JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2005, p. 5435–5439 Vol. 43, No. 11
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.11.5435–5439.2005

Rapid Detection of *Mycobacterium tuberculosis* in Respiratory Samples by Transcription-Reverse Transcription Concerted Reaction with an Automated System

Shunji Takakura,[1]* Shigeo Tsuchiya,[2] Yuichi Isawa,[2] Kiyoshi Yasukawa,[2]† Toshinori Hayashi,[2] Motohisa Tomita,[3] Katsuhiro Suzuki,[3] Tatsuro Hasegawa,[4] Takanori Tagami,[4] Atsuyuki Kurashima,[4] and Satoshi Ichiyama[1]

Department of Clinical Laboratory Medicine, Kyoto University Graduate School of Medicine, 54 Shogoin-Kawaharacho, Sakyo-ku, Kyoto 6068507, Japan[1]; Scientific Instruments Division, Tosoh Corporation, 2743-1 Hayakawa, Ayase, Kanagawa 2521123, Japan[2]; Clinical Research Center, Kinki-chuo Chest Medical Center, 1180 Nagasone-cho, Sakai, Osaka 5918555, Japan[3]; and National Tokyo Hospital, 3-1-1 Takeoka, Kiyoshe-shi, Tokyo 2048585, Japan[4]

Received 6 June 2005/Returned for modification 28 June 2005/Accepted 10 August 2005

The aim of this study was to evaluate the performance of the transcription-reverse transcription concerted (TRC) method for the detection of *Mycobacterium tuberculosis* complex (MTC) 16S rRNA in clinical respiratory samples for the diagnosis of pulmonary tuberculosis. TRC is a novel method that enables the rapid and the completely homogeneous real-time monitoring of isothermal sequence RNA amplification without any post-amplification procedure. The detection limit of the TRC method for MTC was one organism per 100 μl of sputum. The specificity of the method was confirmed by the absence of positive signals for sputum containing 10^6 *M. avium* or *M. kansasii* organisms per 100 μl. A total of 201 respiratory samples from patients diagnosed with or suspected of having tuberculosis were tested. Of the 72 MTC culture-positive samples, the TRC method was positive for 52 (sensitivity, 72.2%), whereas the Roche COBAS AMPLICOR PCR was positive for 58 (sensitivity, 80.6%). Both the TRC method and the COBAS AMPLICOR PCR showed no positive identification for any of the 129 culture-negative samples. The percent agreement between the two methods was 95% (191 of 201 samples). The high sensitivity and specificity together with shorter detection time (within 1 h) of the TRC method allow it to be proposed as a useful method for the rapid detection of MTC in respiratory samples.

The rapid detection and identification of *Mycobacterium tuberculosis* complex (MTC) in respiratory samples are extremely important for optimal diagnosis and effective treatment, as well as for prevention and control of tuberculosis transmission. Various molecular tests based on amplification and detection techniques have been devised for the detection of MTC in clinical samples (2, 14), such as the PCR-based COBAS AMPLICOR Mycobacterium system (Roche Diagnostics, Basel, Switzerland) (4–7), the transcription-mediated amplification-based Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test system (Gen-Probe Inc., San Diego, CA) (5, 15, 16), the strand displacement amplification-based BDProbeTec ET system (Becton Dickinson, Franklin Lakes, NJ) (6, 8), and the ligase chain reaction-based Abbott LCx Mycobacterium tuberculosis assay system (Abbott Laboratories, North Chicago, IL) (1). While they are much more rapid than any culture-based method, these systems still require several hours to get results and involve some complicated procedures (14). Therefore, a faster molecular test with greater ease of manipulation as well as high sensitivity and specificity is desirable.

Recently, we have reported on a novel method designated the transcription-reverse transcription concerted (TRC) method (9). This method, a schematic of which is shown in Fig. 1, is based on isothermal RNA amplification at 43°C with transcriptase and reverse transcriptase in the presence of the intercalation activating fluorescence (INAF) probe (10). Measurement of the fluorescence intensity of the reaction mixture with a dedicated multicolor detector enables completely homogeneous real-time monitoring of the amplification of specific RNA, while it requires only 30 min for simultaneous amplification and detection. We have used the TRC method to establish a system for the detection of specific mRNA transcripts: *tdh* and *trh* of *Vibrio parahaemolyticus* (13), *mecA* of methicillin-resistant *Staphylococcus aureus* (11), and *pab* of *M. tuberculosis* (9, 19).

This report concerns the establishment and evaluation of the TRC method-based targeting of MTC 16S rRNA (hereinafter abbreviated the "TRC method") for the direct detection of MTC in clinical respiratory samples. rRNA was chosen as the target to enable highly selective and sensitive detection because of its multicopy nature in a single cell. The sensitivity and specificity of the TRC method were compared with those of the COBAS AMPLICOR PCR for the direct detection of MTC in sputum samples.

MATERIALS AND METHODS

Preparation of standard RNAs for calibration. Standard RNA containing the target region for TRC amplification was prepared by the in vitro transcription of the SP6 promoter-bearing double-stranded DNA as the template for SP6 RNA

* Corresponding author. Mailing address: Department of Clinical Laboratory Medicine, Kyoto University Graduate School of Medicine, 54 Shogoin-Kawaharacho, Sakyo-ku, Kyoto 6068507, Japan. Phone: 81-75-751-3503. Fax: 81-75-751-3233. E-mail: stakakr@kuhp.kyoto-u.ac.jp.

† Present address: Department of Food Biology, Kyoto University Graduate School of Agricultural Science, Oiwake-cho, Kitashirakawa, Sakyo-ku, Kyoto 6068502, Japan.

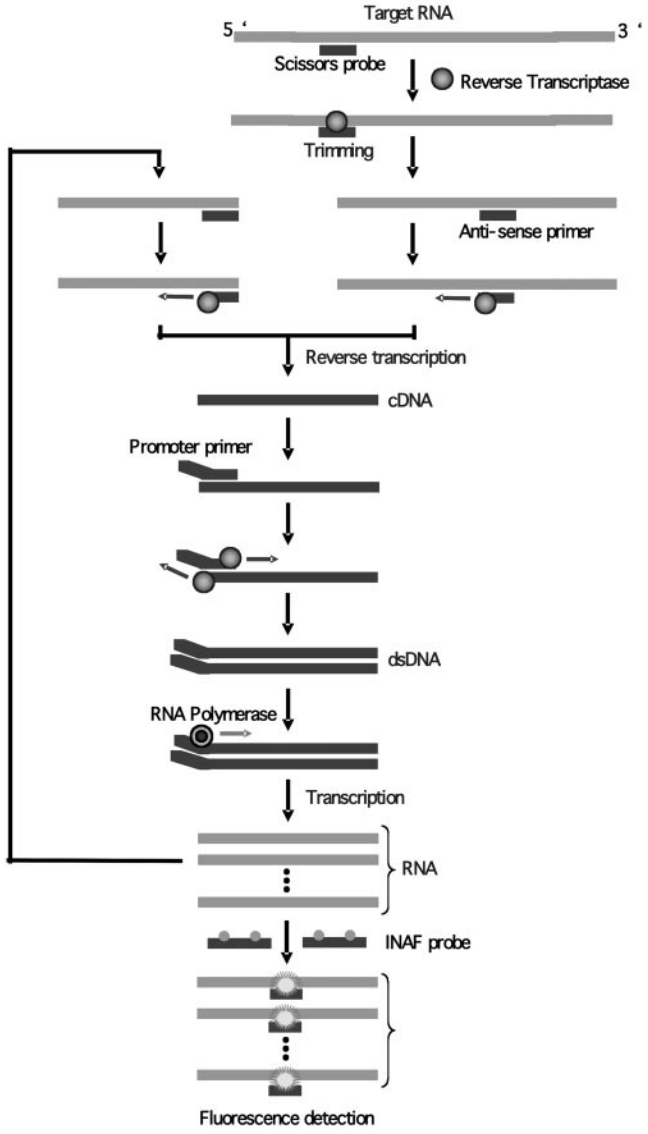

FIG. 1. Schematic description of the elementary steps of the TRC method. The progress of the reaction is monitored by measuring the fluorescence intensity of the reaction mixture. dsRNA, double-stranded RNA.

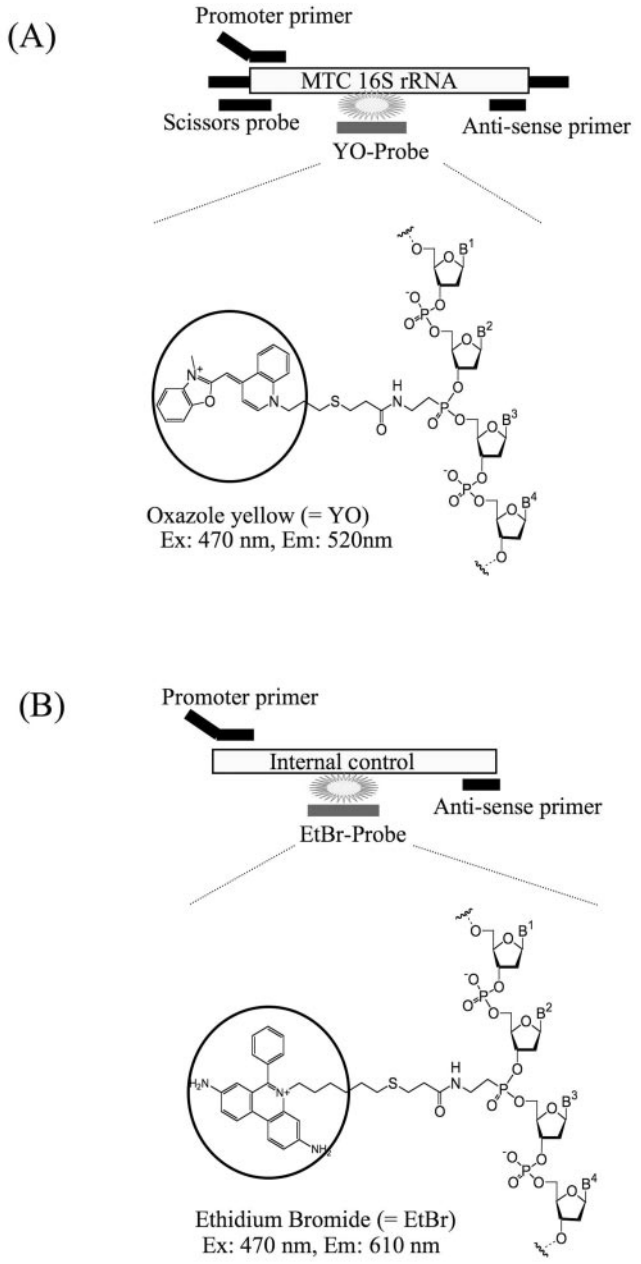

FIG. 2. Primers and probes used in the TRC method designed for amplification and detection of MTC 16S rRNA (A) and internal control (B). Ex, excitation wavelength; Em, emission wavelength.

polymerase. The DNA templates were synthesized from the total DNA extracted from the *Mycobacterium bovis* BCG strain (TOKYO 172; purchased from the National Institute of Infectious Diseases, Tokyo, Japan) by means of PCR (30 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 3 min) with a pair of synthetic oligonucleotide primers, 5′-CGG TAC CCA TTT AGG TGA CAC TAT AGA ATA CAA GTT TTG TTT GGA GAG TTT GAT CC-3′ and 5′-CGG TAC CCC TAC AGA CAA GAA CCC CTC A-3′. The long primer has the SP6 RNA polymerase-binding sequence at its 5′ end (underlined) to provide the preferred transcription initiation site.

The amplified gene sequence was cloned into the EcoRI site of pUC19. The plasmid DNAs were then digested with EcoRI to yield linear DNAs containing the SP6 promoter, followed by in vitro transcription in a reaction mixture composed of 40 mM Tris-HCl (pH 7.5), 6 mM $MgCl_2$, 2 mM spermidine, 10 mM dithiothreitol, 0.5 mM nucleoside triphosphates, 0.1 mg/ml of bovine serum albumin, 1 U/ml of RNase inhibitor, 2.5 U/ml of SP6 RNA polymerase (TaKaRa Bio, Otsu, Shiga, Japan), and 0.025 mg/ml of template DNA. The resultant RNAs were purified by gel filtration with Chromaspin-100 columns (BD Biosciences, Palo Alto, CA). The concentration of the purified RNA was determined spectrophotometrically at an optical density at 260 nm and adjusted to 10^2 to 10^7 copies/5 ml with TE (Tris-EDTA) buffer containing 0.25 U/ml of RNase inhibitor and 5 mM dithiothreitol. The RNAs were then stored at −20°C until use.

Primers, probe, and internal control for the detection of *M. tuberculosis* complex by TRC method. Synthetic oligonucleotides used for the TRC reaction included a pair of amplification primers (designated the promoter primer [5′-AAT TCT AAT ACG ACT CAC TAT AGG GAG ACG GAA AGG TCT CTT CGG AGA TAC-3′] and the antisense primer [5′-ACA AGA CAT GCA TCC CGT-3′]), a scissors probe (5′-TTT CCG TTC GAC TTG CAT GTG TTA-3′) to initiate the TRC reaction, and an INAF probe (5′-CGA AGT GCA GGG C*AG ATC, where the asterisk indicates the base position linked by oxazole yellow) to detect the RNA amplicons. For the specific detection of MTC, primers were designed to amplify base positions 313 to 443 of *M. tuberculosis* 16S rRNA (GenBank accession no. Z83862). As shown in Fig. 2, oxazole yellow-linked and ethidium bromide-linked INAF probes were synthesized for the homogeneous and simultaneous detection of the target 16S rRNA and the internal amplifica-

tion control in the same tube by means of two-color fluorescence monitoring of the reaction mixture at 520 nm and 610 nm, respectively. For the experiments to be able to evaluate the species specificity of the assay, the 16S rRNAs of 16 mycobacterial species other than MTC were prepared in the same manner described above for MTC 16S rRNA. These species comprised *M. avium*, *M. intracellulare*, *M. kansasii*, *M. simiae*, *M. scrofulaceum*, *M. gordonae*, *M. szulgai*, *M. gastri*, *M. xenopi*, *M. nonchromogenicum*, *M. terrae*, *M. triviale*, *M. fortuitum*, *M. chelonae*, *M. abscessus*, and *M. peregrinum*.

TRC method for detection of *M. tuberculosis* complex. The principles of amplification and fluorescence-based detection have been described elsewhere (9). Briefly, 20 μl of the TRC buffer, consisting of a mixture of the substrate solution and the primer solution at a 1:1 (vol/vol) ratio, was added to 5 μl of the nucleic acid extract in a thin-walled PCR tube (Applied Biosystems, Foster City, CA). The basic composition of the reaction mixture was the same as that described previously (9), with the exception of a slight modification consisting of the omission of RNase H. The tube was then set in the dedicated instrument, a TRCR-160 real-time monitor (Tosoh Co., Tokyo, Japan), for 5 min while being preheated to 43°C, followed by the addition of 5 μl of the enzyme solution. The TRCR-160 real-time monitor consists of a square incubator block maintained at 43°C and a sliding fluorescence scanning unit. The latter comprises a light-emitting diode light source to irradiate the excitation light (470 nm) from below into the tube from the bottom and a light guide to collect the fluorescence from the bottom of the reaction tube in two photomultiplier tubes (520 nm and 610 nm) at 1-min intervals. The reaction time required for fluorescence enhancement to reach a cutoff value of 1.2 was adopted as the detection time for the TRC assay. The samples for which the detection time was ≤30 min were considered to have a positive signal, and those with positive signals at 520 nm were judged to be positive. Samples without a positive signal at 520 nm and with a positive signal at 610 nm were considered negative, and those with negative signals at both 520 nm and 610 nm were considered indeterminate.

Sample processing for detection of MTC in experimental sputum samples by TRC method. *Mycobacterium* culture-negative, clinical sputum specimens were obtained from patients diagnosed as not having mycobacterial infection at Kyoto University Hospital, Kyoto, Japan. The samples were mixed and homogenized by vigorous vortexing and were decontaminated by treatment with N-acetyl-L-cysteine (NALC)–NaOH. Various amounts of *M. bovis* BCG (10^0 to 10^4 cells per 100 μl), *M. avium* (10^2 to 10^6 cells per 100 μl), and *M. kansasii* (10^2 to 10^6 cells per 100 μl) were added to these culture-negative sputum specimens. Pretreatment consisted of mixing of 100 μl of the decontaminated samples with 500 μl of 67 mM phosphate buffer (pH 8.6) containing glass beads. After centrifugation at 3,000 × *g* for 5 min at room temperature, the supernatant was removed, followed by the addition of 300 μl of extraction buffer containing 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 0.25 U/μl RNase inhibitor, and 1 mM dithiothreitol. After sonication for 5 min, followed by centrifugation at 3,000 × *g* for 5 min, 5 μl of the supernatant was used as the template.

Sample processing for detection of MTC in clinical sputum samples by culture, TRC method, and COBAS AMPLICOR PCR. Clinical samples were obtained from patients diagnosed with or suspected of having a mycobacterial infection at the National Central Hospital for Chest Disease, Osaka, Japan, and the National Tokyo Hospital, Tokyo, Japan. All samples were decontaminated by treating them by a commercially available NALC-NaOH-based method, CC-E Nichibi (Japan BCG Laboratory, Tokyo, Japan) or BBL Micoprep (Becton Dickinson, Franklin Lakes, NJ). After centrifugation at 3,000 × *g* for 15 min at room temperature, the sediment was resuspended in 1.0 ml of phosphate buffer (pH 7.0). A smear of the decontaminated suspension was stained with the Ziehl-Neelsen stain, and a 500-μl aliquot of the suspension was cultivated in the BACTEC MGIT 960 liquid culture system (Becton Dickinson) for 6 weeks, in accordance with the manufacturer's recommendations. The remaining decontaminated suspension of the samples was immediately used or was stored at −20°C until use. The TRC method was applied to 100 μl of the suspension; the Roche COBAS AMPLICOR PCR was applied to 200 μl of the suspension, which had been treated with the Amplicor Mycobacterium Specimen Pretreatment Set II (Roche Diagnostics). The isolated mycobacteria were identified by an immunochromatographic assay with the anti-MPB64 antibody (Capillia; Becton Dickinson), a chemiluminescent DNA hybridization probe assay (AccuProbe; Gen-Probe Inc.), or a DNA hybridization assay (DDH Mycobacteria; Kyokuto Pharmaceutical Co., Tokyo, Japan).

RESULTS

Sensitivity and specificity of TRC assay with experimental samples. In the experiment with the standard RNA calibrator for the TRC assay (Fig. 3), the time to detection depended on the initial copy number, which ranged from 10^2 to 10^5. For the internal control, the time to detection at 610 nm remained approximately constant at about 10 min, irrespective of the initial copy number. The results for MTC detection in experimental sputum samples containing BCG by the TRC method are shown in Table 1. The detection limit for BCG was one organism per 100 μl of sputum, and the time to detection became shorter for samples with a lower number of BCG organisms. All of the sputum samples containing ≥10^2 *M. avium* or *M. kansasii* organisms per 100 μl were judged to be negative by the TRC method (Table 1). The species specificity of the TRC method was examined by testing the 16S rRNA solution with 16 mycobacterial species other than MTC as the templates. The TRC method did not show positivity for any of the 16S rRNAs

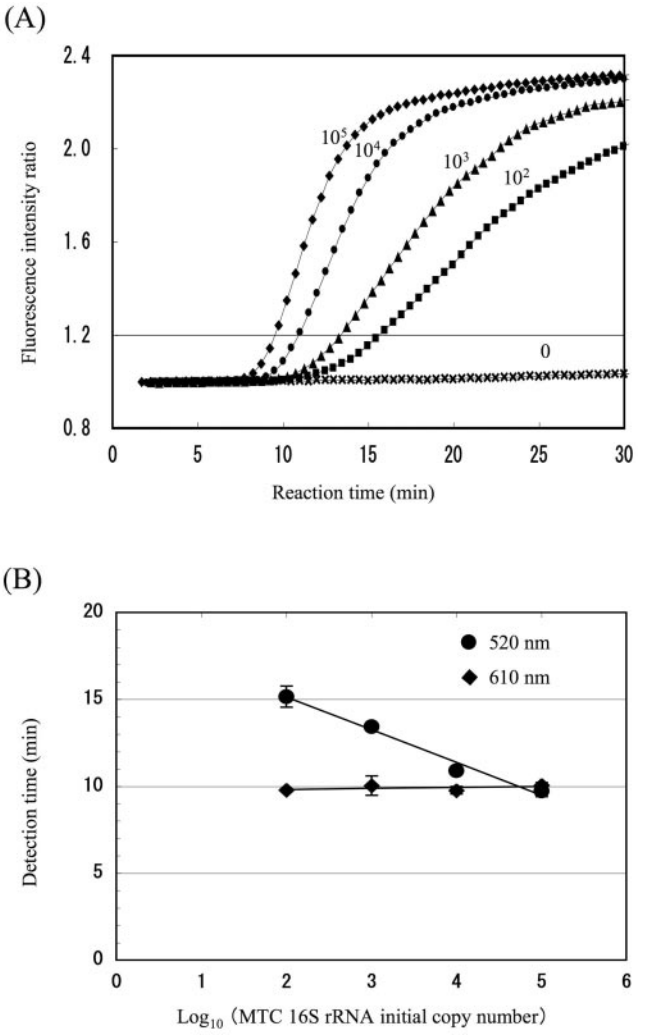

FIG. 3. (A) Fluorescence monitoring of the TRC reaction of the MTC 16S rRNA calibrator. The initial copy numbers of the calibrator are indicated as follows: diamonds, 10^5; circles, 10^4; triangles, 10^3; squares, 10^2; crosses, 0. (B) Initial copy numbers of the calibrator on a logarithmic scale plotted against the time needed to reach the cutoff value of 1.2. Circles indicate the detection time at 520 nm, and diamonds indicate that at 610 nm. The average of the values obtained with triplicates of each sample is plotted.

TABLE 1. Detection of *Mycobacterium tuberculosis* complex-specific 16S rRNA sequence by TRC method in sputum containing BCG (10^0 to 10^4 cells/100 μl), *Mycobacterium avium*, and *M. kansasii* (10^2 to 10^6 cells/100 μl)

No. of organisms/ 100 μl of sputum	Detection time (min)		
	BCG	*M. avium*	*M. kansasii*
10^6	NE[a]	ND[b]	ND
10^5	NE	ND	ND
10^4	10.0	ND	ND
10^3	10.4	ND	ND
10^2	12.5	ND	ND
10^1	15.6	NE	NE
10^0	21.7	NE	NE

[a] NE, not evaluated.
[b] ND, not detected.

(10^6 copies, equivalent to an rRNA content of 10^3 cells, per 100 μl) of the species examined (data not shown).

Evaluation of performance of TRC assay for detection of MTC in clinical sputum samples. Table 2 presents the results of the clinical evaluation of the TRC method and the COBAS AMPLICOR PCR with 201 sputum samples from 173 patients. The TRC method detected as positive 39 of 43 smear-positive, culture-positive samples (sensitivity, 90.7%) and 13 of 29 smear-negative, culture-positive samples (sensitivity, 44.8%), for a total of 52 of 72 culture-positive samples (sensitivity, 72.2%). The COBAS AMPLICOR PCR detected as positive 41 of 43 smear-positive, culture-positive samples (sensitivity, 95.3%) and 17 of 29 smear-negative, culture-positive samples (sensitivity, 58.6%), for a total of 58 of 72 culture-positive samples (sensitivity, 80.6%). The 129 culture-negative samples (Table 2) contained 40 samples culture positive for nontuberculous mycobacteria, such as 13 *M. avium*, 15 *M. intracellulare*, 6 *M kansasii*, 3 *M. abscessus*, and 2 *M. gordonae* isolates and 1 *M. fortuitum* isolate, as well as 89 mycobacteria culture-negative samples. The TRC method did not produce positive results for any of the 129 culture-negative samples (specificity, 100%), none of which were kept suspended, as in the case of in the TRC method, because the fluorescence intensity ratios at 610 nm reached ≥1.2.

Comparison of TRC assay and COBAS AMPLICOR PCR for detection of MTC in clinical sputum samples. As shown in Table 2, the COBAS AMPLICOR PCR detected as positive 41 of 43 smear-positive, culture-positive samples (sensitivity, 95.3%) and 17 of 29 smear-negative, culture-positive samples (sensitivity, 58.6%), for a total of 58 of 72 culture-positive samples (sensitivity, 80.6%). The COBAS AMPLICOR PCR produced no positive results for any of the 129 culture-negative samples (specificity, 100%). There were thus no significant differences in sensitivity and specificity between TRC and PCR, although the sample size was comparatively small for the detection of small differences in sensitivity between the two methods. The correlation of the results of the TRC method and the COBAS AMPLICOR PCR are shown in Table 3, with the overall agreement between TRC and PCR being 95.0% (191 of 201 samples).

TABLE 2. Detection of *Mycobacterium tuberculosis* complex in 201 clinical sputum samples from 173 patients with diagnosed or suspected mycobacterial infection by TRC method, the COBAS AMPLICOR PCR, MGIT[a] culture for MTC, and smear test

Smear result (*n*)	Culture result (*n*)	No. of samples with the indicated result by:			
		TRC method		COBAS AMPLICOR-PCR	
		Positive	Negative	Positive	Negative
Positive (43)	Positive (43)	39	4	41	2
Negative (158)	Positive (29)	13	16	17	12
	Negative (129)	0	129	0	129

[a] MGIT, mycobacterial growth indicator tube.

TABLE 3. Agreement between of results of TRC method and COBAS AMPLICOR PCR for detection of *Mycobacterium tuberculosis* complex in 201 respiratory samples from 173 patients

COBAS AMPLICOR PCR result	No. of samples with the following result by TRC method:	
	Positive	Negative
Positive	50	8
Negative	2	141

DISCUSSION

In this study, we were able to demonstrate that the performance of the TRC method for the detection of MTC from respiratory samples was comparable to that of the COBAS AMPLICOR PCR in terms of sensitivity and specificity. The TRC method detected as little as one BCG organism per 100 μl of sputum. The selection of rRNA as the amplification target, of which a single cell contains multiple copies, may be the primary reason for this favorable result.

In the case of MTC smear-negative and culture-positive samples, the TRC method showed substantially lower rates of positivity than it did for smear- and culture-positive samples, as did the COBAS AMPLICOR PCR. This less favorable result was, however, comparable to the results of previous studies that evaluated nucleic acid amplification assays in similar settings (18). As for the specificity, the TRC method did not show positivity for 10^6 *M. avium* or 10^6 *M. kansasii* organisms per 100 μl of sputum, nor did it show positivity for 10^6 initial copies of 16S rRNA of 16 non-MTC mycobacterial species. The 129 MTC culture-negative samples comprised 40 samples which were culture positive for nontuberculous mycobacterial isolates as well as 89 mycobacterial culture-negative samples. These results clearly demonstrate the excellent specificity of the TRC method for the detection of MTC.

As to sensitivity, the difference between the two methods was not significant, although the comparatively small sample size provided this study with insufficient power to detect small differences in sensitivity. The slightly smaller number of MTC culture-positive samples detected by the TRC method than by the COBAS AMPLICOR PCR may have been due to a reduction in the numbers of viable cells during treatment, since this study included samples from patients undergoing antituberculosis chemotherapy. The levels of mycobacterial rRNA may therefore have decreased faster than the levels of DNA in proportion to the decrease in viable cells. This finding may, rather, indicate some advantage of the TRC method over DNA-based amplification methods in terms of greater correct-

ness in the diagnosis of active disease. Actually, Moore et al. has reported that elimination of MTC rRNA from sputum samples may indicate successful antituberculosis therapy (12).

We found unaccountable false-negative results, four by the TRC method and two by the COBAS AMPLICOR PCR, among the 43 smear- and culture-positive samples. The reason could not be that no viable cells remained in the samples, since MTC was indeed identified by smear as well as culture of these samples. The promptly appearing positive signal of the internal control with the use of either method excluded the possibility that inhibitors were present in the samples. The slightly greater number of false-negative results obtained by the TRC method could be explained by a reduction in the number of viable cells during treatment, although the exact reason is difficult to identify. The fact that these false-negative results were found with the use of both methods together, however, makes it less likely that there are any critical problems specific to the TRC method in terms of false-negative results.

The samples examined in this study showed a prevalence of MTC-positive samples considerably higher than the prevalence in most clinical laboratories in developed nations. This is not surprising, since Japan is characterized by a substantially higher prevalence of tuberculosis compared with that in other developed countries: 24.8 new cases per million population in 2003 (11). In addition, public health policies in Japan have been promoting the concentration of tuberculosis patients in core referral centers specializing in tuberculosis, which include the Kinki-Chuo Chest Medical Center and National Tokyo Hospital.

Of special importance are the several advantages that the TRC method has over the other nucleic acid amplification tests. First, an internal control is amplified to avoid false-negative results; second, the entire procedure is conducted in a completely homogeneous and isothermal format, thus eliminating contamination by postamplification analysis; and third, the results from the time that the samples are decontaminated are available within an hour because amplification and detection require only 30 min. The TRC method also proved to be clinically useful for the rapid identification of MTC in respiratory samples, at least for those smear-positive ones, as is the case for COBAS AMPLICOR PCR.

Pulmonary tuberculosis and nontuberculous mycobacterial infection are sometimes difficult to differentiate based on clinical findings. The nucleic acid amplification test, which could detect MTC only, may add little information in such cases if the result was negative. Development of the TRC method for the detection of *M. avium* complex and *M. kansasii*, which is now in progress in the laboratory at Tosoh Corporation, could further enhance the clinical usefulness of this method for the management of patients with suspected mycobacterial infection especially when the smear result is positive.

In summary, the sensitivity of the TRC method was found to be comparable to that of the COBAS AMPLICOR PCR, and its specificity was excellent. Moreover, less than an hour was required to obtain results after the specimens had been treated with NALC-NaOH. This novel method can thus be expected to be suitable for routine use for the rapid diagnosis of tuberculosis.

ACKNOWLEDGMENTS

We are grateful to Juichi Saito, Ryuichi Horie, Masahide Kondo, and Hideo Suzuki of Tosoh Corporation; Akiko Irie of the Kinki-chuo Chest Medical Center; and Sadao Aoki, Shigeru Takeyama, and Shuichi Ohtawa of National Tokyo Hospital for their contributions.

REFERENCES

1. **Fadda, G., F. Ardito, M. Sanguinetti, B. Posteraro, L. Ortona, C. Chezzi, L. Polonelli, G. Dettori, S. Conti, F. Fanti, and C. Galli.** 1998. Evaluation of the Abbott LCx Mycobacterium tuberculosis assay in comparison with culture methods in selected Italian patients. New Microbiol. **21:**97–103.
2. **Ginesu, F., P. Pirina, L. A. Sechi, P. Molicotti, L. Santoru, L. Porcu, A. Fois, P. Arghittu, S. Zanetti, and G. Fadda.** 1998. Microbiological diagnosis of tuberculosis: a comparison of old and new methods. J. Chemother. **10:**295–300.
3. Reference deleted.
4. **Ichiyama, S., Y. Iinuma, S. Yamori, Y. Hasegawa, K. Shimokata, and N. Nakashima.** 1997. Mycobacterium growth indicator tube testing in conjunction with the AccuProbe or the AMPLICOR-PCR assay for detecting and identifying mycobacteria from sputum samples. J. Clin. Microbiol. **35:** 2022–2025.
5. **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.
6. **Ichiyama, S., Y. Ito, F. Sugiura, Y. Iinuma, S. Yamori, M. Shimojima, Y. Hasegawa, K. Shimokata, and N. Nakashima.** 1997. Diagnostic value of the strand displacement amplification method compared to those of Roche Amplicor PCR and culture for detecting mycobacteria in sputum samples. J. Clin. Microbiol. **35:**3082–3085.
7. **Iinuma, Y., S. Ichiyama, S. Yamori, J. Oohama, N. Takagi, Y. Hasegawa, K. Shimokata, and N. Nakashima.** 1998. Diagnostic value of the Amplicor PCR assay for initial diagnosis and assessment of treatment response for pulmonary tuberculosis. Microbiol. Immunol. **42:**281–287.
8. **Iinuma, Y., K. Senda, N. Fujihara, T. Saito, S. Takakura, M. Shimojima, T. Kudo, and S. Ichiyama.** 2003. Comparison of the BDProbe Tec ET system with the Cobas Amplicor PCR for direct detection of Mycobacterium tuberculosis in respiratory samples. Eur. J. Clin. Microbiol. Infect. Dis. **22:** 368–371.
9. **Ishiguro, T., J. Saitoh, R. Horie, T. Hayashi, T. Ishizuka, S. Tsuchiya, K. Yasukawa, T. Kido, Y. Nakaguchi, M. Nishibuchi, and K. Ueda.** 2003. Intercalation activating fluorescence DNA probe and its application to homogeneous quantification of a target sequence by isothermal sequence amplification in a closed vessel. Anal. Biochem. **314:**77–86.
10. **Ishiguro, T., J. Saitoh, H. Yawata, H. Yamagishi, S. Iwasaki, and Y. Mitoma.** 1995. Homogeneous quantitative assay of hepatitis C virus RNA by polymerase chain reaction in the presence of a fluorescent intercalator. Anal. Biochem. **229:**207–213.
11. **Japan Anti-Tuberculosis Association.** 2004. Japan tuberculosis statistics, annual report: tuberculosis notifications in Japan, 2000–2003. http://www.jata.or.jp/eindex.htm. Accessed 9 July 2005.
12. **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.
13. **Nakaguchi, Y., T. Ishizuka, S. Ohnaka, T. Hayashi, K. Yasukawa, T. Ishiguro, and M. Nishibuchi.** 2004. Rapid and specific detection of *tdh*, *trh1*, and *trh*2 mRNA of *Vibrio parahaemolyticus* using transcription-reverse transcription concerted (TRC) reaction with automated system. J. Clin. Microbiol. **42:**4284–4292.
14. **Piersimoni, C., and C. Scarparo.** 2003. Relevance of commercial amplification methods for direct detection of *Mycobacterium tuberculosis* complex in clinical samples. J. Clin. Microbiol. **41:**5355–5365.
15. **Piersimoni, C., C. Scarparo, P. Piccoli, A. Rigon, G. Ruggiero, D. Nista, and S. Bornigia.** 2002. Performance assessment of two commercial amplification assays for direct detection of *Mycobacterium tuberculosis* complex from respiratory and extrapulmonary specimens. J. Clin. Microbiol. **40:**4138–4142.
16. **Portaels, F., E. Serruys, H. De Beenhouwer, J. Degraux, K. De Ridder, K. Fissette, J. Gomez-Marin, H. Goossens, F. Muhlberger, S. R. Pattyn, F. Nturanye, F. Pouthier, and A. Van Deun.** 1996. Evaluation of the Gen-Probe amplified Mycobacterium tuberculosis direct test for the routine diagnosis of pulmonary tuberculosis. Acta Clin. Belg. **51:**144–149.
17. Reference deleted.
18. **Sarmiento, O. L., K. A. Weigle, J. Alexander, D. J. Weber, and W. C. Miller.** 2003. Assessment by meta-analysis of PCR for diagnosis of smear-negative pulmonary tuberculosis. J. Clin. Microbiol. **41:**3233–3240.
19. **Takakura, S., S. Tsuchiya, N. Fujihara, T. Kudo, Y. Iinuma, S. Mitarai, S. Ichiyama, K. Yasukawa, and T. Ishiguro.** 2005. Isothermal RNA sequence amplification method for rapid antituberculosis drug susceptibility testing of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **43:**2489–2491.