9	0/5	6/10	5/10
101–500	17/26	9/26	21/25	3/22	14/26	10/26
501–1,000	8/9	4/9	6/9	2/8	3/9	2/9
>1,000	8/10	2/10	9/10	4/10	0/10	2/10
Total	40/55	16/55	40/53	9/45	23/55	19/55

method, mycobacteria in clinical specimens can be detected in less than 2 weeks (15).

Following the primary isolation of mycobacteria from a clinical specimen, the organism must be identified to the species level. Initial subgrouping of mycobacteria can be made on the basis of the growth rate and pigment production observed on solid medium. More precise identification of the organism requires additional taxonomic tests. Biochemical tests, such as catalase, niacin, and nitrate reduction, can be used to identify 96% of *M. tuberculosis* isolates; however, because these tests require growth of the organism, results are not available until 3 weeks after primary isolation of the *Mycobacterium* species (10). Newer, more rapid techniques are now available for identification of *M. tuberculosis*. These include nucleic acid probes which specifically bind to *M. tuberculosis* complex RNA; HPLC, which is used to identify species (complex)-specific mycolic acid patterns; gas-liquid chromatography, which identifies mycobacterial cell wall fatty acids; and susceptibility to NAP, which is used in conjunction with the BACTEC system. These methods allow fully grown cultures of *M. tuberculosis* (complex) to be identified in from 8 h to 4 days after primary isolation of the bacillus (2, 3, 9, 12, 15).

The rapid recognition of drug-resistant organisms is essential to the control of multidrug-resistant tuberculosis. Drug susceptibility tests can be performed by using radiometric culture methods, which reduces the time needed after receipt of the specimen to identify drug-resistant organisms from the 7 weeks needed for testing on solid medium to only 3 weeks (11). At this time, however, standardized methods and reagents for testing only five of the antituberculosis drugs (streptomycin, rifampin, isoniazid, ethambutol, and pyrazinamide) are commercially available for use in the BACTEC system. Additional antituberculosis drugs may be tested, but testing requires that appropriate dilutions of the drugs be prepared by the individual laboratories.

The purpose of the survey described here was to assess whether the most rapid methods for AFB microscopy, mycobacterial culture, identification, and susceptibility testing for *M. tuberculosis* were being performed in state and territorial laboratories. The results are summarized in Table 7. While rapid methods for AFB screening and species identification are being used in many of the laboratories surveyed, only 6 of the 19 laboratories that process 500 or more specimens per month use radiometric methods for routine culture, and only 6 of the 18 that test 500 or more specimens per month for drug susceptibility use radiometric methods for such testing (Table 7).

Laboratory delays in diagnosing tuberculosis not only prevent individual patients from receiving appropriate chemotherapy but also seriously hinder public health control efforts. Continued transmission of *M. tuberculosis* by individuals with unrecognized tuberculosis results in exposure and possible infection of additional individuals, thereby creating the potential for outbreaks such as those in New York and Florida (5, 8). Our data indicate that the laboratories that use the most rapid methods for culture, identification, and drug susceptibility testing of *M. tuberculosis* can reduce the time to reporting species identification by 3 weeks and drug susceptibility testing results by almost 2 weeks (Tables 5 and 6).

Managerial strategies can be developed to shorten the turnaround time for tuberculosis testing and reporting. Specimens should be received by the laboratory as quickly as possible, preferably within 1 day of collection. Longer transport times delay the initiation of laboratory tests and could affect specimen viability.

Results of AFB microscopy, culture identification, and drug susceptibility testing should be telephoned to the physician or clinic and followed up with a written report. Such rapid reporting will avoid the delays inherent in preparing and mailing written reports and ensures that the physician receives the test results as quickly as possible.

The recent economic climate and the mistaken perception of tuberculosis as a disease of declining public health significance has resulted in decreased funding for many laboratories. The consequences of this have been to add to the delay in implementation of newer, more rapid diagnostic technologies for tuberculosis.

The goal stated in *A Strategic Plan for the Elimination of Tuberculosis in the United States*, which was published in 1989 (4), is a tuberculosis incidence of less than one case per million population by the year 2010. More recently, the National MDR-TB [multidrug-resistant tuberculosis] Task Force developed a plan to combat multidrug-resistant tuberculosis, including objectives to make the laboratory diagnosis more rapid, sensitive, and reliable (13). To achieve the objectives outlined in those two reports (4, 13), laboratories, clinics, mycobacteriologists, and physicians must be committed to implementing the best possible strategies for the diagnosis, treatment, and management of patients with tuberculosis. Mycobacteriology laboratories that use the most rapid and efficient methods available for the identification and drug susceptibility testing of *M. tuberculosis* can serve to strengthen tuberculosis control efforts.

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