JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1997, p. 2157–2159 Vol. 35, No. 8
0095-1137/97/$04.00+0

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

KIMBERLE CHAPIN* AND TSAI-LING LAUDERDALE

University of South Alabama, Mobile, Alabama

Received 10 February 1997/Returned for modification 8 March 1997/Accepted 29 April 1997

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 IS*6110* 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 microcapillary 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 lytic/extraction procedure used to obtain DNA from clinical specimens followed the procedure of Miller et al. (9). The DNA target for amplification is a 285-bp fragment from the insertion sequence IS*6110*, 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-

* Corresponding author. Mailing address: Department of Pathology, University of South Alabama Medical Center, 2451 Fillingim St., Mobile, AL 36617-2293. Phone: (334) 471-7783. Fax: (334) 471-7884. E-mail: kchapin@jaguar1.usouthal.edu.

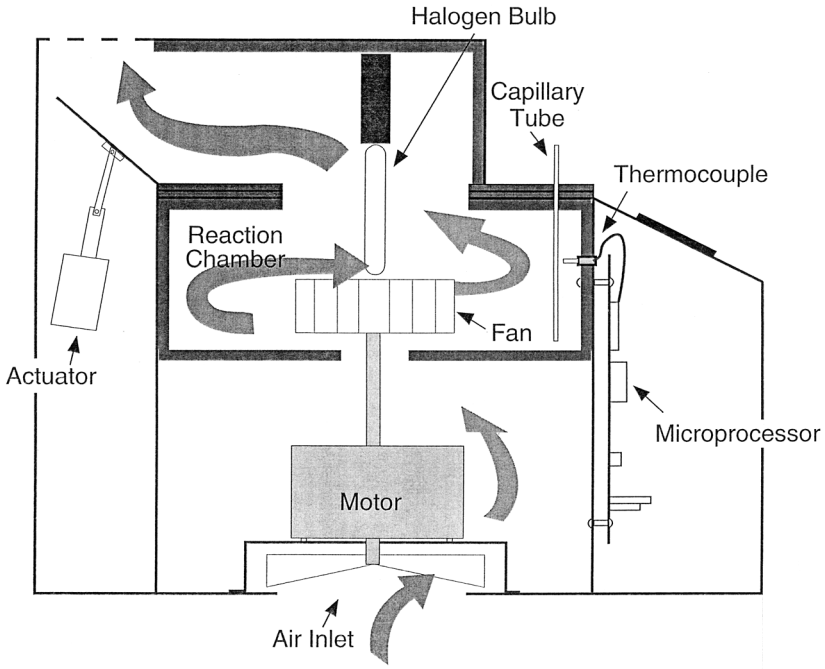

FIG. 1. The ATC. Glass capillary tubes contain the PCR mix. High-velocity air is used for the heating and cooling medium.

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

TABLE 1. PCR mixtures for HBTC and ATC

Method	Total vol (μl)	Sample vol (μl)	Buffer	$MgCl_2$ (mM)	dNTPs[a] (μM each)	Primers (μM each)	AmpliTaq polymerase[b] (U)	External control (fg)
HBTC	50	5	Tris-KCl[c]	2	200	0.5	1	200
ATC	10	1	Tris-BSA-sucrose-cresol red[d]	2	200	1.25	0.4	200

[a] dNTPs, deoxynucleoside triphosphates.
[b] 5 U/μl, diluted 1:12.5 in enzyme diluent which contained 10 mM Tris, pH 8.3, with 2.5 mg of bovine serum albumin per ml before use.
[c] 10 mM Tris (pH 8.3)–50 mM KCl.
[d] 50 mM Tris (pH 8.3)–250 μg of bovine serum albumin (BSA) per ml–2% (wt/vol) sucrose–0.1 mM cresol red.

TABLE 2. PCR cycling parameters

Method	Initial step	No. of cycles	Final step	Total time (min)	
				Cycle	Assay
HBTC	94°C, 5 min	50[a]	72°C, 5 min	165	390
ATC	94°C, 15 s	40[b]	72°C, 30 s	26[c]	240

[a] 94°C for 30 s and 74°C for 1 min.
[b] 94°C for 0 s and 74°C for 30 s; slope = 9.0.
[c] In the retrospective study, the elongation time was 10 s and 50 cycles were performed prior to protocol optimization. The total cycle time was 18 min.

clinical history and chest X ray consistent for *M. tuberculosis*, positive acid-fast bacillus histological tissue examinations, and positive purified protein derivative skin test.

The PCR assay using the ATC had a positive predictive value of 100% and a negative predictive value of 82.1% with all specimens tested. The negative predictive value for respiratory specimens was much better than that for nonrespiratory specimens, 88.7 versus 56.8%. Thus, the PCR amplification technique described here should be used as an adjunct to culture, which is still necessary for susceptibility testing and smear-negative, culture-positive specimens.

The HBTC took approximately 2 1/2 h to complete the 50 PCR cycles, and the ATC took 26 min to complete 40 cycles. The reagent cost per assay was approximately 84 cents for the HBTC and 32 cents for the ATC. The lower reagent cost for the ATC was due mainly to the reduced volume of *Taq* polymerase used (Table 1). The cost for both amplification assays was minimal compared to the Food and Drug Administration-approved kit-formatted assays, Gen-Probe MTD and Roche Amplicor for *M. tuberculosis*. However, use of a "home brew" assay does not allow integration into the clinical microbiology laboratory for many institutions.

The reduced time to perform the entire PCR assay by the ATC (4 h for the ATC versus 6 1/2 h for the HBTC) easily allowed same-day results to be reported to the clinician. Test reporting allowed for appropriate and rapid response to the continued need of isolation and *M. tuberculosis* therapy.

Because the ATC used small glass capillary tubes (0.56-mm inside diameter, 5.1-cm volume), there was a learning curve for 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.

TABLE 3. Summary of PCR results for retrospective and prospective studies[a]

Smear result	Culture result	No. of specimens PCR positive/ no. tested (%)	
		HBTC	ATC
+	*M. tuberculosis*	46/46 (100)	76/76 (100)
−	*M. tuberculosis*	15/35 (42.9)	9/41[b] (22.2)
+	MOTT	0/44	0/63
−	MOTT	0/9	0/17
+	−	0/4	0/5
−	−	0/16	4[c]/68 (5.9)

[a] One hundred and fifty-four specimens were used in the retrospective study, where both the HBTC and the ATC were used. An additional 116 specimens were analyzed in the prospective study using the ATC only.
[b] An additional six specimens tested in the prospective study had 1+ growth in culture (colony counts of 1, 4, 5, 6, 20, and 46).
[c] The four ATC-positive specimens were from three patients who were determined to have had tuberculosis by histological examinations and a positive purified protein derivative test.

We thank Rosemary Farmer for manuscript preparation and Idaho Technology for providing Fig. 1.

REFERENCES

1. **Clarridge, J. E., III, R. M. Shawar, T. M. Shinnick, and B. 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.
2. **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.
3. **Della-Latta, P., E. Waithe, S. Whittier, O. Lungu, I. Weitzman, and S. Silverstein.** 1994. Direct detection of *M. tuberculosis* from sequential specimens by the Gen-Probe amplified system and PCR, abstr. C-336, p. 549. *In* Abstracts of the 94th General Meeting of the American Society for Microbiology 1994. American Society for Microbiology, Washington, D.C.
4. **Eisenach, K. D., M. D. Sifford, M. D. Cave, J. H. Bates, and J. T. Crawford.** 1991. Detection of *Mycobacterium tuberculosis* in sputum samples using a polymerase chain reaction. Am. Rev. Respir. Dis. **144:**1160–1163.
5. **Felmlee, T. A., E. M. Narcisi, D. H. Persing, and K. C. Chapin.** 1994. Comparison of lytic methods for the detection of *M. tuberculosis* (MTB) directly from clinical specimens using PCR, abstr. U-74, p. 185. *In* Abstracts of the 94th General Meeting of the American Society for Microbiology 1994. American Society for Microbiology, Washington, D.C.
6. **Forbes, B. A., and K. E. Hicks.** 1994. Ability of PCR assay to identify *Mycobacterium tuberculosis* in BACTEC 12B vials. J. Clin. Microbiol. **32:** 1725–1728.
7. **Gen-Probe Incorporated.** 1996. Gen-Probe amplified *Mycobacterium tuberculosis* direct test (package insert). Gen-Probe Incorporated, San Diego, Calif.
8. **Idaho Technology Inc.** 1995. Rapidcycler user's guide. Idaho Technology, Idaho Falls, Idaho.
9. **Miller, N., S. G. Hernandez, 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.
10. **National Committee for Clinical Laboratory Standards.** 1994. Molecular diagnostic methods for infectious diseases: proposed guideline, vol. 14, p. 57–59. MM3-P. National Committee for Clinical Laboratory Standards, Villanova, Pa.
11. **Nolte, F. S., and B. Metchock.** 1995. Mycobacterium, p. 409–414. *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.
12. **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.
13. **Roche Diagnostic Systems, Inc.** 1996. Roche Amplicor *Mycobacterium tuberculosis* test (package insert). Roche Diagnostic Systems, Inc., Branchburg, N.J.
14. **Shawar, R. M., F. A. K. El Zaatari, A. Nataraj, and J. E. Clarridge.** 1993. Detection of *Mycobacterium tuberculosis* in clinical samples by two-step polymerase chain reaction and nonisotopic hybridization methods. J. Clin. Microbiol. **31:**61–65.
15. **Thierry, D., A. Brisson-Noël, V. Vincent-Lévy-Frébault, 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.