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Evaluation of a Polymerase Chain Reaction (PCR) – agarose gel electrophoresis assay with a PCR-Hybridization assay for the detection of *Mycobacterium tuberculosis*

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Polymerase Chain Reaction (PCR)–based assays have been established as the most promising tool for the rapid and specific detection of *Mycobacterium tuberculosis* (Mtb) in clinical specimens. PCR-hybridization (HYB) assays have proved to be useful as a confirmatory procedure for PCR-agarose gel electrophoresis (AGE) assays due to their higher specificity. The objective of this study was to evaluate the results generated by a PCR-AGE assay that detects a 123 bp DNA fragment within the IS6110 insertion sequence of Mtb, with a dot-blot PCR-HYB assay which detects an internal sequence of the 123 bp PCR product. PCR products generated from 42 clinical specimens (sputum, pleural aspirate, cerebrospinal fluid, pus, blood, etc) obtained from suspected Mtb cases were selected for testing by PCR-HYB assay which detects a fluorescein -11-dTUP (non-radioactive) label. PCR-AGE detected a band of 123 bp in 19 of the 42 specimens and the 123 bp band was absent in the other 23 specimens. The positive and negative control PCR products obtained from simultaneous PCR assays were also included for testing by PCR-AGE and PCR-HYB. Eleven positive control DNA extracts were derived from acid fast bacilli (AFB) positive sputum specimens and the 16 negative DNA controls included DNA extracts from normal specimens, water, and sterile water PCR controls. All positive controls gave a prominent DNA band at 123 bp position by PCR-AGE while the same band was absent in the negative controls. PCR HYB detected all 11 positive controls. Seventeen of the 19 specimens identified as positive by PCR-AGE, were also found to be positive by PCR-HYB giving a false positivity rate of 10.5% for PCR-AGE. The 23 specimens that were identified as negative by PCR-AGE were also found to be negative by PCR- HYB. All negative controls used in the PCR assays were also negative by both PCR-AGE and PCR-HYB indicating the absence of contamination during PCR. These results demonstrate that PCR-AGE is suitable to be used in developing countries as a rapid detection method for Mtb in clinical specimens. The more expensive PCR-HYB method can be utilized in situations where interpretation of results of PCR-AGE is made difficult. In the two negative samples the band at 123 bp was weak and diffused.

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