DEVELOPMENT OF A MULTIPLEX qPCR FOR THE DETECTION OF ATYPICAL PNEUMONIA USING THE AUTOMATED BD MAX™ SYSTEM

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Introduction

A multiplex qPCR was developed for the detection of pneumonia causing bacteria: Legionella pneumophila (Lpn), Mycoplasma pneumonia (Mpn) and Chlamydophila pneumonia (Cpn) in respiratory specimens such as BAL and sputum on the BD MAX system (Becton, Dickinson and Company; BD, figure 1). The BD MAX is a fully automated molecular platform for the extraction of nucleic acids from various sample types followed by qPCR.

Figure 1. BD MAX™ system.

Methods

A multiplex qPCR was designed for the detection of Lpn, Mpn and Cpn. The analytical limit of detection (LoD) was estimated by testing a dilution of the target sequences cloned into a pUC57 plasmid. Each concentration was tested in triplicates using an input volume of 500µl. The LoD was defined as the lowest concentration at which 100% of all replicates tested positive. The quality of the multiplex assay was confirmed by testing the MP.CP2013 and LPDNA2013 panels from the Quality Control for Molecular Diagnostics (QCMD). This panel consist of specimens with various concentrations of Lpn, Mpn or Cpn and are treated similar as clinical samples.

Table 1. QCMD results atypical pneumonia on the BD MAX™

Sample ID	BD MAX (Cq)	QCMD report (Cq)	Core sample
CPMP13-01		Mpn (36.1)	No
CPMP13-02		Cpn (-)	No
CPMP13-03	Cpn (30.0)	Cpn (32.2)	Yes
CPMP13-04	Cpn (27.0)	Cpn (29.4)	Yes
CPMP13-05	Cpn (31.0)	Cpn (35.1)	No
CPMP13-06		Mpn (38.8)	No
CPMP13-07	Mpn (34.3)	Mpn (38.2)	No
CPMP13-08			Yes
CPMP13-09	Mpn (31.9)	Mpn (35.7)	Yes
CPMP13-10			Yes
CPMP13-11		Mpn (-)	No
CPMP13-12	Cpn (29.1)	Cpn (32.8)	Yes
LPN13-01	Lpn (29.5)	Lpn	Yes
LPN13-02			Yes
LPN13-03	Lpn (27.0)	Lpn	Yes
LPN13-04			No
LPN13-05	Lpn (30.0)	Lpn	Yes
LPN13-06	Lpn (27.0)	Lpn	No
LPN13-07	Lpn (26.7)	Lpn	Yes
LPN13-08	Inhibition	Lpn	No
LPN13-09			Yes
LPN13-10	Lpn (28.7)	Lpn	Yes

Finally, to validate the assay, a retrospective clinical evaluation was performed. For this evaluation specimens were pre-treated using proteinase K. Consequently, DNA was extracted using the ExK DNA-1 extraction kit (BD) and amplification was performed using the BD MMK (SPC) mastermix (BD). Results were compared to our in-house reference method (EasyMAG, Biomerieux; ABI7500fast system, Life Technologies).

Results

Analytical sensitivity for Lpn, Mpn and Cpn were determined at 4, 14 and 5 copies per PCR, respectively.
Results from the QCMD panels gave a 100% concordance to the final report for Cpn and a 100% score for all core samples for Mpn and Lpn (table 1). During the retrospective clinical evaluation a total of 30 respiratory specimens were tested, of those specimens 5 (16%) were positive for Lpn, 3 (10%) were positive for Mpn and 2 (6%) were positive for Cpn. Furthermore, for 2 samples no Cq-value was found for the IC and could therefore not be used in the comparison.

CONCLUSIONS

- A sensitive method for the detection of atypical pneumonia has been developed.
- The ease of use of the BD MAX system makes this assay applicable in a wide range of microbiological laboratories.
- An updated version of this assay with the addition of *C. psittaci* has been developed.

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