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U.S. Hospital Mycobacteriology Laboratories: Status and Comparison with State Public Health Department Laboratories

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In response to the resurgence of tuberculosis, the Centers for Disease Control and Prevention recommended the use of certain mycobacteriology laboratory methods to improve the accuracy of diagnosis and/or minimize times to complete specimen processing. A study to determine the extent to which these recommended methods were being used in hospital laboratories was needed. In 1992, a survey was mailed to infection control and laboratory personnel at 1,076 hospitals with ≥100 beds to determine the mycobacterial laboratory services being performed, the methods being used, the number of specimens being processed, and the times to completion during 1991. In 1995, a 20% sample of hospital laboratories that responded to the initial questionnaire was resurveyed. Responses to the 1992 survey were received from personnel at 756 (70%) hospitals representing 750 laboratories. Among laboratories performing the services, the use of recommended methods was as follows: fluorochrome stain for acid-fast bacillus microscopy (47%); radiometric methods for primary culture (29%); rapid (radiometric methods, use of nucleic acid probes, high-performance liquid chromatography, or gas-liquid chromatography) methods for identification of *Mycobacterium tuberculosis* (59%); and radiometric methods for drug susceptibility testing (55%). Reported times to complete specimen processing were shortest for laboratories that used recommended methods and longest for hospitals that referred specimens to outside laboratories. Only 46% of surveyed laboratories performed at least the minimal number of mycobacterial cultures (20/week) deemed necessary to maintain competence. Among 145 laboratories that performed the services and were resurveyed in 1995, use of recommended techniques increased from 44 to 73% for acid-fast bacillus microscopy, from 27 to 37% for primary culture, from 59 to 88% for *M. tuberculosis* identification, and from 55 to 75% for drug susceptibility testing. These changes were associated with reductions in reported specimen turnaround times. Use of the methods recommended by the Centers for Disease Control and Prevention increased at the resurveyed hospital mycobacteriology laboratories between 1991 and 1995. However, continued efforts are needed to increase the use of recommended methods at moderate- and high-volume laboratories, encourage referral of specimens from low-volume laboratories, and transmit results rapidly from all laboratories.

The number of cases of tuberculosis reported in the United States increased by 20% from 1985 to 1992 (2). Outbreaks of drug-susceptible (7, 14) and multidrug-resistant tuberculosis (9), most of which are associated with human immunodeficiency virus infection, occurred at hospitals, prisons, and homeless shelters. The mycobacteriology laboratory is a vital resource in combatting this resurgence of tuberculosis. The Centers for Disease Control and Prevention has recommended the use of certain laboratory methods to facilitate accurate and prompt diagnosis of tuberculosis (11). For microscopic examination of specimens, fluorescent staining methods were recommended because they allow faster scanning for acid-fast bacillus (AFB) than do conventional Ziehl-Neelsen or Kinyoun methods. For primary culture, radiometric methods (e.g., BACTEC TB; Becton Dickinson, Sparks, Md.) were recommended, since they allow faster detection of mycobacteria than does growth on conventional solid media (10). For species identification, methods such as the use of nucleic acid probes, the BACTEC ρ-nitro-α-acetylamino-β-hydroxypropiophenone (NAP) test, high-performance liquid chromatography, or gas-liquid chromatography were recommended rather than conventional biochemical testing. Finally, for drug susceptibility testing, radiometric methods were recommended, since they can provide results for evaluating first-line antituberculosis drugs more quickly than can conventional testing on solid media (11).

In April 1992, a group of U.S. hospitals was surveyed to assess their capability to diagnose, manage, and prevent nosocomial transmission of tuberculosis. This report presents the results of the mycobacteriology laboratory component of this survey and the results of a 1995 resurvey of a sample of laboratories that responded to the initial survey.

MATERIALS AND METHODS

Surveyed hospitals were selected from the 1990 American Hospital Association data tape of all U.S. (including Puerto Rico) hospitals. A total of 2,841 hospitals were eligible for the survey: 632 city, county, Veterans Administration, and primary medical school-affiliated hospitals, hereafter referred to as public hospitals; and 2,209 privately owned hospitals with ≥100 total hospital beds, hereafter referred to as private hospitals. Of eligible hospitals, 1,159 (41%) had 100 to 199 total hospital beds, 1,127 (40%) had 200 to 399 beds, and 55 (20%) had ≥400 beds. The surveyed group of 1,076 hospitals included all (n = 632) public hospitals and a 20% sample (n = 444) of the private hospitals.

The laboratory component of the survey covered the period 1 January to 31 December 1991. It included the methods used for AFB microscopy, primary culture, *Mycobacterium tuberculosis* identification, drug susceptibility testing, and reporting of results; the numbers of samples processed and *M. tuberculosis*

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isolates identified; and time periods (estimated by laboratory personnel) required for completion of laboratory testing.

Results from surveyed hospital laboratories were compared with those from 56 U.S. state and territorial mycobacteriology laboratories surveyed in December 1991, covering the period January to September 1991 (8); the latter group is referred to as state laboratories. For these laboratories, the number of specimens processed per year was calculated as one-third greater than the number reported for the 9-month period covered by the survey.

In June and July 1995, we resurveyed selected laboratories to determine changes in testing, methods, and completion times. The resurveyed group included a 20% random sample ($n = 150$) of all laboratories that responded to the initial survey. Because some subgroups were underrepresented among the 150 laboratories, we selected an additional 32 that used only biochemical tests for *M. tuberculosis* identification in 1991 and an additional 25 laboratories that used only solid media for drug susceptibility testing in 1991.

Analyses were performed by using the Statistical Analysis System (SAS Institute, Cary, N.C.) program for personal computers. If a specific item of information was missing for a laboratory, that laboratory was excluded only from analyses relating to that item; for this reason, denominators for different analyses may vary. The survey oversampled public versus private hospitals. Estimated statistics for all 2,841 U.S. hospitals with ≥100 total beds were made by using rates specific for public versus private laboratories and weighting responses by the reciprocal of the fraction sampled.

RESULTS

1992 questionnaire. A completed questionnaire was returned from 763 (71%) hospitals. The response rate was 71% for public (450 of 632) and 70% for private (313 of 444) hospitals. The 763 hospitals included 227 (30%) with 100 to 199 total hospital beds, 274 (36%) with 200 to 399 beds, and 262 (34%) with ≥400 beds. Responses were received from hospitals in all 50 states, Washington, D.C., and Puerto Rico. Of the 763 hospitals, 756 completed the laboratory portion of the questionnaire. Because some were served by common laboratories, these 756 responding hospitals were represented by 750 laboratories.

AFB microscopy was performed by 84% (624 of 746) of surveyed hospital laboratories, primary culture was performed by 72% (533 of 741), identification of *M. tuberculosis* was performed by 38% (279 of 738), and drug susceptibility testing of isolates was performed by 13% (94 of 739). Larger hospital laboratories were more likely to perform mycobacteriology procedures. For hospitals with 100 to 199, 200 to 399, and >400 total beds, respectively, microscopy was performed at 60, 89, and 98%; primary culture was performed at 39, 77, and 94%; identification of *M. tuberculosis* occurred at 10, 31, and 68%; and drug susceptibility testing was done at 2, 9, and 26%.

Numbers of specimens processed. Primary culture was performed on >1,000 specimens per year at 46% of surveyed hospital laboratories versus 86% of state laboratories (Table 1). The total numbers of primary cultures performed during 1991 were 831,308 for surveyed hospital laboratories, an estimated 2,110,742 for all U.S. hospitals with ≥100 beds, and 386,400 for state laboratories.

More than 100 *M. tuberculosis* isolates were identified by only 11% of surveyed hospital laboratories compared with 70% of state laboratories (Table 1). Total *M. tuberculosis* isolates identified during 1991 were 21,804 for surveyed hospitals, an estimated 39,874 for all U.S. hospitals with ≥100 beds, and 27,225 for state laboratories.

More than 100 *M. tuberculosis* isolates were tested for drug susceptibility at 15% of surveyed hospital laboratories compared with 51% of state laboratories (Table 1). Susceptibility testing was performed on a total of 6,362 isolates at surveyed hospitals, an estimated 14,120 isolates from all U.S. hospitals with ≥100 beds, and 15,824 isolates at state laboratories. At least one isolate resistant to isoniazid and rifampin was reported from 50% of surveyed hospital laboratories (Table 1).

TABLE 1. Numbers of specimens processed during 1991 by hospital and state health department mycobacteriology laboratories

Specimen type and no.	No. (%)[a] of laboratories performing mycobacteriology test	
	Hospitals	States[b]
Primary culture		
1–500	164 (32)	3 (5)
501–1,000	116 (22)	5 (9)
1001–2000	118 (23)	6 (11)
>2,000	122 (23)	41 (75)
Positive for *M. tuberculosis*		
None	12 (4)	0
1–10	113 (42)	2 (4)
11–50	95 (35)	10 (19)
51–100	19 (7)	4 (8)
≥101	29 (11)	37 (70)
Tested for antimicrobial susceptibility		
None	1 (1)	0
1–10	16 (18)	1 (2)
11–50	43 (49)	8 (18)
51–100	14 (16)	8 (18)
≥101	13 (15)	28 (62)
Resistant to isoniazid and rifampin		
None	44 (50)	—[c]
1–5	28 (32)	
6–10	7 (8)	
11–50	7 (8)	
50–79	2 (2)	

[a] Denominators for percents are the numbers of laboratories which both performed the test and supplied information on the number of specimens.
[b] Results from a separate survey of state public health laboratories (8).
[c] —, data not collected.

Laboratory procedures used. Among laboratories performing microscopy, fluorochrome stain was used at 47% of surveyed hospitals versus 71% of state laboratories (Table 2). Nonradiometric media were the only media used for primary culture at 71% of surveyed hospital and state laboratories (Table 2).

Among the 276 hospital laboratories that provided information on methods used to identify *M. tuberculosis*, biochemical tests were used at 215 (78%), nucleic acid probes were used at 117 (42%), the BACTEC NAP test was used at 64 (23%), and gas-liquid chromatography was used at 4 (1%); high-performance liquid chromatography was not used at any of the laboratories surveyed (total exceeds 100% since some laboratories used more than one method). When each laboratory was assigned to only one category (i.e., so that the total was 100%), biochemical tests alone were used most commonly (41%) at surveyed hospital laboratories, but testing by two or more methods was most common (51%) at state laboratories (Table 2).

Radiometric primary culture was used by 46% (129 of 227) of laboratories that performed *M. tuberculosis* identification but by only 11% (26 of 247) of laboratories that referred isolates for species identification.

Among the hospital laboratories that performed drug susceptibility tests, radiometric methods alone or in combination were used by 56% compared with 20% of state laboratories (Table 2). Among surveyed hospital laboratories, susceptibility testing was performed directly from the specimen concentrate at 31% (25 of 81) and indirectly from cultures at 69% (56 of

TABLE 2. Procedures used at surveyed hospital and state health department mycobacteriology laboratories, 1991

Test or medium	No. (%) of laboratories surveyed	
	Hospitals	States[a]
AFB screening stains		
Fluorochrome[b]	292 (47)	40 (71)
Kinyoun	211 (34)	4 (7)
Ziehl-Neelsen	106 (17)	12 (21)
Other	15 (2)	
Primary culture medium		
Nonradiometric	374 (71)	39 (71)
Radiometric[b]	15 (3)	1 (2)
Both[b]	140 (26)	15 (27)
M. tuberculosis identification tests		
Biochemical tests	113 (41)	13 (25)
Nucleic acid probes[b]	33 (12)	12 (23)
BACTEC NAP[b]	21 (8)	0
HPLC[b,c]	0	1 (2)
≥2 of the above[b]	109 (39)	27 (51)
Drug susceptibility testing medium		
Solid	41 (45)	36 (80)
Radiometric[b]	42 (46)	4 (9)
Both[b]	9 (10)	5 (11)

[a] Results from a separate survey of state public health laboratories (8).
[b] Method recommended by the Centers for Disease Control and Prevention.
[c] HPLC, high-performance liquid chromatography.

81); susceptibility testing was performed on all isolates at 89% (83 of 93), as requested at 2% (2 of 93), or per other algorithms at 9% (8 of 93) of surveyed hospital laboratories.

Use of recommended methods was more common at hospital laboratories that processed larger numbers of specimens (Fig. 1). For example, radiometric methods for primary culture were used by 9% of laboratories processing 1 to 500 specimens per year versus 54% for those processing >2,000 per year.

Since larger-volume laboratories were more likely to use recommended techniques, the use of recommended techniques was generally higher when calculated on a specimens-processed basis than on a laboratory basis. At surveyed hospital laboratories, recommended techniques for primary culture were used for 46% of specimens (versus being used at 29% of laboratories) and for the identification of 92% of *M. tuberculosis* isolates (versus 59% of laboratories). However, recommended techniques were used for susceptibility testing of only 53% of specimens but were used at 55% of laboratories.

Reports of *M. tuberculosis* isolation from cultures were transmitted to the physician by telephone (88%), written report (9%), and other means (4%) and to the state or local health department by telephone (39%), written report (53%), and other means (8%).

Promptness of specimen receipt and processing. Most hospital laboratories reported that specimens arrived in the laboratory on the day of collection (Table 3). Microscopy was completed on the day after specimen arrival in the laboratory (median, day 2), regardless of whether fluorochrome stains were used. *M. tuberculosis* identification was reported most rapidly (median, 21 days) by laboratories that used recommended methods for both primary culture and species identification and least rapidly (median, 42 days) by laboratories either culturing with radiometric media and identifying with biochemical methods or not culturing on radiometric media and referring isolates to outside laboratories for identification. Drug susceptibility testing was completed soonest (median, 21 days) when recommended methods were used for culture, species identification, and susceptibility testing (Table 3). Laboratories referring specimens to outside laboratories for susceptibility testing often had longer times to reporting of results (median, 42 to 60 days); use of recommended methods for culture and/or species identification prior to referral of the isolate for susceptibility testing did not substantially reduce turnaround times (Table 3). Reported completion times were similar for hospital and state laboratories, except for a longer period to arrival in state laboratories (Table 3).

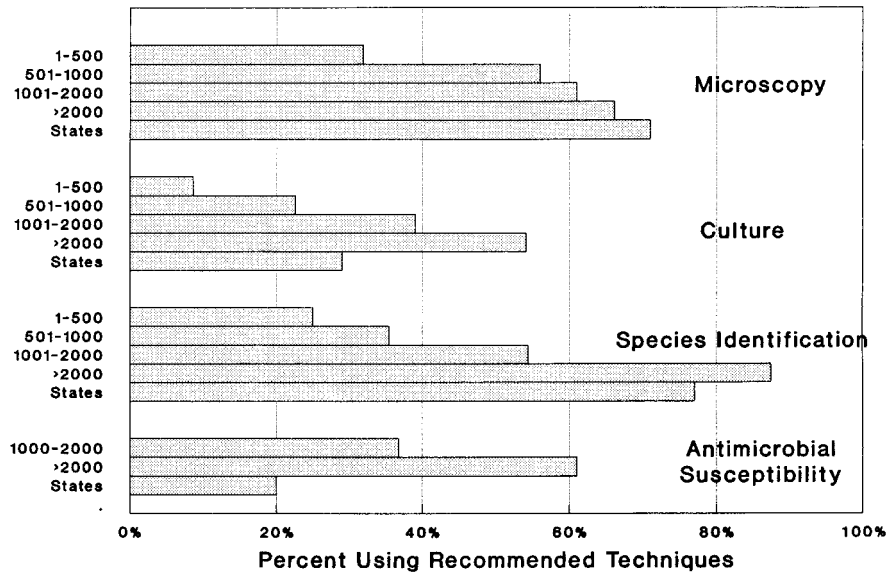

FIG. 1. Proportion of surveyed hospital and state (8) mycobacteriology laboratories using recommended techniques, 1991. Hospital laboratories are stratified by number of specimens processed per year.

TABLE 3. Median time intervals for specimen receipt and processing at surveyed hospital and state health department mycobacteriology laboratories, 1991

Procedure	Laboratory method(s)[a]	Laboratories			
		Hospitals		States[b]	
		No.	Median time interval (days)[c]	No.	Median time interval (days)[c]
Arrival in laboratory		525	1	56	3
Microscopy	Fluorochrome	272	2	40	2
	Other	252	2	16	1
M. tuberculosis identification[d]	Nonradio, biochem	86	35	13	35
	Nonradio, rapid	41	28	24	28
	Radio, biochem	15	42	0	
	Radio, rapid	98	21	16	22
	Nonradio, refer	152	42		
	Radio, refer	13	27		
Drug susceptibility testing[e]	Nonradio, biochem, solid	13	40	10	45
	Nonradio, rapid, solid	15	50	19	45
	Radio, rapid, solid	6	32	7	40
	Radio, rapid, radio	36	21	7	28
	Nonradio, refer, refer	123	60		
	Nonradio, biochem, refer	43	60		
	Radio, refer, refer	13	42		
	Radio, rapid, refer	41	60		
	Other referral	41	56		

[a] Nonradio, nonradiometric; radio, radiometric; biochem, biochemical; rapid, BACTEC NAP, nucleic acid probes, gas-liquid chromatography, and/or high-performance liquid chromatography; refer, referral of isolates to outside laboratories. In addition to the methods designated rapid, fluorochrome microscopy and radiometric methods are also rapid methods.
[b] Results from separate survey of state public health laboratories (8).
[c] For arrival in laboratory, day 1 is the day of specimen collection; for other categories, day 1 is the day of arrival in laboratory.
[d] Methods of isolation and species identification, respectively.
[e] Methods of isolation, species identification, and susceptibility testing, respectively.

Resurvey in 1995. Among 150 laboratories (20% random sample) selected for resurvey in 1995, 145 (97%) were contacted. Between 1991 and 1995 the use of recommended methods among laboratories performing the service increased from 44 to 73% for AFB microscopy, from 27 to 37% for primary culture, from 59 to 88% for *M. tuberculosis* identification, and from 55 to 75% for drug susceptibility testing (Table 4).

For primary culture, use of the radiometric BACTEC TB system increased from 29 laboratories in 1991 to 36 in 1995. An additional 19 laboratories used broth media for primary culture in 1995. Of these laboratories, nine used Septi-Chek (Becton Dickinson), eight used the mycobacterium growth indicator tube (Becton Dickinson), and two used the fluorescent BACTEC 9000 (Becton Dickinson). Three resurveyed laboratories planned to acquire the BACTEC 9000 by September 1995.

Use of nucleic acid probes for *M. tuberculosis* identification increased from 24 to 38 laboratories, while the use of biochemical tests decreased from 42 to 21 laboratories and the use of the BACTEC NAP test decreased from 12 to 7 laboratories from 1991 to 1995.

The 145 resurveyed laboratories included 66 that used conventional stains for AFB microscopy in 1991; by 1995, 6 (9%) did not perform microscopy, 24 (36%) continued to use only conventional stains, and 36 (55%) used fluorescent stains with or without conventional stains. In 1995, among 77 laboratories that did not use radiometric methods for primary culture in 1991, 9 (12%) did not perform culture, 60 (78%) continued to culture without radiometric methods, and 8 (10%) used radiometric methods.

TABLE 4. Comparison of procedures performed at a sample of hospital mycobacteriology laboratories in 1991 and 1995

Procedure	No. of laboratories in category/total (%)[a]	
	1991	1995
AFB microscopy		
Not performed	26/143 (18)	31/145 (21)
Kinyoun or Ziehl-Neelsen	66/117 (56)	31/114 (27)
Fluorochrome stain	51/117 (44)	83/114 (73)
Primary culture		
Not performed	38/144 (26)	48/145 (33)
Nonradiometric	77/106 (73)	61/97 (63)
Radiometric	29/106 (27)	36/97 (37)
M. tuberculosis identification		
Not performed	86/144 (60)	95/145 (66)
Biochemical tests	24/58 (41)	6/50 (12)
Rapid methods	34/58 (59)	44/50 (88)
Drug susceptibility testing		
Not performed	123/145 (85)	125/145 (86)
Solid media	10/22 (45)	5/20 (25)
Radiometric	12/22 (55)	15/20 (75)

[a] Denominators for the category "not performed" are total laboratories responding to the question. Denominators for other categories are laboratories performing the test.

Among the resurvey sample of 145 laboratories, only 24 used biochemical tests for *M. tuberculosis* identification and 10 used solid media for drug susceptibility testing in 1991; therefore, additional laboratories in these categories were contacted. Of a total of 52 laboratories in 1991 that performed *M. tuberculosis* identification by biochemical tests only, in 1995 21 (40%) no longer performed species identification, 14 (27%) continued to use biochemical tests only, and 17 (33%) used one or more rapid methods. Of a total of 34 laboratories in 1991 that performed drug susceptibility testing on solid media only, in 1995 9 (26%) no longer performed susceptibility testing, 16 (47%) continued to use solid media only, and 9 (26%) used radiometric methods.

Specimen turnaround times were compared for laboratories reporting data in both 1991 and 1995. Median times to report decreased from day 2 to day 1 for AFB microscopy (103 laboratories), from day 40 to 21 for *M. tuberculosis* identification (79 laboratories), and from day 45 to 35 for drug susceptibility testing (54 laboratories).

DISCUSSION

We report the mycobacteriology laboratory capabilities and practices of 750 laboratories at U.S. hospitals with ≥100 total hospital beds. In 1991, AFB microscopy was performed by 84% of laboratories, primary mycobacterial culture was performed by 72%, *M. tuberculosis* identification was conducted by 38%, and drug susceptibility testing was done by 13%. As would be expected, laboratories at larger hospitals were more likely to perform these services. Of the surveyed hospital laboratories that performed these services in 1991, 47% used recommended techniques for AFB microscopy, 29% used them for primary cultures, 59% used them for *M. tuberculosis* identification, and 55% used them for drug susceptibility testing. These figures understate the use of recommended techniques, which were used more commonly by higher-volume laboratories; 92% of *M. tuberculosis* isolates were identified by surveyed hospital laboratories that used recommended techniques.

In 1995, we resurveyed a group of the laboratories that responded to the original questionnaire. The proportion of laboratories providing mycobacteriology services decreased modestly. Among resurveyed laboratories performing the services, use of recommended techniques increased from 44 to 73% for AFB microscopy, from 27 to 37% for primary culture, from 59 to 88% for *M. tuberculosis* identification, and from 55 to 75% for drug susceptibility testing. Among laboratories that were not using recommended methods in 1991, the proportion still not using recommended methods in 1995 was 36% for microscopy, 78% for primary culture, 27% for species identification, and 47% for drug susceptibility testing. Changes in mycobacteriology methods in recent years have been reported in other studies (13).

Few data on the capabilities of hospital mycobacteriology laboratories are available. Data from laboratory proficiency testing services for 1994 indicated that 506 to 683 laboratories performed AFB microscopy only, 1,126 to 1,166 performed primary culture but not species identification, 568 to 699 performed species identification but not drug susceptibility testing, and 259 to 314 performed drug susceptibility testing (5). These figures include both hospital and nonhospital laboratories and include only 85 to 90% of the laboratories enrolled with the College of American Pathologists (CAP), the largest proficiency testing service.

The CAP Mycobacteriology E survey covers only laboratories that perform mycobacterial culture, includes a small number of foreign laboratories, and of course excludes laboratories not subscribing to the CAP testing service. For 1991, the CAP survey showed that 42% of 1,029 responding hospital laboratories processed 1 to 50 specimens per month in 1991 (12); in comparison, 31% of the hospitals responding to our 1991 survey performed primary culture on 1 to 500 specimens per year.

It is vital that optimal laboratory methods be used at state health department (8, 11) and other reference laboratories. However, total specimen volume during 1991 was estimated to be higher among all 2,841 U.S. hospitals with ≥100 beds than among state laboratories for primary cultures performed (2,110,742 versus 386,400, respectively) and *M. tuberculosis* isolates identified (39,874 versus 27,225). Multiple isolates may be identified from a given patient, so the estimated number of isolates exceeds the 26,283 cases of tuberculosis reported in 1991 (4). These figures emphasize the importance of optimizing laboratory methods at hospital as well as at reference laboratories.

The American Thoracic Society has suggested that performing at least 10 to 15 AFB microscopies and ≥20 primary cultures per week is needed to maintain competence (1). In 1991, approximately 46% of surveyed hospital laboratories performed ≥20 cultures per week. These findings suggested a need for greater referral of isolates to outside laboratories. The results of our 1995 resurvey suggest that there has been a modest increase in such referral.

The Center for Disease Control and Prevention has published suggested laboratory turnaround times (3). In 1991, specimen receipt within 1 day of collection was reported by 92% of surveyed laboratories, microscopic examination within 1 additional day by 86%, *M. tuberculosis* identification within 21 days by 32%, and drug susceptibility testing within 28 days by 39%. As in a survey of state laboratories for the same year (8), the use of recommended techniques was associated with minimum specimen turnaround times for both identification (median, 21 days) and susceptibility testing (median, 21 days) of *M. tuberculosis*. Our 1995 resurvey of hospital laboratories revealed not only substantial increases in the use of recommended methods but also substantial decreases in estimated turnaround times.

Among hospital laboratories referring specimens to outside laboratories, turnaround times in 1991 were often longer (medians of up to 42 days for *M. tuberculosis* identification and 60 days for drug susceptibility testing) than at laboratories performing testing in-house. This suggests that improvements in laboratory techniques at referral laboratories and/or better management are needed to ensure rapid transmittal of specimens and timely reporting of results from referred specimens.

Weaknesses of this study include the fact that hospitals with fewer than 100 beds, comprising >50% of U.S. hospitals, were not surveyed. However, the larger hospitals that were studied are far more likely to treat patients with tuberculosis. Unfortunately, the specimen processing times were estimates provided by laboratory personnel and were not derived by averaging a sample of report times from each laboratory. Additionally, the 1995 resurvey included a relatively small number of laboratories.

These results are encouraging. Among a sample of hospital laboratories surveyed in both 1991 and 1995, use of recommended mycobacteriology techniques increased and reported specimen turnaround times decreased. Nevertheless, there is clearly a need for continued expansion of recommended technologies at hospitals processing moderate to large numbers of specimens. Especially if the number of cases of tuberculosis resumes its decades-long decline (6), regionalization or combination of low-volume laboratories may be an increasingly attractive strategy. All laboratories, especially those receiving

referred specimens, should adopt administrative procedures to ensure the most rapid handling of specimens and transmittal of results to clinical, infection control, and public health personnel.

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