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# Changing Practices in Mycobacteriology: a Follow-Up Survey of State and Territorial Public Health Laboratories

BILLIE R. BIRD,[1]* MAXINE M. DENNISTON,[1] ROBIN E. HUEBNER,[2] AND ROBERT C. GOOD[3]

*Division of Laboratory Systems, Public Health Practice Program Office,[1] Division of Tuberculosis Elimination, National Center for Prevention Services,[2] and Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases,[3] Centers for Disease Control and Prevention, Atlanta, Georgia 30333*

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**The resurgence of tuberculosis, which includes an increase in the isolation of multidrug-resistant strains of *Mycobacterium tuberculosis*, emphasizes the need for more rapid laboratory testing for identification of the etiological agent of the disease. In December 1991, state and territorial public health laboratories were surveyed to determine the methods that they were using for testing and reporting of *M. tuberculosis*. A follow-up survey was conducted in June 1994 to measure changes in the testing and reporting practices that had occurred as a result of efforts focused on the disease and on laboratory improvement. Completed questionnaires were received from 51 of 55 laboratories. Comparative data indicate that the proportion of laboratories reporting testing results within the number of days recommended by the Centers for Disease Control and Prevention has increased. Starting from the time at which the laboratory receives the specimen, the proportion of laboratories reporting the results of microscopic smear examination within the recommended 24 h has increased from 52.1 to 77.6%; the proportion reporting isolation and identification within 21 days has increased from 22.1 to 72.9%; and the proportion reporting results of isolation, identification, and drug susceptibility testing within 28 days has increased from 16.7 to 48.9%. Use of the recommended rapid testing methods has also increased: the proportion of laboratories using fluorescence staining for acid-fast microscopy has increased from 71.4 to 85.7%, the proportion using BACTEC for primary culture has increased from 27.1 to 79.6%, the proportion using rapid methods for *M. tuberculosis* identification has increased from 74.5 to 100.0%, and the proportion using BACTEC for primary drug susceptibility testing has increased from 26.2 to 73.3%. By implementing the recommended methods for *M. tuberculosis* testing and reporting, state and territorial public health laboratories are now able to transmit results to physicians more rapidly.**

From the time at which national reporting of tuberculosis began in 1953 until 1984, the United States experienced a steady decline in the incidence of cases (2). During these years of declining incidence, fewer resources were channeled into studies and control of tuberculosis (4a) and consequently into public health mycobacteriology laboratories. The need for diagnostic efficiency was not recognized as a major public health issue. In 1985, however, a steady increase in the reported incidence of tuberculosis began (2). Early in the 1990s, outbreaks of multidrug-resistant tuberculosis that were characterized by high fatality rates (up to 89%) occurred (7). Public health laboratories using conventional testing methods and traditional reporting practices were often incapable of providing results of diagnostic testing in time for effective patient management.

At the 1992 conference Meeting the Challenge of Multidrug-Resistant Tuberculosis, held at the Centers for Disease Control and Prevention (CDC), the critical role of the laboratory in controlling multidrug-resistant tuberculosis was described. The laboratory issues work group emphasized the crucial need for more rapid turnaround times (TATs) for *Mycobacterium tuberculosis* testing and reporting. It recommended that clinical specimens reach the laboratory within 24 h of collection, that physicians receive acid-fast microscopy smear results within 24 h of the laboratory's receipt of the specimen, that positive cultures be detected by the laboratory within 14 days of specimen receipt, that the laboratory identify *M. tuberculosis* isolates within 17 to 21 days of specimen receipt, and that drug susceptibility test results be received by the physician within 28 days of the laboratory's receipt of the specimen (5). The use of rapid testing and reporting methods was recognized as essential for laboratories to achieve these goals. The recommended testing methods were fluorochrome staining for acid-fast bacillus (AFB) microscopy; radiometric culture or an equivalent rapid method for culture; a rapid method (i.e., high-performance liquid chromatography [HPLC], nucleic acid probes, or BACTEC/NAP) for identification of *M. tuberculosis* isolates; and the radiometric system using a panel of the five primary drugs—isoniazid, rifampin, pyrazinamide, ethambutol, and streptomycin—for drug susceptibility testing isolates of *M. tuberculosis* (5). On the basis of the discussions at this conference, a TB Task Force convened by the director of the CDC developed and published the "National Action Plan to Combat Multidrug-Resistant Tuberculosis" (2). A goal of the action plan is to make the laboratory diagnosis of tuberculosis more rapid, sensitive, and reliable.

A 1991 survey of state and territorial public health laboratories showed that laboratories using the more rapid testing methods had an average TAT of 22 days for isolation, identification, drug susceptibility testing, and reporting of *M. tuberculosis*. In contrast, laboratories using conventional methods (primarily solid media) had an average TAT of 40 days (6).

Intensive efforts to upgrade the capability of state, territo-

* Corresponding author. Mailing address: Centers for Disease Control and Prevention, Public Health Practice Program Office, Division of Laboratory Systems, Laboratory Practice Training Branch, 4770 Buford Hwy., NE, Mailstop A-16, Atlanta, GA 30341. Phone: (770) 488-4071. Fax: (770) 488-7677. Electronic mail address: brb2@phpdls1.em.cdc.gov.

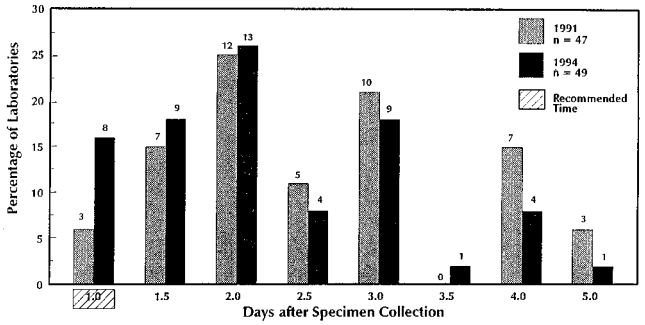


FIG. 1. Amount of elapsed time from specimen collection to receipt in the laboratory. Actual numbers of laboratories are shown above the bars.

rial, and local public health mycobacteriology laboratories by providing additional funding and focused training were initiated by the CDC. The present study was conducted to determine if the program for upgrading mycobacteriology laboratories was successful. Reported here are the results of a follow-up survey examining testing and reporting practices of state and territorial public health mycobacteriology laboratories.

## MATERIALS AND METHODS

In June 1994, as a follow-up to the 1991 survey, a study was designed to assess changes that had occurred in the *M. tuberculosis* testing and reporting practices of the state and territorial public health mycobacteriology laboratories. The follow-up survey used the same questionnaire that was used initially (6). Additional information concerning specific changes that had been made since January 1992 was requested. A total of 55 surveys were mailed to 51 state mycobacteriology laboratories and to the laboratories in Puerto Rico, Guam, the Virgin Islands, and the District of Columbia.

Both the initial and follow-up surveys were conducted by the Association of State and Territorial Public Health Laboratory Directors (ASTPHLD). Completed questionnaires were returned to the ASTPHLD, and the data were subsequently analyzed by the CDC. For the follow-up study, information from both the initial and the follow-up questionnaires was analyzed by using SAS (9). Only results from laboratories that responded to both surveys are reported here.

## RESULTS

Completed questionnaires were received from 51 of the 55 state and territorial laboratories included in the follow-up survey (a 92.7% response rate). Of the 51 laboratories that responded to both the initial survey and the follow-up survey, 1 was not doing any mycobacterial testing at the time of either survey; 2 other laboratories were doing testing at the time of only one of the surveys. Forty-nine of the 51 laboratories were performing AFB microscopy at the time of both surveys. Forty-eight laboratories were performing primary culture for *M. tuberculosis* in 1991, compared with 49 in 1994, and 47 laboratories were identifying *M. tuberculosis* isolates in 1991, compared with 48 in 1994.

The number of laboratories receiving specimens within the recommended 24 h after collection increased from three (6.4%) in 1991 to eight (16.3%) in 1994 (Fig. 1). Nineteen laboratories (38.7%), however, reported in 1994 receiving specimens more than 2 days after collection. The number of laboratories generating reports of microscopic examination within the recommended 24 h of specimen receipt increased from 25 (52.1%) to 38 (77.6%). By June 1994, all laboratories were generating AFB microscopy reports within 2 days except for one laboratory which reported commonly taking 8 days.

The number of laboratories generating reports of *M. tuberculosis* isolation and identification within 21 days of receiving the clinical specimens increased from 10 (22.1%) to 35 (72.9%). Thirteen laboratories (27.0%) took ≥22 days to generate reports. Two of these 13 laboratories reported taking in excess of 42 days.

The number of laboratories generating reports of isolation, identification, and drug susceptibility testing of *M. tuberculosis* within 28 days after receiving clinical specimens increased from 7 (16.7%) to 22 (48.9%). Of the 23 laboratories not reporting within 28 days, 9 (20%) required 29 to 35 days, 6 (13.3%) required 36 to 42 days, and 8 (17.7%) required 43 days or longer.

The methods used by the laboratories for detecting and identifying *M. tuberculosis* are shown in Table 1. An increase in the proportion of laboratories using the recommended methods occurred in each testing category. By June 1994, no laboratory was relying solely on conventional biochemical testing for identification of *M. tuberculosis* isolates.

Forty-three of the 51 laboratories were performing drug susceptibility testing on *M. tuberculosis* isolates in December 1991 compared with 45 in June 1994. Table 2 shows the practices that the laboratories used for drug susceptibility testing. In both surveys, no laboratory reported using only the direct testing method. A small increase was seen in the proportion of laboratories using the recommended practice of testing all *M. tuberculosis* isolates for drug susceptibilities.

The proportion of those laboratories performing identification tests for *M. tuberculosis* using the telephone to report a positive test result to hospitals, clinics, or physicians increased from 38.3 to 75.0%. A much smaller increase, however, from 29.7 to 37.5%, was seen in the proportion of laboratories using the telephone to make the same report to the state tuberculosis

TABLE 1. Methods used by laboratories for detecting and identifying *M. tuberculosis*

| Method(s) | % of laboratories using method(s) | |
|---|---|---|
| | 1991[a] | 1994[a] |
| AFB microscopy | | |
| Fluorochrome staining[b] | 68.6 | 82.4 |
| Ziehl-Neelsen | 21.6 | 9.8 |
| Kinyoun | 5.9 | 3.9 |
| Don't perform | 3.9 | 3.9 |
| Primary culture | | |
| Solid medium only | 68.6 | 17.7 |
| Radiometric medium only | 2.0 | 0.0 |
| Solid and radiometric media[b] | 23.5 | 76.5 |
| Other | 0.0 | 2.0 |
| Don't perform | 5.9 | 3.9 |
| Species identification | | |
| Biochemical tests only | 23.5 | 0.0 |
| Nucleic acid probes only[b] | 17.7 | 43.1 |
| BACTEC/NAP only[b] | 0.0 | 0.0 |
| HPLC only[b] | 2.0 | 2.0 |
| More than one of the above[b] | 49.0[c] | 49.0[d] |
| Don't perform | 7.8 | 5.9 |

[a] $n = 51$.

[b] Recommended method(s).

[c] Fifteen laboratories were using biochemicals and probes; three were using biochemicals, probes, and HPLC; three were using biochemicals and HPLC; two were using biochemicals, BACTEC/NAP, and probes; and two were using biochemicals, BACTEC/NAP, and HPLC.

[d] Twelve laboratories were using biochemicals and probes; three were using probes and HPLC; seven were using biochemicals, probes, and HPLC; one was using biochemicals and HPLC; one was using biochemicals, BACTEC/NAP, and probes; and one was using biochemicals and BACTEC/NAP.

TABLE 2. Methods used for *M. tuberculosis* drug susceptibility testing

| Test method(s) | % of laboratories using method | |
|---|---|---|
| | 1991[a] | 1994[b] |
| Type of test[c] | | |
| Direct test only | 0.0 | 0.0 |
| Indirect test only | 41.9 | 51.1 |
| Both methods | 58.1 | 48.9 |
| Medium | | |
| Solid only | 72.1 | 26.7 |
| Radiometric only | 9.3 | 40.0 |
| Solid and radiometric | 16.3 | 33.3 |
| Not reported | 2.3 | 0.0 |
| Isolates tested | | |
| All isolates | 39.5 | 45.5 |
| As requested | 14.0 | 4.6 |
| Other[d] | 46.5 | 50.0 |
| Not reported | 0.0 | 2.2 |

[a] $n = 43$.
[b] $n = 45$.
[c] Direct testing is done with samples of specimen concentrates; indirect testing is done with samples of pure cultures of the organisms.
[d] Testing done by a predetermined schedule, such as one test per patient every 3 months.

control program. An increase was also seen in the use of other methods, such as electronic or fax transmission, for reporting to the state tuberculosis control program.

Figure 2 summarizes the increases in the proportions of laboratories that were using the methods recommended by the CDC for *M. tuberculosis* testing and reporting. Laboratories not responding to the question or not performing the type of testing or reporting described in the question are not included in the denominators.

Tables 3 and 4 compare the 22 laboratories that by June 1994 were generating reports of *M. tuberculosis* isolation, identification, and drug susceptibility testing within 28 days with the 23 laboratories that were taking longer than 28 days to make the same reports. Only the 45 laboratories that were perform-

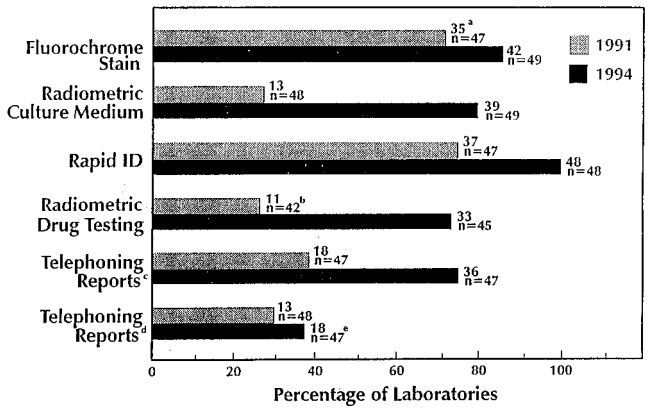


FIG. 2. Summary of proportions of laboratories using rapid methods. a, actual number of laboratories; b, one laboratory did not report the medium used; c, telephoning of reports to clinics, hospitals, and physicians; d, telephoning of reports to tuberculosis control programs; e, one laboratory did not give its method for reporting to hospitals, etc.

TABLE 3. Comparison of TATs for those laboratories that generate reports of *M. tuberculosis* isolation, identification, and drug susceptibility testing within the recommended 28 days and those laboratories that take longer than 28 days[a]

| Laboratory TAT (days) | No. of laboratories processing the following no. of specimens/mo | | | | % of laboratories | | | |
|---|---|---|---|---|---|---|---|---|
| | <100 | 101–500 | 501–1,000 | >1,000 | Receiving specimens within 1 day of collection | Receiving specimens within 2 days of collection | Reporting AFB microscopy results within 1 day of receiving specimen | Reporting *M. tuberculosis* identification within 21 days of receiving specimen | Reporting *M. tuberculosis* identification and drug testing results within 28 days of receiving specimen |
| ≤28 ($n = 22$)[b] | 2 | 14 | 3 | 2 | 4.5 | 59.1 | 86.4 | 100.0 | 100.0 |
| >28 ($n = 23$) | 2 | 8 | 5 | 8 | 26.1 | 65.2 | 78.3 | 52.2 | 0.0 |

[a] Only laboratories that perform AFB microscopy, isolation, identification, and drug susceptibility testing are included in this comparison.
[b] Information concerning the number of specimens is not available for one laboratory in this group.

TABLE 4. Comparison of testing methods and reporting practices of those laboratories that generate reports of *M. tuberculosis* isolation, identification, and drug susceptibility testing within the recommended 28 days and those laboratories that take longer than 28 days[a]

| Laboratory practice | % of laboratories with the following TAT by practice: | |
|---|---|---|
| | ≤28 days[b] | >28 days[c] |
| Use of fluorochrome staining for AFB microscopy | 86.4 | 87.0 |
| Use of BACTEC and solid medium for primary culture | 95.5 | 69.6 |
| Use of rapid identification methods | 100.0 | 100.0 |
| Use of BACTEC for primary drug testing | 90.9 | 56.5 |
| Telephoning of results to hospitals, clinics, and physicians | 81.8 | 68.1 |

[a] Only laboratories that perform AFB microscopy, isolation, identification, and drug susceptibility testing are included in this comparison.
[b] $n = 22$.
[c] $n = 23$.

ing AFB microscopy, isolation, identification, and drug susceptibility testing were included in the comparison.

The 47 laboratories that provided information concerning specimen volume were grouped by the number of clinical specimens and referred isolates combined that were processed per month for mycobacteria from 1 January to 30 September 1991 (Table 5). Regardless of specimen volume, the extent of testing was the same for both surveys except that two more laboratories had initiated drug susceptibility testing by 1994. In 1994, 50% (2 of 4) of the laboratories processing <100 specimens per month, 63.6% (14 of 22) of the laboratories processing 101 to 500 specimens per month, 37.5% (3 of 8) of the laboratories processing 501 to 1,000 specimens per month, and 20% (2 of 10) of the laboratories processing >1,000 specimens per month were able to generate reports of AFB microscopy, isolation, identification, and drug susceptibility testing within the recommended 28 days.

## DISCUSSION

From December 1991 to June 1994, increases occurred in the number of state and territorial laboratories that reported receiving specimens for mycobacterial testing and generating reports of *M. tuberculosis* testing results within the recommended times. Concurrently, more laboratories were using the rapid testing methods required for achieving the recommended TATs (Fig. 2).

As encouraging as these data are, only 72.9% of the laboratories had a TAT of 21 days for reporting *M. tuberculosis* isolation and identification. Apparently, more than the mere implementation of rapid methods for isolating and identifying *M. tuberculosis* is necessary to attain the recommended TAT of ≤21 days. Other factors that may be involved include the "batching" of specimens for processing and testing, the number of days per week on which testing services are provided, and the quality of the specimen.

Batching is the holding of specimens or testing samples for several days until a group of samples is obtained or a specified day of the week is reached before proceeding with the testing process. Batching can cause increases of days or weeks in the turnaround time. The fewer the days per week on which testing services or laboratory coverage is provided, the greater the necessity for batching. For example, if laboratory coverage for reading of drug susceptibility tests is not provided on weekends, all drug susceptibility testing performed in the recommended radiometric system can only be initiated on Friday, producing a delay of 4 days for isolates identified as *M. tuberculosis* on Monday. Thus, ideally, laboratories should provide complete coverage 5 days per week and modified coverage on the weekends.

Also an element in the total TAT is the time following the completion of a laboratory test before the results are received by the physician. The number of laboratories using only written reports has decreased significantly. As might be expected, a large increase has occurred in the proportion of laboratories using the telephone for transmitting results to hospitals, clinics, and physicians. A much smaller increase in the use of the telephone, however, was seen for reporting to state tuberculosis control programs.

Although written reports remain the ultimate method for reporting results to state tuberculosis control programs, the use of electronic equipment such as the computer and the fax machine is becoming more common for rapid notification.

Great strides have been made in upgrading the capabilities of state and territorial laboratories for *M. tuberculosis* testing and reporting; however, only 22 of these laboratories have been able to achieve the recommended 28-day TAT for isolating, identifying, drug susceptibility testing, and reporting of results. The most conspicuous difference between the two groups of laboratories is in their use of the BACTEC radiometric system for culturing and drug susceptibility testing. Twenty-six percent more laboratories in the group with TATs of ≤28 days than in the group taking longer than 28 days used BACTEC for culturing, and 34% more used BACTEC for drug susceptibility testing. Use of the BACTEC system appears to be a major factor in reducing a laboratory's TAT.

Over the 2½ years between the two surveys, the smallest amount of improvement was in the time between the collection of a clinical specimen and the arrival of that specimen in the testing laboratory (Fig. 1). In a hospital setting, keeping the elapsed time from collection of the specimen to its arrival in the hospital's laboratory within the recommended 24 h is rarely an issue. For state laboratories, however, specimens must often be transported from a remote site. In spite of some improvement in the time for specimens to arrive, 32 (65%) of the laboratories reported in 1994 an average of 2 days or more for receiving specimens. Delays in specimen receipt by the laboratory cause an increase in the total time before the physician can receive the testing results.

TABLE 5. Extent of *M. tuberculosis* testing in state and territorial laboratories in 1994

| No. of specimens per month processed for mycobacteria | No. of laboratories performing the following type of testing | | | |
|---|---|---|---|---|
| | AFB microscopy | Primary culture | Identification of *M. tuberculosis* | Drug susceptibility testing |
| ≤100[a] | 6 | 6 | 6 | 4 |
| 101–500[b] | 23 | 23 | 22 | 22[c] |
| 501–1,000[d] | 8 | 8 | 8 | 8 |
| >1,000[e] | 10 | 10 | 10 | 10 |

[a] $n = 6$.
[b] $n = 23$.
[c] Two more laboratories were performing drug susceptibility testing in 1994 than in 1991.
[d] $n = 8$.
[e] $n = 10$.

Receipt of specimens within 24 h of collection not only minimizes delays in initiating laboratory testing but also avoids possible adverse effects—decreases in the number of viable organisms and growth of contaminants—on specimen quality that might be caused by a longer transport time. Decreased specimen quality can cause an unnecessary increase in the amount of time that it will take the laboratory to recover and identify the infecting organism.

Clearly, for state and territorial laboratories to achieve the recommended time of 24 h, additional efforts will be required. Laboratories need to communicate to their clients the importance of forwarding specimens in a more timely manner. Achieving the most efficient mechanism possible for transport and delivery of specimens should be a cooperative effort between specimen submitters and the testing laboratory. Once specimens have arrived at the facility, the laboratory needs to ensure that they are rapidly routed to the mycobacteriology laboratory.

An official statement adopted in June 1982 by the American Thoracic Society asserted that to maintain proficiency in reading smears, laboratories doing AFB microscopy should examine a minimum of 10 to 15 specimens per week. It was also asserted that to maintain proficiency in culture and identification of *M. tuberculosis*, laboratories should perform digestion and culture on a minimum of 20 specimens per week (1). The CDC manual "Public Health Mycobacteriology—a Guide for the Level III Laboratory" states that "proficiency and cost-effectiveness may best be maintained. . .where at least 10 drug susceptibility test patterns are done each week" (8). In spite of these statements, the study data show that laboratories processing a few mycobacterial specimens (less than 100 per month) have not decreased the extent of their *M. tuberculosis* testing. Instead, these small-volume laboratories have instituted the recommended rapid testing methods.

A higher percentage of laboratories that process ≤500 specimens per month generate reports of isolation, identification, and drug susceptibility testing within the recommended 28 days compared with those that process >500 specimens per month. This fact suggests the importance of other factors in addition to the use of rapid testing and reporting methods for achieving recommended TATs. Although not measured in this study, factors that can affect a laboratory's total TAT include the ratio of personnel performing testing to the number of specimens processed, the amount and breakdown of allocated laboratory space, the number of replicates of essential equipment (e.g., the BACTEC instrument), and the experience and training of the laboratory staff.

The major concern with small-volume laboratory testing, however, is one not of TAT but of proficiency. Of the laboratories reporting the number of *M. tuberculosis* isolates that they tested for drug susceptibility over the 9 months covered by the questionnaire, 36 (81%; $n = 44$) were testing an average of less than 10 isolates per week. The 1982 American Thoracic Society recommendations concerning the minimum number of specimens for culturing and identification of isolates were based on conventional testing methods using solid media and biochemical reactions. At this time, proficiency testing data available for the new testing systems using liquid media for culture and molecular methods for identification are insufficient to justify modifying the 1982 recommendations. A study correlating proficiency testing data using the newer testing methods with the number of specimens tested is needed.

Clearly, achieving the optimal balance of all factors, from the number of specimens processed to methods of reporting, so that TATs and testing proficiency are maximized requires delicate integration of multiple elements.

Some limitations related to estimates of TATs apply to the data. First, it is difficult to assess the accuracy of the estimated TATs. From the responses, it was evident that some laboratories had performed careful calculations to arrive at their estimates. For other laboratories, it was not possible to discern the amount of effort expended in providing estimated times. Second, a few respondents provided estimates in terms of both calendar days and working days. Since most respondents did not indicate whether their estimates were in calendar or working days, it is not possible to determine how often only working days were reported instead of calendar days. Third, for 22.5% of the laboratories, the person who completed the follow-up survey was not the same individual who responded to the initial questionnaire; thus, for a given laboratory, inconsistencies between the manners in which TATs were estimated in the two surveys could exist.

The laboratory plays a significant role in the control of tuberculosis. Rapid reporting of *M. tuberculosis* isolates and results of drug susceptibility testing are essential to effective patient management and to minimizing unwarranted exposure of the population to infected persons. The present study shows that from December 1991 to June 1994, state and territorial public health laboratories increased their use of rapid mycobacterial testing methods and reduced the time needed to generate reports of isolation, identification, and drug susceptibility testing of *M. tuberculosis*. After annual increases in the number of reported cases of tuberculosis from 1985 to 1992, a 5.1% decline in incidence was reported in 1993 (25,313 cases in 1993 compared with 26,673 cases in 1992) (3). By 1994, the number of cases reported annually had decreased 8.7% (4). We believe that changes in testing practices by state and territorial public health laboratories, as reported in this paper, have contributed to the decreased incidence of tuberculosis in the United States. The answer to the question posed by Tenover et al. in their article "The Resurgence of Tuberculosis: Is Your Laboratory Ready?" (10) is yes, many of the state and territorial public health laboratories are now ready.

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