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## Evaluation of a Novel PCR-Based Diagnostic Assay for Detection of *Mycobacterium tuberculosis* in Sputum Samples

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**We report on a PCR-based assay we have developed for the detection of *Mycobacterium tuberculosis* in sputum samples. One hundred sputum specimens, which included 34 culture-positive and 66 culture-negative specimens, were evaluated with this system. Of the 34 culture-positive specimens, 31 were PCR positive, and 60 of the culture-negative specimens were PCR negative. An internal standard has been included in the assay system to monitor PCR inhibition and to confirm the reliability of the PCR assay.**

Several research groups have described PCR assays for the sensitive and specific detection of *Mycobacterium tuberculosis* in clinical samples (1, 2, 3a, 6, 8, 14, 16). Direct identification of mycobacteria in clinical samples by PCR amplification (17) of a specific target nucleic acid sequence offers the potential for same-day diagnosis of infection (10).

An initial study was conducted to design PCR primers yielding a 298-bp product, a DNA probe, and an internal standard yielding a 192-bp product from the sequence of the 16S-23S rRNA intergenic spacer region of *M. tuberculosis* and to assess their sensitivity and specificity of detection (7). The specificity of the complex specific primer set MBT3 (19 bp) and MB23S (21 bp) was confirmed by screening a large panel of mycobacterial species as previously described (7). In this clinical evaluation study, 100 sputum samples were analyzed with a PCR assay developed in our laboratory. PCR amplification was performed with lysed sputum specimens with the internal standard added to an additional PCR for each sample to monitor PCR inhibition within that specimen. Post-PCR amplification, the samples were analyzed with a colorimetric DNA probe detection technology, which includes a capture and reporter probe utilizing solution hybridization and magnetic separation techniques (12). The results obtained with the PCR assay outlined were compared with the culture results.

One hundred sputum specimens were donated from three centers for this investigation. The culture-positive specimens were collected sequentially and combined with randomly selected negative specimens and submitted for culture to the University College Hospital, Galway, Ireland. The specimens were decontaminated by standard procedures (5). The Ziehl-Neelsen smear results and culture status of these specimens were unknown to us at the time of testing. The sputum samples were lysed for PCR amplification according to the following protocol. A 50- $\mu$ l aliquot of sputum was withdrawn and centrifuged at 6,000  $\times$  g for 5 min to sediment the mycobacterial cells. The supernatant was removed, and the sediment was resuspended in 50  $\mu$ l of lysis solution containing a final concentration of 100 mM NaOH, 50  $\mu$ g of proteinase K, and 0.05% Triton, incubated at 60°C for 30 min, and then incubated at 95°C for 10 min. This mixture was then neutralized by the addition of Tris-HCL (pH 7.5). For PCR amplification, 5  $\mu$ l of the lysed sputum was added to 95  $\mu$ l of master cocktail

containing a final concentration of 100 mM KCl, 20 mM Tris-HCL (pH 8.0), 0.2% Triton X-100, 5 mM MgCl<sub>2</sub>, 1.25 mM deoxynucleoside triphosphates (dATP, dGTP, dCTP, and dUTP-dTTP [2:1]), 30 pmol of the *M. tuberculosis* complex specific primers MBT3 and MB23S, 2.5 U of *Taq* polymerase (Promega), and 1 U of uracil-*N*-glycosylase (supplied by Cambio BioSciences). The 192-bp internal standard was added at a concentration of 1.25 fg ( $5 \times 10^3$  molecules) where appropriate. The PCR mixtures were incubated at 37°C for 10 min, followed by being heated to 95°C to destroy any dUTP-containing DNA. The reactions were then cycled through 45 cycles of denaturing at 95°C for 30 s, annealing at 69°C for 30 s, extension at 72°C for 30 s, and holding at 72°C after amplification. Post-PCR, the amplified target DNA was detected by the previously described method (12). Briefly, the PCR product was captured by solution hybridization to complementary capture and reporter probes. The capture and reporter probes, designed from the sequence of the 16S-23S rRNA intergenic spacer region of *M. tuberculosis*, are specific for members of the *M. tuberculosis* complex. The capture probe was conjugated to fluorescein isothiocyanate, which can be captured by a magnetic bead coated with anti-fluorescein isothiocyanate antibody. The reporter probe was conjugated to alkaline phosphatase. Alkaline phosphatase mediates the hydrolysis of phenolphthalein monophosphate to chromogenic phenolphthalein, a pink solution which is read at  $A_{550}$ . In order to minimize the risk of contamination or PCR carryover, uracil-*N*-glycosylase was included at the amplification stage of the assay (11). Each stage of the assay was physically separated, and dedicated pipettes and plugged tips were used to control contamination.

In this investigation, each sputum sample was analyzed in parallel with the PCR-based assay for the presence of *M. tuberculosis*, with and without addition of the internal standard to monitor the level of PCR inhibition in that specimen. Of the 34 culture-positive specimens, 33 were smear positive and 31 were PCR positive according to our assay system. A false-negative result was obtained for one specimen which was both culture and Ziehl-Neelsen smear positive and PCR positive for the internal standard but PCR negative for *M. tuberculosis*. Two culture-positive samples were PCR negative for both the internal standard and *M. tuberculosis*. This may indicate the presence of PCR inhibitors in these samples or may reflect the limitations of the current sample preparation procedure. The use of the internal standard to monitor PCR inhibition in these

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samples was valuable in preventing false-negative results from being reported. Of the 66 culture-negative specimens, all were PCR positive for the internal standard, and 60 samples were PCR negative for *M. tuberculosis*. The six samples which were PCR positive but culture negative were from tuberculosis patients undergoing chemotherapy at the time of testing. Similar observations have been previously reported (4, 9, 13, 15).

The results obtained in this study compare well with those reported by other investigators (2, 3, 9, 13, 15), indicating that our assay is capable of detecting *M. tuberculosis* in sputum. Because of the variable nature of sputum specimens, the inclusion of an internal standard in the PCR assay represents an important tool for assessing the reliability of the assay and can be valuable in preventing false-negative results from being reported. Further optimization of the assay at the sample preparation and detection steps may lead to the development of a reliable, sensitive, and specific assay for the detection of *M. tuberculosis* in sputum.

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#### REFERENCES

- Altamirano, M., M. T. Kelly, A. Wong, E. T. Bessuille, W. A. Black, and J. A. Smith. 1992. Characterization of a DNA probe for detection of *Mycobacterium tuberculosis* complex in clinical samples by polymerase chain reaction. *J. Clin. Microbiol.* **30**:2173-2176.
- Beavis, K. G., M. B. Lichy, D. L. Jungkind, and O. Giger. 1995. Evaluation of Amplicor PCR for direct detection of *Mycobacterium tuberculosis* from sputum samples. *J. Clin. Microbiol.* **33**:2582-2586.
- Beige, J., J. Lokies, T. Schaberg, U. Finckh, M. Fischer, H. Mauch, H. Lode, B. Köhler, and A. Rolfs. 1995. Clinical evaluation of a *Mycobacterium tuberculosis* PCR assay. *J. Clin. Microbiol.* **33**:90-95.
- Cho, S. N., G. M. E. van der Vliet, S. Park, S.-H. Baik, S.-K. Kim, Y. Chong, A. H. J. Kolk, P. R. Klatser, and J.-D. Kim. 1995. Colorimetric microwell plate hybridization assay for detection of amplified *Mycobacterium tuberculosis* DNA from sputum samples. *J. Clin. Microbiol.* **33**:752-754.
- Clarridge, J. E., III, R. M. Shanwar, T. M. Shinnick, and B. B. Plikaytis. 1993. Large-scale use of polymerase chain reaction for the detection of *Mycobacterium tuberculosis* in a routine mycobacteriology laboratory. *J. Clin. Microbiol.* **31**:2049-2056.
- Collins, C. H., J. M. Grange, and M. D. Yates. 1985. *Bacteriology*, p. 44-54. Butterworth, London.
- De Wit, D., L. Steyn, S. Shoemaker, and M. Sogin. 1990. Direct detection of *Mycobacterium tuberculosis* in clinical specimens by DNA amplification. *J. Clin. Microbiol.* **28**:2437-2441.
- Glennon, M., T. Smith, M. Cormican, D. Noone, T. Barry, M. Maher, M. Dawson, J. J. Gilmartin, and F. Gannon. 1994. The ribosomal intergenic spacer region: a target for the PCR diagnosis of tuberculosis. *Tubercle Lung Dis.* **75**:353-360.
- Jonas, V., M. J. Alden, J. I. Curry, K. Kamisango, C. A. Knott, R. Lankford, J. M. Wolfe, and D. F. Moore. 1993. Detection and identification of *Mycobacterium tuberculosis* from sputum specimens by amplification of rRNA. *J. Clin. Microbiol.* **31**:2410-2416.
- Kolk, A. H. J., A. R. J. Schuitema, S. Kuijper, J. van Leewen, P. W. M. Hermans, J. D. A. van Embden, and R. A. Hartskeerl. 1992. Detection of *Mycobacterium tuberculosis* in clinical samples by using polymerase chain reaction and a nonradioactive detection system. *J. Clin. Microbiol.* **30**:2567-2575.
- Kox, L. F. F., D. Rhienthong, A. M. Medo, N. Udumsantisuk, K. Ellis, J. van Leewen, S. van Heusden, S. Kuijper, and A. H. J. Kolk. 1994. A more reliable PCR for detection of *Mycobacterium tuberculosis* in clinical samples. *J. Clin. Microbiol.* **32**:672-678.
- Longo, M. C., M. S. Berninger, and J. L. Hartley. 1990. Use of uracil DNA glycosylase to control contamination in polymerase chain reaction. *Gene* **93**:125-128.
- Martinazzo, G., R. Bichi, S. Marcolini, A. Pelliccia, and E. Turchetti. 1994. A novel nucleic acid detection system (C-TRAK) for detecting the t(14:18) and t(9:22) chromosomal translocations associated with haematological malignancies, p. 221-239. In E. C. Borden, J. M. Goldman, and F. Grignani (ed.), *Molecular diagnosis and monitoring of leukemia and lymphoma*. Ares-Serono Symposia Publications, Rome.
- Miyazaki, Y., H. Koga, S. Kohno, and M. Kaku. 1993. Nested polymerase chain reaction for detection of *Mycobacterium tuberculosis* in clinical samples. *J. Clin. Microbiol.* **31**:2228-2232.
- Moore, D. F., and J. I. Curry. 1995. Detection and identification of *M. tuberculosis* directly from sputum sediments by Amplicor PCR. *J. Clin. Microbiol.* **33**:2686-2691.
- Pao, C. C., T. S. B. Yen, J.-B. You, J.-S. Maa, E. H. Fiss, and C.-H. Chang. 1990. Detection and identification of *Mycobacterium tuberculosis* by DNA amplification. *J. Clin. Microbiol.* **28**:1877-1880.
- Reischi, U., M. Pulz, W. Ehret, and H. Wolf. 1994. PCR-based detection of mycobacteria in sputum samples using a simple and reliable DNA extraction protocol. *BioTechniques* **17**:844-845.
- Saiki, R. K., D. H. Gelfand, and S. Stoffel. 1988. Primer directed enzymatic amplification of DNA with a thermostable polymerase. *Science* **239**:487-491.