Development of a new diagnostic tool for the detection of Chlamydia pneumoniae and Mycoplasma pneumoniae in a duplex real-time PCR

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Introduction
Chlamydia pneumoniae and Mycoplasma pneumoniae are two atypical respiratory pathogens. Both bacteria are an important cause of community-acquired pneumonia (CAP), between 10% and 20% of cases approximately. Symptoms may be mild but the most common Upper and Lower Respiratory Infections (URI and LRTI) in children and adults include tracheobronchitis, pharyngitis, laryngitis, sinusitis and also more severe illness like atypical pneumonia. Chlamydia pneumoniae and Mycoplasma pneumoniae can be also implicated in chronic pulmonary diseases such as bronchial asthma. A large number of respiratory agents involved in respiratory tract infections, including viruses and bacteria share similar clinical features and symptoms. Identification and differentiation of Chlamydia pneumoniae and Mycoplasma pneumoniae from other agents are important to choose or to adapt an appropriate and effective antibiotic therapy. Culture, serological and antigen detection techniques are currently being used for the diagnosis but these methods have many drawbacks such as cost, time to result and low sensitivity. Now, the Nucleic Acid Amplification Techniques (NAATs) with Real-Time PCR techniques have many benefits for the detection of respiratory pathogens like high sensitivity and specificity and are much quicker. We propose a new real-time PCR based diagnostic tool for Chlamydia pneumoniae and Mycoplasma pneumoniae diagnosis. Our duplex Real-Time PCR kit Chla/Mycopro pneumonia r-gene® allows the simultaneous detection of both bacteria in a single tube reaction.

Materials & Methods
Extraction:
Respiratory samples are pre-treated with 10μl (for 200μl of sample) of Proteinase K (Novagen) at 20mg/ml and incubated for 15 min at 56°C. Nuclease/EasyMAG extraction (MALogene) are validated for a volume of 400μl of sample eluted in 100μl, or 200μl of sample eluted in 50μl. For both volumes, 50μl of magnetic silica is used.

Amplification:
15μl of extracted sample are added to 15μl of ready-to-use Chla/Mycopuro amplification premix. Signal is read at 531nm for Chlamydia pneumoniae and at 560nm for Mycoplasma pneumoniae.

QCMD EQA Programme 2011 Chlamydia pneumoniae & Mycoplasma pneumoniae:
Chlamydia pneumoniae and Mycoplasma pneumoniae panels 2011 are extracted on Nuc/SESS® easyMAG® of 200μl of sample eluted in 10μl. PK pre-treatment is performed. QCMD CPMP 2011 panel is amplified with Chla/Mycopuro pneumonia r-gene® kit (ref 71-044) on ABI 7500 Fast (Applied Biosystem), Dx Real-Time System (Bio-Rad) and LC480 (Roche).

Analytical Sensitivity:
The analytical sensitivity of the Chla/Mycopuro pneumonia r-gene® kit is determined experimentally on quantified samples of Chlamydia pneumoniae (at 4.9 RFU/10μl) and Mycoplasma pneumoniae (at 5.000 CCU/10μl) from the panel QCMD CPMP 2010. For each bacteria, serial dilutions are performed in a nasopharyngeal sample negative for both bacterial species. Each dilution is extracted 15 times using the Nuc/SESS® easyMAG extraction with 20μl of sample eluted in 5μl, PK pre-treatment is performed. Each extract is amplified with Chla/Mycopuro pneumonia r-gene® kit (ref. 71-044) on ABI 7500 Fast.

Specificity:
The specificity of the Chla/Mycopuro pneumonia r-gene® kit is determined experimentally on a panel of various viruses/bacteria representing respiratory pathogens or present in respiratory samples. Nuc/SESS® easyMAG extraction of 400μl of sample eluted in 100μl is performed then amplification is done on a Veran KPCR AD (Siemens).

Intra/Inter-assay reproducibility:
The reproducibility is performed on different concentrations of ATCC cultures (ATCC VR-1355 and ATCC29342) or quantified samples from the panel QCMD CPMP 2011 of Chlamydia pneumoniae and Mycoplasma pneumoniae. For the intra-assay reproducibility, both bacteria are diluted at 10x, 5x and 2x the LOD in a nasopharyngeal negative sample. Each dilution is extracted 10 times on Nuc/SESS® easyMAG with 20μl of sample eluted in 5μl. PK pre-treatment is performed. Each extract is amplified in a same run with Chla/Mycopuro pneumonia r-gene® kit (ref. 71-044) on LC480. For the inter-assay reproducibility, both bacteria are diluted at 100x, 10x and 5x the LOD in a nasopharyngeal sample negative. Each dilution is extracted 10 times on 10 independent runs of extraction using extraction Nuc/SESS® easyMAG with 20μl of sample eluted in 5μl. PK pre-treatment is performed. Each extract is amplified in 10 independent runs with Chla/Mycopuro pneumonia r-gene® kit (ref. 71-044) on DX Real Time System.

Results
QCMD 2011 Chlamydia pneumoniae & Mycoplasma pneumoniae EQA programme

Analytical Sensitivities of Chlamydia pneumoniae and Mycoplasma pneumoniae

Specificity
None of following viruses or bacteria is amplified with Chla/Mycopuro pneumonia r-gene®, which attests of the good specificity of the assay.

Conclusion
Results presented in this study show the sensitivity, robustness and reliability of 71-044 Chla/Mycopuro pneumonia r-gene® kit. The high quality associated with its compatibility with the major extraction and real time PCR platforms allows an immediate integration of Chla/Mycopuro pneumonia r-gene® in most routine diagnostic laboratories. This tool belongs to the respiratory MWS r-gene® brand new range of product which represents an innovative solution in response to the challenges in respiratory infections diagnosis. These PCR assays can assist clinical laboratories in identifying 12 respiratory pathogens or families of respiratory pathogens in hospitalized patients and aid in patient therapy management.