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Evaluation of a Rapid Air Thermal Cycler for Detection of Mycobacterium tuberculosis

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The Air Thermal Cycler (ATC) (Idaho Technology, Idaho Falls, Idaho) utilizes the unique technology of small-volume glass capillary tubes and high-velocity air for the heating and cooling medium for the PCR. Standard heat block thermal cycler (HBTC) and ATC performance characteristics were compared for the detection of Mycobacterium tuberculosis. Sensitivity was 100% for all smear-positive, M. tuberculosis culture-positive specimens for both the HBTC and the ATC. Of smear-negative, M. tuberculosis culture-positive specimens, sensitivity was 42.9% with the HBTC and 22.0% with the ATC. Specificity was 100% for both assay systems. Total assay time was 6.5 and 4 h and the reagent cost was 84 and 32 cents for the HBTC and ATC, respectively. The ATC offered an excellent alternative to the traditional HBTC for diagnosis of M. tuberculosis in smear-positive specimens by PCR.

Due to the resurgence of tuberculosis, the emergence of multiple-drug-resistant Mycobacterium tuberculosis strains, and a need to differentiate between M. tuberculosis and other mycobacteria for isolation and treatment purposes, rapid and accurate detection of M. tuberculosis in clinical specimens is critical. PCR using IS6110 primers has been shown to be a rapid, sensitive, and specific procedure for the detection of M. tuberculosis complex in clinical samples (1, 4, 6, 12, 14, 15).

Thermocyclers commonly used for PCR utilize metal blocks or water for thermal equilibration, and samples are contained in plastic microcentrifuge tubes or microtiter wells. Average temperature transition rates for heat block thermocyclers (HBTC) are about 1°C/s; thus, a considerable amount of time is spent heating and cooling the sample (8).

The air thermocycler (ATC) uses high-velocity air for heating and cooling. Samples are contained in micropipetted tubes which provide a high surface area-to-volume ratio for temperature cycling. Temperature transition rates of 5 to 10°C/s can be obtained in an ATC, thus reducing the cycling time (8).

In a retrospective study, performance characteristics of the HBTC and the ATC for sensitivity, total assay time, and overall cost per assay for the detection of M. tuberculosis from clinical specimens were compared. The study used residual processed specimens that had been cultured for mycobacteria and frozen at −70°C. In a prospective study, further performance data using the ATC only for detection of M. tuberculosis and clinical impact of rapid detection was then obtained by using freshly processed specimens.

Clinical specimens used in the retrospective study were from the University of South Alabama Medical Center (USAMC). Specimens used in the prospective study were obtained from patients at four Mobile area medical centers (USAMC, 89.6%; other sites, 10.4%). All specimens were cultured for mycobacteria by standard laboratory procedures (11). All PCR assays were performed at USAMC.

A total of 154 specimens were tested in the retrospective study, and 116 specimens were tested in the prospective study. The types of specimens included 229 (84.8%) respiratory specimens (sputum, bronchial washing, and bronchoalveolar lavage) and 41 (15.2%) nonrespiratory specimens.

The DNA target for amplification is a 285-bp fragment from the insertion sequence IS6110, specific for the M. tuberculosis complex (5).

The DNA amplification reaction mixture and cycling parameters with total assay times are listed in Tables 1 and 2, respectively. Both reactions were optimized 5, 8; also, unpublished data. For the ATC, 10 µl of the reaction mixture was aspirated into a glass capillary tube and ends were sealed with a flame. After PCR, the 10 µl was loaded directly onto the gel. For the HBTC, 50 µl of the reaction mixture was pipetted into a microcentrifuge tube, from which 10 µl was loaded onto the gel after PCR.

The HBTC used for PCR was the TwinBlock System EasyCycler (Ericomp Inc., San Diego, Calif.). The PCR reagents used with the HBTC were from Perkin-Elmer (Norwalk, Conn.). The ATC used was the Rapidcycler (Idaho Technology, Idaho Falls, Idaho) (Fig. 1). Reagents used with the ATC were from Idaho Technology with the exception of the Taq polymerase. AmpliTaq polymerase (Perkin-Elmer) was used in reaction mixes for both thermocyclers. Ten microliters of amplified product was examined by ethidium bromide staining after agarose (2%) gel electrophoresis.

Contamination control measures followed published recommendations (10). An external control using the same primer set with a 400-bp target was added to each sample to check for specimen inhibition (5). Positive and negative DNA extraction controls plus high and low positive PCR controls were used for each run.

The results for both thermocyclers are summarized in Table 3. For smear-positive, M. tuberculosis culture-positive specimens, sensitivity was 100% for both the HBTC and the ATC. Sensitivity for smear-negative, M. tuberculosis culture-positive specimens was significantly lower with both the HBTC and the ATC, 42.9 and 22.0%, respectively.

A number of possibilities for the lower sensitivities of the amplification methods for smear-negative, culture-positive specimens exist and are consistent with other amplification studies with smear-negative, culture-positive specimens (2, 9). One possibility is sample distribution with paucibacillary spec-
imens. Sensitivity may have improved had a series of three appropriately collected specimens from a patient who was smear-negative been analyzed. Sensitivities of amplification assays have been shown to improve with multiple sequential specimens from a single patient (1, 3, 9). The current protocol in our laboratory for all acid-fast bacillus smear-negative specimens where PCR for *M. tuberculosis* is requested is consultation with the laboratory director and the request for three specimens from the patient before an interpretation is rendered.

Specimen types other than respiratory also may have contributed to the lower sensitivity of smear-negative, *M. tuberculosis* culture-positive specimens. In the prospective study, there were six smear-negative, *M. tuberculosis* culture-positive specimens that were PCR negative by the ATC. These specimens had low colony counts in culture (Table 3), and none of them were respiratory. When the ATC PCR results from smear-negative, *M. tuberculosis* culture-positive specimens were analyzed further, sensitivity was 33.3% (8 of 24) for respiratory specimens and 5.9% (1 of 17) for all other specimens. However, none of the specimens used in the study showed inhibition in PCR.

The lower sensitivity of the ATC compared to the HBTC for smear-negative, *M. tuberculosis* culture-positive specimens could be due to smaller specimen input in the PCR (1 versus 5 μl for ATC and HBTC, respectively). Use of the HBTC allowing higher specimen volume may be appropriate for smear-negative specimens. As a comparison, the current Food and Drug Administration approved-amplification tests for *M. tuberculosis* use the following: in the Roche Amplicor assay, 100 μl of the specimen is processed with a final output of 200 μl, of which 50 μl is used for amplification, and in the Gen-Probe MTD test, 50 μl of the specimen is processed with a final output of 250 μl, of which 50 μl is used for amplification (7, 13). Overall, the majority of patients had a single specimen from a single source tested. Preliminary in-house data showed that multiple specimens regardless of specimen type or smear result increased PCR sensitivity. The improved sensitivity is most likely attributed to improving the combination of limiting factors previously addressed: sample number, specimen type, and specimen volume.

Specificity was determined to be 100% for both the ATC and the HBTC for specimens with mycobacteria other than *M. tuberculosis* (MOTT) and culture-negative specimens (Table 3). In our laboratory PCR was performed on any specimen that had a positive smear, and the results were included in the prospective study. There were four smear-negative, *M. tuberculosis* culture-negative specimens (from three patients) that were PCR positive in the prospective study. All cases were resolved as PCR true positives based on chart review with...
manipulating the tubes in specimen aspiration, tube sealing, and loading. Specimen tracking was more difficult due to the
inability to label the capillary tubes. The disadvantages of the ATC are breakable glass capillary tubes and use of a flame to
seal ends, which can be minimized by not using prescored capillary tubes and not overflaming during sealing. Another
disadvantage of the ATC is the smaller volume available for sample input (10 μl total).

The advantages of the ATC compared to the HBTC are the decreased assay time and the lower cost per assay due to the
decreased reaction volume. In addition, premixing of the tracking dye in the buffer facilitates easy loading of samples directly
from the capillary tubes onto the gel. Overall, the ATC offered an excellent alternative to the HBTC for the diagnosis of M.
tuberculosis in smear-positive specimens by PCR.

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