* Identification of *Mycobacterium tuberculosis* in PCR products of clinical samples by using an oligonucleotide based ELISA detection method

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Abstract

The present study was focused on the early definitive laboratory identification of *Mycobacterium tuberculosis*. The research design was based on the specific detection of biotinylated PCR products, which were captured in a microtiter plate coated with streptavidin, with a digoxigenin labeled oligonucleotide probe.

M. tuberculosis DNA was PCR amplified using the forward primer Pt18 and the reverse primer InS2 biotinylated at the 5'end. PCR was based on the amplification of a 249 base pair fragment of the *M. tuberculosis* complex-specific insertion element IS6110. The sensitivity of PCR-ELISA was found to be10 times higher than PCR alone, where the amplified product was detected by agarose gel electrophoresis (AGE). 10 fg of *M.* tuberculosis DNA could be detected in the ELISA test in comparison to a detection level of 100 fg in PCR-AGE.

67 clinical samples were tested for acid-fast bacilli by direct microscopy and culture and for the presence of *M. tuberculosis* complex DNA by PCR-AGE and PCR-ELISA. All 67 clinical samples were negative for acid-fast bacilli by Ziehl-Neelsen staining. Out of the 67 clinical samples, 63 were negative while 4 were positive for *M. tuberculosis* complex DNA in both the PCR-AGE and PCR-ELISA tests. PCR-ELISA can detect as little as 2 bacterial cells in comparison to the detection limit of 20 bacterial cells by PCR alone. Nevertheless, since the results obtained in the ELISA test for the clinical samples were in complete agreement with those of PCR-AGE, further studies are needed to confirm whether this test could be used to detect *M. tuberculosis* in clinical samples that give an ambiguous or negative result with PCR-AGE.

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