Confirmation of the Presence of *Mycobacterium tuberculosis* and Other Mycobacteria in Mycobacterial Growth Indicator Tubes (MGIT) by Multiplex Strand Displacement Amplification

F. ZUHRE BADAK,[1]† DEANNA L. KISKA,[1]‡ MICHAEL O'CONNELL,[2] COLLEEN M. NYCZ,[2]
CAMI HARTLEY,[1] SHARON SETTERQUIST,[1] AND ROY L. HOPFER[1,3]*

*Becton Dickinson Research Center, Research Triangle Park,[2] and Clinical Microbiology-Immunology Laboratories,
University of North Carolina Hospitals,[1] and Departments of Microbiology-Immunology and Pathology,
University of North Carolina School of Medicine,[3] Chapel Hill, North Carolina*

Multiplex strand displacement amplification (mSDA) is capable of amplifying three distinct DNA sequences simultaneously. These include sequences present in most genera of mycobacteria, a sequence specific for *Mycobacterium tuberculosis*, and an internal control. mSDA was used to detect the presence of these target sequences in 154 (72 positive, 76 negative, and 6 failed) clinical specimens cultured in the mycobacterial growth indicator tube (MGIT) system. A wide variety of specimen types were processed and cultured. Once these cultures were deemed positive by MGIT fluorescence or were deemed negative after 8 weeks of incubation, MGIT culture aliquots were processed for mSDA analyses. A chemiluminescent microwell assay was used to detect the amplified products. The procedure was relatively simple and took less than 6 h to complete. The sensitivity of mSDA for detecting acid-fast bacilli was 96.4% compared to that of MGIT culture. Sensitivity and specificity were 97.2 and 96.1%, respectively, when all clinical criteria were considered. mSDA was shown to be a rapid and effective method for confirming the presence of *M. tuberculosis* and other mycobacteria in positive MGIT cultures.

The rise in the incidence of tuberculosis seen in this country since 1985 coupled with outbreaks of multidrug-resistant isolates of *Mycobacterium tuberculosis* has generated considerable interest and research in molecular diagnostic techniques for the mycobacteriology laboratory. Ideally, an amplification system should be more sensitive than microscopic examination, should be at least as sensitive as culture methods, should accurately confirm the identity of acid-fast bacilli (AFB) present in positive specimens and cultures, and should provide the information in a timely manner. Many clinical laboratories (5, 7, 10, 13, 18) have used in-house assays to amplify specific nucleotide sequences for the detection of *M. tuberculosis* in clinical specimens. Commercially available amplification systems (1, 2, 14, 22) are being developed and evaluated for the detection and identification of AFB in clinical specimens and in culture. The application of these assays for culture identification is gaining popularity because of the observed sensitivity, specificity, and timeliness of testing (9). It should be possible to design a panel of primers to identify commonly isolated AFB to the species level, thereby eliminating time-consuming biochemical identification. Most laboratories will continue to culture AFB regardless of the sensitivity of the amplification assay in order to determine the drug susceptibility of the causative organism.

Strand displacement amplification (SDA) has been used to amplify the *M. tuberculosis*-specific IS*6110* element from purified DNA. Recently (6), SDA was used to detect *M. tuberculosis* in clinical specimens by using this same *M. tuberculosis*

marker. This study was designed to determine if multiplex SDA (mSDA) could be used to accurately identify AFB present in the mycobacterial growth indicator tube (MGIT) system as soon as the MGIT becomes positive (fluorescent). mSDA is a DNA amplification technology in which three target sequences are amplified simultaneously. The three target gene sequences amplified in this study were 16S ribosomal DNA (rDNA), IS*6110*, and an internal control (IC). The 16S rDNA mycobacterial genus target (MGEN) is present in most clinically significant mycobacteria, including *M. tuberculosis* complex, *M. avium*, *M. intracellulare*, *M. paratuberculosis*, *M. abscessus*, *M. fortuitum*, *M. chelonae*, *M. gordonae*, *M. kansasii*, *M. flavescens*, *M. gastri*, *M. scrofulaceum*, *M. ulcerans*, *M. marinum*, *M. leprae*, *M. terrae*, *M. malmoense*, *M. szulgai*, and *M. asiaticum*. The MGEN target is not present in *M. genevense*, *M. celatum*, and *M. simiae*. Amplification of the insertion element IS*6110* indicates the presence of mycobacteria of the *M. tuberculosis* complex (21). The IC is used as an amplification control to indicate the presence of inhibitors in the prepared specimen which may yield a false-negative result. The four possible outcomes obtained from mSDA are (i) the specimen is negative (MGEN negative, *M. tuberculosis* negative, and IC positive); (ii) the specimen contains a mycobacterium species other than *M. tuberculosis* (MGEN positive, *M. tuberculosis* negative, and IC positive); (iii) the specimen contains *M. tuberculosis* (*M. tuberculosis* and IC positive); and (iv) the reaction failed (IC negative), indicating that inhibitors are present and the presence or absence of AFB cannot be determined.

(This study has been previously presented in part [12].)

* Corresponding author. Mailing address: UNC Hospitals, Clinical Microbiology, CB 7600, Chapel Hill, NC 27514. Phone: (919) 966-7894. Fax: (919) 966-0486.
† Present address: Ege University, Izmir, Turkey.
‡ Present address: State University of New York, Health Science Center, Syracuse, N.Y.

MATERIALS AND METHODS

Specimens. All clinical specimens, except blood, submitted for AFB culture were included in this study. Standard *N*-acetyl-L-cysteine sodium hydroxide digestion, decontamination, and concentration methods (16) were used on nonsterile specimens. All stool specimens and respiratory specimens from patients with cystic fibrosis were further decontaminated with oxalic acid (26). MGITs

and BACTEC 460 bottles (B460) each received 0.5 ml of the specimen, while two Lowenstein-Jensen (LJ) slants were each inoculated with 0.1 ml of specimen. The majority of specimens were processed within 24 h, and smears were prepared by fluorochrome staining (16). MGITs were read daily for 8 weeks, B460 readings were performed twice weekly for 2 weeks and once weekly for 2 additional weeks, and LJ slants were examined weekly for 8 weeks. MGITs and LJ slants from smear-positive specimens were held for up to 12 weeks. Isolates of AFB were identified by standard biochemical tests (16) and DNA-RNA hybridization (Gen-Probe AccuProbe, San Diego, Calif.).

Culture preparation for mSDA. Aliquots were taken for mSDA analysis when the MGIT culture became fluorescent. In addition, an aliquot was taken from the MGIT when the B460 culture became positive if this occurred prior to the MGIT culture becoming positive. Aliquots of negative MGIT cultures were taken at the end of 8 weeks. At the indicated time, a 1-ml aliquot of the MGIT culture was processed for mSDA. The aliquot was centrifuged for 1 min at $13,000 \times g$. The supernatant was discarded, and the pellet was resuspended in 1 ml of KPDG (32.5 mM K_2HPO_4 [pH 7.6], 7.5% dimethyl sulfoxide, 3% glycerol, 0.01% NaN_3). The sample was centrifuged again at $13,000 \times g$ for 1 min. The supernatant was discarded, and the pellet was resuspended in 1 ml of KPDG. A third centrifugation for 1 min at $13,000 \times g$ was performed, and the supernatant was discarded. The pellet was then resuspended in 1 ml of KPDG containing 1 mg of proteinase K (Sigma Chemical Co.)/ml. The sample was incubated at 55°C for 30 min and then incubated at 100°C for 30 min in order to kill any mycobacteria present in the sample (27) and to release DNA for amplification. The sample was cooled to room temperature, vortexed, and stored at −70°C until the mSDA procedure was performed.

SDA. SDA is an isothermal in vitro DNA amplification technique. This amplification technique employs a restriction enzyme and a DNA polymerase with displacement properties to generate multiple copies of a target DNA sequence. Adapter-mediated mSDA simultaneously amplifies three target sequences, resulting in the detection of the mycobacterium genus (16S rDNA), *M. tuberculosis* (IS6110), and an IC while only using two primers. A complete explanation of SDA and mSDA is offered by Walker et al. (23–25).

mSDA assay. mSDA was performed essentially as described by Walker et al. (25) except that dUTP was used instead of TTP to allow removal of contaminating amplicons. The final concentrations or amounts of components were as follows: 45 mM K_2HPO_4 (pH 7.6); 7.5% dimethyl sulfoxide; 3% glycerol; 0.1 mg of bovine serum albumin/ml; 0.5 μM (each) primer; 0.05 μM (each) adapter; 0.025 μM (each) bumper; 0.5 mM 2′-deoxyadenosine 5′-*O*-(1-thiotriphosphate), 0.2 mM (each) dGTP, dCTP, and dUTP; 500 copies of the internal control sequence; 150 U of *Hinc*II; 4 U of exo-Klenow polymerase; 0.5 U of uracil-*d*-glycosylase (UDG); 2 U of UDG inhibitor; and 6.5 mM $Mg(C_2H_3O_2)_2$. With each mSDA batch run, controls containing various copy numbers of *Mycobacterium bovis* genomic DNA were also tested. This provided a run-to-run calibration and a within-run control on the efficiency of mSDA. The genomic controls were diluted in KPDG containing 10 ng of human DNA/μl such that 0, 50, 100, 250, or 500 copies of *M. bovis* genomic DNA were contained in 25 μl. *M. bovis* controls were tested in triplicate. All components except enzymes, magnesium acetate, and the UDG inhibitor were combined with 25 μl of the mSDA-processed MGIT sample or the *M. bovis* genomic control. This mixture was vortexed and heated for 2 min at 95°C and then incubated at 41°C for 5 min. UDG was added, and the sample was incubated at 41°C for 10 min. A mixture containing the enzymes, UDG inhibitor, and magnesium acetate was then added, and the resulting mixture was incubated for 2 h at 41°C. The final reaction volume was 50 μl. The reaction was stopped by heating for 2 min at 95°C. The sample was immediately assayed in the microwell assay or was stored at −20°C until tested. Frozen mSDA samples were heated for 2 min at 95°C prior to being assayed.

Chemiluminescent assay. The chemiluminescent microwell assay was performed as described by Spargo et al. (20). The microwell assay is a chemiluminescent enzyme-linked immunosorbent assay utilizing a streptavidin-coated microwell plate and biotin-labeled DNA probes for capture of the amplified product. An alkaline phosphatase-labeled DNA probe binds to the mSDA product and produces light in the presence of a chemiluminescent substrate. The light produced is detected with a photomultiplier tube, and the results are given in relative light units (RLU). For each microwell batch run, synthetic DNA assay controls were tested along with the mSDA reaction mixtures. The controls were single-stranded synthetic-target DNA containing dU for dT and were designed to match the sequence of the mSDA-generated products. The controls were diluted in a buffer containing all components of the mSDA reaction mixture except the enzymes and the inhibitor since they do not affect the mSDA result.

The assay was performed as follows. Completed mSDA reaction mixtures and synthetic target dilutions were heated at 95°C for 2 min to denature the DNA. The solutions were cooled for 5 min at room temperature, and 10 μl of the denatured sample was added to each well. Assay synthetic target controls were tested in duplicate; mSDA reactions were tested as single points. Immediately thereafter, 90 μl of hybridization buffer (0.05 M Trizma base [Sigma], 0.9 M NaCl, 0.05 mM $ZnCl_2$ [Sigma], 0.1% bovine serum albumin [Sigma], 0.2% NaN_3, 10.2 μg of salmon sperm DNA [Sigma]/ml, 2% trehalose [Quadrant; pH 7.0], capture and detector probes [concentrations optimized for high signal and low backgrounds]) was added. The microwell plate was covered and incubated for 45 min at 37°C. Three stringency washes (10 mM Trizma base, 0.1% bovine serum albumin, 0.01% Nonidet P-40 (Sigma), 250 mM NaCl, 0.1% NaN_3 [pH 7.5]) of
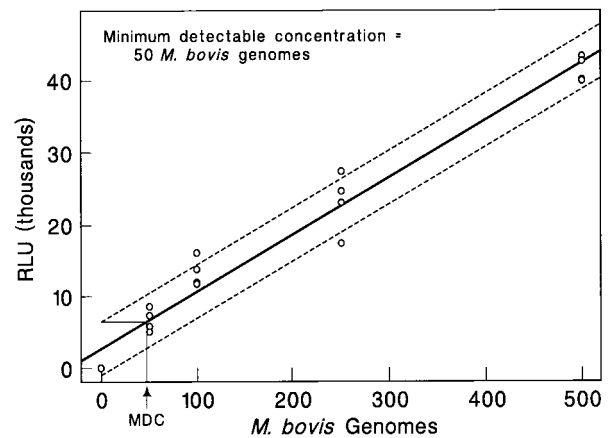

FIG. 1. SDA positive-control response curve. Shown is a plot of RLU for samples spiked with fixed quantities of *M. bovis* genomes. Samples were treated identically to clinical specimens with regard to both amplification and detection. The solid line indicates a least-squares linear regression fit. The dotted lines indicate 95% confidence limits for fit. The MDC of 50 *M. bovis* genomes is marked on the plot. The MDC is the lowest concentration of *M. bovis* genomes which results in an expected response (RLU value) that is significantly greater than the response for zero *M. bovis* genomes.

300 μl each were performed at room temperature. One hundred microliters of the chemiluminescent substrate Lumiphos 530 (Lumigen Inc.) was added to each well. The plates were covered and incubated at 37°C for 30 min. Luminescence (RLU) was read on a microwell plate luminometer (Labproducts TM, Research Triangle Park, N.C.) at 37°C, with a 2 s/well integration time.

Statistical analysis. The mSDA, MGIT, and B460 results for the 154 specimens were compiled and stored along with sample type (sputum, urine, etc.) and additional therapy data as available. The mSDA assay produces a quantitative luminescence result (RLU) which does not immediately fall into defined categories of positive and negative. In fact, there are an infinite number of possible cutoff values above which one could declare a positive result. Also, the mSDA assay procedure was done in seven batches of about 20 samples per batch plus controls. Thus, the RLU response for the clinical samples may be calibrated via the controls included with each batch. This provides a way of removing day-to-day variability in the mSDA assay, as captured by the controls, to assist in deciding whether a clinical sample is positive or negative by mSDA. A plot of RLU values obtained for several levels of mSDA controls is given in Fig. 1. This also shows reproducibility and minimum detectable concentration (MDC) of the mSDA assay detection system. The MDC is the minimum number of *M. bovis* genomes that can be significantly ($P < 0.05$) distinguished from zero genomes based on the variability in replicate assays done for each of the positive controls (17). The MDC is labeled in Fig. 1.

The RLU data were evaluated on several scales, including raw RLU and RLU normalized by various combinations of the negative, 50-genome and 100-genome controls. For example, normalizing to the 50-genome positive control was achieved by dividing RLU values obtained for a particular batch of clinical samples by the mean RLU of the 50-genome positive control run with that batch of clinical samples and multiplying the result by 50. Fluctuations in RLU values for different batches are accounted for by this process. Actual cutoff values for each method were chosen from a cutoff plot as described below. Receiver operator characteristic (ROC) curves were used to compare the accuracies and discrimination abilities of the methods. An ROC curve is a plot of true-positive rate versus false-positive rate. The true-positive rate is equivalent to sensitivity (expressed as a proportion) and is the probability of a positive (mSDA) test for individuals in whom the presence of mycobacteria has been shown by one of the culture systems: MGIT, B460, or LJ slants. The false-positive rate is equivalent to one minus the specificity and is the probability of a positive (mSDA) test for individuals in whom the absence of mycobacteria has been shown by MGIT, B460, and LJ slants. The ROC curve captures the trade-off between sensitivity and specificity and quantifies the accuracy of diagnostic test methods (6, 11).

The area under the ROC curve may be used to quantify and compare ROC curves. Often, smooth curves are fit to ROC curves when comparing diagnostic tests (11). In our application we compared ROC curves to assess different cutoff strategies for the same set of data. The area under the empirical ROC curve thus provided a more accurate comparison and was used to identify a satisfactory scale for summarizing the RLU data.

A cutoff plot for the mSDA results, normalized to the 50-genome control and with the culture result as the reference, is given in Fig. 2. This is a plot of sensitivity and specificity versus cutoff values scaled to the 50-genome *M. bovis*
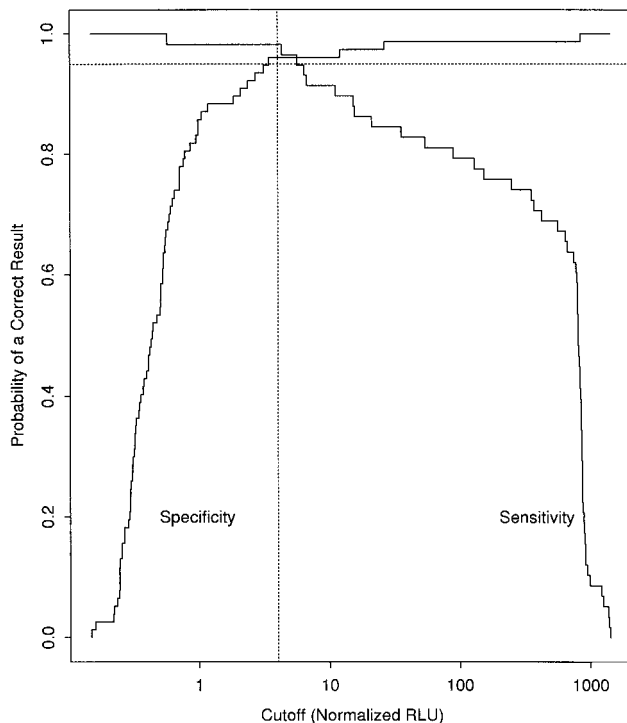
FIG. 2. ROC cutoff plot of mSDA test for the *Mycobacterium* genus, with positive culture from either MGIT, B460, or LJ slants as the reference method. The intersection of the horizontal dotted line with the sensitivity and specificity curves indicates 95% cutoffs for sensitivity and specificity. The vertical dotted line represents the chosen cutoff at 4 normalized RLU. These normalized units are proportional to the number of *M. bovis* genome equivalents per mSDA reaction.

standard. The cutoff plot allows visual selection of a cutoff value for a desired value of either sensitivity or specificity.

RESULTS

Culture and mSDA findings. During this study we performed mSDA analysis on 154 samples (Table 1) selected from 800 MGIT cultures that had been inoculated with a variety of clinical specimens. As shown, the majority of specimens were from respiratory sources (133 of 154, 86%). MGIT cultures were selected for mSDA analysis based on one of the five categories shown in Table 2. As shown, 55 of the 154 MGIT

TABLE 1. Sources of 154 specimens used to inoculate MGIT

Specimen source	No. tested (failed)
Respiratory	133
Sputum	94 (1)
Bronchoalveolar lavage	16 (1)
Bronchial wash	13
Tracheal aspirate	5
Biopsy	5
Nonrespiratory	21
Stool	9
Bone marrow	4 (4)
Urine	3
Gastric aspirate	2
Peritoneal aspirate	1
Pericardial aspirate	1
Abscess (wound)	1

TABLE 2. MGIT versus SDA results

MGIT category[a]	No. of samples[b]	No. of samples SDA positive
1. F+ C+ (total true positives)	55	53
1.1. MGIT culture grew MOTT	44	43
1.2. MGIT culture grew *M. tuberculosis*	11	10
2. F+ C−	52 (5)	17
2.1. Subsequent MGIT culture positive	5	5
2.2. Positive B460 or LJ culture	7	7
2.3. Patient on TB medication	3	3
2.4. MGIT false-positive fluorescence[c]	37 (5)	2
3. F+ C+ (culture grew bacteria or fungi)	33	1
4. F− C− (control true negative)	13 (1)	0
5. F− C− (AFB cultured in B460 or LJ slants)	1	1
Total specimens tested	154 (6)	72

[a] F+, fluorescence positive; F−, fluorescence negative; C+, culture positive; C−, no organisms cultured; TB, tuberculosis.
[b] Numbers in parentheses indicate numbers of failed SDA reactions; these were not included in the statistical analysis.
[c] Majority of MGITs became fluorescent in ≤3 days.

samples tested (category 1) were from MGIT cultures which grew *M. tuberculosis* (11 isolates) or other AFB (MOTT; 44 isolates). Of the 55 culture-positive MGIT specimens tested, 53 were mSDA positive for the appropriate organism, resulting in a sensitivity of 96.4%. There were 19 additional mSDA-positive findings. Seventeen (category 2) of these were from MGIT cultures which became fluorescent but did not grow AFB. A chart review indicated that only 2 (category 2.4) of these 17 were likely mSDA false-positive results. The other 15 mSDA positives were from patients who had had other cultures positive for AFB or who were receiving antimycobacterial therapy. The majority of the 37 specimens from category 2.4 which fluoresced but did not grow AFB were from MGIT cultures that had been incubated ≤3 days. Most were fluorescence negative by day 5 of incubation. The 33 specimens from category 3 which fluoresced but grew bacteria or fungi are also relevant to the testing of MGIT fluorescent cultures since AFB may or may not be present in cultures contaminated with bacteria or fungi. All but 1 of the 33 specimens in category 3 were mSDA negative and were from patients with no cultural or clinical evidence of mycobacterial infection. The 13 specimens in category 4 could be considered negative controls; however, these specimens would not be tested if one were to use mSDA on positive MGIT cultures only. Considering all clinical and cultural findings, the overall sensitivity and specificity were 97.2 and 96.1%, respectively, when mSDA was used to test MGIT fluorescent cultures. The positive predictive value was 95.8%, and the negative predictive value was 97.4%. The sensitivity of mSDA was calculated as tp/(tp + fn), where tp is the number of true positives and fn is the number of false negatives. From Table 2, the number of true positives is 53 + 5 + 7 + 3 + 1 = 69 and the number of false negatives is 2; thus, the sensitivity of mSDA compared to culture is 69/71, or 97.2%. The specificity of mSDA was calculated as tn/(tn + fp), where tn is the number of true negatives and fp is the number of false positives. From Table 2, the number of true negatives is 30 + 32 + 12 = 74 and the number of false positives in 2 + 1 = 3; thus, the specificity of mSDA is 74/77, or 96.1%. The positive predictive value of mSDA was calculated as tp/(tp +

TABLE 3. RLU readings obtained by mSDA

Culture no.	mSDA signal (RLU)[a] for:			Result		
	M. tuberculosis	MGEN	IC	SDA	Culture	MGIT category[b]
345	25	36	14,273	—[c]	—	3
382	23	19	1,646	—	—	3
354	24	73,771	13,573	MOTT	MAC[d]	1.1
111	43,792	66,800	33,372	MTB[e]	MTB	2.2
150	29	32	12,632	—	—	2.4
305	25	6,908	11,273	MOTT	MAC	1.1
343	27	25	13,559	—	—	2.4
160	28	21	10,802	—	—	2.4
171	25	21	11,587	—	—	4
364	26	47	10,127	—	—	4
357	21	117	13,992	—	—	3
29	26	57,189	12,323	MOTT	MAB[f]	1.1
12	44,362	83,302	28,646	MTB	MTB	1.2
180	26	29	8,591	—	—	2.4
40	36	30	10,432	—	—	2.4
50	29	23	2,042	—	Bacteria	3
282	25	1,796	11,462	MOTT	MAC	2.2
65	28	23	8,945	—	—	4
229	29	55,197	10,839	MOTT	MAB	1.1
22	27	48	10,593	—	—	4
66	37	45	12,865	—	—	4
424	26	28	11,460	—	—	3
9	50,057	84,846	35,726	MTB	MTB	1.2
186	39	49	15.4	Failed	—	2.4
476	48	24	10,302	—	—	4

[a] Cutoffs for this batch are as follows: *M. tuberculosis*, 2,000 RLU; MGEN, 320 RLU; IC, 100 RLU.
[b] MGIT categories are as defined in Table 2.
[c] —, negative result.
[d] MAC, *M. avium* complex.
[e] MTB, *M. tuberculosis*.
[f] MAB, *M. abscessus*.

fp) = 69/72, or 95.8%. The negative predictive value of mSDA was calculated as tn/(tn + fn) = 74/76, or 97.4%.

There were six specimens (Table 1) that contained inhibitor(s) to the mSDA reaction. No AFB were isolated from the six MGIT cultures with a failed mSDA result. All four bone marrow specimens selected for mSDA testing failed to amplify the internal signal (control sequence).

The RLU results for a typical mSDA batch run are shown in Table 3. All possible results are shown, i.e., when *M. tuberculosis* is present, when MOTT are present, when the specimen is negative for AFB, and when the reaction fails due to internal inhibitors present within the test specimen.

Statistical analysis. Normalizing the RLU values of the clinical specimens to the 50-genome positive control gave a slightly more discriminating decision procedure than choosing a single fixed cutoff value as measured by the area under the ROC curve. Note that with just seven batches of mSDA data one would not expect much difference. This may, however, be more relevant in a repeat-use clinical setting with multiple locations and batches, provided the controls track variability over such locations and batches. The cutoff plot for data normalized to the 50-genome positive control is given in Fig. 2. Subsequent summaries and analyses are based on data normalized to the 50-genome control.

The cutoff value that allows detection of 95% of culture-positive specimens is given by the intersection of the sensitivity curve with the dotted line in Fig. 2 and corresponds to about 4 U on the normalized RLU scale. This scale is proportional to the number of genome equivalents in the clinical samples. In general, cutoff selection for the mSDA procedure requires

assessment of the relative importance of sensitivity and specificity. For example, one may choose to minimize the risk function $R_c = L_1(1 - \text{sens}_c)p + L_2(1 - \text{spec}_c)(1 - p)$, where R_c is the overall risk; sens_c is the sensitivity and spec_c is the specificity as a function of the cutoff, c; L_1 is the loss associated with a false-negative result; L_2 is the loss associated with a false-positive result; and p is an estimate of the probability, in the laboratory population, that a sample will contain an AFB.

DISCUSSION

The use of metabolic indicators of growth such as CO_2 production, change in atmospheric pressure, or utilization of O_2 has reduced the incubation time needed to detect the presence of microorganisms in clinical specimens. This is especially true for slowly growing organisms such as mycobacteria. Molecular techniques such as PCR and other nucleic acid amplification techniques (NAAT) are capable of detecting and identifying mycobacteria in a few hours. The sensitivities of these NAAT when applied to direct clinical specimen testing have been found to be higher than that of microscopic examination but somewhat lower than that of conventional culture methodology (3, 8, 19). The cost-effectiveness of performing NAAT on smear-positive specimens seems justifiable since therapeutic and isolation issues can be resolved in a timely manner. The cost-effectiveness of performing NAAT on all incoming specimens has yet to be established. Besides cost-per-test issues, the major factors related to this uncertainty include (i) the lack of sensitivity of NAAT due to inhibitors in the clinical specimen or due to inefficient cell breakage and release of target DNA, (ii) the rare incidence of tuberculosis and other mycobacterial diseases in many patient populations, and (iii) the lack of specificity of NAAT due to the presence of environmental contaminants or due to the presence of dead mycobacteria following antimycobacterial therapy. These concerns, coupled with antimycobacterial susceptibility testing requirements, allow NAAT to supplement rather than replace routine culture for AFB.

In addition, NAAT should be useful for testing cultured specimens of AFB that have become positive. We applied one such NAAT (mSDA) for rapid evaluation of a particular mycobacterial growth detection system (MGIT). The time to detection for the MGIT system is comparable to that of the B460 system (4, 15). We used MGIT fluorescence as a selective criterion for inclusion in the mSDA analysis. Historically, specimens submitted for AFB cultures have a positivity rate near 7% at our institution. *M. tuberculosis* accounts for less than 15% of those positive cultures (<30 patients/year). During this study 11 of the 55 positive cultures were positive for *M. tuberculosis* (20%). The 55 positives were selected from 800 MGIT cultures during this study period. We chose to test MGIT positives as well as a few negatives rather than test all 800 MGIT cultures. The sensitivity of mSDA for testing fluorescence-positive MGIT specimens obtained in this study is probably accurate; however, the specificity might have been different had all 800 specimens been tested. The specificity might have improved since no false positives were detected in categories 4 and 5. The three false positives occurred in categories 2.4 and 3 in which MGIT specimens did become fluorescence positive.

Although these three results were presumed to be false-positive mSDA reactions, one cannot rule out the presence of low numbers (negative smears) of dead mycobacteria derived from environmental sources (food or water). Concentrated preparations from these three MGIT specimens were AFB negative by microscopic examination. Regardless of the three false-positive mSDA results, which lower the specificity, there were no false reactions between the *M. tuberculosis* and

MGEN mSDA results. For all specimens positive for AFB by culture, whether grown in MGIT, B460, or LJ slants, when the mSDA result was positive, it was correct (*M. tuberculosis* versus MOTT). Thus, the mSDA system does have a major advantage over other NAAT that are designed to detect only *M. tuberculosis* and an IC. Detection of MGEN and the IC with a negative *M. tuberculosis* result by mSDA gives a high degree of confidence that the patient does not have tuberculosis and can be removed from isolation. Although one can argue that the patient may have both MOTT and a small amount of *M. tuberculosis*, a condition resulting in a negative *M. tuberculosis* mSDA signal, this is true for any NAAT or culture method used in the mycobacteriology laboratory. The six mSDA failures represented a 4% failure rate, which leads to increased laboratory expenses in terms of labor and materials. It may be possible to reduce the failure rate by additional processing steps prior to mSDA analysis of bone marrow specimens.

Another area of concern for our laboratory is how adaptable the mSDA assay might be for a clinical mycobacteriology laboratory. In this study, five different individuals performed the mSDA assay. After being processed, the MGIT culture aliquots were stored and batch processed at Becton Dickinson Research Center (BDRC). Each technologist performed the mSDA and the chemiluminescence assays on a minimum of 40 MGIT samples plus controls. All stock reagents were available at BDRC; therefore, there was a minimum of pipetting, preparation of buffers, etc. In addition, the microwell chemiluminescence trays were already prepared. This manual hands-on method provided results in approximately 4 h. Routine testing of 1 to 30 specimens each day would prove cumbersome given the manual nature of the current assay methods. This technology can presumably be automated, thus making it more attractive to most clinical laboratories. With a cost-effective automated system one could also argue that AFB culture incubation times might be greatly reduced by using NAAT technology. For instance, mSDA or other NAAT might allow incubation of AFB cultures to end after 7 to 14 days if the sensitivity is sufficiently high and the cost per test is sufficiently low. Compared to the alternative of 8 weeks of incubation, NAAT would represent significant improvement in labor, work flow, and instrumentation issues in the AFB laboratory. Considerable research remains to be done before the value of mSDA or other NAAT becomes clearly established in the mycobacteriology laboratory.

REFERENCES

1. **Abe, C., K. Hirano, M. Wada, Y. Kazumi, M. Takahashi, Y. Fukasawa, T. Yoshimura, C. Miyagi, and S. Goto.** 1993. Detection of *Mycobacterium tuberculosis* in clinical specimens by polymerase chain reaction and Gen-Probe Amplified Mycobacterium tuberculosis Direct Test. J. Clin. Microbiol. **31:**3270–3274.
2. **An, Q., D. Buxton, A. Hendricks, L. Robinson, J. Shah, L. Lu, M. Vera-Garcia, W. King, and D. M. Olive.** 1995. Comparison of amplified Qβ replicase and PCR assays for detection of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **33:**860–867.
3. **Bennedsen, J., V. O. Thomsen, G. E. Pfyffer, G. Funke, K. Feldmann, A. Beneke, P. A. Jenkins, M. Hegginbothom, A. Fahr, M. Hengstler, G. Cleator, P. Klapper, and E. G. L. Wilkins.** 1996. Utility of PCR in diagnosing pulmonary tuberculosis. J. Clin. Microbiol. **34:**1407–1411.
4. **Bowles, E. J., T. R. Wisniewski, J. M. Makowski, K. K. Frantz, and J. A. Kiehlbauch.** 1995. A clinical comparison of the BBL MGIT mycobacteria growth indicator tube with Bactec, Septicheck, and conventional media, abstr. U-142, p. 142. *In* Abstracts of the 95th General Meeting of the American Society for Microbiology 1995. American Society for Microbiology, Washington, D.C.
5. **Clarridge, J. E., III, R. M. Shawar, T. M. Shinnick, and B. B. Plikaytis.** 1993. Large-scale use of polymerase chain reaction for detection of *Mycobacterium tuberculosis* in a routine mycobacteriology laboratory. J. Clin. Microbiol. **31:**2049–2056.
6. **Down, J. A., M. A. O'Connell, M. S. Dey, A. H. Walters, D. R. Howard, M. C. Little, W. E. Keating, P. Zwadyk, Jr., P. D. Haaland, D. A. McLaurin III, and G. Cole.** 1996. Detection of *Mycobacterium tuberculosis* in respiratory specimens by strand displacement amplification of DNA. J. Clin. Microbiol. **34:**860–865.
7. **Eisenach, K. D., M. D. Sifford, M. D. Cave, J. H. Bates, and J. T. Crawford.** 1991. Detection of *Mycobacterium tuberculosis* in sputum samples using a polymerase chain reaction. Am. Rev. Respir. Dis. **144:**1160–1163.
8. **Forbes, B. A., and K. E. Hicks.** 1993. Direct detection of *Mycobacterium tuberculosis* in respiratory specimens in a clinical laboratory by polymerase chair reaction. J. Clin. Microbiol. **31:**1688–1694.
9. **Forbes, B. A., and K. E. Hicks.** 1994. Ability of PCR assay to identify *Mycobacterium tuberculosis* in BACTEC 12B vials. J. Clin. Microbiol. **32:**1725–1728.
10. **Hance, A. J., B. Grandchamp, V. Lévy-Frébault, D. Lecossier, J. Rauzier, D. Bocart, and B. Gicquel.** 1989. Detection and identification of mycobacteria by amplification of mycobacterial DNA. Mol. Microbiol. **3:**843–849.
11. **Hanley, J. A.** 1989. Receiver operator characteristic (ROC) methodology: the state of the art. Crit. Rev. Diagn. Imaging **29:**307–335.
12. **Hopfer, R. L., F. Badak, D. Kiska, C. Hartley, S. Setterquist, C. Nycz, and M. O'Connell.** Use of strand displacement amplification (SDA) for confirmation of mycobacterium and *Mycobacterium tuberculosis* in clinical specimens cultured in mycobacterial growth indicator tubes (MGIT), abstr. C-21, p. 4. *In* Abstracts of the 96th General Meeting of the American Society for Microbiology 1996. American Society for Microbiology, Washington, D.C.
13. **Kirschner, P., J. Rosenau, B. Springer, K. Teschner, K. Feldmann, and E. C. Böttger.** 1996. Diagnosis of mycobacterial infections by nucleic acid amplification: 18-month prospective study. J. Clin. Microbiol. **34:**304–312.
14. **Moore, D. F., and J. I. Curry.** 1995. Detection and identification of *Mycobacterium tuberculosis* directly from sputum sediments by Amplicor PCR. J. Clin. Microbiol. **33:**2686–2691.
15. **Neumann, M. A., J. Rogers, R. Williams, and S. Maxwell.** 1995. Comparison of the new mycobacterial growth indicator tube (MGIT) with the BACTEC 460 TB system for the rapid detection of mycobacteria from clinical specimen, abstr. C-138, p. 24. *In* Abstracts of the 95th General Meeting of the American Society for Microbiology 1995. American Society for Microbiology, Washington, D.C.
16. **Nolte, F. S., and B. Metchock.** 1995. Mycobacterium, p. 400–437. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology. American Society for Microbiology, Washington, D.C.
17. **O'Connell, M., B. Belanger, and P. Haaland.** 1993. Calibration and assay development using the four-parameter logistic model. Chem. Intell. Lab. Syst. **20:**97–114.
18. **Pao, C. C., T. S. Benedict Yen, J.-B. You, J.-S. Maa, E. H. Fiss, and C.-H. Chang.** 1990. Detection and identification of *Mycobacterium tuberculosis* by DNA amplification. J. Clin. Microbiol. **28:**1877–1880.
19. **Pfyffer, G. E., P. Kissling, E. M. I. Jahn, H.-M. Welscher, M. Salfinger, and R. Weber.** 1996. Diagnostic performance of Amplified *Mycobacterium tuberculosis* Direct Test with cerebrospinal fluid, other nonrespiratory, and respiratory specimens. J. Clin. Microbiol. **34:**834–841.
20. **Spargo, C. A., P. D. Haaland, S. R. Jurgensen, D. D. Shank, and G. T. Walker.** 1993. Chemiluminescent detection of strand displacement amplified DNA from species comprising the *Mycobacterium tuberculosis* complex. Mol. Cell. Probes **7:**395–404.
21. **Thierry, D., M. D. Cave, K. D. Eisenach, J. T. Crawford, J. H. Bates, B. Gicquel, and J. L. Guesdon.** 1990. *IS6110*, an IS-like element of *Mycobacterium tuberculosis* complex. Nucleic Acids Res. **18:**188.
22. **Vuorinen, P., A. Miettinen, R. Vuento, and O. Hällström.** 1995. Direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens by Gen-Probe Amplified Mycobacterium tuberculosis Direct Test and Roche Amplicor Mycobacterium tuberculosis Test. J. Clin. Microbiol. **33:**1856–1859.
23. **Walker, G. T., M. S. Fraiser, J. L. Schram, M. C. Litle, J. G. Nadeau, and D. P. Malinowski.** 1992. Strand displacement amplification—an isothermal *in vitro* DNA amplification technique. Nucleic Acids Res. **20:**1691–1696.
24. **Walker, G. T., M. C. Little, J. G. Nadeau, and D. D. Shank.** 1992. Isothermal, *in vitro* amplification of DNA by a novel restriction enzyme/DNA polymerase system. Proc. Natl. Acad. Sci. USA **89:**392–396.
25. **Walker, G. T., J. G. Nadeau, P. A. Spears, J. L. Schram, C. M. Nycz, and D. D. Shank.** 1994. Multiplex strand displacement amplification (SDA) and detection of DNA sequences from *Mycobacterium tuberculosis* and other mycobacteria. Nucleic Acids Res. **22:**2670–2677.
26. **Whittier, S., R. Hopfer, M. Knowles, and P. Gilligan.** 1993. Improved recovery of mycobacteria from respiratory secretions of patients with cystic fibrosis. J. Clin. Microbiol. **31:**861–864.
27. **Zwadyk, P., Jr., J. A. Down, N. Myers, and M. S. Dey.** 1994. Rendering of mycobacteria safe for molecular diagnostic studies and development of a lysis method for strand displacement amplification and PCR. J. Clin. Microbiol. **32:**2140–2146.