Introduction

Mycoplasma pneumoniae (Mpn), Chlamydia pneumoniae (Cpn) and Legionella pneumophila (Lpn) can cause both epidemic and endemic occurrences of acute respiratory disease and are responsible for 25–40% of cases of community acquired pneumonia. We developed and evaluated a multiplexed, real-time PCR assay for the detection of these agents in oropharyngeal swabs (OP) and broncho-alveolar lavage (BAL) specimens on the Luminex ARIES® instrument. This is a fully integrated, walk-away instrument for RT-PCR.

Methods

OP, collected in Universal Transport Medium (UTM), and BAL specimens were spiked with 10-fold dilutions of Mpn, Lpn or Cpn. Two hundred ul of each specimen was added to cassettes and then primer containing readymix loaded onto ARIES cassettes.

Primer pairs were obtained from Luminex. Internal (MHV 5) control primers were used to amplify the sample processing control (SPC) in each cassette. ReadyMix and cassettes were purchased from Luminex and contained all reagents for extraction, purification, amplification and detection.

The ARIES instrument generates a Ct value, including the SPC, as well as a confirmatory Tm value, for each reaction.

Instrument: ARIES® is an in vitro diagnostic medical device for detection of nucleic acids by fluorescence based PCR (Figure 1).

Instrument Preparation: 6 μl of Primers mix were added to each ReadyMix tube, attached to the sample added cassettes and loaded on the ARIES®. After loading, the reaction then proceeded to completion in a hand-off manner. The Luminex ARIES® generates RT-PCR amplification and melt curves for each target, with a resulting Ct value calculated for both the sample processing control and the targets (Figure 2 and 3).

Results

Limit of detection (LOD) for Mpn, Cpn and Lpn, using data from both specimen types, were 200 cfu/mL, 200 cfu/mL and 20 cfu/mL, respectively. Twenty-five of 23 Mpn-positive samples tested positive (either OP or BAL specimens), giving a 100% accuracy. All Cpn-positive samples (either OP or BAL specimens) were likewise positive using the ARIES® assay (100% accuracy) and 20 Lpn-positive samples (all BAL specimens) were confirmed positive using the ARIES® system (100% accuracy). All previously negative samples were confirmed negative using the ARIES® assay. No specimens were positive for Cpn. Reproducibility of the resultant Ct values, did not change over the course of the different testing period by different operator No reactivity was seen during any of the specificity testing.

Stability of the collected specimen, in terms of reproducibility of the resultant Ct value, did not change over the course of the 8-day testing period. Since November 2016, a total of 836 patient specimens have been processed in our reference laboratory, with six positive Mpn and two positive Lpn.

| Table 1: Mpn Accuracy | Twenty-three MPN positive samples were positive out of 23 samples with ARIES assay, giving a 100% accuracy. |
| Table 2: Cpn Accuracy | All Cpn-positive samples (either OP or BAL specimens) were likewise positive using the ARIES® assay (100% accuracy). |
| Table 3: Lpn Accuracy | 20 Lpn-positive samples (all BAL specimens) were confirmed positive using the ARIES® system (100% accuracy). |

Conclusion

The availability of a real-time PCR assay greatly enhances the ability to rapidly diagnose infection caused by these agents.

The Luminex ARIES® system involves minimal technologist time in sample preparation, and the cassettes contain all necessary reagents for extraction, amplification and detection.

The ARIES® software generates the resulting curves, assigns the corresponding Ct values and confirmatory Tm values. This instrument is easily adapted to additional target sequences.

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