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Item Type	Journal Article
Authors	Doern, Gary V.
Citation	J Clin Microbiol. 1996 Aug;34(8):1873-6.
Download date	2025-02-08 13:20:45
Link to Item	https://hdl.handle.net/20.500.14038/38133

GUEST COMMENTARY

Diagnostic Mycobacteriology: Where Are We Today?

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INTRODUCTION

Tuberculosis has reemerged as a significant public health problem in certain parts of the United States. In addition, strains of *Mycobacterium tuberculosis* with various patterns of resistance to antituberculosis chemotherapeutic agents have been recovered with increasing frequency (17, 18). The continuation of the human immunodeficiency virus (HIV) epidemic has contributed to both of these problems. In an attempt to address the concurrent problems of the increasing prevalence of tuberculosis and more frequent recognition of multi-drug-resistant strains of *M. tuberculosis*, the Centers for Disease Control and Prevention (CDC) in Atlanta, Ga., has recently developed and now promulgates recommendations for controlling the transmission of tuberculosis (2, 3).

Obviously, an essential component of any tuberculosis control program is rapid and accurate identification of infected individuals, especially those most likely to transmit viable organisms. In this regard, the CDC has also developed recommendations for standards of laboratory practice regarding detection, identification, and susceptibility testing of *M. tuberculosis* in clinical specimens (17). The three most fundamental aspects of these recommendations are provision of acid-fast bacillus smear results within 24 h of specimen collection, isolation and identification of *M. tuberculosis* within 10 to 14 days, and provision of susceptibility test results within a total of 15 to 30 days. All three of these objectives significantly impact on the function of mycobacteriology sections of clinical microbiology laboratories. Hours of operation, staffing patterns, technology decisions, and the cost of providing service are all profoundly influenced by these proposed standards of laboratory practice.

In particular, the second recommendation of the CDC, isolation and identification of *M. tuberculosis* within 10 to 14 days, would have profound ramifications on the function of clinical microbiology laboratories. A question arises: can this standard be reasonably achieved using current "state-of-the-art" diagnostic methods? An important corollary to this question pertains to the recent licensure of a commercial molecular diagnostic test that will make it possible for even the smallest laboratory to detect and identify *M. tuberculosis* directly in clinical specimens in a matter of hours. What will be the role of direct molecular diagnostic tests in mycobacteriology? What are the performance characteristics of molecular detection assays? Use of such technologies would clearly permit laboratories to meet the standard of 10 to 14 days for the isolation and definitive species identification of *M. tuberculosis*. Will molecular-probe based methods become broadly applied in clinical microbiology laboratories? Should they be used routinely or selectively? What are the fiscal implications of direct molecular

detection and identification? These issues serve as the basis for this guest commentary.

Question: Are current CDC recommendations defining 10- to 14-day limits on the recovery and definitive species identification of *M. tuberculosis* achievable by methods other than direct nucleic acid amplification methods?

Answer: Probably not, at least in most laboratories.

Currently, the fastest and most reliable approach to detecting and identifying *M. tuberculosis* in clinical specimens other than use of a direct molecular detection test is use of a broth-based radiometric or continuous monitoring detection system with identification of clinical isolates recovered in culture using a nucleic acid probe-based culture confirmation identification system (8). Seen in Table 1 are the lengths of times to recovery and identification of *M. tuberculosis* obtained in 10 U.S. laboratories during 1993. All 10 laboratories employed a radiometric detection system and nucleic acid probes for identification of isolates. Only 2 of these 10 laboratories, laboratories E and G, met the CDC standard of 10 to 14 days for isolation and identification of *M. tuberculosis*. The wide ranges observed for mean lengths of time to detection and species identification among the 10 laboratories described in Table 1 were explained by differing staffing patterns, variable scheduling of mycobacteria specimen processing and workup of positive cultures, and whether probe-based identification was applied directly to growth in primary cultures or used only to characterize organisms following at least one subculture. Both of the laboratories that met the CDC's recommended standard processed mycobacteriology specimens 7 days per week and aggressively attempted probe-based identification whenever possible directly on growth from primary cultures. In addition, laboratory E worked up positive cultures with probe identification tests 7 days per week.

Question: Would application of nucleic acid amplification assays for detection and identification of *M. tuberculosis* directly in clinical specimens permit laboratories to meet the CDC's recommended standard?

Answer: Possibly, at least for certain specimens.

One nucleic acid amplification assay for the detection and identification of *M. tuberculosis* directly in clinical material, the MTD test (Gen-Probe, Inc., San Diego, Calif.), has recently received Food and Drug Administration (FDA) approval and is now available commercially. The MTD assay is a 4-h procedure based on transcription-mediated amplification of a 16S rRNA target sequence. Results are read with a luminometer. A second amplification method, the Amplicor MTB procedure (Roche Diagnostics Systems, Somerville, N.J.), is currently awaiting FDA approval. The Amplicor MTB assay is predicted on PCR amplification of a 584-bp segment of 16S rRNA; it utilizes a thermocycler and requires 6 h to complete.

In addition to the MTD test and the Amplicor MTB assay, three other direct nucleic acid amplification methods for detecting *M. tuberculosis* are in various stages of development.

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TABLE 1. Recovery of *Mycobacterium* spp. in 10 U.S. laboratories during 1993

Medical center	No. of specimens	Isolation of <i>M. tuberculosis</i>			No. of isolates of:	
		No. of isolates	Mean no. (range) of days to:		MAC	Other mycobacteria
			Detection	Identification ^a		
A	1,880	15	14 (6-24)		57	31
B	3,384	19	21 (8-39)	40 (9-76)	83	67
C	7,560	49	15 (3-43)	27 (7-72)	160	80
D	2,640	10	16 (7-36)	28	153	15
E	5,612	40	11 (3-22)	13	57	10
F	3,678	34	18 (4-37)	22	206	28
G	4,345	28	11 (4-25)	12 (5-25)	156	47
H	1,177	15	11 (3-27)	16	40	14
I	5,042	40	17 (4-26)	22	162	119
J	4,462	102	12 (1-50)	20 (4-57)	253	98

^a In all cases, the length of time to identification listed in this table is predicated on when the specimen was received. In laboratories D, E, F, H, and I, the length of time to identification was provided to the author as the period following detection. Therefore, no determination of ranges was possible.

These include an assay predicted on ligase chain reaction amplification of target nucleic acid segments (Abbott Diagnostics), a second method based on PCR (Johnson and Johnson Clinical Diagnostics), and finally, a technique which employs strand displacement amplification technology (Becton-Dickinson Microbiology Systems).

Both the MTD test and the Amplicor MTB assay have been extensively evaluated in clinical laboratories, and the results of such evaluations have been described in the literature. The overall sensitivity of the MTD test for detecting *M. tuberculosis* appears to be 88 to 90%, with a specificity of ca. 98% (1, 10, 14, 15, 20). The Amplicor MTB assay appears to have slightly lower levels of sensitivity (i.e., 75 to 80%) and a slightly higher level of specificity (i.e., ca. 99%) (3, 20). Perhaps not surprisingly, both assays demonstrate much higher levels of sensitivity when applied to smear-positive specimens rather than to smear-negative specimens: MTD test, 96 to 97% versus 75%; Amplicor MTB assay, 95% versus 60%. This is undoubtedly due to the fact that smear-positive specimens usually contain larger numbers of organisms than do smear-negative specimens.

With certain caveats, use of direct amplification assays for *M. tuberculosis* would easily permit laboratories to meet the CDC's recommended standard for turnaround time for isolating and identifying *M. tuberculosis*, at least in specimens that yield a positive probe result. Results are available the same day the test is performed. The point can be made that direct nucleic acid amplification assays will occasionally fail to detect positive specimens in view of their lack of absolute sensitivity. This, however, is not a valid reason to avoid use of these assays, since the same statement can be made for culture. Indeed, culture, which has generally been considered the definitive test for detecting *M. tuberculosis*, is now recognized as having lower levels of sensitivity than the MTD test and perhaps the Amplicor MTB assay. It is the very existence of direct molecular assays that has permitted studies that demonstrate the lack of absolute sensitivity of culture to be performed. At least part of the enhanced sensitivity of amplification assays versus culture for detecting *M. tuberculosis* may be explained by the ability of such methods to detect nonviable organisms that would be missed by culture. It should be noted, however, that positive results obtained with the MTD test, the first direct test to receive FDA approval, should be considered presumptive according to the dictates of the FDA. An ancillary culture must be performed in an attempt to recover an organism for definitive identification.

Question: How should direct nucleic acid amplification assays be applied in the clinical microbiology laboratory?

Answer: This is a very complicated question with no obvious single answer. However, in many settings the answer may be "Not at all."

The following discussion is predicated on the MTD test, since it was the first to become commercially available. Fifty tests are packaged in a single kit with this system. One MTD kit costs \$1,500 (i.e., \$30 per test) when purchased in volumes of less than one kit per month. The cost is reduced to \$1,387 per kit when volumes of ≥ 1 kit per month are purchased (i.e., \$27.74 per test). With the 1993 experience of the 10 laboratories described in Table 1 as an indicator, a mean of 3,978 specimens were processed annually in individual laboratories to yield a mean of 35 isolates of *M. tuberculosis*. On the basis of the assumption that an individual laboratory would perform the probe assay 5 days per week, i.e., 260 days per year, on average 15 specimens would be processed during a given day. This specimen volume would actually necessitate utilization of 19 tests, since ancillary tests (i.e., a positive and a negative control and two internal standards) must be performed when any number of clinical specimens are analyzed by the MTD test. On the basis of this volume, the laboratory would qualify for the volume discount, yielding pricing of \$27.74 per test. The actual cost per specimen would be slightly higher (i.e., \$35.14), however, because of the requisite controls and standards.

Over the course of an entire year, the laboratory would spend more than \$137,000 in supply costs alone for direct detection of *M. tuberculosis*. Furthermore, among the 35 specimens that would be expected to be positive during the year, 3 or 4 would be missed by the MTD test because of its 88 to 90% sensitivity, resulting in 31 or 32 positive specimens by probe. Typically, multiple positive specimens are received for individual patients. On the basis of a general estimate of 2 to 4 positive specimens per patient, the 31 or 32 positive probe specimens would really represent only about 10 patients. In other words, the cost associated with directly identifying an individual patient as positive would be approximately \$13,700, and this figure pertains only to supply costs. If these costs were amortized over the entire U.S. mycobacteriology testing experience, the financial burden on an already tenuous health care economy would be staggering. Clearly, because of the way the MTD test has been priced, in the context of its cost implications for individual laboratories as well as its fiscal impact globally, it cannot be employed as a routine screen, irrespective of how desirable its use might be. Simply put, it is unaffordable.

What about selective use of the MTD test? One approach that is intuitively appealing is performance of the probe assay only with smear-positive specimens. Indeed, the MTD test has received FDA approval only for this application. Unfortunately, because of the packaging employed in the MTD test, this usage may also be cost-prohibitive, at least in many laboratories. The 50 tests in the MTD kit are contained in a single reagent vial that has a shelf life of 4 weeks once opened. Again using estimates derived from the 1993 experience of the 10 laboratories outlined in Table 1, during the course of a year, an average of 219 positive mycobacteria specimens (35 *M. tuberculosis*, 133 *M. avium* complex (MAC), and 51 isolates of other mycobacteria) would be derived from a mean of 3,978 specimens processed. Using 60% as the aggregate detection sensitivity of acid-fast bacillus smears for all three mycobacteria groups (1, 5, 7, 10–13, 14–16, 20), roughly 131 of these 219 specimens would be expected to be smear positive. These 131 smear-positive specimens are likely to have come from approximately 44 patients, again on the basis of the notion that 2 to 4 positive specimens per patient are generally submitted. If these 44 patients were distributed evenly over the course of 1 year, on average ca. 3.4 unique patients with positive smears would be seen during a given 4-week period. Since each reagent vial from an MTD 50-test kit has a 4-week outdate, the entire vial cost of \$1,500 would be expended on those 3.4 patients: in other words, the cost per smear-positive patient would be \$441. The annualized cost of applying the MTD test only to smear-positive specimens from unique patients would be approximately \$19,500. This \$19,500 would presumably permit direct same-day identification of an average of about 10 patients with *M. tuberculosis* infections each year, i.e., \$1,950 per patient.

Obviously, this per-patient cost estimate is based on a laboratory experience in which a mean of 35 *M. tuberculosis* isolates are recovered annually along with 133 isolates of MAC and 51 isolates of other mycobacteria. These numbers represent averages derived from the collective experience of the 10 laboratories described in Table 1 in which the overall prevalence of *M. tuberculosis* among all positive specimens was ca. 16%. A second assumption is that the sensitivity of acid-fast bacillus smears for *M. tuberculosis*, i.e., ca. 60% (1, 5, 7, 10–13, 14–16, 20), is also applicable to mycobacteria other than *M. tuberculosis*. This may or may not be true. It is clear, however, that by changing the relative prevalence of *M. tuberculosis*, the cost-effectiveness of applying the MTD test to smear-positive specimens also changes. Five of the laboratories in Table 1 (laboratories B, D, F, G, and I) had relative isolation rates of *M. tuberculosis* of less than the mean of 16%, perhaps because of disproportionately large HIV-positive patient populations with attendant high relative rates of MAC recovery. Two laboratories (laboratories A and C) were very close to the 16% average *M. tuberculosis* recovery rate, while the remaining three laboratories (laboratories E, H, and J) had high *M. tuberculosis* relative isolation rates. Clearly, the higher the relative prevalence of *M. tuberculosis*, the more cost-effective the test becomes. To wit, the MTD test would be easiest to justify in laboratories E, H, and J. Similarly, increasing the absolute number of smear-positive patients with tuberculosis who are investigated also would enhance cost-effectiveness.

The cost of the MTD test applied only to smear-positive specimens could be dramatically decreased if the shelf life of the reagent vial from the 50 test kit could be lengthened. This vial contains a mixture of *Mycobacterium* enzymes and probe reagent. One possibility would be to freeze small aliquots of this mixture following reconstitution at -70°C in an attempt to lengthen the shelf life. Indeed, one anecdotal observation suggests that the MTD amplification reagent is good for at least 6

months when frozen and stored at -70°C (19). It must be cautioned, however, that FDA approval of the MTD test was predicated on handling of the amplification reagent precisely as described by the manufacturer, i.e., with no freezing.

The foregoing cost analyses has been restricted to costs incurred by the laboratory. Although such costs are extremely high, it is possible that they might be offset by other institutional savings that would be realized through use of a nucleic acid amplification assay for the direct detection of *M. tuberculosis*. In view of the high-level sensitivity of the MTD test when applied exclusively to smear-positive specimens and its resultant high negative predictive value, when the MTD test is found to be negative with a smear-positive specimen it is highly unlikely that the patient from whom the specimen was derived has tuberculosis. A negative result, therefore, could be construed as indicating that precautions aimed at diminishing the risk of transmission such as protective isolation would be unnecessary. This could result in significant institutional cost savings.

Two institutional cost analyses pertaining to direct nucleic acid amplification tests for *M. tuberculosis* have recently been presented in abstract form (4, 6). Both were predicated on a PCR-based assay for the direct detection of *M. tuberculosis*. Interestingly, one study concluded that institutional cost savings clearly offset the cost of using PCR for direct detection of smear-positive specimens (4), while the other study concluded that the cost of direct PCR assay was not justifiable (6). Clearly, more extensive studies are necessary before the overall institutional cost-effectiveness of direct nucleic acid amplification assays can be ascertained. Obviously, because of geographic and institutional differences in costs, such studies are best conducted in one's own institution. In the absence of such data, it is difficult to justify the cost of at least the MTD test for direct detection of *M. tuberculosis*.

CONCLUSIONS

The CDC has recently developed recommendations regarding standards of practice in U.S. mycobacteriology laboratories (15). One of these standards, the ability to isolate and definitively identify *M. tuberculosis* from human clinical specimens within 10 to 14 days, appears to be difficult to achieve in even large academic medical center laboratories using any existing technology other than direct molecular probe-based assays. One such assay, the MTD test, has recently become commercially available. Unfortunately, this test has been configured and priced in such a way as to preclude its use in most laboratories even for application only to smear-positive specimens pending the availability of institutional cost savings data. Furthermore, use of probe-based assays for direct detection of *M. tuberculosis* in clinical specimens would have to be accompanied by use of culture so as viable organisms would be available for performance of antimicrobial susceptibility studies, another CDC mandate.

These conclusions lead to two obvious assertions. First, the CDC might consider revising its recommended standard for length of time to isolation of *M. tuberculosis*. Second, the manufacturer of the MTD test is urged to reconsider its pricing of this product. There exist numerous factors that influence the pricing of a diagnostic reagent. In the case of the MTD test, clearly considerable development costs were incurred by the manufacturer. Also, the process whereby the materials which comprise this test are manufactured, packaged, and quality controlled is likely to be very expensive. In addition, at an enormous cost to the manufacturer, the MTD test languished in the FDA for more than 4 years prior to approval. For these

reasons, it may be that the current price per test of the MTD test, \$27 to \$30, is necessary and justifiable. The reality is, however, that this pricing will mitigate against extensive use of this exciting new technology. If the MTD assay cost \$5 per test, this entire discussion would be moot. Arguably every laboratory performing mycobacteriology would adopt this test for all specimens. One would think that nearly 100% of an entire market at \$5 per test would be considered more desirable than virtually no market at a cost of \$27 to \$30 per test.

Finally, it should be emphasized that this discussion was predicated on the MTD test because it is the first direct probe-based assay to receive FDA approval and become commercially available. The Amplicor MTB assay is currently pending FDA approval. When it is licensed for distribution, depending on its pricing, the foregoing analysis will be equally applicable.

ACKNOWLEDGMENTS

I am indebted to the following individuals for provisions of their 1993 mycobacteriology data: Melodie Beard, Rush-Presbyterian St. Lukes Medical Center, Chicago, Ill.; David Bruckner, UCLA Medical Center, Los Angeles, Calif.; Marie Coyle, Harborview Medical Center, Seattle, Wash.; Paola DeGirolami, Deaconess Hospital, Boston, Mass.; Franklin Koontz, University of Iowa Hospital and Clinics, Iowa City, Iowa; Patrick Murray, Barnes Hospital, St. Louis, Mo.; Michael Saubolle, Good Samaritan Hospital, Phoenix, Ariz.; David Sewell, Veterans Administration Hospital, Portland, Ore.; Benedict Wasilauskas, North Carolina Baptist Hospital, Winston-Salem, N.C.; and Judith Westerling, University of Massachusetts Medical Center, Worcester, Mass. In addition, the excellent secretarial assistance of Debbie McQuaid is acknowledged.

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