JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1996, p. 860–865 Vol. 34, No. 4
0095-1137/96/$04.00+0

Detection of *Mycobacterium tuberculosis* in Respiratory Specimens by Strand Displacement Amplification of DNA

JAMES A. DOWN,[1]* MICHAEL A. O'CONNELL,[1] MARGARET S. DEY,[1] ADRIANN H. WALTERS,[2] DEBORAH R. HOWARD,[1] MICHAEL C. LITTLE,[2] WILLIAM E. KEATING,[2] PETER ZWADYK, JR.,[3,4] PERRY D. HAALAND,[1] DANIEL A. MCLAURIN III,[2] AND GENEVIEVE COLE[5]

Becton Dickinson Research Center, Research Triangle Park, North Carolina 27709[1]; Becton Dickinson Diagnostic Instrument Systems, Sparks, Maryland 21152[2]; Pathology and Laboratory Medicine, VA Medical Center, Durham, North Carolina 27705[3]; Department of Pathology, Duke University Medical Center, Durham, North Carolina 27710[4]; and Mycobacteriology Laboratory, State Laboratory of Public Health, North Carolina Department of Environment Health and Natural Resources, Division of Laboratory Services, Raleigh, North Carolina 27601[5]

Received 28 June 1995/Returned for modification 14 October 1995/Accepted 19 December 1995

A total of 294 clinical respiratory specimens, including 75 with culture-positive results, were tested for the presence of *Mycobacterium tuberculosis* by strand displacement amplification (SDA) of DNA. A region of the IS*6110* insertion element and an internal control sequence were amplified and then detected by a chemiluminescence assay. Receiver operator-characteristic curves were used to evaluate three methods for declaring specimens positive for *M. tuberculosis*. By the preferred method, SDA chemiluminescence results were converted to theoretical numbers of *M. tuberculosis* organisms. A positive threshold (PT) value, above which 95% of the SDA results were judged to be *M. tuberculosis* positive (sensitivity = 95%), was found to be 2.4 *M. tuberculosis* organisms per SDA reaction. The analogous PT value for 95% sensitivity on smear-positive specimens was 3.6 *M. tuberculosis* organisms per reaction. The PT of 2.4 *M. tuberculosis* organisms per reaction detected 100% of culture-positive, smear-positive specimens (sensitivity = 100%), while 95% sensitivity was achieved with a PT of 15.5 *M. tuberculosis* organisms per reaction. Specificities, which were calculated with respect to culture- and smear-negative specimens, ranged from 96% at a PT of 15.5 *M. tuberculosis* organisms to 84% at a PT of 2.4 *M. tuberculosis* organisms per reaction. The *M. tuberculosis*-negative specimens were also segregated according to whether the patients received antituberculosis chemotherapy. SDA specificity ranged from 90% (PT = 2.4 *M. tuberculosis* organisms) to 98% (PT = 15.5 *M. tuberculosis* organisms) for the *M. tuberculosis*-negative specimens from patients who had not received chemotherapy. SDA specificity in the *M. tuberculosis*-negative specimens from patients who received chemotherapy was lower (85 to 94%). This study represents the first large-scale demonstration of *M. tuberculosis* detection in clinical sputum specimens by isothermal DNA amplification with SDA.

DNA amplification may shorten clinical detection of *Mycobacterium tuberculosis* from weeks to less than a day. However, numerous technical hurdles have yet to be overcome before the technology becomes routine enough to supplant culture-based diagnosis of tuberculosis (TB). Since its first discovery (25), various authors have tested the use of PCR (1, 2, 3, 6–13, 17, 18, 21, 22–24, 27, 29, 30, 32, 37) for detection of *M. tuberculosis* in clinical specimens. Other DNA amplification methods (15, 26) have also been tried. In this study, we assessed the ability of strand displacement amplification (SDA) to detect *M. tuberculosis* in sputa.

SDA has been previously shown to amplify purified *M. tuberculosis* DNA (20, 28, 33–37); thus, the purpose of this work was to develop a method for amplification and detection of *M. tuberculosis* DNA in clinical specimens. The IS*6110* element, a well-documented *M. tuberculosis*-specific sequence (2, 10–13, 17, 20, 22, 27, 30–32), was used as the DNA target. An internal control was used to monitor SDA suppression, as has previously been done for PCR (5, 8). For each batch of clinical specimens processed, standard curves were constructed by using various levels of *M. tuberculosis* genomic DNA in conjunction with negative and positive controls for specimen processing, SDA, and assaying. This enabled conversion of SDA results to theoretical numbers of *M. tuberculosis* and allowed monitoring of background contamination. These calibrators and controls also provided different strategies for selecting cutoffs or positive thresholds (PTs) used in declaring specimens positive for *M. tuberculosis*.

The specimens were divided into aliquots that were individually processed and amplified, which allowed assessment of the variability of SDA results within specimens. DNA was released from the *M. tuberculosis* by a simple method that also rendered the specimens safe to handle (37). Finally, a sensitive chemiluminescence microtiter plate assay (28, 36, 37) was used to provide a quantitative, nonradiometric SDA result. PTs for *M. tuberculosis* detection were calculated from these quantitative assay data via joint assessment of sensitivity and specificity of SDA relative to culture and smear results. In this paper, we report the results of a study in which the methodology for sample processing, SDA, chemiluminescence assaying, and numeric analysis were combined to analyze 294 sputum samples for the presence of *M. tuberculosis*.

MATERIALS AND METHODS

Specimen processing. Sputum specimens were processed for culture on Lowenstein Jensen medium with the *N*-acetyl-L-cysteine–sodium hydroxide–sodium citrate (NALC) protocol (16), and the concentrated specimens were stained for

* Corresponding author. Mailing address: Becton Dickinson Research Center, 21 Davis Dr., Research Triangle Park, NC 27709-2016. Phone: (919) 990-2206. Fax: (919) 549-7572. Electronic mail address: Down@bdrc.bd.com.

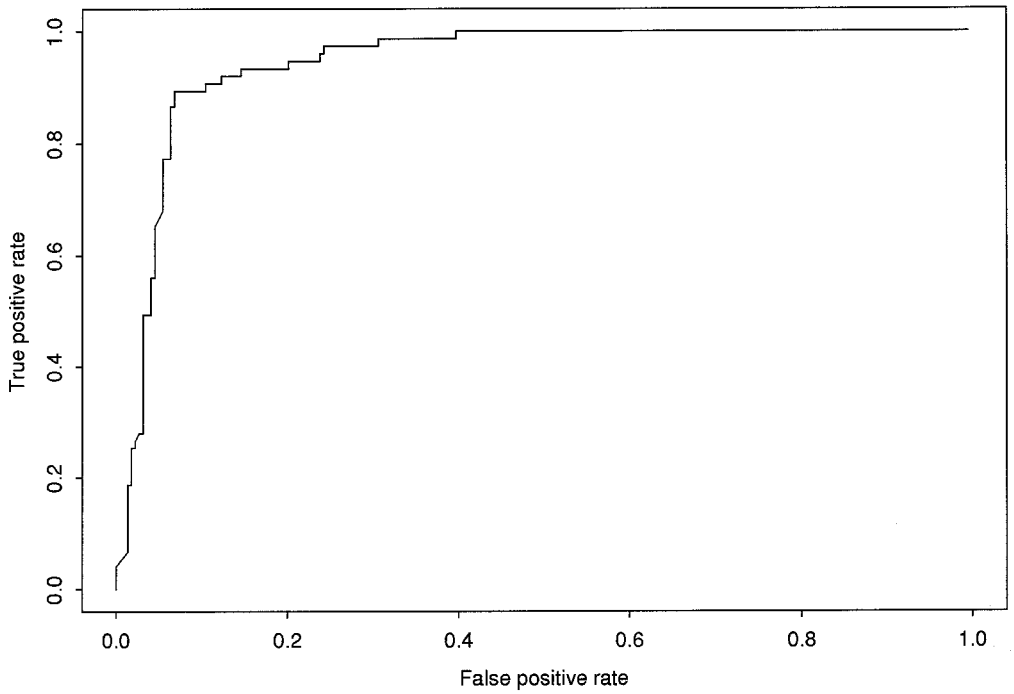

FIG. 1. ROC curve for SDA test of *M. tuberculosis* with culture as the reference method.

acid-fast bacteria (AFB) by using Auramine O. Identification of *M. tuberculosis* cultures was confirmed by high-pressure liquid chromatography (4) and a DNA probe test (GenProbe, San Diego, Calif.) specific for TB complex mycobacteria. The unprocessed portions of the clinical samples were stored at −20°C until they were processed for analysis by SDA. The samples remained frozen approximately 2 months to a year before being tested. Patients were not identified in this study, and, therefore, it was not known how many of the specimens represented repeated samplings from the same patient.

After culture results were obtained, the frozen specimens were processed by the NALC procedure. SDA inhibitors were removed from the NALC sediments by two successive 1-min washes with 1 ml of 25 mM K_2HPO_4 (pH 7.6; wash buffer) in a Microfuge (Fisher, Pittsburgh, Pa.) as previously described (36). The washed pellets were resuspended in 1 ml of wash buffer and heated for 30 min at 100°C to release the DNA from the mycobacteria (37).

SDA. In each experiment, the sample processing, SDA, and assays were done in separate locations to avoid cross-contamination. In initial experiments, four replicates from each specimen were individually processed and amplified to assess the intraspecimen variability of the SDA amplification and detection system. Specimens that were strongly SDA positive were omitted from the analysis of variability because their replicates produced relative light unit (RLU) values at the maximum detection limit of the luminometer (20,000 RLU). In later experiments, only a single test was performed on each specimen. Sensitivity and specificity calculations were done with one sample from each specimen, and these were randomly selected in cases in which multiple aliquots were processed.

To perform SDA, 25 μl of each processed, heat-lysed specimen was combined with 45 μl of SDA reaction mixture (36, 37). The mixture included 25,000 copies of a synthetic internal control (SIC) to control for amplification suppression. The IS*6110* DNA and SIC were simultaneously amplified at 41°C, and the products were measured in RLU by the method described previously (28, 36).

Amplification and assay controls were run along with each experimental batch of samples. Standard curves were constructed by using synthetic IS*6110* DNA targets equivalent to 0, 5, 10, 25, or 50 *M. tuberculosis* organisms. Multiple replicates of the 25-organism DNA control were also amplified to assess reaction variability. Controls for the chemiluminescence assay consisted of 0, 500, 2,000 or 8,000 amol of purified *M. tuberculosis* DNA. Negative controls consisted of wash buffer aliquots which were carried through the processing procedure along with the specimen NALC sediments and then combined with SDA reaction mixture containing 250, 500, or 750 ng of human DNA (Sigma, St. Louis, Mo.). Every SDA reaction mixture was assayed twice.

Analysis of results. The SDA, culture, and AFB smear results for the 294 specimens as well as antibiotic chemotherapy data, when known, were entered into a Paradox 4.0 relational database (Borland International, Scotts Valley, Calif.). The SDA assay produces a quantitative 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. Three methods were considered for choosing cutoff values. Actual cutoff values for each method were determined via cutoff plots. These cutoff values were based on raw RLU values (method 1), on a multiple of the background RLU as determined for each experiment (method 2), or on the five-*M. tuberculosis* organism DNA standard as determined for each experiment (method 3). For methods 2 and 3, all SDA results were scaled to the negative and five-genome control, respectively. Method 3 thus provided the attractive feature of expressing SDA assay results for clinical specimens as a theoretical number of *M. tuberculosis* organisms.

Receiver operator-characteristic (ROC) curves were used to quantify and compare the accuracy and discrimination abilities of the methods. An ROC curve is a plot of true-positive rate versus false-positive rate. True-positive rate is equivalent to sensitivity (expressed as a proportion) and is the probability of a positive SDA test in samples which had shown presence of *M. tuberculosis* in culture and/or smear tests. False-positive rate is equivalent to 1 minus specificity and is the probability of a positive SDA test in samples which had shown the absence of *M. tuberculosis* in culture and/or smear results. The ROC curve captures the trade-off between sensitivity and specificity.

ROC curves and associated cutoff decision plots allow quantification of the accuracy of diagnostic tests and associated cutoff strategies. The ROC curve for the SDA test of *M. tuberculosis* by cutoff method 3 with culture as the reference is given in Fig. 1. An ROC curve that is located closer to the top left corner of the plot indicates a more accurate test method. This 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 (14). In our application, we compared ROC curves to assess different cutoff strategies on the same set of samples and areas under the empirical ROC curve provided a more accurate comparison.

The *M. tuberculosis*-positive specimens were segregated into several groups: AFB smear positive and culture positive, AFB smear positive, and culture positive. The thresholds required to detect 95% of the smear-positive or culture-positive specimens (sensitivity = 95%) were then determined via cutoff curves and were designated the PTs. The negative specimens (i.e., culture negative and smear negative) altogether, or after segregation according to antibiotic chemotherapy, were tested with the same PTs to determine the corresponding SDA specificities. Cutoff curves for the SDA detection of *M. tuberculosis* with culture as the reference are given in Fig. 2. These are plots of sensitivity and specificity versus cutoff as determined via method 3 whereby all RLU values are scaled to the five-genome *M. tuberculosis* standard.

The effect of reaction inhibition was included as follows. (i) specimens that produced RLU values that were greater than the PT were judged to be *M. tuberculosis* positive. (ii) Specimens that produced RLU values that were less than the PT and that gave a SIC result that was less than a SIC cutoff were rejected as having suppressed amplification reactions. The SIC cutoff was 0.36× the mean value of the SIC results of negative controls from each experiment.

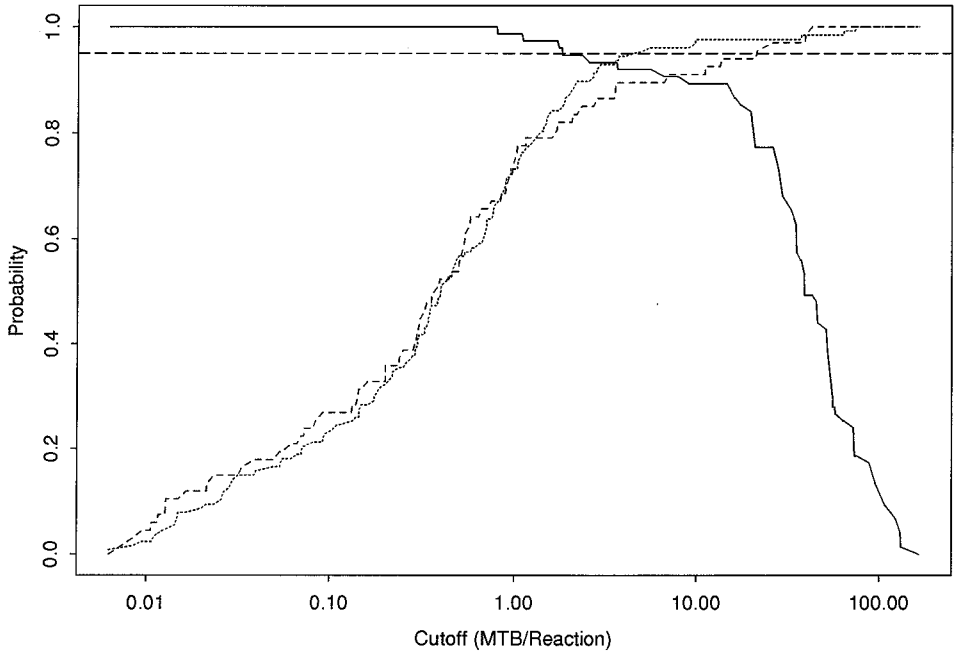

FIG. 2. Cutoff plot for SDA detection of *M. tuberculosis* with culture as the reference for positive determination. Solid line, sensitivity; dashed SDA specificity for chemotherapy-positive specimens; dotted line, chemotherapy-negative specimens. The intersection of the dashed horizontal line with the sensitivity and specificity curves indicates 95% cutoffs for sensitivity and specificity. MTB, *M. tuberculosis* organisms.

This value resulted in acceptance of 95% of the culture-negative specimens as negative. (iii) Specimens that produced RLU values that were less than the PT and that had a SIC result greater than the SIC cutoff were considered *M. tuberculosis* negative.

RESULTS

Culture and smear results. In total, 304 sputum specimens were analyzed by SDA. Ten samples that grew MOTTs (mycobacteria other than *M. tuberculosis*) were broken down as follows: six specimens cultured *Mycobacterium avium-M. intracellulare* complex bacteria, of which four were smear positive, while the remaining four specimens cultured *Mycobacterium gordonae*, none of which was smear positive. Of the remaining 294 specimens, 75 grew *M. tuberculosis* and 219 were culture negative. Thirteen of the culture-negative and 60 of the culture-positive specimens were AFB smear positive. A breakdown of the specimens, including whether the patients were receiving antituberculosis chemotherapy, is presented in Table 1.

Variability of SDA results within specimens. Among the 83 specimens from which two or more replicates were taken, the average percent coefficient of variation (%CV) for the SIC RLU result was 24.7%, while the average %CV for the *M. tuberculosis* RLU values was 41.6%. Five of these specimens showed internally discordant results whereby some aliquots were *M. tuberculosis* positive and others were *M. tuberculosis* negative by SDA, depending on which PT was used. The apparent discordance resulted from the fact that associated RLU values were on the boundary between SDA-positive and SDA-negative determinations.

TABLE 1. Breakdown of culture and smear specimen results according to anti-TB chemotherapy

Specimen type	No. with no therapy	No. with therapy	Total[a]
Positive culture and smear	26	29	60
Positive culture only	9	6	15
Positive smear only	3	7	13
Negative culture and smear	127	67	206
Totals	165	109	294

[a] Chemotherapy data could not be recovered for 20 of the 294 specimens, accounting for the apparent discrepancies in totals.

Comparison of methods for discriminating *M. tuberculosis*-positive specimens. Method 2, which used multiples of the background signal for the threshold, was rejected because of low sensitivity and specificity as quantified by the area under the ROC curve. Sensitivity and specificity were 81% at a cutoff of 12 *M. tuberculosis* organisms per reaction. As quantified by the area under the ROC curve, methods 1 and 3 had similar sensitivities and specificities and provided a more accurate and discriminating cutoff strategy than method 2. Subsequent analyses were based on method 3, which also has the intuitive appeal of expressing SDA results as theoretical numbers of *M. tuberculosis* organisms by scaling to the five-genome *M. tuberculosis* standard.

Monitoring SDA suppression with the internal control. The SIC was included in the SDA reactions to avoid false-negative results caused by amplification inhibitors in the clinical specimens. We defined inhibition as a SIC RLU result that was less than a cutoff which rejected the lowest 5% of SIC RLU values obtained for SDA reactions from culture-negative specimens. This cutoff was found to be 0.36× the mean SIC RLU values of the negative controls of the respective experiments. We also found that *M. tuberculosis* in specimens also caused suppression of the SIC. As shown in Table 2, the smear-positive and culture-positive specimens contained higher percentages of samples with SIC results that were less than 0.36× negative-control SIC RLU values. This was not a problem, because the

TABLE 2. Suppression of the SDA internal control by *M. tuberculosis* in clinical specimens

Result	No. (%) of suppressed specimens[a]	Total no. of specimens
Culture results		
Negative	11 (5.0)	219
M. tuberculosis positive	22 (29.3)	75
Smear results		
Negative	14 (6.3)	221
<1 bacillus per field	3 (11.5)	26
1–10 bacilli per field	3 (15.0)	20
>10 bacilli per field	13 (48.1)	27

[a] Suppression is defined as RLU values of less than 0.36× the SIC control for each experiment.

SIC result was disregarded in specimens that were *M. tuberculosis* positive by SDA.

Two culture-positive specimens gave SIC RLU results that were below the SIC cutoff and were considered inhibited. All four aliquots from one of these specimens gave negative SDA and SIC results; therefore, it was a legitimate false-negative result. The other specimen had three aliquots that gave clear *M. tuberculosis*-positive results without suppressed SIC results. However, by chance, the one failed SDA was used in the sensitivity and specificity calculations because it had been randomly selected for the analysis, as described in Materials and Methods. Thus, the initial analysis showed a false-negative rate of 2 of 75 (2.7%) attributed to SDA inhibition, which we reconciled to be 1 of 75 (1.3%).

Sensitivity and specificity of SDA versus specimen population. PTs (i.e., cutoffs providing sensitivities of 95%) and specificities with respect to culture and smear specimens, culture-only specimens, and smear-only specimens are given in Table 3. Since culture-positive, smear-positive specimens were expected to be *M. tuberculosis* positive by SDA, we also determined the PT that declared 100% of these specimens as SDA positive (sensitivity = 100%). This was found to be 2.4 *M. tuberculosis* organisms per reaction. The same PT detected 95% of the culture-positive-only specimens. The SDA specificity was 84% for all culture-negative, smear-negative specimens for this PT. Broken down according to anti-TB treatment, this represented a specificity of 90% for specimens of patients not on anti-TB chemotherapy and a specificity of 85% for specimens from patients receiving chemotherapy (Table 3). A higher PT of 15.5 *M. tuberculosis* organisms per reaction gave a sensitivity of 95% in the culture-positive, smear-positive specimens. This PT produced higher values for specificity ranging from 94 to 98% (Table 3). Regardless of which PT from Table 3 was used, SDA specificities for the culture- and smear-negative specimens from patients who had not received anti-TB chemotherapy were greater than for specimens from patients who received treatment.

Among the 75 culture-positive specimens, the 4 specimens that were not detected by SDA at the 95% sensitivity level were also smear negative. Thus, 11 of 15 (73%) of the culture-positive, smear-negative specimens were detected by SDA. None of the SDA results from the 10 MOTT specimens exceeded the PTs mentioned above (i.e., equivalent to 2.4, 3.6, or 15.5 *M. tuberculosis* organisms per reaction), and therefore none gave false-positive determinations.

DISCUSSION

Specimens and specimen processing. Rendering *M. tuberculosis*-containing specimens compatible with culture and SDA proved challenging because the reagents used to process sputum samples for culture are inhibitory to DNA amplification. Researchers using PCR have reported similar specimen-processing challenges (e.g., see references 11 and 12), and, as seen here for SDA, components of some specimens have been found to inhibit PCR (3).

The sample processing method described herein used two successive high-speed washes to remove inhibitors from the NALC sediments. It was found to be simpler than methods that include phenol-chloroform extraction (2, 3, 7, 9) or other DNA isolation procedures (10, 18). In contrast to previous workers (2, 3, 11, 22, 23), we did not use a complex lysis mixture to disrupt the *M. tuberculosis* because heating in 25 mM K_2HPO_4 produced enough DNA to permit detection of the *M. tuberculosis* and it did not have a detrimental effect on SDA. Since a quarter of each NALC pellet was processed into a final volume of 1 ml and 25 μl was amplified, the SDA results reported in this paper represented 1/160 of each original specimen. Use of the whole specimen should allow greater sensitivity in future SDA experiments.

Use of PTs to identify *M. tuberculosis*-positive specimens. The minimum concentration of purified *M. tuberculosis* DNA detectable by SDA and the chemiluminescence assay was previously found to be equivalent to 1.2 *M. tuberculosis* organisms per reaction, while the reliable detection limit (i.e., 95% confidence level) was 3 *M. tuberculosis* organisms per reaction (28). Variability introduced by specimen inhibitors may reduce the ability to discriminate low positive results from the background. However, we found that the SIC RLU results of multiple aliquots processed from single specimens gave a mean

TABLE 3. Analysis of SDA sensitivity and specificity

Type of specimen	PT (*M. tuberculosis*)	SDA sensitivity (%)[a]	SDA specificity (%) for the following specimen types[b]:		
			Total culture and smear negative[c]	Without therapy	With therapy
Culture and smear positive	2.4	100 (60/60)	84 (173/206)	90 (114/127)	85 (57/67)
	15.5	95 (57/60)	96 (198/206)	98 (124/127)	94 (63/67)
Culture positive	2.4	95 (71/75)	84 (173/206)	90 (114/127)	85 (57/67)
Smear positive	3.6	95 (69/73)	89 (183/206)	94 (119/127)	90 (60/67)

[a] A PT of 2.4 *M. tuberculosis* organisms per reaction detected 100% of culture and smear-positive specimens or 95% of culture-positive specimens, and the respective SDA specificities are therefore identical. The values are repeated in the separate rows for clarity. Numbers of specimens are given in parentheses.

[b] Numbers of specimens are given in parentheses.

[c] Includes specimens for which chemotherapy data were not known.

%CV of 25%, which was considered reasonable. In contrast, the higher value for the *M. tuberculosis* results (41.6%) was attributed to clumping of the *M. tuberculosis* and the difficulty of evenly distributing the organisms throughout the aliquots.

The calculated values for SDA sensitivity and specificity were similar to, or in some cases better than, those reported for PCR (10–13, 18, 21, 22, 27, 30). For example, Beige et al. (1) detected 48 of 49 TB patients (sensitivity = 98%) using PCR amplification of two *M. tuberculosis*-specific primer systems to detect TB-positive patients. With this system, apparent *M. tuberculosis* DNA was detected in 16 of 54 patients presumed not to have TB (specificity = 70%). Miyazaki et al. (21) used nested PCR to detect *M. tuberculosis* in 62 of 64 specimens (sensitivity = 97%) from TB-positive patients. Among 351 *M. tuberculosis*-negative specimens, 28 gave *M. tuberculosis*-positive signals by PCR (specificity = 92%). Eisenach's group (11) used PCR to detect IS*6110 M. tuberculosis* DNA with a sensitivity of 98% (50 of 51 specimens) and a specificity of 98% for MOTT specimens (41 of 42), whereas they obtained a specificity of 100% (26 of 26) from nontuberculous specimens. Similarly, other groups using PCR to detect *M. tuberculosis* DNA reported sensitivities of 87.2% (13) and 91% (22), with respective specificities of 97.7 and 100%. Using the GenProbe rRNA amplification test for *M. tuberculosis*, Jonas et al. (15) observed a sensitivity of 82% and a specificity of 99% in clinical specimens. The SDA PTs of 2.4 and 3.6 *M. tuberculosis* organisms per reaction used in this study detected 95% of the culture-positive-only or smear-positive-only specimens, respectively. In addition, as seen for PCR (1, 18, 22), sensitivity was higher for culture-positive, smear-positive samples than for culture-positive, smear-negative samples.

Suppression of the internal control. The SIC was included in the SDA reactions to flag false-negative reactions due to SDA inhibition. However, as previously reported (37), SDA reactions with low internal-control results tended to be from *M. tuberculosis* culture-positive specimens. This is not surprising, since smear- or culture-positive specimens may contain thousands of *M. tuberculosis* organisms, each with about 10 IS*6110* elements (30), which compete with the SIC for the amplification reagents (such as enzymes and nucleotides, etc.).

The cutoff used for rejecting suppressed SDA reactions was empirically determined to eliminate the 5% of culture-negative specimens with the lowest SDA SIC RLU results. Using the SIC cutoff on the 75 MTB culture-positive specimens gave a reconciled false-negative rate due to SDA inhibition of 1.3%.

False-positive results. Previous workers (1, 26) have pointed out that DNA amplification may give false-positive determinations because of DNA from nonculturable *M. tuberculosis*. This suggests that SDA false-positive results should be more prevalent among specimens from recovering TB patients who are on anti-TB chemotherapy but who are still shedding dead *M. tuberculosis*. This is indeed what was seen; however, our findings must be interpreted with some caution since SDA false-positive reactions may result from numerous causes, as previously discussed for PCR (1) and Q beta replicase (26).

Amplicon contamination may produce false-positive results, as seen in a recent multilaboratory study of PCR detection of *M. tuberculosis* in spiked clinical specimens. The sensitivity of detecting 1,000 *M. tuberculosis* organisms spiked into clinical specimens ranged from 2 to 90%, and these workers also found false-positive rates of as high as 77% because of cross-contamination (23). However, in the experiments reported herein, separate negative controls for 0 *M. tuberculosis* organisms and sample processing monitored levels of background reaction in each experiment. High backgrounds were factored out of the analysis since they were additive to the standard curves. Standard laboratory practices for DNA amplification were strictly followed (19). As such, we do not think that DNA carryover was a major contributor to the false-positive rate. We were also aware of the potential problem of cross-contamination by *M. tuberculosis* organisms and took care to avoid contact between pipettes and adjacent specimen tubes during processing.

Growth on solid medium, as done in these experiments, may be less sensitive than growth in liquid culture. In addition, the NALC sample processing method (16) is known to kill a fraction of mycobacteria in each specimen. Therefore, the culture results may have underrepresented the true number of *M. tuberculosis*-positive specimens.

The chemiluminescence assay probe presented another potential source of signal contamination. If general contamination occurred, the resulting high backgrounds would have been subtracted from the results. In contrast, well-to-well contamination in the microtiter plate would have been hard to detect and control for. Pilot studies indicated that it rarely occurred.

Finally, an erroneous standard curve that resulted in conversion of the SDA RLU results to an incorrect number of equivalent *M. tuberculosis* organisms might have resulted in SDA false-positive or false-negative results. If this happened, whole experiments would have been incorrectly scaled. However, since the ROC analysis in RLUs (method 1) gave results similar to those for the analysis of the same data after conversion to *M. tuberculosis* (method 3), a scaling error seems unlikely.

Since the factors that may cause false-positive determinations should have had equal effects on all of the specimens, they do not account for the differences in specificity that were seen between specimens of patients on anti-TB chemotherapy and those who were not. Therefore, we think the difference may have been due to the presence of DNA from nonviable *M. tuberculosis* in the specimens.

In summary, this is the first large-scale demonstration that SDA is capable of detecting *M. tuberculosis* in sputa. The finding of higher SDA specificity from culture-negative specimens of patients who did not receive anti-TB chemotherapy versus the specimens from patients undergoing treatment is consistent with previous PCR studies and suggests that *M. tuberculosis* detection by DNA amplification may be more accurate in specimens from newly identified patients who are not yet on therapy. However, our findings represent a limited set of specimens used in this study and as such must be confirmed by rigorous evaluation in a larger clinical population. This study also underscores the value of patient data for the validation of new *M. tuberculosis* detection systems.

ACKNOWLEDGMENTS

We thank our colleagues at the North Carolina Public Health Laboratory, the Durham Veterans' Administration Hospital, the Becton Dickinson Research Center, and Becton Dickinson Diagnostic Instrument Systems for their contributions.

REFERENCES

1. **Beige, J., J. Lokies, T. Schaberg, U. Finckh, M. Fischer, M. Mauch, H. Lode, B. Kohler, and A. Rolfs.** 1995. Clinical evaluation of a *Mycobacterium tuberculosis* PCR assay. J. Clin. Microbiol. **33:**90–95.
2. **Brisson-Noel, A., C. Aznar, C. Chureau, S. Nguyen, C. Pierre, M. Bartoli, R. Bonete, G. Pialoux, B. Gicquel, and G. Garrigue.** 1991. Diagnosis of tuberculosis by DNA amplification in clinical practice evaluation. Lancet **338:**364–366.
3. **Brisson-Noel, A., B. Gicquel, D. Lecossier, V. Levy-Frebault, X. Nassif, and A. J. Hance.** 1989. Rapid diagnosis of tuberculosis by amplification of mycobacterial DNA in clinical samples. Lancet **ii:**1069–1071.
4. **Butler, W. R., K. C. Jost, and J. O. Kilburn.** 1991. Identification of mycobacteria by high-performance liquid chromatography. J. Clin. Microbiol. **29:**2468–2472.

5. **Cone, R., A. C. Hobson, and M.-L. W. Huang.** 1992. Coamplified positive control detects inhibition of polymerase chain reactions. J. Clin. Microbiol. **30:**3185–3189.
6. **Cousins, D. V., S. D. Wilton, B. R. Francis, and B. L. Gore.** 1992. Use of polymerase chain reaction for rapid diagnosis of tuberculosis. J. Clin. Microbiol. **30:**255–258.
7. **Del Portillo, P., L. A. Murillo, and M. E. Patarroyo.** 1991. Amplification of a species-specific DNA fragment of *Mycobacterium tuberculosis* and its possible use in diagnosis. J. Clin. Microbiol. **29:**2163–2168.
8. **DeWit, D., M. Wooton, B. Allan, and L. Steyn.** 1993. Simple method for production of internal control DNA for *Mycobacterium tuberculosis* polymerase chain reaction assays. J. Clin. Microbiol. **31:**2204–2207.
9. **DeWit, D., S. Shoemaker, and M. Sogin.** 1990. Direct detection of *Mycobacterium tuberculosis* in clinical specimens by DNA amplification. J. Clin. Microbiol. **28:**2437–2441.
10. **Eisenach, K. D., M. D. Cave, J. H. Bates, and J. T. Crawford.** 1990. Polymerase chain reaction amplification of a repetitive DNA sequence specific for *Mycobacterium tuberculosis*. J. Infect. Dis. **161:**977–981.
11. **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.
12. **Folgueira, L., R. Delgado, E. Palenque, and A. R. Noriega.** 1993. Detection of *Mycobacterium tuberculosis* in clinical samples by using a simple lysis method and polymerase chain reaction. J. Clin. Microbiol. **31:**1019–1021.
13. **Forbes, B. A., and K. E. S. Hicks.** 1993. Direct detection of *Mycobacterium tuberculosis* in respiratory specimens in a clinical laboratory by polymerase chain reaction. J. Clin. Microbiol. **31:**1688–1694.
14. **Hanely, J. A.** 1989. Receiver operator characteristic (ROC) methodology: the state of the art. Crit. Rev. Diagn. Imaging **29:**307–335.
15. **Jonas, V., M. J. Alden, J. I. Curry, K. Kamisango, C. A. Knott, R. Lankford, J. M. Wolfe, and D. F. Moore.** 1993. Detection and identification of *Mycobacterium tuberculosis* directly from sputum sediments by amplification of rRNA. J. Clin. Microbiol. **31:**2410–2416.
16. **Kent, P. T., and G. P. Kubica.** 1985. Public health mycobacteriology, a guide for the level III laboratory. U.S. Department of Health and Human Services, Centers for Disease Control, Atlanta, Ga.
17. **Kocagoz, T., E. Yilmaz, S. Ozkara, S. Kocagoz, M. Hayran, M. Sachedeva, and H. F. Chambers.** 1993. Detection of *Mycobacterium tuberculosis* in sputum samples by polymerase chain reaction using a simplified procedure. J. Clin. Microbiol. **31:**1435–1438.
18. **Kolk, A. J. H., A. R. J. Schuitema, S. Kuijuper, J. Leeuwen, P. W. M. Hermans, J. D. A. Embden, and R. A. Hartskeerl.** 1992. Detection of *Mycobacterium tuberculosis* in clinical samples by using polymerase chain reaction and a nonradioactive detection system. J. Clin. Microbiol. **30:**2567–2575.
19. **Kwok, S., and R. Higuchi.** 1989. Avoiding false positives with PCR. Nature (London) **339:**237–238.
20. **Little, M. C., P. A. Spears, and D. D. Shank.** 1994. Nucleotide sequence and strand displacement amplification of the 70K protein gene from mycobacteria. Mol. Cell. Probes **8:**375–384.
21. **Miyazaki, Y., H. Koga, S. Kohno, and M. Kaku.** 1993. Nested polymerase chain reaction for detection of *Mycobacterium tuberculosis* in clinical samples. J. Clin. Microbiol. **31:**2228–2232.
22. **Nolte, F. S., B. Metchock, J. E. McGowan, A. Edwards, O. Okwumabua, C. Thurmond, P. S. Mitchell, B. Plikaytis, and T. Shinnick.** 1993. Direct detection of *Mycobacterium tuberculosis* in sputum by polymerase chain reaction and DNA hybridization. J. Clin. Microbiol. **31:**1777–1782.
23. **Noordhoek, G. T., A. H. J. Kolk, G. Bjune, D. Catty, J. W. Dale, P. E. M. Fine, P. Godfrey-Faussett, S.-N. Cho, T. Shinnick, S. B. Svenson, S. Wilson, and J. D. A. van Embden.** 1994. Sensitivity and specificity of PCR for detection of *Mycobacterium tuberculosis*: a blind comparison study among seven laboratories. J. Clin. Microbiol. **32:**277–284.
24. **Plikaytis, B., K. D. Eisenach, J. T. Crawford, and T. M. Shinnick.** 1991. Differentiation of *Mycobacterium tuberculosis* and *Mycobacterium bovis* BCG by a polymerase chain reaction assay. Mol. Cell. Probes **5:**215–219.
25. **Saiki, R. K., S. Scharf, F. Faloona, K. B. Mullis, G. T. Horn, H. A. Erlich, and N. Arnheim.** 1985. Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science **230:**1350–1354.
26. **Shah, J. S., J. Liu, D. Buxton, B. Stone, R. Nietupski, D. M. Olive, W. King, and J. D. Klinger.** 1995. Detection of *Mycobacterium tuberculosis* directly from spiked human sputum by Q-beta replicase amplified assay. J. Clin. Microbiol. **33:**322–328.
27. **Shawar, R. M., F. A. K. El-Zaatari, A. Nataraj, and J. E. Clarridge.** 1993. Detection of *Mycobacterium tuberculosis* in clinical samples by two-step polymerase chain reaction and nonisotopic hybridization methods. J. Clin. Microbiol. **31:**61–65.
28. **Spargo, C., 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.
29. **Sritharin, V., and R. Barker.** 1991. A simple method for diagnosing *M. tuberculosis* infection in clinical samples using PCR. Mol. Cell. Probes **5:**385–395.
30. **Thierry, D., A. Brisson-Noel, V. Vincent-Levy-Frebault, S. Nguyen, J.-L. Guesdon, and B. Gicquel.** 1990. Characterization of a mycobacterium tuberculosis insertion sequence, IS*6110*, and its application in diagnosis. J. Clin. Microbiol. **28:**2668–2673.
31. **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.
32. **Thierry, D., C. Chureau, C. Aznar, and J. L. Guesdon.** 1992. The detection of *Mycobacterium tuberculosis* in uncultured clinical specimens using the polymerase chain reaction and a non-radioactive probe. Mol. Cell. Probes **6:**181–191.
33. **Walker, G. T., M. S. Fraiser, J. L. Schram, M. C. Little, J. G. Nadeau, and D. P. Malinowski.** 1992. Strand displacement amplification—an isothermal, *in vitro* DNA amplification technique. Nucleic Acids Res. **20:**1691–1696.
34. **Walker, G. T., M. C. Little, J. G. Nadeau, and D. D. Shank.** 1992. Isothermal *in vitro* amplification of DNA by a restriction enzyme/DNA polymerase system. Proc. Natl. Acad. Sci. USA **89:**392–396.
35. **Walker, G. T., J. G. Nadeau, P. 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.
36. **Walker, G. T., C. A. Spargo, C. M. Nycz, J. A. Down, M. S. Dey, A. H. Walter, D. R. Howard, W. E. Keating, M. C. Little, J. G. Nadeau, S. R. Jurgensen, V. R. Neece, and P. Zwadyk, Jr.** 1995. A chemiluminescent DNA probe test based upon strand displacement amplification, p. 329. *in* D. L. Wiedbrauk and D. H. Farkas (ed.), Molecular methods for virus detection. Academic Press, San Diego, Calif.
37. **Zwadyk, P., 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.