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Clinical Evaluation of TRCRapid M.TB for Detection of *Mycobacterium tuberculosis* Complex in Respiratory and Nonrespiratory Specimens[▽]

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The rapid and accurate diagnosis of tuberculosis is crucial to providing optimal treatment and reducing the spread of infection. We evaluated respiratory and nonrespiratory clinical specimens using a new automated *Mycobacterium tuberculosis* complex (MTBC) rRNA detection kit (TRCRapid M.TB; Tosoh Bioscience, Tokyo, Japan), which is based on the transcription-reverse transcription concerted reaction (TRC). TRC enables the rapid and completely homogeneous real-time monitoring of isothermal RNA sequence amplification without any postamplification procedures. The results were compared with those obtained by *M. tuberculosis* culture. A total of 1,155 respiratory specimens and 420 nonrespiratory specimens collected from 1,282 patients were investigated. Of the 45 specimens culture positive for MTBC, 42 were TRC positive, and of the 1,530 specimens culture negative for MTBC, 1,523 were TRC negative. Compared to the results of culture, the overall sensitivity and specificity of TRC were 96.6% and 99.9%, respectively, for respiratory specimens and 87.5% and 98.5%, respectively, for nonrespiratory specimens. The sensitivities of TRC were 100% for smear-positive respiratory and nonrespiratory specimens, 88.9% for smear-negative respiratory specimens, and 80% for smear-negative nonrespiratory specimens. No significant differences in test performance between respiratory and nonrespiratory specimens were observed. The TRC method proved to be clinically useful for the rapid identification of MTBC in respiratory and nonrespiratory specimens and in both smear-positive and smear-negative samples.

A recent World Health Organization survey estimated that there were 9 million new cases of tuberculosis (TB) in 2006 and that the incidence of multidrug-resistant TB was increasing worldwide. In Japan, the number of patients affected by TB shows a decreasing trend; however, the spread of TB in Japan is still greater than that observed in many European and American developed nations. Despite this decreasing trend, TB remains the most prevalent infectious disease in Japan (8). Therefore, the rapid and accurate diagnosis of *Mycobacterium tuberculosis* complex (MTBC) infections and discrimination between MTBC and nontuberculous mycobacteria are important for the optimal treatment and prevention of group and hospital-acquired TB infections.

An acid-fast bacillus smear test result can be obtained in a short time and is important for the diagnosis of these infectious diseases, but it cannot identify the species of acid-fast bacilli. Three to 6 weeks are required to confirm infection with the tubercle bacillus by culturing of specimens. Therefore, various molecular tests based on nucleic acid amplification and detection techniques have been devised for the rapid detection of MTBC in clinical specimens, such as techniques that use the PCR-based Cobas Amplicor Mycobacterium system (Roche Diagnostics, Basel, Switzerland) (2–4, 11, 12, 15–17); the transcription-mediated, amplification-based Amplified Mycobacterium Tuberculosis Direct Test system (Gen-Probe Inc., San Diego, CA) (5, 13, 18); and the strand-displacement amplification-based BD ProbeTec ET system (Becton Dickinson, Franklin Lakes, NJ) (1, 6, 9, 14). Recently, a new assay based on the transcription-reverse transcription concerted reaction (TRC), TRCRapid M.TB (TRC kit), has also been released.

TRC is an isothermal RNA amplification technology (7), and the TRC kit can detect MTBC within 30 min (19). We previously reported on the utility of the TRC kit for the detection of MTBC (20); however, the efficacy of the TRC kit for the detection of MTBC in nonrespiratory specimens has not yet been reported. The study described here aimed to evaluate the performance of the TRC kit in comparison with that of culture for the rapid detection of MTBC in both respiratory and nonrespiratory specimens, following 2 years of its use as a routine diagnostic test in a clinical laboratory.

MATERIALS AND METHODS

Patients and clinical specimens. This study was approved by the Yokohama City University Medical Center Ethics Committee. From December 2006 to November 2008, specimens were obtained from 1,282 patients, including children and adults, hospitalized in the Yokohama City University Medical Center, Kanagawa, Japan, with clinical signs or symptoms of pulmonary or extrapulmonary TB or for exclusion of the possibility of *M. tuberculosis* infection. Patients who had received treatment for tuberculosis within the past 5 years were excluded from this study. A total of 1,155 respiratory specimens (1,012 sputum, 81 bronchoalveolar lavage fluid, and 62 bronchial aspirate specimens) and 420 nonrespiratory specimens (114 gastric aspirate, 114 pleural effusion, 56 cerebrospinal fluid [CSF], 50 urine, 24 lymph node, 19 lower gastrointestinal tract, 12 skin, 7 lung, 6 abscess fluid, 6 ascitic fluid, 5 aspirate, 4 pericardial fluid, and 3 biopsy specimens) were examined for the presence of mycobacteria.

Processing of specimens. Only the sputum specimens were treated with semi-alkaline protease (SAP), a mucolytic agent. The other specimen types were not

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treated with SAP. All types of specimens, including sputum, were equally divided into two groups: one for the smear culture test and the other for the nucleic acid amplification test. Except for CSF specimens, all specimens selected for the nucleic acid amplification test were processed by the *N*-acetyl-L-cysteine (NALC)–NaOH procedure with an SDL Snap'n Digest Reagent kit (Scientific Device Laboratory, Des Plaines, IL). The specimens were centrifuged at 3,000 × *g* for 20 min at 4°C. The supernatant was removed, and the sediment (pellet) was resuspended in 5 ml of phosphate-buffered saline (PBS; pH 6.8) and decontaminated with 10 ml of NALC-NaOH solution (4% NaOH, 2.9% trisodium citrate, 0.5% NALC). The mixtures were allowed to stand for 15 min at room temperature. The mixtures were diluted in up to 50 ml of PBS and were subsequently centrifuged at 3,000 × *g* for 20 min at 4°C. The supernatant was removed. The sediment was resuspended in approximately 500 μl of PBS, and the mixtures were centrifuged at 500 × *g* for 1 min at 4°C. A 500-μl aliquot of the suspension was then directly processed for TRC.

CSF specimens were diluted with 50 ml of PBS and centrifuged at 3,000 × *g* for 15 min at 4°C. The supernatant was removed. The sediment was suspended in approximately 500 μl of PBS, and the mixtures were centrifuged at 500 × *g* for 1 min at 4°C. A 500-μl aliquot of the suspension was then directly processed for TRC.

RNA extraction. RNA was extracted with an Extragen MB (Tosoh Bioscience) extraction kit for mycobacterial nucleic acid. The manufacturer's directions were followed. In brief, a 200-μl aliquot of specimen suspension (NALC-NaOH-treated, resuspended sediment) was mixed with an RNA-stabilizing reagent (RNAsafer stabilizer reagent; Omega Bio-Tek, Inc., Norcross, GA), and the mixture was centrifuged at 16,000 × *g* for 5 min at 4°C. The supernatant was removed, and the pellet was dissolved in 1,000 μl of wash buffer. After the suspension was mixed, it was incubated at 70°C for 3 min to solubilize and remove inhibitors of the gene amplification reaction that may have been present in a specimen. The suspension was centrifuged at 16,000 × *g* for 5 min at 4°C. The supernatant was removed, and the pellet was dissolved in 50 μl of bacteriolysis buffer containing zirconium oxide beads. The suspension was then placed in a sonic water bath for bacterial cell destruction, and the nucleic acid was released in the solution. The suspension was centrifuged at 16,000 × *g* for 3 min at 4°C. The supernatant (30 μl) was then transferred into a new tube and used for TRC amplification.

TRCRapid M.TB. The principles of amplification and fluorescence-based detection have been described elsewhere (7); and descriptions of the primers, probe, and internal control used for the detection of MTBC by TRC have also been published previously (19). 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 on a thin-walled PCR tube. The tube was set in a dedicated instrument, the TRCRapid-160 real-time monitor (Tosoh Bioscience), for 5 min while it was being preheated to 43°C, followed by addition of 5 μl of the enzyme solution. The TRCRapid-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 for irradiation of the excitation light (470 nm) into the tube from below and a light guide to collect the fluorescence emitted from the bottom of the reaction tube in two photomultiplier tubes (520 nm and 610 nm) at 30-s intervals. MTBC rRNA amplification is detected by measuring the fluorescence emitted at 520 nm, whereas that of the internal control nucleic acid is detected by measuring the fluorescence emitted at 610 nm. 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. Samples with detection times of ≤30 min were considered to have a positive signal, and those with positive signals at 520 nm were judged to be positive. Samples with a positive signal at 610 nm and no positive signal at 520 nm were considered negative, and those with negative signals at both 520 nm and 610 nm were considered to be ones in which the amplification reaction was inhibited.

Microscopy. Specimens selected for testing by the smear culture test were centrifuged at 3,000 × *g* for 15 min at 4°C. The supernatant was removed and the sediment was used for acid-fast smear testing. Fixed smears were stained with auramine O fluorochrome as a screening method. Slides positive for acid-fast bacilli were confirmed to be positive by Ziehl-Neelsen staining.

Mycobacterial culture. The mycobacterial culture test was conducted by SRL, Inc. (Tokyo, Japan). The major portion of the processed sediment was cultured in a Bactec MGIT 960 apparatus (Becton Dickinson). After the smear test, the specimens were decontaminated with NALC-NaOH and incubated in MGIT medium at 37°C for up to 6 weeks. Isolates of mycobacteria were identified by the AccuProbe *Mycobacterium tuberculosis* complex colony identification test (Gen-Probe, Inc., San Diego, CA) or a DNA hybridization assay (DDH Mycobacteria assay; Kyokuto Pharmaceuticals Co., Tokyo, Japan) (10). The DDH Mycobacteria assay can be used to identify 18 mycobacterial species (*M. tuberculosis* complex, *M. kansasii*, *M. marinum*, *M. simiae*, *M. scrofulaceum*, *M. gordonae*, *M. szulgai*, *M. avium*, *M. intracellulare*, *M. gastri*, *M. xenopi*, *M. nonchromogenicum*, *M. terrae*, *M. triviale*, *M. fortuitum*, *M. chelonae*, *M. abscessus*, and *M. peregrinum*).

Analysis of discrepant results. The diagnostic culture test was considered the "gold standard." In cases in which the culture results were discrepant from the TRC results, clinical data and other results obtained with additional specimens from the patient were considered. The clinical data assessed included the patient's history, symptoms, chest X-ray findings, tuberculin skin test result, and history of drugs administered, whenever these data were available.

Statistical analysis. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of TRC were calculated by using the culture results as the reference standards. Statistical comparisons were performed by using Fisher's exact probability test; a P value of <0.05 was considered significant.

RESULTS

Identification of mycobacteria. Eighty respiratory specimens (6.9%) and 25 nonrespiratory specimens (6.0%) showed the growth of mycobacteria in culture. Of the 80 respiratory specimens culture positive for mycobacteria, 29 grew MTBC; 40 grew *M. avium*; 2 grew *M. intracellulare*; 3 grew *M. fortuitum*; 2 grew *M. chelonae*; and 1 each grew *M. gordonae*, *M. kansasii*, and *M. szulgai*. The mycobacteria in one specimen could not be typed by the DDH Mycobacteria test. A total of 1,075 respiratory specimens (three of which were smear positive) were culture negative.

Of the 25 nonrespiratory specimens culture positive for mycobacteria, 16 showed the growth of MTBC (7 isolates were cultured from gastric aspirate specimens, 5 from lymph node specimens, 1 from an aspirate specimen, 1 from a biopsy specimen, 1 from a lung tissue specimen, and 1 from a CSF specimen), 4 specimens (3 gastric aspirate specimens and 1 pleural effusion specimen) grew *M. avium*, 1 (a gastric aspirate specimen) grew *M. avium* and *M. fortuitum*, 1 (a gastric aspirate specimen) grew *M. abscessus*, 1 (a gastric aspirate specimen) grew *M. intracellulare*, 1 (a gastric aspirate specimen) grew *M. kansasii*, and 1 (a skin specimen) grew *M. marinum*. A total of 395 nonrespiratory specimens (8 of which were smear positive) were culture negative.

TRC. The TRC results were obtained within 3 h (90 min for NALC-NaOH treatment, 60 min for RNA extraction, and 30 min for TRC amplification and detection).

Respiratory specimens. The amplification results for all specimens are summarized in Table 1. Of the 1,155 respiratory specimens tested, 29 specimens (21 smear positive and 8 smear negative) were TRC positive and 1,126 specimens (32 smear positive and 1,094 smear negative) were TRC negative. Inhibition of TRC occurred with 16 respiratory specimens (1.4%) (Table 2). After the specimens were frozen for approximately 1 week, none were inhibitory to TRC when they were retested, and no specimens were positive for MTBC by culture and/or TRC.

Of the 29 culture-positive respiratory specimens (20 smear positive and 9 smear negative), 28 (96.6%) were positive by the TRC test. The one remaining culture-positive, TRC-negative specimen was classified false negative by TRC (Table 3). The specimen was a smear-negative sputum specimen, and retesting by TRC led to a positive result. We also observed one TRC-positive, culture-negative respiratory specimen (smear positive) from which *M. avium* grew during culture; this specimen was classified false positive by TRC.

After analysis of the discrepancies obtained for the 1,155

TABLE 1. Comparison of confirmed results by TRCRapid M.TB assay and culture for respiratory and nonrespiratory specimens

Specimen, TRC result, and smear result	No. of specimens with the following culture result for MTBC:			Sensitivity (%)[a]	Specificity (%)[b]	PPV (%)	NPV (%)
	Positive	Negative	Total				
Respiratory							
Total							
Positive	28	1	29	96.6	99.9	96.6	99.9
Negative	1	1,125	1,126				
Total	29	1,126	1,155				
Smear positive							
Positive	20	1	21	100	97.0	95.2	100
Negative	0	32	32				
Total	20	33	53				
Smear negative							
Positive	8	0	8	88.9	100	100	99.9
Negative	1	1,093	1,094				
Total	9	1,093	1,102				
Nonrespiratory							
Total							
Positive	14	6	20	87.5	98.5	70.0	99.5
Negative	2	398	400				
Total	16	404	420				
Smear positive							
Positive	6	5	11	100	66.7	54.5	100
Negative	0	10	10				
Total	6	15	21				
Smear negative							
Positive	8	1	9	80.0	99.7	88.9	99.5
Negative	2	388	390				
Total	10	389	399				

[a] Sensitivity was calculated on the basis of the results for the culture-positive specimens.
[b] Specificity was calculated on the basis of the results for the culture-negative specimens.

respiratory specimens tested, the overall sensitivity, specificity, PPV, and NPV of TRC compared to the culture results were 96.6%, 99.9%, 96.6%, and 99.9%, respectively (Table 1). The sensitivity, specificity, PPV, and NPV of TRC for smear-positive specimens were 100%, 97.0%, 95.2%, and 100%, respectively; and those for smear-negative specimens were 88.9%, 100%, 100%, and 99.9%, respectively.

Nonrespiratory specimens. Of the 420 nonrespiratory specimens tested, 20 specimens (11 smear positive and 9 smear negative) were TRC positive and 400 specimens (10 smear positive and 390 smear negative) were TRC negative. Inhibition of TRC occurred with five nonrespiratory specimens (1.2%) (Table 2). After the specimens were frozen for approximately 1 week, none were inhibitory to TRC when they were retested, and no specimens were positive for MTBC by culture and/or TRC.

Of the 16 culture-positive nonrespiratory specimens (6 smear positive and 10 smear negative), 14 (87.5%) were TRC positive. The remaining two culture-positive, TRC-negative specimens were classified false-negative by TRC (Table 3). These specimens included one gastric aspirate specimen and one lymph node specimen. Both specimens were smear negative and TRC positive when they were retested.

Of the six TRC-positive culture-negative nonrespiratory specimens, four were smear-positive specimens obtained from five patients who had positive histological examination results and/or who had received therapy for TB. One smear-negative pleural effusion specimen was obtained from a patient who had a high adenosine deaminase (ADA) score and who was therefore considered to have clinically active TB (Table 4). The one remaining specimen was a smear-positive skin specimen and grew *M. marinum* in culture. It was classified false positive by TRC.

After the analysis of the discrepancies obtained for the 420 nonrespiratory specimens tested, the overall sensitivity, specificity, PPV, and NPV of TRC compared to the results of culture were 87.5%, 98.5%, 70.0%, and 99.5%, respectively (Table 1). The sensitivity, specificity, PPV, and NPV of TRC for smear-positive specimens were 100%, 66.7%, 54.5%, and 100%, respectively; and those for smear-negative specimens were 80.0%, 99.7%, 88.9%, and 99.5%, respectively.

DISCUSSION

In 2005, Takakura et al. published the results of the first study of TRC for the detection of MTBC in respiratory specimens (19). We have also reported the utility of TRC for the detection of MTBC by using 157 clinical specimens (20); however, in that study, the specimens were not classified as respi-

TABLE 2. Distribution of species and comparison of initial TRCRapid M.TB assay results with culture results

Type of specimen	No. of specimens	No. of specimens with the following result:				
		TRC Positive	TRC Negative	Inhibition	False positive[a]	False negative[b]
Respiratory						
Sputum	1,012	23	989	15	1	1
Bronchoalveolar lavage fluid	81	4	77	1		
Bronchial aspirates	62	2	60			
Nonrespiratory						
Gastric aspirates	114	6	108	4		1
Pleural effusion	114	1	113	1	1	
Cerebrospinal fluid	56	1	55			
Urine	50	0	50			
Lymph node	24	5	19		1	1
Lower gastrointestinal tract	19	0	19			
Skin	12	1	11		1	
Lung	7	4	3		3	
Abscess fluid	6	0	6			
Ascitic fluid	6	0	6			
Aspirate	5	1	4			
Pericardial fluid	4	0	4			
Biopsy	3	1	2			
Total	1,575	49	1,526	21	7	3

[a] TRC-positive, culture-negative specimens were initially classified as false positive.
[b] TRC-negative, culture-positive specimens were initially classified as false negative.

ratory and nonrespiratory specimens. Here, we report on our 2-year experience with TRC for the detection of MTBC in both respiratory and nonrespiratory clinical specimens.

We observed TRC inhibition rates of 1.4% for respiratory specimens and 1.2% for nonrespiratory specimens. These rates are similar to or better than those of previous studies that used the Cobas Amplicor PCR (inhibition rates, 1.0% to 4.7% for respiratory specimens and 2.6% to 3.4% for nonrespiratory specimens) (2, 11, 16, 17, 21).

For respiratory specimens, the sensitivity of TRC was 96.6%, similar to that reported previously (90.7%) (19). This level of sensitivity is also similar to or slightly better than the sensitivities obtained in previous studies that used the Cobas Amplicor PCR (83.0% to 94.2%) (2, 11, 12, 15, 16). Separate analyses of smear-negative specimens have shown that the sensitivity of TRC (88.9%) is lower than that for smear-positive specimens (100%), but our observations did show an improvement in sensitivity in comparison to that indicated in a previous report of a study that used TRC (44.8%) (19). A possible explanation for this is that we used a different extraction kit, which may have produced a higher RNA concentration. We also observed the sensitivity of TRC for smear-negative specimens to be higher than that recorded in previous studies that used the Cobas Amplicor PCR (48.6% to 75.0%) (2, 16, 17, 19), which may be due to the differences in the extraction kits used as well as the different underlying amplification principles of the two assays.

For nonrespiratory specimens, the sensitivity of TRC was 87.5%, which is also higher than that previously reported by use of the Cobas Amplicor PCR (61.5% to 85.0%) (12, 17). Separate analyses demonstrated that sensitivity is lower for smear-negative specimens (80.0%) than for smear-positive specimens (100%) but still higher than that previously reported when the Cobas Amplicor PCR was used (61.5% to 68.7%) (16, 17).

Discrepancies in the results between culture and the TRC test were observed for both respiratory and nonrespiratory specimens. One respiratory specimen (a smear-negative sputum specimen) was TRC negative and culture positive (Table 3). The specimen was positive when it was retested by TRC, and the detection time was 964 s. Two nonrespiratory specimens, one gastric aspirate specimen and one lymph node specimen, were also TRC negative and culture positive. Both specimens were smear negative and became TRC positive upon retesting (detection times, 908 s and 955 s, respectively). The detection time by TRC is roughly correlated with the smear result. Usually, detection times are rapid (389 s to 875 s) for smear-positive specimens. In the cases described above, the detection times were comparatively long, and an unequal distribution of bacilli may have been responsible for the discrepant result.

In contrast, one respiratory specimen and six nonrespiratory specimens in our study were TRC positive and culture negative (Table 4). One TRC-positive, culture-negative respiratory specimen, a sputum specimen, grew *M. avium* in culture. Detection times are usually rapid (389 s to 875 s) for smear-

TABLE 3. Analysis of three specimens from three patients culture positive for *Mycobacterium tuberculosis* complex and negative by TRCRapid M.TB assay

Type of specimen[a]	Comment(s)
Respiratory, sputum	Clinical diagnosis of active pulmonary TB
Nonrespiratory	
Gastric aspirate	Clinical diagnosis of active pulmonary TB
Lymph node	TB was diagnosed histologically; the clinical diagnosis was cervical lymph node TB

[a] All specimens had negative smear results, and the final interpretation of the results for all specimens was false negative.

TABLE 4. Analysis of seven specimens from seven patients culture negative for *Mycobacterium tuberculosis* complex and positive by TRCRapid M.TB assay

Type of specimen	Smear result	Comment(s)	Final interpretation of TRC result
Respiratory, sputum	Positive	*M. avium* grew by culture	False positive
Nonrespiratory			
Lung	Positive	Histological diagnosis of TB; the patient had been diagnosed with TB and received chemotherapy at another hospital 6 years earlier	True positive
Lung	Positive	Histological diagnosis of TB; no history of TB	True positive
Lung	Positive	Histological diagnosis of TB; no history of TB	True positive
Lymph node	Positive	The patient was diagnosed with pulmonary TB and had received chemotherapy at another hospital 9 years earlier	True positive
Pleural effusion	Negative	The patient was diagnosed with tuberculosis-related pleurisy, as the patient had a high ADA score and positive TRC result; the patient was started on chemotherapy	True positive
Skin	Positive	*M. marinum* grew by culture	False positive

positive specimens. The detection time for this specimen was 1,756 s, which is extraordinarily slow, even though it was smear positive (the positive value was 3 plus, corresponding to a Gaffky scale score of 9). Subsequently, the specimen grew *M. avium* and not MTBC in culture. This specimen was considered false positive by TRC. The TRC assay does not usually judge a large quantity of *M. avium* in a specimen to be false positive over a long detection time. However, false-positive results are repeatedly reported for all nucleic acid amplification test systems that rely on quite different amplification systems. Tosoh Bioscience is going to release a second-generation TRC kit with enhanced specificity; it is expected that this will reduce the incidence of false-positive results caused by a cross-reaction with *M. avium*.

Of the six TRC-positive, culture-negative nonrespiratory specimens, one pleural effusion specimen was also smear negative, but its ADA value was high and its tuberculin test result was positive. The patient also experienced an improvement in breathing after taking an antituberculous drug. The other five specimens were smear positive. Three patients from whom lung tissue specimens were obtained were histologically diagnosed with TB. Two of these three patients had no history of TB and were considered to have active pulmonary TB. The third patient had a history of TB and was clinically diagnosed with relapsing pulmonary TB. By histopathological analysis, the lymph node specimen obtained from one patient did not test positive for TB; however, 9 years earlier, the patient had been diagnosed with pulmonary TB and had received chemotherapy at another hospital. The lymph node tissue specimen examined in the present study had been surgically removed on the basis of swelling and a flare-up thought to be associated with active lymph node TB. Because these four specimens were smear positive and the patients who provided the specimens were clinically diagnosed with active TB, the specimens should have contained a sufficient quantity of bacilli for culture. Therefore, for these four cases, the culture results may be considered false negative. However, these discrepancies may be caused by the detection of nonviable bacilli by TRC. rRNA is a stable RNA target with a long half-life; hence, in the case of these specimens, TRC would be expected to detect nonviable bacilli. This can cause discrepancies in culture results and affect the calculations of specificity.

The final TRC-positive, culture-negative specimen, a skin specimen, grew *M. marinum* in culture. The TRC instruction manual states that *M. marinum* does elicit positive TRC results; this bacterium tends to infect humans through skin lesions. Therefore, in addition to MTBC, *M. marinum* infection should be considered when TRC results for skin specimens are positive, and culture results are necessary for a definite diagnosis.

Significant differences were noted between respiratory and nonrespiratory specimens in terms of the overall specificity ($P < 0.05$) but not in the overall sensitivity ($P = 0.25$). The differences in the overall specificity were attributable to six TRC-positive, culture-negative nonrespiratory specimens. When we excluded TRC-positive specimens in which *M. marinum* grew during culture, significant differences were noted in the overall specificity ($P < 0.05$). These findings may suggest that the sensitivity of TRC is greater than that of culture in the case of nonrespiratory specimens or that *Mycobacterium* cells isolated from tissues such as the lungs or lymph nodes may not survive for long in culture.

In summary, TRC is a sensitive, specific, and rapid technique for the detection of MTBC in respiratory and nonrespiratory specimens. TRC is also effective for the detection of MTBC in both smear-positive and smear-negative specimens, and the TRC test performs very well when it is used for routine clinical diagnosis. However, cross-reactions are possible when a high number of *M. avium* and *M. marinum* isolates are present in skin specimens.

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