

INTRODUCTION

The proportion of lower respiratory tract infections in children and adults, including community-acquired pneumonia, associated with *M. pneumoniae* infection during the past 10 years has ranged from 0% to more than 50%, varying with age, the geographic location of the population examined and the diagnostic methods used. Serological methods, in particular such as the complement fixation test (CFT) and EIAs, are most widely used to diagnose a *M. pneumoniae* infection. The application of PCR is more and more accepted as a rapid diagnostic test since culture is too slow and too insensitive to be therapeutically relevant. The aim of this study was to evaluate the performance of a new molecular assay based on the Loop Mediated Isothermal amplification (LAMP) technology, the Illumigene Mycoplasma DNA amplification assay on dilution series from titrated reference strains, on the 2014 *M. pneumoniae* Quality Control for Molecular Diagnostics (QCMD) panel and on 12 archived *M. pneumoniae* positive specimens.

Sample nr	Sample type	Result Illumigene	Result in-house PCR
LBA nr 12	BAL	POS	POS
10085296	pleural fluid	POS	POS
10083565	throat swab	POS	POS
11104290	NPA	POS	POS
11103869	NPA	POS	POS
11096404	NPA	POS	POS
11106798	BAL	POS	POS
12111037	BASP	POS	POS
11123005	SPUT	POS	POS
13033300	BAL	POS	POS
14025919	BAL	POS	POS
12040168	throat swab	POS	POS

Table 2. Result archived samples

MATERIALS & METHODS

Dilution series of 5 titrated reference strains (PI1428, and JAP377, 3996PL, 4972BRA, 6303DES kindly provided by C. Bébéar) were made. The stock concentration of the strains varied between $1,1 \cdot 10^7$ and $6,5 \cdot 10^7$ colour changing unit per ml (CCU/ml). In addition, the 2014 QCMD *M. pneumoniae* panel for External Quality assessment (EQA) was tested. Finally, twelve archived (-70°C) respiratory specimens, previously found to be *M. pneumoniae* positive by in-house real-time PCR were included in this study. Nucleic acids were extracted by using the NucliSens EasyMag (BioMérieux). Eluates were tested immediately using the Illumigene Mycoplasma DNA amplification assay. The same extract was amplified the day after by the in-house real-time PCR targeting the P1-gene.

QCMD panel	Result Illumigene	Result in-house PCR (original)	Result in-house PCR (repeat)
CPMP14-01	NEG	NEG	NEG
CPMP14-02	NEG	NEG	NEG
CPMP14-03	POS	POS	POS
CPMP14-05	NEG	NEG	NEG
CPMP14-06	NEG	NEG	NEG
CPMP14-07	NEG	NEG	NEG
CPMP14-08	NEG	NEG	NEG
CPMP14-09*	NEG	POS	NEG
CPMP14-10	POS	POS	POS
CPMP14-11	NEG	NEG	NEG
CPMP14-12*	NEG	POS	NEG

* low bacterial load in original sample

Table 1. Result 2014 QCMD panel

RESULTS

When analysing the dilution series of the *M. pneumoniae* reference strains, 1 discordant result between the in-house real-time PCR and the Illumigene Mycoplasma DNA assay was obtained with the lowest dilution of the reference strain with the lowest stock concentration ($1,1 \cdot 10^7$ CCU/ml). This concentration was at the limit of detection for both assays. After retesting the archived QCMD2014 *M. pneumoniae* panel by in-house PCR no discordant results were obtained (Table 1). All 12 clinical samples were *M. pneumoniae* positive by the Illumigene Mycoplasma DNA amplification assay as well (Table 2).

CONCLUSIONS

The Illumigene Mycoplasma DNA amplification assay is comparable in sensitivity and specificity to the in-house real-time PCR, with 1 discordant result, probably at the limit of detection. The assay is rapid and easy to use. Further testing on a larger number of positive and negative specimens is recommended.

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