Establishing and validating the molecular identification of mycobacterial infection on direct patient samples

KMA Karunathilaka[1], KGRA Kumara[1], D Yasaratne[2], CN Ratnatunga[1]

Introduction and Objectives: Tuberculosis (TB) is a communicable disease for which early diagnosis is critical for disease control. It can manifest in both pulmonary and extra pulmonary forms. In addition, there is an unrecognized burden of non-tuberculous mycobacterial disease in Sri Lanka. The identification of mycobacterial species from clinical samples is usually done utilizing standard culturing methods, whichtakes 4 – 8 weeks. Thus, a simple and cost-efficient diagnostic and differentiation method is needed.

Methods: Samples (from suspected site of infection) collected from patients with suspected tuberculosis were divided, and subjected to culturing (Lowenstein–Jensen medium, incubated at 37 ºC) and DNA extraction. For the DNA extraction, QIAamp DNA mini kit was used. The identification of the genus *Mycobacteria* was done using the *Hsp65* gene amplification. *IS6110* and *GyrB* region amplification was used to separate *Mycobacterium tuberculosis* complex (MTBCs) from non-tuberculosis mycobacteria. Culture results were used as the gold standard and the cultures were also subjected to *IS6110* and *GyrB* PCR for confirmation of MTBC.

Results:A total of 48 samples were analyzed consisting of sputum (5), pleural fluid (8), pus/ abscess (2), peritoneal fluid (3), BAL (27), urine (1), lymph node (1), and bone marrow aspirate (1).Thirteen samples were found to be positive for culture. *Mycobacterial* DNA was successfully extracted from samples by the extraction kit as evidenced by the positive bands given for *hsp65* PCR.*Hsp65* PCR had a specificity and sensitivity of 100% and 61.5%, respectively compared to culture (Table). The *IS6110* and *GyrB*PCR didn't give reliable bands for direct sample extractions. In contrast the *GyrB*PCR performed on pure culture DNA extracts gave positive bands for the *Hsp65*PCR positive samples confirming that they were all MTBC isolates.

Table: Culture/ smear (confirmed by *hsp65* and gyrBPCR on DNA from culture) - Preliminary results.

		Culture/ smear (confirmed by *hsp65* and *gyrB*PCR identification on DNA from culture)	
		Positive	Negative
Hsp65 PCR on direct sample DNA extracts	Positive	8	0
	Negative	5	35

Conclusion: Of the 48 patient samples, 13 samples were identified as belonging to the MTBC. There were no NTM isolates in these samples. The *IS6110* and *GyrB*PCR needs to be optimized for direct patient samples.

Keywords: Mycobacterium tuberculosis complex, Hsp65, IS6110, GyrB, PCR

[1]*1Department of Microbiology, Faculty of Medicine, University of Peradeniya, Sri Lanka*
2Department of Medicine, Faculty of Medicine, University of Peradeniya, Sri Lanka

Address for correspondence: Dr. CN Ratnatunga. Telephone: +94776631106 Email: champa26@gmail.com
https://orcid.org/0000-0002-2684-002X