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Evaluation of the Quantamatrix Multiplexed Assay Platform system for simultaneous detection of *Mycobacterium tuberculosis* and the rifampicin resistance gene using cultured mycobacteria

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ABSTRACT

Background: The differentiation of *Mycobacterium tuberculosis* complex (MTBC) from non-tuberculous mycobacteria (NTM) is of primary importance for infection control and the selection of anti-tuberculosis drugs. Up to date data on rifampicin (RIF)-resistant tuberculosis (TB) is essential for the early management of multidrug-resistant TB. The aim of this study was to evaluate the usefulness of a newly developed multiplexed, bead-based bioassay (Quantamatrix Multiplexed Assay Platform, QMAP) for the rapid differentiation of 23 *Mycobacterium* species including MTBC and RIF-resistant strains.
Methods: A total of 314 clinical *Mycobacterium* isolates cultured from respiratory specimens were used in this study.
Results: The sensitivity and specificity of the QMAP system for *Mycobacterium* species were 100% (95% CI 99.15–100%, $p < 0.0001$) and 97.8% (95% CI 91.86–99.87%, $p < 0.0001$), respectively. The results of conventional drug susceptibility testing and the QMAP Dual-ID assay were completely concordant for all clinical isolates (100%, 95% CI 98.56–100%). Out of 223 *M. tuberculosis* (MTB) isolates, 196 were pan-susceptible and 27 were resistant to RIF according to QMAP results. All of the mutations in the RIF resistance-determining region detected by the QMAP system were confirmed by *rpoB* sequence analysis and a REBA MTB-Rifa reverse blot hybridization assay. The majority of the mutations ($n = 26$, 96.3%), including those missing wild-type probe signals, were located in three codons (529–534, 524–529, and 514–520), and 17 (65.4%) of these mutations were detected by three mutation probes (531TTG, 526TAC, and 516GTC).
Conclusions: The entire QMAP system assay takes about 3 h to complete, while results from the culture-based conventional method can take up to 48–72 h. Although improvements to the QMAP system are needed for direct respiratory specimens, it may be useful for rapid screening, not only to identify and accurately discriminate MTBC from NTM, but also to identify RIF-resistant MTB strains in positive culture samples.

Introduction

The genus *Mycobacterium* comprises more than 125 species, some of which are pathogenic or potentially pathogenic to humans and some of which are saprophytes (Forrellad et al., 2013). The most important pathogen of this genus is *Mycobacterium tuberculosis* complex (MTBC). *Mycobacterium tuberculosis* (MTB) infection leads to tuberculosis (TB), which is associated with significant morbidity and mortality and remains one of the deadliest diseases worldwide (Zaman, 2010). The World Health

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Organization (WHO) estimated that there were 10.4 million new TB cases and 1.4 million TB deaths in 2015 (World Health Organization, 2016).

Although there has recently been a decreasing trend in new TB infections, the incidence and mortality rate for TB are still excessively high. In particular, the Republic of Korea has an intermediate TB burden, with a higher TB prevalence compared to developed countries (Bae et al., 2008; Lee et al., 2012a). In addition, the incidence of infections caused by non-tuberculous mycobacteria (NTM), including pulmonary, skin and soft tissue, bone, bloodstream, and central nervous system infections, has increased markedly over the past few decades (Marras et al., 2007; Thanachartwet et al., 2014). Species identification of NTM is recommended because the clinical significance and drug resistance patterns of NTM differ by species (Thanachartwet et al., 2014; Maurya et al., 2012).

The most effective means of protection against mycobacterial infections is early diagnosis and treatment. The preliminary diagnosis of mycobacterial infection is based on clinical presentations and radiological findings, but a definite diagnosis depends on laboratory tests (Maurya et al., 2012). TB treatment and control have been severely compromised in recent years due to the increasing prevalence of drug-resistant TB (Abubakar et al., 2013). The WHO estimated that the global burden of multidrug-resistant TB (MDR-TB), defined as combined resistance to rifampicin (RIF) and isoniazid (INH), increased from 500 000 cases per year to nearly 2 000 000 cases in 2015 (World Health Organization, 2016). Failure to consider the possibility of drug-resistant TB until drug-susceptibility test (DST) results become available after weeks to months can result in unnecessarily inadequate drug regimens, worsening of the disease, and further transmission of MDR-TB.

The standard identification and drug resistance diagnostic methods for mycobacterial infections are based on different biochemical tests and phenotypic characteristics, such as growth rate, pigmentation, and colony morphology. However, these methods have well-known limitations in that they are complex, labor-intensive, and time-consuming (Bae et al., 2008). The introduction of molecular diagnostic methods has overcome some of these disadvantages and improved the speed and accuracy of identification and resistance detection.

Various molecular techniques for the diagnosis of MTBC and NTM have been developed and evaluated (Ichiyama et al., 1997; Lee et al., 2000; Kiraz et al., 2010; Bloemberg et al., 2013; Pérez-Osorio et al., 2012; Lotz et al., 2010; Karimi and Amanati, 2016; Quinlan et al., 2015). These include real-time PCR, hybridization, the use of chemiluminescent DNA probes, high-performance liquid chromatography, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), PCR-restriction fragment length polymorphism (RFLP) analysis, and sequence analysis.

A new diagnostic system using a multiplexed, bead-based bioassay (Quantamatrix Multiplexed Assay Platform; QMAP), with shape-encoded silica microparticles (Kim et al., 2015), known as QMAP Dual-ID (Quantamatrix, Seoul, Republic of Korea), has been developed for the rapid identification of MTBC and NTM, and for the accurate detection of RIF resistance-associated mutations in the *rpoB* gene of MTB. The 24 multi-probes targeting *Mycobacterium* species are specific to the following: the *Mycobacterium* genus, MTBC, *M. avium*, *M. intracellulare*, *M. scrofulaceum*, *M. abscessus* complex, *M. chelonae*, *M. fortuitum* complex, *M. ulcerans*/*M. marinum*, *M. kansasii*, *M. genavense*/*M. simiae*, *M. terrae*, *M. nonchromogenicum*, *M. celatum*, *M. gordonae*, *M. szulgai*, *M. mucogenicum*, *M. aubagnense*, *M. malmoense*, *M. smegmatis*, *M. phlei*, *M. xenopi*, *M. flavescens*, and *M. peregrinum*/*M. septicum*. The QMAP diagnostic platform uses proprietary encoded microdisk technology. One disk has a 50-μm-thick silica-coated surface and a graphical bar-coded, carboxyl-functionalized magnetic disk that allows a >1000-plex coding capacity in high-throughput analysis (Kim et al., 2015). A capacity of 1000 multiplex has been shown to be possible in a single microwell system, meaning that it is possible to test 1000 types of pathogen in one microwell for one sample (Figure 1).

The purpose of this study was to evaluate the usefulness of the QMAP system for the differentiation of MTBC from NTM and for the rapid detection of RIF resistance by using 314 clinical *Mycobacterium* spp. isolates from respiratory specimens.

Methods

Clinical isolates

Clinical isolates were obtained from the Asan Medical Center (Seoul, Republic of Korea). Inclusion criteria included suspected TB or NTM disease based on abnormal chest X-rays or clinical findings. In total, 314 DNA samples from patients suspected of MTBC or NTM pulmonary disease were examined.

This study was approved by the Institutional Ethics Committee of Asan Medical Center (approval number 2009-0170) and all participants provided written informed consent.

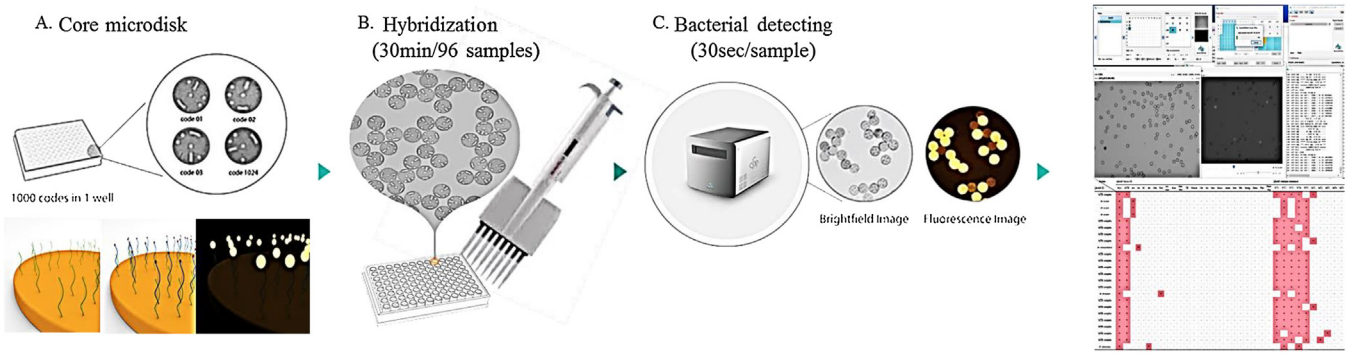

Figure 1. Working principle of the Quantamatrix Multiplexed Assay Platform (QMAP) system. (A) Core microdisk, different types of disk-shaped graphically encoded carboxyl-functionalized magnetic microparticles. (B) Target species genes are synthesized using a PCR process for amplification and labeled using biotin-dCTP. Denatured PCR products and magnetic microdisks are mixed on a microwell plate and hybridized, and R-phycoerythrin-conjugated streptavidin is added to produce fluorescent signals that are analyzed automatically (40 min/96 samples). (C) QMAP analysis, the fluorescence intensity of all microdisks in the image is automatically measured using the QMAP software provided (ver 2. 0) and auto-interpreted by a Microsoft Excel program (30 s/sample). Positive fluorescence intensity signals (>500) for the identification of *Mycobacterium* species and RIF-resistant MTB strains are highlighted in red.