Technical report

A rapid polymerase chain reaction technique for detecting *M tuberculosis* in a variety of clinical specimens

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Abstract

A rapid in-house polymerase chain reaction (PCR) assay is described for the direct detection of *Mycobacterium tuberculosis* complex in clinical material. Its performance is compared with two kit based systems. The results of the in-house assay were comparable with the commercial assays, detecting *M tuberculosis* in 100% of smear positive, culture positive samples. The in-house assay proved to be rapid, easy, and inexpensive to perform, and the inclusion of an internal inhibitor control permitted validation of the PCR results.

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The polymerase chain reaction (PCR) can be used for the direct detection and identification of *Mycobacterium tuberculosis* complex in clinical material. In this study the assay described by Tan *et al.*¹ based on the repetitive sequence IS6110 of *M tuberculosis*, was modified as follows. It was adapted for use with a capillary air thermal cycler (CATC; Rapidcycler, BioGene) which is more efficient and faster than conventional heated block thermal cyclers.² In addition, since endogenous inhibitors in clinical samples may give rise to a significant number of false negative results,¹⁻⁶ an internal control of 169 base pairs (bp) was designed (Novocastra Laboratories), which coamplified with the same primers as for the 123 bp target.

Methods

The DNA extraction procedure included a boiling step, followed by ultrasonication in the presence of glass beads.¹ The mastermix consisted of 3 mM MgCl₂ /bovine serum albumin/Ficoll/dye (BioGene), 200 µM dNTPs, 15 pmol each primer, 25 fg internal control, and 0.25 U Taq polymerase. The reaction mixture consisted of 1 µl DNA extract and 9 µl mastermix. The cycling profile comprised an initial step of 94°C for one minute, followed by 45 cycles of 94°C for 0 seconds, 50°C for 0 seconds, and 72°C for two seconds.² Amplified

products were electrophoresed through 3% agarose gel and visualised using a transilluminator. The entire assay, including DNA extraction, amplification, and detection, took three hours. Samples found to contain PCR inhibitors, as evidenced by non-amplification of the internal control, were subjected to a simple chloroform extraction⁷ and retested as above.

The performance of the in-house assay was evaluated in a limited comparison with two commercially available PCR assays licensed for the detection of *M tuberculosis* complex organisms in respiratory samples, namely Amplicor[®] MTB (Roche Diagnostic Systems)⁴ and C-TRAK[®] MTB (Raggio-Italgene Srl).⁶ The Amplicor and C-TRAK kit based assays were performed according to the manufacturers' instructions. Both methods took approximately nine hours to perform, and only the C-TRAK assay included an inhibitor control.

Results

The results of all three PCR assays obtained with a range of clinical samples were compared with those of acid-fast smear and culture. Thereafter, the in-house PCR assay was compared further with the results of acid-fast smear and culture on a greater number and variety of clinical samples.

Details of the clinical samples studied are shown in table 1. Of the total of 115 specimens, 31 were tested using all three PCR assays and a further 84 by the in-house assay alone. Smears of all samples were prepared and stained using standard auramine fluorochrome staining. Specimens were cultured onto Löwenstein Jensen slopes or into MB/BacT liquid culture medium.⁸ Cerebrospinal fluid samples were cultured and PCR tested without decontamination. All other samples were digested and decontaminated,⁸ then neutralised before culture and PCR testing (table 1).

The results of the in-house PCR assay are shown in table 2, together with the smear and culture results. Inhibitors were detected in two of 92 respiratory samples (2.2 %); a sample of pus was also found to contain inhibitors, giving an overall inhibitor rate of 2.6% (3/115). Simple chloroform extraction of the prepared extract overcame the inhibition in all of these.

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Table 1 Types and numbers of specimens studied

Sample type	Specimen	In-house alone	In-house v kits	Decontamination method
Respiratory	Sputum	59	22	1N NaOH
	BAL	8	3	1N NaOH
Non-respiratory	Biopsy*	9	2	N/2 H ₂ SO ₄
	CSF	5	3	None
	Pus	1	1	1N NaOH
	Urine	2	0	N/2 H ₂ SO ₄
Total		84	31	

BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid.

*Including spinal disc; leg and face tissue; pleural, lung, lymph node, rectal, and liver biopsies.

Table 2 Results of in-house polymerase chain reaction (PCR) assay for the detection of *M tuberculosis* complex in 115 samples compared with conventional smear and culture results

Smear	Culture	PCR	Specimen	Number tested
+	+	+	Sputum	34
+	+	+	BAL	4
+	+	+	Biopsy	3
+	+	+	Urine	2
–	+	+	Sputum	8
–	+	+	Pus	1
–	+	–	Sputum	10
–	+	–	BAL	3
–	+	–	Biopsy	1
–	+	–	CSF	2
+	–	+	Sputum	2*
+	–	–	Sputum	6†
+	–	–	BAL	1‡
–	–	–	Sputum	21
–	–	–	BAL	3
–	–	–	Biopsy	7
–	–	–	CSF	6
–	–	–	Pus	1
			Total	115

BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid.

*Specimens from patients on treatment.

†Two specimens grew *M avium* complex; two grew *M malmoense*; and two grew *M szulgai*.‡Specimen grew *M szulgai*.

The overall sensitivity for smear positive, culture positive samples was 100% (43/43), and for smear negative, culture positive samples, 36% (9/25). The in-house assay detected *M tuberculosis* in two smear positive, culture negative specimens from patients on antituberculous chemotherapy. *M tuberculosis* was detected in two lymph node samples which were smear positive and culture positive. There was no evidence of cross reaction with samples subsequently yielding *M avium* complex, *M malmoense*, or *M szulgai*, and no smear negative, culture negative sample gave a positive PCR result.

The results of the kit based assays were concordant with those of the in-house assay with the following exceptions. Neither of the commercial assays detected *M tuberculosis* in the two smear positive, culture positive lymph node biopsies. Only the Amplicor system detected *M tuberculosis* in one of two smear negative, culture positive CSF samples. However, Amplicor failed to detect *M tuberculosis* in one smear positive, culture positive sputum sample which was positive by both other PCR assays.

Discussion

Endogenous inhibitors have been reported to occur in 3.2–52% of respiratory samples.^{1–6} In

this study, the in-house assay detected PCR inhibitors in three (2.6%) of 115 samples. The C-TRAK assay did not detect inhibition in 31 samples. The Amplicor kit used in this work did not include an internal standard, although this is now available.

The low inhibitor rate obtained with the in-house assay suggests not only that the simple DNA release method employed may have been capable of removing inhibitors effectively, but also highlights some of the advantages of using a CATC. We have previously reported that the low DNA sample input (1 µl/10 µl PCR) used with the CATC gave a 10-fold reduction in inhibitor rate compared with the use of a more conventional block thermocycler, despite a similar sample input ratio (5 µl/50 µl PCR).² Further, the smaller sample input did not appear to adversely affect the sensitivity of the in-house PCR. In addition, the low inhibitor rate may be partly accounted for by the fact that the PCR buffer for the CATC contains BSA (to prevent denaturation of the polymerase on the surface of the glass capillary), which has been reported to help overcome inhibitors in respiratory samples.⁵

Owing to differences in DNA extraction procedures between the three PCR systems compared in this study, assays were carried out on separate aliquots of clinical material which may cause problems in sampling error. Nevertheless, the performance of the in-house assay was comparable with those of the kit based assays in this limited study. All three assays successfully detected *M tuberculosis* in the majority of smear positive sputum samples, the only exception being one sample which tested PCR negative using the Amplicor system. All assays performed poorly with smear negative, culture positive samples, detecting *M tuberculosis* in three of 13 samples (23%). However, a further 12 smear negative, culture positive samples were tested by the in-house assay alone and overall nine of 25 (36%) were PCR positive. This level of sensitivity has been documented by others and variously ascribed to the presence of endogenous inhibitors, non-homogeneity of samples, sampling error, and low mycobacterial load.^{9–10}

The in-house assay results, as well as those of the kit based assays, therefore support the Food and Drug Administration observation that with PCR assays acceptable accuracy is currently only obtained with smear positive, culture positive respiratory samples. Further work needs to be directed towards improving the DNA extraction techniques, monitoring for the presence of inhibitors by the use of internal controls, improving amplicon detection methods, and the inclusion of suitable internal and external QC procedures before these methods can be extended to smear negative, culture positive samples.

Nonetheless, the in-house assay as described in this study performed at least as well as commercially available systems. The inclusion of an inhibitor control was valuable in preventing false negative results being reported. The test could be completed within three hours of the receipt of the sample. It was also less labour

intensive than the kit based assays. Therefore, it offers a rapid PCR based system for the direct detection of *M tuberculosis* complex in smear positive samples, which assists patient management by providing prompt supportive evidence of *M tuberculosis* infection, as opposed to that caused by other mycobacteria which could yield a smear positive result on microscopy.

- 1 Tan MF, Ng WC, Chan SH, *et al.* Comparative usefulness of PCR in the detection of Mycobacterium tuberculosis in different clinical specimens. *J Med Microbiol* 1997;46:164–9.
- 2 Kearns AM, Freeman R, Steward M. Evaluation of a rapid air thermal cyclor for the detection of Mycobacterium tuberculosis. *J Clin Microbiol* 1998;36:604–5.
- 3 Brisson-Noel A, Aznax C, Chureau C, *et al.* Diagnosis of tuberculosis by DNA amplification in clinical practice evaluation. *Lancet* 1991;338:364–6.
- 4 Dalovisio JR, Montenegro-James S, Kemmerly SA, *et al.* Comparison of the amplified Mycobacterium tuberculosis (MTB) direct test, Amplicor MTB PCR, and IS6110-PCR for detection of MTB in respiratory specimens. *Clin Infect Dis* 1996;23:1099–106.
- 5 Forbes BA, Hicks KE. Substances interfering with direct detection of Mycobacterium tuberculosis in clinical specimens by PCR: effects of bovine serum albumin. *J Clin Microbiol* 1996;34:2125–8.
- 6 Maher M, Glennon M, Martinazzo G, *et al.* Evaluation of a novel PCR-based diagnostic assay for detection of Mycobacterium tuberculosis in sputum samples. *J Clin Microbiol* 1996;34:2307–8.
- 7 Miller N, Hernandez SG, Cleary TJ. Evaluation of Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test and PCR for direct detection of Mycobacterium tuberculosis in clinical specimens. *J Clin Microbiol* 1994;32:393–7.
- 8 Magee JG, Freeman R, Barrett A. Enhanced speed and sensitivity in the cultural diagnosis of pulmonary tuberculosis using a continuous automated mycobacterial liquid culture system. *J Med Microbiol* 1998;47:1–7.
- 9 Chapin K, Lauderdale T-L. Evaluation of a rapid air thermal cyclor for detection of Mycobacterium tuberculosis. *J Clin Microbiol* 1997;35:2157–9.
- 10 Yuen K-Y, Yam W-C, Wong L-P, *et al.* Comparison of two automated DNA amplification systems with a manual one-tube nested PCR assay for the diagnosis of pulmonary tuberculosis. *J Clin Microbiol* 1997;35:1385–9.