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Rapid detection of four non-fermenting Gram-negative bacteria directly from cystic fibrosis patient's respiratory samples on the BD MAX™ system

Talita T. Rocchetti[a,b], Suzane Silbert[a,*], Alicia Gostnell[a], Carly Kubasek[a], Robert Jerris[c], Jennifer Vong[c], Raymond Widen[a]

[a] Esoteric Testing Laboratory, Pathology Department, Tampa General Hospital, Tampa, FL, USA
[b] Laboratório Especial de Microbiologia Clínica (LEMC), Federal University of São Paulo/UNIFESP, São Paulo, SP, Brazil
[c] Children's Healthcare of Atlanta, Atlanta, GA, USA

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ABSTRACT

The aim of this study was to develop a multiplex PCR test to detect *Achromobacter xylosoxidans* (AX), *Burkholderia cepacia* (BC), *Pseudomonas aeruginosa* (PSA) and *Stenotrophomonas maltophilia* (SM) directly from CF patient's respiratory samples using the open mode of the BD MAX™ System. A total of 402 CF respiratory samples were evaluated by culture and PCR. Specific sets of primers and probes for each target were designed in-house. Out of 402 samples tested, 196 were identified as negative and 206 as positive by culture for AX, PSA, BC and SM. Among culture positive samples, PCR detected 21/27 AX, 4/5 BC, 138/140 PSA and 29/34 SM. In addition, PCR assay identified 35 samples as positive that were initially negative by culture for those 4 targets. The CF BDM test proved to be an excellent tool to detect AX, BC, PSA and SM by real-time PCR on an automated platform.

1. Introduction

Cystic fibrosis (CF) is a recessive genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator protein (CFTR) [1]. In consequence, transport of chloride and sodium across epithelial cells is compromised resulting in secretions containing a higher concentration of salt and subsequently more viscosity [2]. Dense mucus precludes mucociliary clearance and favors bacterial colonization. As a result, respiratory infection is the most serious complications that CF patients can present and represent the major cause of morbidity and mortality on this population [3]. For this reason, CF care involves the use of intensive antimicrobial therapy in younger patients to eradicate initial infection and, in older patients to suppress chronic infection or to treat the intermittent exacerbations of respiratory symptoms characteristic of CF [1].

Pseudomonas aeruginosa (PSA) remains the most common non-fermenting gram-negative bacteria isolated from CF respiratory infections. In the past years, some opportunistic non-fermenting gram-negative bacteria, such as *Burkholderia cepacia* complex (BCC), *Achromobacter xylosoxidans* (AX) and *Stenotrophomonas maltophilia* (SM) have been increasingly reported as causing respiratory infections in these patients [4]. Together, these four microorganisms consist of the most prevalent non-fermenting gram-negative bacteria isolated in respiratory infection of CF patients. Respiratory infections caused by these microorganisms are closely related to loss of the lung function and this fact, added to the emergence of multidrug-resistant bacteria, makes it important to

* Correspondence to: Esoteric Testing Laboratory, Tampa General Hospital, 1 Tampa General Circle, Tampa, FL 33606-3571, USA.
E-mail address: ssilbert@tgh.org (S. Silbert).

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rapidly and correctly identify these microorganisms [5].

Culture-based microbiological approaches easily identify pathogens, such as PSA, which are common and prevalent in CF lung infections. On the other hand, it might be challenging for non-pseudomonas bacterial identification, especially in laboratories that are not specialized in CF respiratory examination [6]. For this reason, colonization and infection by these microorganisms can go undetected resulting in negative consequences. In addition, phenotypic techniques are time consuming and results from a traditional culture can take up to 72 h to be available. Early detection of initial respiratory infection, however, allows successful treatment to eradicate or, at least, delay the onset of chronic infections [7,8].

Several techniques have been introduced in the routine of clinical laboratories to accelerate the detection of these pathogens. Polymerase Chain Reaction (PCR) amplification, specifically real-time PCR (qPCR), appears to be an excellent alternative for the diagnosis of bacterial infection directly from respiratory samples of CF patients. Many strategies of qPCR have already been suggested to improve detection [9–11]; none of them, however, use a multiplex test for a concomitant diagnostic of different bacterial strains directly from CF respiratory samples.

The use of automated DNA extraction combined with qPCR avoid the risk of process contamination by reducing sample handling and decrease the test turnaround time which collaborate with early therapy decisions [11]. The BD MAX™ (BDM) System (BD Diagnostic Systems, Sparks, MD) is an automated platform that combines extraction and qPCR on the same instrument. It offers the choice to use BD MAX™ FDA-cleared assays [12] or the open platform mode for user developed tests [13,14].

The aim of this study was to develop a multiplex PCR test to detect *Achromobacter xylosoxidans*, *Burkholderia cepacia* complex, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* directly from CF patient's respiratory samples using the open mode of the BD MAX™ System (BDM).

2. Material and methods

2.1. Cystic fibrosis BD MAX user developed test (CF BDM Test)

2.1.1. Primers and probes

A multiplex real-time PCR was developed to detect AX, BCC, PSA, SM and *Beta-globin* (*BG*, internal control) on the BD MAX System (Table 1). A set of primers and probe to detect PSA, previously described were evaluated in this study [15]. Primers and probes for AX, SM, BCC and BG were designed in-house and were selected based on alignments with sequences collected from the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov). Sequences were aligned using SeqMan II (DNASTAR 5.0 software) to obtain consensus sequences for each target. The AX alignment included all types of $bla_{OXA-114}$ sequences available on NCBI (types a-n, p-v). The BCC alignment included 9 species from *B. cepacia* complex (*B. cepacia*, *B. multivorans*, *B. cenocepacia*, *B. stabilis*, *B. vietnamiensis*, *B. dolosa*, *B. ambifaria*, *B. anthinia* and *B. pyrrocina*). Specific primers and probes were selected by using the Primer3 Program (http://simgene.com/Primer3) and all of them were checked with Oligo-Analyzer 3.0 (http://biotools.idtdna.com.analyzer), an online service of IDT Biotools (Coralville, Iowa) to ensure good parameters (melting temperature, % of Cytosine (C) and Guanine (G) nucleotide, size), minimal self-complementary and to prevent the presence of secondary structures. The binding site was confirmed by Mfold web Server (http://unafold.rna.albany.edu/?q=mfold) to make sure regions that could impede primers and probes to anneal were not present. An NCBI BLAST was performed to check the specificity of the DNA sequences of primers and probes. Primers and probes were synthesized at IDT (Coralville, Iowa) and BG probe at LGT Biosearch Technologies (Petaluma, California).

Table 1
Primers and probes used for the CF BDM Test.

Target	Sequence (5′ to 3′)	Gene
BCC	PF: TCCGGAAAGAAAWCCTTGGY PR: AATGCAGTTCCCAGGTTGAG Pb: **FAM** CGTGCGCAGGCGGTTTGCTA **BHQ1**	16S *rRNA*
AX	PF: CACGAGCCGGTCTGGAA PR: GTGAATACCAGACCACCGAATAC Pb: **JOE** TACCAGCCYGCCTATCCCGACT **BHQ1**	*blaOxa*-114
PSA	PF: ACGACGGTCATGGGCAACT PR: GTGATAGTAGCCGGAGTAGTAGCTGT Pb: **ROX** AAGCTGCTCTCGGAACAGGT **BHQ1**	*regA*
SM	PF: ACTGCGCGTGTARTCGTA PR: GGCATCGATCGKGACACC Pb: **CY5** AAGGCTTCGGCAACAAGCGC **BHQ1**	*metB*
BG	PF: GCAAGGTGAACGTGGATGAA PR: AACCTGTCTTGTAACCTTGATACCAA Pb: **Quasar 705** TTGGTGGTGAGGCCCTGGGC **BHQ3**	*Beta-Globin*

PF: Primer Forward; PR: Primer reverse; Pb: Probe; BHQ 1: Black Hole Quencher 1; BHQ 3: Black Hole Quencher 3.