

# Comparison between two RealTime PCR methods in respiratory infections from *Cp. pneumoniae* and *M. pneumoniae*

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## Introduction

*Cp. pneumoniae* (*Cp*) and *M. pneumoniae* (*Mp*) account for frequent causes of community acquired respiratory infections. In the United States the incidence of pneumonia caused by these pathogens is 20-40% with 600.000 hospital patients each year<sup>[1,2]</sup>.

Diagnosis of these pathogens is normally performed through direct (coltures and NAAT) and indirect (serology) techniques with different sensitivity and specificity and different turn-around-time. In particular molecular biology techniques such as RealTime PCR (RT-PCR) show a higher specificity and sensitivity for the detection of *Cp* and *Mp* by comparison to other diagnostic techniques.

In this study we compared two RT-PCR techniques (LightCycler RealTime PCR Roche and affigene Cp/Mp tracer Alfa Wassermann Diagnostics) for the detection of *Cp* and *Mp* in biological samples.

## Methods 1

In 2007 50 biological samples coming from hospital patients have been investigated (AFN and BAL). These patients showed acute respiratory symptoms. After preparing the cellular pellet, samples have been extracted using NucliSens easyMag Biomerieux, according to manufacturer's procedures.

## Methods 2

### Amplification using LightCycler RealTime PCR Roche Technique

The extracted material has been amplified with RT-PCR technique using LightCycler II with *Mycoplasma pneumoniae* LCSet Kit and *Chlamydia pneumoniae* LCSet kit (TIB molbiol for Roche). The method consisted in the use of glass capillaries where 15 µl of master mix and 5 µl of DNA extracted from the sample have been put into contact (Fig.1). The amplification mix has been prepared adding to primers and probes the hot start Taq DNA polymerase present in the LightCycler FastStart DNA Master<sup>plus</sup> Hybridization Probes (Roche). During the reaction, a genomic fragment (target gene) of the pathogen (polymorphic membrane protein G family of 14bp for *Cp* and cytoadhesin P1 gene of 342bp for *Mp*) has been amplified using FRET probes and specific primers<sup>[3]</sup> (Fig. 2) and detected using fluorescence coming from the couple of specific hybridization probes. The detection has been performed with a real time reading of the fluorescence from amplification products, directly inside the capillary with a detection limit of 5 genomic equivalents/PCR reaction in biological samples for both pathogens. The reading is performed at each cycle on six channels, using probes labelled with different fluorophores (ex.: LC-Red 640) (Fig.3). The use of this test is for scientific research only.

## Methods 3

### Affigene Cp/Mp Tracer Alfa Wassermann Diagnostic Technique

The extracted material has been amplified with a RT-PCR multiplex technique which allows for the simultaneous detection of *Cp* (Hex) and *Mp* (Fam) and an Internal Control DNA IC Carrier (Rox), certified CE-IVD, using the Stratagene Mx3000P system for the amplification with the affigene Cp/Mp tracer kit which allows for results analysis using the affigene analysis software (Fig. 4). The affigene *Cp/Mp* tracer kit foresees the use of scorpions primers<sup>[4]</sup> which act through a unimolecular mechanism amplifying the signal and preventing aspecific signals from happening. These aspecific signals come from the amplification reaction of the genomic fragment (target gene) of the pathogenic agent Major Outer Membrane Protein (MOMP) for *Cp* and P1 adhesion protein for *Mp* (Fig. 5). During the reaction, strips with 8 optical caps and strips with 8 microtubes are used. Inside them 25 µl of master mix and 25 µl of DNA extracted from the clinical sample are put into contact. The limit of detection in biological samples (Limit of Detection, LOD) is 3.7 genomic equivalents/PCR reaction of *Cp* (interval 2.5-8.7) and 4.2 genomic equivalents/PCR reaction of *Mp* (interval 2.5-11.1). The material used is disposable (Fig.6).

## Results

Though both technique showed a different sensitivity while detecting the presence of *Mp* and *Cp* genetic material, following what has been stated by the two manufacturers, both techniques used here showed compliant results except for a biological sample, positive for *Mp* only with the RT-PCR affigene Cp/Mp tracer Alfa Wassermann Diagnostics method. By the way, such a result has been confirmed by serology performed for the research of IgG and IgM anti-*Mp* antibodies with an Immunoenzymatic ELISA technique (Novagnost<sup>TM</sup> *Mycoplasma pneumoniae* IgG and IgM, Dade Behring), showing seroconversion, by comparison to a previous sample (Tab. 1). All results obtained were in

compliance with the clinical suspicion of such infections and in the majority of cases with the presence of IgG and IgM specific anti-Cp (Microimmunofluorescence technique) and anti-Mp antibodies (ELISA Novagnost™ Mycoplasma pneumoniae IgG and IgM Dade Behring technique).

**Conclusions**

Both RT-PCR techniques were easy to perform and simple while analysing results obtained. Both were very sensitive and therefore very useful for lab. diagnosis of respiratory infections caused by Cp and Mp. In particular Affigene Cp/Mp tracer Alfa Wassermann Diagnostic technique showed a considerable diagnostic applicability since it is CE-IVD certified, easy to perform (RT-PCR Multiplex) and with relatively low costs.

Fig. 1 Test Materials and Methods

Fig.2 The Principle of FRET Probes

Fig. 3 Amplification Curve

Fig. 4 Test Materials and Methods

Fig. 5 The Principle of SCORPIONS Probes

Fig. 6 Amplification Curve

Tab.1 Results from the comparison between RT-PCR techniques

Kit used	Pathogens investigated			
	Cp		Mp	
	Pos	Neg	Pos	Neg
Roche	10	40	1	49
Alfa Wassermann	10	40	2	48