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Rapid Detection of Pyrazinamide Resistant Mycobacterium tuberculosis by High Resolution Melting Curve Analysis

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Abstract

Objective: To develop a high-resolution melting curve analysis (HRM) assay for detection of PZA resistance.

Material and Method: Thirty samples of PZA-susceptible *M. tuberculosis* and eight isolates of PZA-resistant *M. tuberculosis* were included in the experiment. Five sets of primers were designed to cover the *pncA* gene and its upstream nucleotides. The *pncA* gene fragments were amplified by the PCR method. Determination of *pncA* mutation in the sample by comparing their melting behavior of the PCR products with the *M. tuberculosis* wild type by using Gene scanning software of the LightCycler® 480 instrument.

Results: Mutations were clearly detected in all PZA resistant samples by the HRM, whereas all PZA susceptible samples showed no mutation in the *pncA* gene. Results were concordant with the drug susceptibility testing by using BACTEC™ MGIT™ 960 PZA kit and mutation detection by the DNA sequencing method.

Conclusion: This HRM method offers a rapid and reliable screen for PZA resistant *M. tuberculosis*.

Keywords: Mycobacterium, Pyrazinamide, Drug resistant, *pncA* gene, High resolution melting curve analysis

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