

The use of the polymerase chain reaction test in the diagnosis of tuberculosis

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Summary

Current techniques for laboratory diagnosis of tuberculosis have some serious limitations. These include the high cost and time required for the current assays. The development of a rapid, sensitive, specific and low-cost assay is therefore of considerable importance. We report here the development and laboratory testing of a polymerase chain reaction DNA-based diagnostic test for the presence of *Mycobacterium tuberculosis* in sputum. The assay shows a high level of sensitivity and specificity and requires considerably less capital, consumables and time inputs than existing laboratory tests. We believe this technology is ready for large-scale evaluation and use, particularly in hospital-based laboratories.

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In a country such as South Africa, which has a considerable Third-World component, many argue that we should not be doing high-tech research at all. In this debate, words such as relevance, need, affordability and applications are often heard. However, there are instances where high-tech research can be applied to community health problems, an example of which is presented here: the rapid diagnosis of infectious diseases such as tuberculosis.

Basic research has led to a detailed understanding of DNA synthesis and replication. Researchers have used this knowledge to develop an *in vitro* method for DNA synthesis, known as polymerase chain reaction (PCR),^{1,2} in which a specific fragment of DNA can be amplified, possibly a million-fold. While this technology was developed as an aid to basic research, it has been used for the diagnosis of infectious diseases including tuberculosis.^{3,4}

Where existing methods are insensitive, provide only indirect evidence of infection (e.g. IgM), or require extended culture (e.g. *Mycobacterium tuberculosis*) PCR may prove useful. Since the PCR technique detects organism-specific DNA it can be used in both active and latent disease. It is very sensitive (detection limit as low as one organism,³) highly specific, inexpensive and relatively rapid. The starting material can be any tissue or body fluid or even archival material (e.g. paraffin blocks).

We report our use of this technology for detection of *M. tuberculosis* in patients presenting at Tygerberg Hospital, and propose that this is a good example of the use of a so-called

high-tech method for obtaining a low-cost, valid diagnosis for a high-priority disease.

Materials and methods

Samples

Samples from patients suspected of having tuberculosis were obtained from the TB laboratory in the Department of Medical Microbiology, Tygerberg Hospital. Sputum samples were prepared as for routine BACTEC⁵ analysis and a small aliquot was removed for PCR analysis. The liquefied sputum sample (up to 0,5 ml) was overlaid on 1 ml sterile 50% sucrose and centrifuged at 12 000 *g* for 5 minutes. The resulting pelleted material was resuspended in sterile saline, centrifuged at 12 000 *g* for 10 minutes and the final pellet was suspended in 50 μ l saline.⁶

PCR amplification of DNA

Oligonucleotide primers are described elsewhere.⁷ For amplification, a 10 μ l sample of the saline suspension was combined in a total volume of 99 μ l of a premixture containing 1 \times PCR buffer (10 mM tris-HCl (pH 9,0 at 25°C), 1,5 mM MgCl₂, 50 mM KCl, 0,01% gelatin, 0,01% Triton X-100), 200 μ M each of dATP, dGTP, dCTP and dTTP and primers of 0,4 μ M each. The reaction mixtures were heated at 95°C for 5 minutes to lyse cells and denature DNA. Taq polymerase (1 unit) was added, the mixture overlaid with 40 μ l mineral oil and a heating cycle of 93°C for 1 minute, 68°C for 1 minute and 72°C for 2 minutes was repeated 30 times in a PCR machine built in our own laboratories. Re-amplification of negative samples was done by taking a 5 μ l aliquot of previously amplified material and initiating a new PCR cycle in a separate tube and mixture.

Detection of product

The amplified DNA product was visualised after gel electrophoresis on 12% polyacrylamide or 1,7% agarose gels by ethidium bromide staining and UV transillumination. A 123 base-pair fragment is detectable after PCR amplification of *M. tuberculosis* DNA.^{6,7} The high degree of specificity of the reaction is shown by the absence of any product from amplification of other *Mycobacterium* species (*M. avium*, *M. intracellulare*, *M. kansasii* and *M. fortuitum* (result not shown)).⁶⁻⁸

Comparative techniques

Ziehl-Neelsen (ZN) staining for detection of acid-fast bacilli and BACTEC culturing was done according to established protocols.

Results

The results of assays for the presence of *M. tuberculosis* using the method described here, are shown in Table I.

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TABLE I. COMPARISON OF EXISTING AND PCR TESTS FOR DIAGNOSIS OF *M. TUBERCULOSIS*

		PCR+	PCR-	Totals
ZN+	BACTEC+	190	3	193
	BACTEC -	2	0	2
		192	3	195
ZN-	BACTEC+	9	0	9
	BACTEC -	0	15	15
		9	15	24
Totals		201	18	219

Samples: Sputum from normal, apparently healthy persons (15) and from putative TB patients (204). ZN — Ziehl-Neelsen stain; BACTEC — culture system; PCR — as described here. Positive and negative reactions to the tests are shown.

Discussion

BACTEC-positive samples numbered 202, of which 199 (sensitivity 98,5%) were also PCR-positive. Of 17 BACTEC-negative samples, 15 were PCR-negative (specificity 88,2%). It should be noted that the 2 samples that were BACTEC-negative and PCR-positive reacted ZN-positive. We suggest the possibility of the failure of the BACTEC system in these cases; possibly chemotherapy of the patient prior to sampling affected the BACTEC test, which is not able to detect non-dividing organisms (e.g. *M. tuberculosis* in response to drug injury).

Three BACTEC-positive samples were PCR-negative, even after sucrose treatment of sputum. Although this method removes most of the Taq polymerase inhibitors, carry-over may occur in a small number. Another explanation for this discrepancy is a false-positive result due to contamination of the BACTEC system, but since all 3 were also ZN-positive, this is unlikely, unless the ZN test detected the presence of other *Mycobacterium* species.

Twenty-one samples tested PCR-negative on first-stage amplification and PCR-positive after second-stage amplification (re-amplification). In the case of PCR-negative samples,

we re-amplified only negative samples. However, in a real situation, we would recommend routine re-amplification.⁹

The PCR tests are currently being done by a medical technologist with no specific background experience in DNA technology. The test is usually completed in just less than 1,5 days, but could be done in 1 day.

Developments in PCR technology are likely to shorten the time even further. This contrasts with results obtained from culture or BACTEC, which take 8 - 40 days, and has immediate implications for patient treatment, particularly as regards hospitalisation cost and compliance. Furthermore, the capital expenditure is approximately one-fifth (or less) that of the BACTEC detection system (approximately R25 000 for PCR and R140 000 for BACTEC). The PCR test uses no isotopes and costs approximately R10 per test for PCR against approximately R85 per test for BACTEC. In addition, the PCR test poses fewer hazards and waste-disposal problems.

The PCR has not been extensively tested in a low-prevalence situation, such as community screening, and our results suggest that negative predictive value is not yet adequate for this use. However, it is clear that in a hospital laboratory situation, the PCR test should be considered for wider evaluation and use.

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