

Evaluation of TBc Identification Immunochromatographic Assay for Rapid Identification of *Mycobacterium tuberculosis* Complex in Samples from Broth Cultures[∇]

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Received 18 September 2010/Returned for modification 21 December 2010/Accepted 3 March 2011

Tuberculosis (TB) is a disease of major public health concern worldwide, especially in developing countries. In addition, the human immunodeficiency virus (HIV) epidemic has increased the incidence of infection with nontuberculous mycobacteria (NTM). Rapid, accurate, and simple methods for differentiation of *Mycobacterium tuberculosis* complex (MTBC) isolates from NTM is greatly needed for successful control of the TB epidemic. This study was done to evaluate the clinical performance of the BD MGIT TBc identification test (TBc ID) for rapid identification of MTBC in samples from broth cultures. A total of 229 Ziehl-Neelsen (ZN) stain-positive MGIT cultures were tested using the TBc ID test, and the results were compared with those of the AccuProbe MTBC identification test (GenProbe, San Diego, CA). The agreement between the TBc ID test and the AccuProbe assay was 96% (kappa = 0.92; confidence interval [CI] = 0.869 to 0.971). The sensitivity, specificity, and negative and positive predictive values of the TBc ID test compared to the AccuProbe assay were 100%, 92.4%, 100%, and 92.2%, respectively. After additional molecular testing, the agreement between the two methods increased to 97.8% (kappa = 0.96; CI = 0.917 to 0.994), and the specificity and positive predictive value increased to 95.6% and 95.7%, respectively. The TBc ID test is a simple, sensitive, and specific test for identification of MTBC in samples from acid-fast bacillus (AFB) smear-positive cultures. The TBc ID test could be a good alternative to the AccuProbe test in TB diagnostic laboratories.

Tuberculosis (TB) remains a major public health problem worldwide. The World Health Organization (WHO) estimates that 9.2 million new cases of TB occurred in 2007 (16). Ninety-five percent of the estimated incidence occurs in developing countries, with 80% of cases occurring in only 22 countries (15). The TB problem is further compounded by the increasing prevalence of human immunodeficiency virus (HIV) infections. In addition, the incidence of infections by nontuberculous mycobacteria (NTM) has increased significantly due to the HIV epidemic (4). Tuberculosis and NTM infections account for the majority of deaths in HIV patients (11). Therefore, early diagnosis, adequate therapy, and prevention measures against further transmission are essential for TB control programs. Control measures and treatment of patients with tuberculosis are different from those for patients infected with NTM, and thus detection and accurate identification are increasingly important for appropriate management.

Conventional methods of culture and identification of mycobacteria are very slow, taking up to 6 to 8 weeks for growth and another several weeks to months for biochemical identification. The introduction of liquid-based methods, however, has shortened the times to both detection and identification. Even though these methods require less time for the detection of positive cultures, accurate identification of samples is crucial. Current rapid methods for the identification of mycobac-

teria in samples from liquid culture include high-performance liquid chromatography (HPLC) (2) and the use of nucleic acid probes (6). HPLC analysis is used mainly in research and reference laboratories, as the method requires expensive equipment and skilled personnel, which reduces its applicability, especially in resource-limited settings. The AccuProbe assay (Gen-Probe, San Diego, CA) is a commonly used method for species identification in diagnostic laboratories. However, the method is laborious, expensive, and technically demanding, requiring relatively large amounts of specimen (2 to 3 ml) (5). Other recently developed molecular methods used in diagnostic laboratories include the Inno LiPA Mycobacteria v2 test (Innogenetics, Ghent, Belgium), the GenoType Mycobacterium CM (common mycobacteria) test (Hain Lifesciences GmbH, Nehren, Germany), and the GenoType Mycobacterium AS (additional species) test (Hain Lifesciences GmbH, Nehren, Germany) for NTM (8). However, the turnaround time is approximately 5 h. Therefore, rapid, inexpensive, and simple alternative methods are greatly needed for identification of mycobacteria.

Recently, a number of rapid MPT64 antigen assays were developed to discriminate between *Mycobacterium tuberculosis* complex (MTBC) isolates and NTM by immunochromatography (ICT). MPT64 is a mycobacterial protein secreted only by MTBC and has been shown to differentiate MTBC from NTM (1, 3, 10, 12, 13). The MPT64 gene is well characterized, and the antigen has been studied widely (7, 9).

In this study, we evaluated the performance of a new rapid ICT test, the BD MGIT TBc identification test (TBc ID) (Becton Dickinson, Sparks, MD), for the identification of MTBC in samples from positive MGIT broth cultures. The test

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[∇] Published ahead of print on 16 March 2011.

uses monoclonal anti-MPT64 antibodies to detect MTBC in samples from acid-fast bacillus (AFB) smear-positive MGIT cultures. It is a simple and rapid manual test with no sample preparation. The total assay time is 15 min, with reactivity determined by visual color development of test and control lines.

MATERIALS AND METHODS

Study design. This study was conducted at the National Health Laboratory Service (NHLS) Diagnostic Microbiology Laboratory, Tshwane Academic Division, Pretoria, South Africa. The laboratory receives specimens from an academic hospital, two district hospitals, and a number of primary health care clinics. This study comprised three distinct segments: proficiency, reproducibility, and clinical performance. The proficiency and reproducibility segments were conducted using panels of seeded samples provided by Becton Dickinson. Clinical performance was determined with approximately equal numbers of MTBC and NTM samples. Ethical approval was obtained from the ethics committee of the Faculty of Health Science, University of Pretoria.

Proficiency. Proficiency was determined using panels of seeded samples with various amounts of recombinant MPT64 antigen (0, 1, 3, and 7 ng/ml) to demonstrate various intensities of the test line. The expected test results were either positive (strong, moderate, or weak) or negative. The panels of seeded samples were provided by the company (Becton Dickinson) and were randomized and coded prior to shipping to the clinical sites for testing. The tests were carried out in a blinded fashion by three laboratory staff members; each person was given 20 seeded samples.

Reproducibility. For reproducibility, 108 seeded samples were tested by three laboratory staff members. The antigen panels were run on three consecutive days, using the same lot of TBc ID devices, in order to determine the reproducibility of the TBc ID test and readers. Panels consisted of 12 blinded and randomized samples, with 6 positive (3 ng/ml) and 6 negative (0 ng/ml) samples.

Clinical performance. To assess the clinical performance of the TBc ID test for the detection of MTBC, a total of 229 consecutive MGIT culture medium tubes which gave a positive signal in the Bactec MGIT 960 mycobacterial detection system (Becton Dickinson, Sparks, MD) were enrolled in this study. All cultures were examined by Ziehl-Neelsen (ZN) staining on the day of detection to confirm the presence of AFB. The sample size was calculated based on the number of samples necessary for the widths of the 95% confidence intervals (CI) of the positive and negative percent agreements to be ≤ 10 percentage points. The enrolled MGIT medium tubes were for all specimen types, as recommended in the TBc ID package insert, with the exception of urine and blood specimens. All of the MGIT cultures in this study were identified using the AccuProbe *Mycobacterium tuberculosis* complex culture identification test (GenProbe, San Diego, CA) for MTBC as described by the manufacturer. However, solid medium was not used, so mixed infections could not be excluded definitively.

BD MGIT TBc ID testing was performed according to the manufacturer's instructions. In brief, 100 μ l of the positive MGIT culture was placed into the TBc ID well, followed by a 15-min waiting period for the test reaction to occur. Results were read as positive for MTBC by observing the presence of pink to red lines on the control area as well as the test area of the device. The AccuProbe results were not known at the time that the TBc ID test was performed. The AccuProbe assay was used as a reference standard in this study. The clinical trial site uses the AccuProbe assay as one of its routine identification methods. All of the MGIT culture tubes were tested within 1 to 10 days of reaching a positive signal in the MGIT 960 instrument. Prior to being tested, the MGIT cultures were stored at room temperature. During the clinical study, positive and negative quality control tests were included in every run of AccuProbe and TBc ID tests.

Additional testing of discrepant results. The TBc ID test was repeated within 24 h if discrepant results were obtained between the TBc ID test and the AccuProbe assay. The AccuProbe test could not be repeated because of an insufficient remaining MGIT culture medium volume (AccuProbe testing required a volume of 2 ml). Discrepant results were also retested using two different molecular methods: GenoType MTBDR Plus (Hain Lifesciences GmbH, Nehren, Germany) and a Roche LightCycler mycobacterium detection kit (Roche Applied Science, Mannheim, Germany), using the manufacturers' instructions. These two molecular assays are also used as identification methods in the diagnostic laboratory. True positive results were defined as those where the sample was positive with both molecular assays.

Statistical analysis. The data were tabulated using AccuProbe results (gold standard) to categorize the TBc ID results as truly positive, truly negative, false positive, or false negative, and the sensitivity, specificity, and positive and neg-

ative predictive values for the TBc ID test were calculated. The agreement between the two methods was estimated by the kappa value, interpreted as follows: < 0.2 , poor; 0.21 to 0.4, fair; 0.41 to 0.6, moderate; 0.61 to 0.8, substantial; and ≥ 0.81 , excellent.

RESULTS

The present study evaluated the performance of a new rapid ICT test, the TBc ID test, for the identification of MTBC in samples from positive MGIT broth cultures. The study comprised three distinct segments: proficiency, reproducibility, and clinical performance. Three laboratory staff members participated in proficiency testing. All three technicians passed proficiency criteria prior to enrolling clinical specimens. Two demonstrated 100% proficiency and one demonstrated 95% proficiency (due to an error in recording of results) with a panel of 20 MPT64-positive and -negative samples. Reproducibility was 100% across all samples and days and for the three laboratory staff members.

Of the 229 cultures, 4 were not compliant to the study enrollment criteria and were excluded from the analysis. A total of 225 compliant positive MGIT culture tubes were used to determine the final performance characteristics of the TBc ID test. Among the 225 total specimens, 185 were sputum specimens, 24 were gastric aspirates, 6 were cerebrospinal fluid samples, 4 were pus samples, 3 were pleural fluid samples, 2 were tracheal aspirates, and 1 was a nasopharyngeal aspirate. Of the 225 samples, 112 samples were MTBC positive and 113 samples were NTM positive by AccuProbe assay. All MGIT tubes tested in the TBc ID test produced interpretable results, and the control line appeared clearly. The time to positivity for the MGIT cultures did not affect the performance of the TBc ID test.

The agreement between the TBc ID test and the AccuProbe assay was 96% ($\kappa = 0.92$; CI = 0.869 to 0.971). The sensitivity and specificity of the TBc ID test compared to the AccuProbe assay were 100% and 92.4%, respectively. The negative and positive predictive values were 100% and 92.2%, respectively. Among the 225 samples, 9 had results for the TBc ID test that did not match the AccuProbe results (discrepant results). These 9 samples yielding discrepant results were retested by the TBc ID test and two molecular assays: GenoType MTBDR Plus and a Roche LightCycler mycobacterium detection kit. For 8/9 retested discrepant samples, at least one molecular assay agreed with the TBc ID test. For 4/9 (A177A, A011A, A090A, and A104A) retested discrepant samples, both molecular assays agreed with the TBc ID test. For 1/9 (A028) retested discrepant samples, both molecular assays agreed with the AccuProbe assay (Table 1).

For one discrepant sample (A056A), the culture was positive in less than 2 days, three of the five tests identified it as NTM, and the repeat TBc ID test did not agree with the first TBc ID test. Even though the signal detection at less than 2 days was early, the culture was ZN smear positive. The probable explanation is that the specimen was mixed, containing both MTBC and NTM. However, contamination is likely to have flagged the signal earlier.

After additional molecular testing, the agreement between the two methods increased to 97.8% ($\kappa = 0.96$; CI = 0.917

TABLE 1. Results of additional molecular testing for discrepant samples

Sample	Specimen type	MGIT time to detection (days:h)	AccuProbe result	TBc ID test result ^a	TBc ID test repeat result ^a	Genotype MTBDR Plus result	Roche LightCycler detection result
A177A	Sputum	04:08	NTM	P	P	MTBC	MTBC
A011A	Sputum	05:17	NTM	P	P	MTBC	MTBC
A090A	Sputum	06:16	NTM	P	P	MTBC	MTBC
A104A	Sputum	05:18	NTM	P	P	MTBC	MTBC
A056A	Gastric aspirate	01:20	NTM	P	N	MTBC	NTM
A127A	Gastric aspirate	29:13	NTM	P	P	MTBC	NTM
A176A	Sputum	05:16	NTM	P	P	NTM	MTBC
A020A	Sputum	05:15	NTM	P	P	NTM	MTBC
A028A	Sputum	36:02	NTM	P	P	NTM	NTM

^a P, positive; N, negative.

to 0.994) and the specificity and positive predictive value increased to 95.6% and 95.7%, respectively.

DISCUSSION

The incidence of NTM infection has increased substantially with the HIV/AIDS epidemic (4). Differentiation of MTBC from NTM is therefore of increased importance for appropriate patient management. The TB-HIV coinfection rates in South Africa are above 60% (16). Microbiological laboratories in South Africa are faced with the challenge of rapid and accurate identification of *M. tuberculosis*. Thus, there is a need for fast, effective, and inexpensive methods for detection and identification of MTBC to allow for early treatment.

In this study, we evaluated the performance of the TBc ID test for differentiation of MTBC and NTM in samples from AFB-positive cultures. In contrast with the AccuProbe assay, the TBc ID test is a one-step, rapid (15 min) test, and it is less expensive and does not require any sample preparation or any instrumentation. Such a decrease in turnaround time may have important implications for the control of TB, as it will reduce the chance for further transmission events.

An excellent agreement ($\kappa = 0.96$) was found between the TBc ID test and the AccuProbe assay, with an excellent sensitivity (100%) and very good specificity (95.6%). Other published studies reported similar sensitivities and specificities, such as 98.6% and 97.9%, respectively, for the Capilia TB assay (Tauns, Numazu, Japan) and 99.0% and 100%, respectively, for the SD TB Ag MPT64 rapid test (Standard Diagnostics, Seoul, South Korea) (3, 10, 13).

The TBc ID test was evaluated with MGIT culture medium and a Bactec MGIT 960 system, as this is the most common culture method used by the majority of diagnostic laboratories. The assay also demonstrated similar good results for testing on solid media (14).

Additional molecular testing of samples that showed discrepant results demonstrated that the AccuProbe assay cannot be relied upon on as an absolute gold standard for this type of comparative study. Interestingly, the additional molecular tests did not always agree fully. The two molecular assays had discrepant results suggesting mixed infections, and a possible reason for this could be related to competition for the PCR targets. Despite these limitations, the TBc ID test performed favorably against the molecular assays.

A limitation of the study was that only the MTBC probe was used in the AccuProbe assay and the identification of NTM was based on a negative MTBC probe test. It is possible that a low copy number of the *M. tuberculosis* target in the MGIT tube could give a negative result by the AccuProbe assay.

In conclusion, the TBc ID test is an easy, very sensitive, and specific diagnostic tool which accurately detects the presence or absence of MTBC in samples from AFB smear-positive cultures. Considering that the BD MGIT TBc ID test is rapid, less labor-intensive, and inexpensive, it could be a good alternative to the AccuProbe test for use in TB diagnostic laboratories.

ACKNOWLEDGMENTS

We thank the staff members of NHLS/University of Pretoria, Tshwane Academic Division, and Ann Graziosi and Paula Bauer of Becton Dickinson for their assistance during the study.

This project was supported by Becton Dickinson and Company, Sparks, MD.

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