Detection of *Mycobacterium tuberculosis* from Sputum Impregnated on Filter Paper for 5 Days using PCR Technique

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ABSTRACT

With the increased incidence of tuberculosis and the advent of multiple-drug-resistant strains, more rapid detection of *M. tuberculosis* has important public health significance that may improve management of infected patients and facilitate infection control procedures. As many laboratories are incapable of performing culture and drug susceptibilities, a performance of diagnostic assay at central reference laboratories may be desirable. However, the shipment of sputum specimens over long distances from peripheral health centers to central laboratories may affect the culture recovery. Therefore, a rapid PCR-based method was considered since it can detect both viable and non-viable organisms. This preliminary study was conducted to evaluate the efficacy of detection by PCR and by culture from sputum impregnated on filter paper for 5 days at room temperature, and the results were compared with those of staining and conventional culture before storage which used as the "gold standard".

Of the 231 sputum specimens examined, *M. tuberculosis* were recovered from 124 samples by culture before storage. The culture positivity rate was significantly decreased to 70% after 5 days storage. For PCR assay, a fragment of 377-bp of the IS*6110* sequence was amplified and detected using three methods : first PCR combined with agarose gel electrophoresis (AGE), first PCR with dot blot hybridization (DBH), and nested PCR with AGE. Compared with culture, the sensitivity, specificity, and efficiency for first PCR with AGE were 71.8, 100 and 84.9%, respectively and with DBH were 89.5, 96.3 and 92.6%, respectively. The same values for nested PCR were 96.0, 97.2, and 96.5%, respectively. Of these methods, the nested PCR showed excellent sensitivity and specificity with no significant different ($p = 0.727$) from conventional culture. Thus the storage of sputum on filter paper and kept at room temperature for 5 days had no apparent effect on the performance of nested PCR.

We recommend to use this PCR method in combination with culture for detection of *M. tuberculosis* from mailing sputum specimens storage on filter paper. This sputum collection and storage has the advantage that samples can be sent by post with a minimum space and without refrigeration, convenient for culturing, less culture contamination, and remain viable for PCR assay. Future modifications to PCR protocol on sputum samples collected and stored on filter paper, especially at the sample preparation step and at the detection step will make the assay more reliable, and suitable for use in routine clinical practice or in epidemiological study for analysis of large number of samples collected in the field to be sent by post to the well-established central laboratories for PCR analysis.

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