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Poster Session V
Molecular diagnosis of respiratory tract bacterial infections

DEVELOPMENT AND VALIDATION OF A REAL-TIME MULTIPLEX PCR METHOD FOR DETECTION OF COMMON ATYPICAL RESPIRATORY TRACT BACTERIAL PATHOGENS.

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Objective:
Atypical bacterial pathogens, including *Chlamydophila pneumoniae*, *Mycoplasma pneumoniae*, and Legionella pneumophila, and some other uncommon Legionella species are involved with lower respiratory tract infections. The diagnosis of these organisms are usually performed by serology and culture. The serology is insensitive and culture remains difficult, time-consuming, and insensitive. The rates of respiratory infections in relation to these pathogens are probably underestimated due to the difficulty in identifying them. Rapid and accurate identification of these causative agents is a key component for establishment of proper prognosis, and facilitate early treatment. Nucleic acid amplification techniques are suitable for the rapid detection of these microbial agents. Over the past decade several PCR assays have been described that allow the detection of these pathogens. We have developed and validated a simple, rapid, and cost-effective multiplex real-time PCR assay to detect *C. pneumoniae*, *M. pneumoniae*, and *L. pneumophila* directly in respiratory samples.

Methods:
A collection of 410 nasopharyngeal swab (NPS) and broncoalveolar lavage (BAL) specimens send for routine testing for respiratory pathogens were used for the method validation. Two hundred microliters of BAL or Universal Viral Transport Medium (UTM) was used to extract and purify DNA using EasyMag (Biomereux) automated instrument. Five microliter of 55ul total eluate was used as the template for PCR. A 87bp *mip* gene of *L. pneumophila* (LP), 135 bp 16S rDNA of *M. pneumoniae* (MP), and 118bp 16S rDNA of *C. pneumoniae* (CP) was used as PCR targets. The bacteriophage lambda DNA (144bp) was used as an internal control (IC). Primers and TaqMan probes were designed by SciTool (Integrated DNA Technology) using gene sequences obtained from Genbank. All oligonucleotides were synthesized by BioSearch Technologies. Genes were amplified and detected using the QuantiTect Multiplex PCR kit (Qiagen) supplimented with respective primers and TaqMan probes in a Rotorgene 6500 (Qiagen). A reference laboratory PCR method was used to validate and to determine test performance characteristics.

Results:
Out of 410 specimen tested 363 were negative for all target organisms by both reference and new PCR method. Forty six specimens were positive for either LP (n=20), MP (n=8), or CP (n=18). None of the positive specimens had more than one organism. The reference method detected one additional LP positive specimens. The test performance characteristics showed a 100% specificity, 97.9% sensitivity, 100% positive predictive value (PPV), and 99.7% negative predictive value (NPV) for the new method as compared to the reference method. The cost per test is Cdn$ 10 and the turn-around-time is under 3 hrs.

Conclusions:
The new real-time multiplex PCR method is very specific, sensitive and cost-effective and can be used to detect C. pneumoniae, *M. pneumoniae*, and *L. pneumophila* in respiratory specimens accurately and timely. This will facilitate appropriate utilization of antimicrobial therapy.