Diagnostic Microbiology and Infectious Disease xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Detection of *Mycobacterium avium* complex DNA directly in clinical respiratory specimens: opportunities for improved turn-around time and cost savings

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ARTICLE INFO

Article history:
Received 11 October 2013
Received in revised form 22 January 2014
Accepted 23 January 2014
Available online xxxx

Keywords:
Mycobacterium tuberculosis complex
Mycobacterium avium complex
Real-time PCR
Multiplex, AccuProbe

ABSTRACT

We developed, evaluated, and implemented a Taqman multiplex real-time polymerase chain reaction (PCR) assay for the detection of *Mycobacterium avium* complex (MAC), targeting the 16S-23S rRNA internal transcribed spacer, which we have combined with an existing *Mycobacterium tuberculosis* complex assay for use directly in clinical respiratory specimens. Evaluation of the performance of this assay for MAC detection included 464 clinical respiratory specimens tested prospectively. This real-time PCR assay was found overall to have a sensitivity of 71.1%, a specificity of 99.5%, a positive predictive value of 98.0%, and a negative predictive value of 90.2% for MAC. The assay provides results prior to the availability of cultured material and identification, most within 24 h of specimen receipt, and may reduce the need to culture MAC-PCR–positive specimens when susceptibility testing is not requested. Additionally, we have found significant cost savings of approximately $21.00 per specimen and staff time reductions of 3.75 h per specimen with implementation of this assay.

1. Introduction

Infections caused by *Mycobacterium tuberculosis* complex (MTBC) remain a significant health problem today. The World Health Organization (WHO) estimates that one-third of the world's population is infected with MTBC (WHO, 2013a). Tuberculosis (TB), the active form of infection caused by MTBC, is a leading cause of morbidity and mortality, primarily in developing countries (Maartens and Wilkinson, 2007; WHO, 2013b). In developed countries, such as the United States, the incidence of TB is comparatively low. However, in countries with low TB incidence, the prevalence of nontuberculous mycobacteria (NTM) is increasing and exceeding the prevalence of TB (Cassidy et al., 2009; Hoefsloot et al., 2013; Winthrop et al., 2010). Among infections caused by NTM, *Mycobacterium avium* complex (MAC) is the most frequently diagnosed (Cassidy et al., 2009; Glassroth, 2008; Griffith et al., 2007; Hoefsloot et al., 2013). MAC infections are opportunistic and can severely affect those infected with HIV (Cassidy et al., 2009; Winthrop et al., 2010). Infections with MAC present similar clinical symptoms to TB including cough, fever, and weight loss but require different medical treatment as members of MAC have been found to be resistant to most drugs used to treat TB (Glassroth, 2008; Herzmann and Lange, 2010). Additionally, MAC infections may result in severe pulmonary disease or death if left untreated or wrongly treated (Ito et al., 2012), especially among immunocompromised individuals. Thus, rapid methods of detection and differentiation between MTBC and MAC should ideally be used to ensure proper and accurate initiation of treatment to patients with mycobacterial infections.

Since 2007, rapid detection of MTBC is performed in our laboratory using a highly specific and sensitive real-time polymerase chain reaction (PCR) assay targeting the insertion sequence 6110 (IS*6110*) (Halse et al., 2010). The IS*6110* is the most commonly used target for the identification of MTBC and has been shown to provide the highest sensitivity for MTBC (Drouillon et al., 2007; Pounder et al., 2006; Savelkoul et al., 2006). However, progress in the development of rapid molecular techniques for rapid detection of MAC has been minimal. Current methods used for MAC identification include conventional PCR to detect the 16S-23S rRNA internal transcribed spacer (ITS) (Ngan et al., 2011; Park et al., 2006). This target has been utilized due to its greater genetic discrimination between MAC members and non-MAC members (De Smet et al., 1995); however, the *hsp65* gene (Bensi et al., 2013) has also been evaluated. Assays utilizing real-time PCR and melt-curve analysis have also been described for clinical isolates (Lim et al., 2008; Richardson et al., 2009) for identifying many *Mycobacterium* species, but it is not clear if these tests are sensitive enough to be utilized directly on clinical specimens and some specificity issues exist. Other methods for identifying MAC include AccuProbe (Gen-Probe, San Diego, CA, USA) and DNA sequence analysis of ribosomal or housekeeping genes, but these methods are time consuming and costly.

Here, we describe a Taqman multiplex real-time PCR assay targeting the ITS region for the detection of MAC directly in respiratory specimens combined with a previously published real-time PCR assay for detection of MTBC. The utilization of this MTBC-

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http://dx.doi.org/10.1016/j.diagmicrobio.2014.01.019

MAC real-time PCR assay has reduced both the cost and turn-around time (TAT) for the identification of MTBC and MAC in clinical respiratory samples in our laboratory, often providing early detection of MAC and a reduction in MAC culture for MAC-PCR–positive specimens when susceptibility testing is not performed.

2. Materials and methods

2.1. MTBC-MAC real-time PCR assay

The MTBC-MAC real-time PCR assay was developed by combining 2 singleplex real-time PCR assays. The singleplex PCR assay for MTBC was previously described (Halse et al., 2010). Primers and TaqMan® minor groove binder (MGB) probe sets specific for MAC detection were designed by manual sequence alignment of the ITS region for all MAC members and selected using the Primer Express software (version 3.0) (Applied Biosystems, Foster City, CA, USA). One forward primer, 5 reverse primers, and 2 probes were designed to assure detection of all MAC strains (Fig. 1).

Each clinical sample was tested in duplicate in 25-μL volumes in an optical 96-well reaction plate. Each reaction contained components of the PerfeCTa qPCR ToughMix (Quanta Biosciences, Inc., Gaithersburg, MD, USA) that included 2× reaction buffer with optimized concentrations of magnesium chloride included, primers targeting the IS*6110* element, and primers targeting the ITS region of MAC: 900 nM MACITS-F and a mix of 5 MACITS-R primers at a final concentration of 1000 nM each. A mix of 2 MGB probes, labeled with the reporter dye VIC, designated as MACITS-P, was included at a final concentration of 250 nM along with an MGB probe labeled with the reporter dye 6-carboxyfluorescein (FAMTM) targeting IS*6110*. Finally, a Texas Red®-X probe, designated as bicoid-P, was used at a concentration of 250 nM to test a duplicate patient sample for the presence of inhibitors as described previously. The MGB probes were obtained from Applied Biosystems, and the Texas Red®-X probe and oligonucleotides were obtained from Integrated DNA Technologies (Coralville, IA, USA).

Cycling conditions on the ABI 7500 FAST were: 95 °C for 3 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 45 s. Threshold cycle (C_T) values less than 37 were reported as positive, and samples with C_T values greater than 37 were retested; if the results remained the same, the specimen was reported as positive, and if they were not, the specimen was reported as inconclusive. Specimens were reported as indeterminate if PCR inhibition was observed.

2.2. Analytical sensitivity and specificity

The analytical sensitivity of the assay was determined by triplicate testing of a series of 10-fold dilutions containing DNA isolated from *M. avium* (ATCC 15769), with known concentrations in colony-forming units (CFU). These dilutions were tested with the MAC singleplex assay and with the MTBC-MAC multiplex assay. The efficiency of the PCR was calculated from the r^2 value generated by the standard curve. The limit of detection of the assay for sputum specimens was determined by seeding the dilutions described above into known negative processed sputum specimens and testing each dilution in triplicate with the MTBC-MAC real-time PCR assay.

The specificity of the assay was determined by testing approximately 1×10^6 genome copies of DNA purified from MTBC organisms, MAC organisms, and other respiratory pathogens that can cause similar clinical symptoms. This testing included the following bacterial strains: *Achromobacter xylosoxidans*, *Acinetobacter baumannii*, *Acinetobacter lwoffii*, *Bordetella bronchiseptica*, *Bordetella parapertussis*, *Bordetella pertussis*, *Burkholderia cepacia*, *Burkholderia multivorans*, *Chlamydophila pneumoniae*, *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Moraxella catarrhalis*, *Mycobacterium abscessus*, *Mycobacterium africanum* (n = 2), *Mycobacterium arosiense*, *M. avium*, *M. avium* subsp. *avium* (n = 2), *M. avium* subsp. *paratuberculosis* (n = 2), *M. avium* subsp. *silvaticum*, *M. avium* subsp. *suis*, *Mycobacterium bovis* (n = 3), *M. bovis BCG* (n = 2), *Mycobacterium chelonae*, *Mycobacterium chimaera*, *Mycobacterium colombiense*, *Mycobacterium flavescens*, *Mycobacterium fortuitum*, *Mycobacterium gastri*, *Mycobacterium gordonae* (n = 2), *Mycobacterium haemophilum*, *Mycobacterium intracellulare*, *Mycobacterium kansasii*, *Mycobacterium marseillense*, *Mycobacterium microti* (n = 2), *Mycobacterium peregrinum*, *Mycobacterium phlei*, *Mycobacterium scrofulaceum*, *Mycobacterium seoulense*, *Mycobacterium simiae*, *Mycobacterium szulgai*, *Mycobacterium terrae*, *M. tuberculosis* (n = 5), *Mycobacterium ulcerans*, *Mycobacterium xenopi*, *Mycoplasma pneumoniae*, *Neisseria meningitidis*, *Neisseria mucosa*, *Neisseria sicca*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, *Stenotrophomonas*

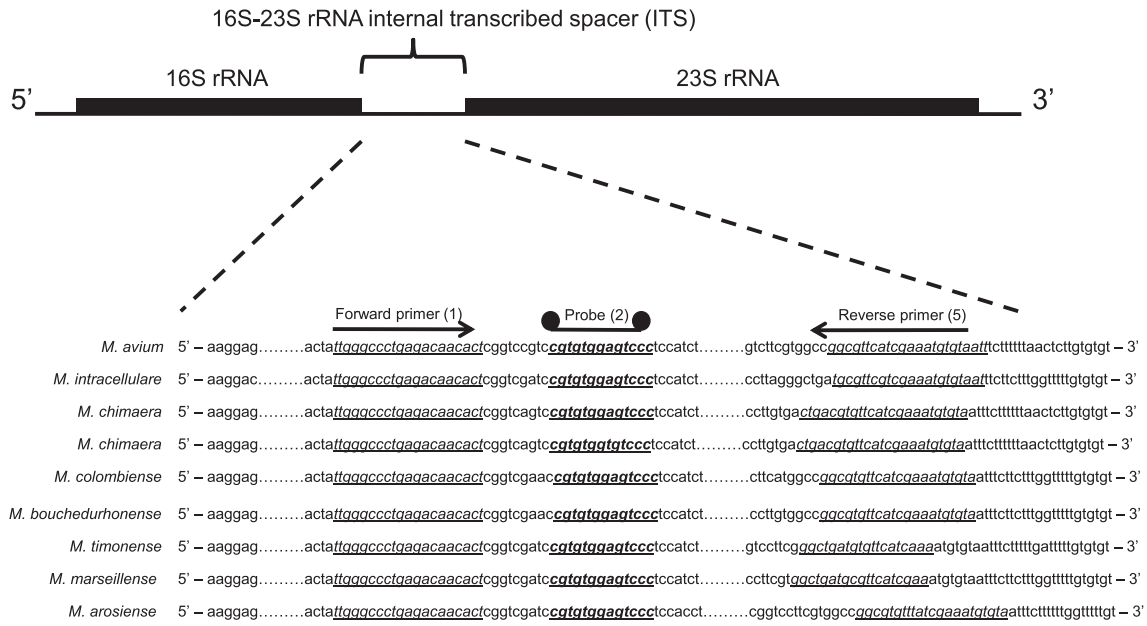

Fig. 1. Sequence alignment of the forward primers, reverse primers, and probes to the ITS region for 9 representative MAC members.

Please cite this article as: Tran AC, et al, Detection of *Mycobacterium avium* complex DNA directly in clinical respiratory specimens: opportunities for improved turn-around time..., Diagn Microbiol Infect Dis (2014), http://dx.doi.org/10.1016/j.diagmicrobio.2014.01.019