JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1997, p. 3082–3085 Vol. 35, No. 12
0095-1137/97/$04.00+0

Diagnostic Value of the Strand Displacement Amplification Method Compared to Those of Roche Amplicor PCR and Culture for Detecting Mycobacteria in Sputum Samples

SATOSHI ICHIYAMA,[1,2]* YOSHIKA ITO,[1] FUMIYOSHI SUGIURA,[3] YOSHITSUGU IINUMA,[2,3] SADAAKI YAMORI,[3] MASAHIRO SHIMOJIMA,[4] YOSHINORI HASEGAWA,[2] KAORU SHIMOKATA,[5] AND NOBUO NAKASHIMA[1]

Departments of Clinical Laboratory Medicine[1] and Clinical Preventive Services,[5] Nagoya University Hospital, and First Department of Internal Medicine, Nagoya University School of Medicine,[2] Showa-ku, Nagoya 466, Department of Internal Medicine, Chubu National Hospital, Obu 474,[3] and Department of Bacteriology, BML Research Laboratories, Sendagaya, Shibuya-ku, Tokyo 151,[4] Japan

Received 31 March 1997/Returned for modification 31 May 1997/Accepted 28 August 1997

We compared the ability of the semiautomated BDProbeTec-SDA system, which uses the strand displacement amplification (SDA) method, with that of the Roche Amplicor-PCR system and the Septi-Chek AFB culture system to directly detect *Mycobacterium tuberculosis* complex (MTB) and other mycobacteria in sputum samples. A total of 530 sputum samples from 299 patients were examined in this study. Of the 530 samples, 129 were culture positive for acid-fast bacilli with the Septi-Chek AFB system; 95 for MTB, 29 for *M. avium-M. intracellulare* complex (MAC), and 5 for other mycobacteria. The BDProbeTec-SDA system detected 90 of the 95 samples culture positive for MTB (sensitivity, 94.7%), and the Amplicor-PCR system detected 85 of the 95 samples culture positive for MTB (sensitivity, 89.5%). The specificity of each system, based on the clinical diagnosis, was 99.8% for SDA and 100% for PCR, respectively. Among the 29 samples culture positive for MAC, the BDProbeTec-SDA system detected MAC in 24 samples (sensitivity, 82.8%), whereas the Amplicor-PCR system detected MAC in 23 samples (sensitivity, 79.3%). The specificities of the systems were 98.3 and 100%, respectively. The high degrees of sensitivity and specificity of the BDProbeTec-SDA system suggest that it should be very useful in clinical laboratories for the rapid detection of mycobacteria in sputum samples.

Various amplification methods for the direct detection and identification of mycobacteria in clinical samples have been developed. PCR is one of the most important features of these methods. The sensitivities and specificities of in-house PCRs have varied significantly, reflecting not only the operational skills of each laboratory but also the target regions of nucleic acids and the amplified products detected by each system (1, 7, 16, 20–22, 24). In attempts to standardize the laboratory application of such methods, several amplification kits for detecting mycobacteria have been developed. The PCR-based Roche Amplicor Mycobacterium system (Roche, Basel, Switzerland) for detecting *Mycobacterium tuberculosis* complex (MTB), *M. avium*, and *M. intracellulare* (2–4, 6, 8, 9, 13, 17, 22, 24, 30, 32) and the rRNA amplification-based Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test system (MTD; Gen-Probe Inc., San Diego, Calif.) for detecting MTB (1, 13, 15, 16, 18, 22, 23, 29, 30) are now commercially available. However, the reported sensitivities of these kits are insufficient for smear-negative samples (1, 3, 4, 8, 16, 17, 32). Other amplification methods, such as ligase chain reaction (Abbott Laboratories, Chicago, Ill.) (14), Q-beta replicase-amplified assay (GENE-TRAK, Framingham, Mass.) (25), and a nucleic acid sequence-based amplification assay (Organon Teknika, Amsterdam, The Netherlands) (28) are now being clinically evaluated for their efficacies at detecting mycobacteria.

A new amplification method for detecting mycobacteria, strand displacement amplification (SDA; Becton Dickinson Microbiology Systems, Sparks, Md.) (11, 12, 26, 31), has recently been developed. SDA is a novel method in which DNA segments of the IS*6110* repetitive element specific to MTB (27), together with a segment of the 16S rRNA gene common to all members of genus *Mycobacterium* (5), are amplified isothermally. The BDProbeTec-SDA system (Becton Dickinson), which uses this method, is capable of automatically performing rapid detection of mycobacteria. In the present study, we compared the ability of the BDProbeTec-SDA system to directly detect MTB and other mycobacteria in sputum samples with those of the Amplicor-PCR system and a liquid culture method, the Septi-Chek AFB system (Becton Dickinson).

MATERIALS AND METHODS

Sample processing and culture. Clinical samples were obtained from patients being followed during antituberculosis chemotherapy and from those suspected of having mycobacterial infection or other pulmonary diseases. A total of 530 sputum samples from 299 patients were examined in this study: 265 samples from Chubu National Hospital, Aichi Prefecture, Japan, collected between October 1995 and February 1996; 99 samples from Nagoya University Hospital, Nagoya, Japan, collected between September and December 1996; and 166 samples from the BML Research Laboratories, Tokyo, Japan, collected between March and May 1996. All samples were decontaminated by treatment with an equal volume of *N*-acetyl-L-cysteine (NALC)-NaOH (final concentration, 2%) for 15 min at room temperature and were neutralized with sterile 0.067 M phosphate buffer (pH 6.8). After centrifugation at 3,000 × *g* for 15 min, each pellet was resuspended in 2.0 ml of sterile 0.067 M phosphate buffer (pH 6.8). The 265 specimens from Chubu National Hospital were subjected to auramine-rhodamine staining for the detection of acid-fast bacilli. Culture for mycobacteria was performed by inoculation of 0.5 ml of the decontaminated material into a Septi-Chek AFB vial at 35°C, and the vials were monitored for growth of mycobacteria for 8 weeks. Growth- positive cultures were confirmed to contain acid-fast bacilli by Ziehl-Neelsen staining. The remaining 265 specimens from Nagoya University Hospital and the BML laboratory were screened, and growth-positive cultures were confirmed to contain acid-fast bacilli by Ziehl-Neelsen staining. Mycobacterial cul-

* Corresponding author. Mailing address: Department of Clinical Laboratory Medicine, Nagoya University Hospital, 65 Tsurumai-cho, Showa-ku, Nagoya 466, Japan. Phone: (81)-52-744-2614. Fax: (81)-52-744-2613. E-mail: sichiyam@tsuru.med.nagoya-u.ac.jp.

TABLE 1. Detection of MTB in sputum samples with BDProbeTec-SDA and Amplicor-PCR systems[a]

Culture result (no. of samples)	No. of samples with the indicated result with the following system:			
	BDProbeTec-SDA system		Amplicor-PCR system	
	Positive	Negative	Positive	Negative
Positive (95)	90	5	85	10
Negative (401)	55	346	50	351

[a] Samples yielding initially discrepant results between the BDProbeTec-SDA and Amplicor-PCR systems were retested with each system to confirm the results. However, the test results from assays with one or the other system for 19 of these samples could not be reproduced. Due to our limited sample volumes, the result of the initial or repeat test could not be confirmed, and, hence, the results for these 19 samples were excluded from the analyses in Tables 1 and 2. Samples from which mycobacteria other than MTB had been cultured were excluded from this analysis. These samples included sputa from patients during or after antituberculosis chemotherapy, patients with tuberculosis before chemotherapy, and patients who did not have tuberculosis.

ture in these centers was performed by inoculation into a Septi-Chek vial and two plates of Ogawa egg medium. The isolated mycobacteria were identified with chemiluminescent DNA probes (AccuProbe; Gen-Probe), by the DNA-DNA hybridization method (DDH-Mycobacteria; Kyokuto Pharmaceutical Co., Tokyo, Japan), or by standard biochemical tests (19). The remaining decontaminated suspension of each sample was stored at −80°C prior to amplification testing (from several days to 5 months).

BDProbeTec-SDA system. Tests with the BDProbeTec-SDA system involve four steps: sample preparation with NALC-NaOH and heating, decontamination to remove contaminating amplicons, amplification by SDA, and detection reactions. Each NALC-NaOH-treated specimen (250 μl) was transferred to a sample processing tube into which 1.0 ml of the sample diluent had been placed. After mixing and centrifugation at 12,200 × *g* for 3 min, the supernatant was decanted. A sample processing capsule and 1.0 ml of the sample diluent were added to the tube, which was vortex mixed for 5 s. Positive and negative controls were prepared in the same manner. The tube was placed in the Lysolyzer (Becton Dickinson) for incubation at 105°C for 1 h. The tube was then agitated in FastPrep (Becton Dickinson) for 45 s to break the capsule containing zirconium and silica for mechanical disruption of the cell walls of the mycobacterial organisms in the sample by the zirconium and silica. If the capsule in the tube failed to rupture, agitation was repeated. The specimen was then subjected to analysis with the automated bench-top BDProbeTec analyzer for amplification and hybridization-detection reactions. This analyzer automatically handles the reagents, performs the amplification, and detects the amplified products. The basis and details of the entire system have been described in previous papers (11, 12, 26, 31). Decontamination with uracil DNA glycosylase is included in this system to reduce the carryover of previously amplified products. Each NALC-NaOH-treated and heat-lysed specimen (55 μl) was subjected to a test for the coamplification of the IS*6110* element for the detection of MTB and the 16S rRNA gene for the detection of other mycobacteria. In addition, positive and negative controls for the detection of MTB and other mycobacteria and an internal control for monitoring SDA suppression were tested. The amplified products were allowed to hybridize with probes in solution and were detected with a solid-phase solution and detected with a solid-phase chemiluminescent capture probe. Samples positive for MTB were IS*6110* positive and 16S rRNA positive, whereas samples positive for mycobacteria other than MTB were IS*6110* negative and 16S rRNA positive. The total time required by the instrument for decontamination, amplification, and detection was less than 6 h. Up to 48 specimens could be processed in a run.

Amplicor-PCR system. Each NALC-NaOH-treated specimen (100 μl) was examined with the Amplicor-PCR system according to the instructions of the manufacturer.

RESULTS AND DISCUSSION

Among the 530 sputum samples, 129 were culture positive for acid-fast bacilli with the Septi-Chek AFB system: 95 for MTB, 29 for *M. avium-M. intracellulare* complex (MAC), 3 for *M. kansasii*, 1 for *M. abscessus*, and 1 for an unidentified mycobacterium.

Table 1 presents the results for the detection of MTB by the two amplification systems, the BDProbeTec-SDA and Amplicor-PCR systems, from 496 samples, excluding 34 samples culture positive for mycobacteria other than MTB. The BDProbeTec-SDA system detected MTB in 90 of the 95 samples culture positive for MTB (sensitivity, 94.7%). Among the 401 culture-negative samples, 55 were SDA positive (specificity, 86.3%). The Amplicor-PCR system detected MTB in 85 of the 95 samples culture positive for MTB (sensitivity, 89.5%) and 50 of the 401 samples culture negative for MTB (specificity, 87.5%). With the exception of one sample positive with the BDProbeTec-SDA system, these amplification-positive but culture-negative samples had been obtained from patients with tuberculosis during or after antituberculosis chemotherapy. Therefore, on the basis of the clinical diagnosis the revised specificities were 99.8% for SDA and 100% for PCR. These discrepant results might have been due to the detection of nonviable organisms by PCR or SDA and to the lack of use of solid media for culture for the 265 samples from Chubu National Hospital. The agreement between the results obtained with the BDProbeTec-SDA system and the Amplicor-PCR system for the detection of MTB was 96.4% (478 of 496 samples). There were 4 SDA-negative but PCR-positive samples and 14 SDA-positive but PCR-negative samples. Two of the 4 SDA-negative but PCR-positive samples and 7 of the 14 SDA-positive but PCR-negative samples were positive for MTB by culture.

Table 2 presents the results of detection of MAC by the two systems in 430 samples, excluding 100 culture-positive samples for mycobacteria other than MAC. Among the 29 samples culture positive for MAC, the BDProbeTec-SDA system detected MAC in 24 samples (sensitivity, 82.8%), whereas the Amplicor-PCR system detected MAC in 23 samples (sensitivity, 79.3%). The specificities of the systems were 98.3% (394 of 401 samples) and 100% (401 of 401 samples), respectively. Since the BDProbeTec-SDA system amplifies a segment of the 16S rRNA gene common to all members of the genus *Mycobacterium*, some of the seven SDA-positive but culture-negative samples might have had those results because of the detection of mycobacteria other than MTB or MAC. The level of agreement between these systems for the detection of MAC was 96.7% (416 of 430 samples). There were 3 SDA-negative but PCR-positive samples and 11 SDA-positive but PCR-negative samples. All 3 SDA-negative but PCR-positive samples and 4 of the 11 SDA-positive but PCR-negative samples were culture positive for MAC. Of the five samples which yielded positive results by culture for mycobacteria other than MTB or MAC, only one sample culture positive for *M. kansasii* was detected with the BDProbeTec-SDA system.

The application of amplification assays to the diagnosis of tuberculosis has been widely reported in the literature. The rRNA-based MTD system (Gen-Probe) and the Amplicor-PCR system (Roche) are commercially available in the United States, Europe, and Japan. The U.S. Food and Drug Admin-

TABLE 2. Detection of MAC in sputum samples with the BDProbeTec-SDA and Amplicor PCR systems[a]

Culture result (no. of samples)	No. of samples with the indicated result with the following system:			
	BDProbeTec-SDA system		Amplicor-PCR system	
	Positive	Negative	Positive	Negative
Positive (29)	24	5	23	6
Negative (401)	7	394	0	401

[a] Samples from which mycobacteria other than MAC had been cultured were excluded from this analysis.

istration has recommended the clinical use of the MTD system only with smear-positive samples because of the lack of sufficient sensitivity with smear-negative samples and the high cost of this system. The cost implications of using amplification test kits are still an issue in clinical laboratories (10).

For the detection of MTB, we found that the BDProbeTec-SDA system had an excellent sensitivity (94.7%). This system also showed a sensitivity of 75%, as an initial detection test, for the eight smear-negative, culture-positive samples from patients with no history of antituberculosis chemotherapy. The high sensitivity of this system might be due in part to the mechanical disruption of organisms in the processing steps, but it is more likely to be principally due to the amplification of sequences in the repetitive IS*6110* element in MTB. Differences in the volumes of the original specimen do not account for the differences in sensitivity, since the BDProbeTec-SDA system actually tests less (13.75 μl) of the original NALC-NaOH-treated specimen than the Amplicor-PCR system does (25 μl). For the detection of MAC, in which both systems amplify a single copy of the 16S rRNA gene sequence, the sensitivities of the two systems were similar, 82.8% for the BDProbeTec-SDA system and 79.3% for the Amplicor-PCR system. With the exception of one sample, no false-positive result due to amplicon contamination was obtained with the BDProbeTec-SDA or the Amplicor-PCR system. In both systems, a single-tube method is used for lysis of the organisms and decontamination with uracil DNA glycosylase. These methods for protection against cross-contamination might contribute to the extremely low rates of false-positive results.

The BDProbeTec-SDA system is semiautomated and can process up to 48 samples in a single run in under 6 h (for decontamination, amplification, and detection). Because of its high sensitivity and specificity, the BDProbeTec-SDA system can be recommended for use in clinical laboratories for the rapid detection of mycobacteria from sputum samples.

ACKNOWLEDGMENT

We thank Nippon Becton Dickinson Co. Ltd. for supplying the BDProbeTec-SDA kits and supporting us financially.

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. **Beavis, K. G., M. B. Lichty, D. L. Jungkind, and O. Giger.** 1995. Evaluation of Amplicor PCR for direct detection of *Mycobacterium tuberculosis* from sputum specimens. J. Clin. Microbiol. **33:**2582–2586.
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. **Bergmann, J. S., and G. L. Woods.** 1996. Clinical evaluation of the Roche AMPLICOR PCR *Mycobacterium tuberculosis* test for detection of *M. tuberculosis* in respiratory specimens. J. Clin. Microbiol. **34:**1083–1085.
5. **Böddinghaus, B., T. Rogall, T. Flohr, H. Blocker, and E. C. Böttger.** 1990. Detection and identification of mycobacteria by amplification of rRNA. J. Clin. Microbiol. **28:**1751–1759.
6. **Carpentier, E., B. Drouillard, M. Dailloux, D. Moinard, E. Vallee, B. Dutilh, J. Maugein, E. Bergogne-Berezin, and B. Carbonnelle.** 1995. Diagnosis of tuberculosis by Amplicor Mycobacterium Tuberculosis test: a multicenter study. J. Clin. Microbiol. **33:**3106–3110.
7. **Clarridge, J. E., III, R. M. Shawar, T. M. Shinnick, and R. 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.
8. **D'Amato, R. F., A. A. Wallman, L. H. Hochstein, P. M. Colaninno, M. Scardamaglia, E. Ardila, M. Ghouri, K. Kim, R. C. Patel, and A. Miller.** 1995. Rapid diagnosis of pulmonary tuberculosis by using Roche AMPLICOR *Mycobacterium tuberculosis* PCR test. J. Clin. Microbiol. **33:**1832–1834.
9. **Devallois, A., E. Legrand, and N. Rastogi.** 1996. Evaluation of Amplicor MTB test as adjunct to smears and culture for direct detection of *Mycobacterium tuberculosis* in the French Caribbean. J. Clin. Microbiol. **34:**1065–1068.
10. **Doern, G. V.** 1996. Diagnostic mycobacteriology: where are we today? J. Clin. Microbiol. **34:**1873–1876.
11. **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.
12. **Hellyer, T. J., T. W. Fletcher, J. H. Bates, W. W. Stead, G. L. Templeton, M. D. Cave, and K. D. Eisenach.** 1996. Strand displacement amplification and the polymerase chain reaction for monitoring response to treatment in patients with pulmonary tuberculosis. J. Infect. Dis. **173:**934–941.
13. **Ichiyama, S., Y. Iinuma, Y. Tawada, S. Yamori, Y. Hasegawa, K. Shimokata, and N. Nakashima.** 1996. Evaluation of Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test and Roche PCR-microwell plate hybridization method (AMPLICOR MYCOBACTERIUM) for direct detection of mycobacteria. J. Clin. Microbiol. **34:**130–133.
14. **Iovannisci, D. M., and E. S. Winn-Deen.** 1993. Ligation amplification and fluorescence detection of *Mycobacterium tuberculosis* DNA. Mol. Cell. Probes **7:**35–43.
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. **Miller, N., S. G. Hernandes, and T. J. Cleary.** 1994. Evaluation of Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test and PCR for direct detection of *Mycobacterium tuberculosis* in clinical specimens. J. Clin. Microbiol. **32:**393–397.
17. **Moore, D. F., and J. I. Curry.** 1995. Detection and identification of *Mycobacterium tuberculosis* directly from sputum samples by Amplicor PCR. J. Clin. Microbiol. **33:**2686–2691.
18. **Moore, D. F., J. I. Curry, C. A. Knott, and V. Jonas.** 1996. Amplification of rRNA for assessment of treatment response of pulmonary tuberculosis patients during antimicrobial therapy. J. Clin. Microbiol. **34:**1745–1749.
19. **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, 6th ed. American Society for Microbiology, Washington, D.C.
20. **Nolte, F. S., B. Metchock, J. E. McGowan Jr., 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.
21. **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.
22. **Noordhoek, G. T., J. D. A. van Embden, and A. H. J. Kolk.** 1996. Reliability of nucleic acid amplification for detection of *Mycobacterium tuberculosis*: an international collaborative quality control study among 30 laboratories. J. Clin. Microbiol. **34:**2522–2525.
23. **Pfyffer, G. E., P. Kissling, R. Wirth, and R. Weber.** 1994. Direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens by a target-amplified test system. J. Clin. Microbiol. **32:**918–923.
24. **Schirm, J., L. A. B. Oostendorp, and J. G. Mulder.** 1995. Comparison of Amplicor, in-house PCR, and conventional culture for detection of *Mycobacterium tuberculosis* in clinical samples. J. Clin. Microbiol. **32:**3221–3224.
25. **Shah, J. S., J. Liu, D. Buxton, A. Hendricks, L. Robinson, G. Radcliffe, W. King, D. Lane, D. M. Olive, and J. D. Klinger.** 1995. Q-Beta replicase-amplified assay for detection of *Mycobacterium tuberculosis* directly from clinical specimens. J. Clin. Microbiol. **33:**1435–1441.
26. **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.
27. **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.
28. **Van der Vliet, G. M. E., R. A. F. Schukkink, B. van Gemen, P. Schepers, and P. R. Klatser.** 1993. Nucleic acid sequence-based amplification (NASBA) for the identification of mycobacteria. J. Gen. Microbiol. **139:**2423–2429.
29. **Vlaspolder, F., P. Singer, and C. Roggeveen.** 1995. Diagnostic value of an amplification method (Gen-Probe) compared with that of culture for diagnosis of tuberculosis. J. Clin. Microbiol. **33:**2699–2703.
30. **Vuorinen, P., A. Miettinen, R. Vuento, and O. Hallstrom.** 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.

31. **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.
32. **Wobeser, W. L., M. Krajden, J. Conly, H. Simpson, B. Yim, M. D'Costa, M. Fuksa, C. Hian-Cheong, M. Patterson, A. Phillips, R. Bannatyne, A. Haddad, J. L. Brunton, and S. Krajden.** 1996. Evaluation of Roche Amplicor PCR assay for *Mycobacterium tuberculosis*. J. Clin. Microbiol. **34:**134–139.