Real-time PCR detection of *Streptococcus pneumoniae* in respiratory specimen BAL, whole blood, cerebrospinal fluid and urine using the Luminex ARIES® platform

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INTRODUCTION

- Community-acquired pneumonia (CAP) and complications such as bacteremia and meningitis due to Streptococcus pneumoniae infection still occurs in at risk populations, despite the availability of effective vaccines.
- Laboratory confirmation of *S.pneumoniae* remains challenging in cases of CAP despite advances in blood culture techniques and the availability of nucleic acid amplification tests.
- In our experience, current microbiological methods and diagnostic assays leave up to 80% of in-hospital patients without an identified pathogen causing pneumonia, potentially leading to misuse of antibiotic.
- In this study, we are targeting a portion of the *S. pneumoniae* specific gene (autolysin, lytA) for detection. Using RT-PCR MultiCode® Technology, we tested four different clinical samples types: bronchoalveolar lavage (BAL), whole blood (WB), cerebrospinal fluid (CSF) and urine to cover the various presentations of invasive pneumococcal infections.

MATERIALS AND METHODS

- S. pneumoniae primer pairs were designed to include one fluorescent reporter labeled primer with an MultiCode® isoC on the 5' end with an unlabeled primer, both obtained from IDT. The primer sequences are /56-FAM//iMe-isodC/A CGC AAT CTA GCA GAT GAA GCA and CTC CCT GTA TCA AGC GTT TTC GGC
- The sample processing control primer murine hepatitis virus 2 (MHV 2), ReadyMix® and cassettes were purchased from Luminex. The cassette with ReadyMix® contains all components needed to extraction of nucleic acid and perform PCR.
- Sensitivity (Limit of Detection, or LOD), precision, accuracy and stability for BAL, WB, CSF and urine were tested to validate the assay to meet CLIA requirements. Waste clinical samples were used and spiked with *S. pneumoniae* when necessary (Figure 1).

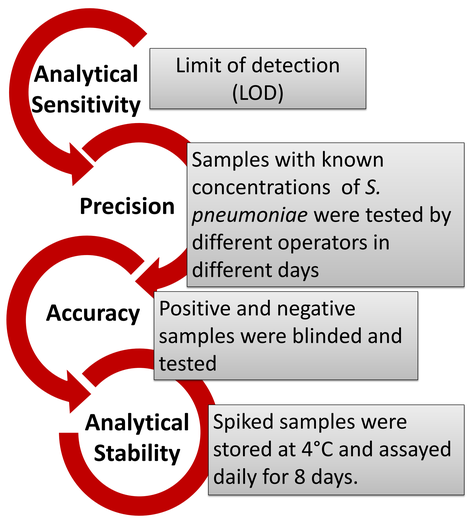

Figure 1. Testing scheme

INSTRUMENT

- The ARIES® is an in vitro diagnostic medical device for detection of nucleic acids by fluorescence based PCR.
- The lytA and MHV 2 primer pool were added to the ReadyMix® tubes, attached to the cassettes. Next, the clinical samples were added to the cassette and loaded in the ARIES®.
- The ARIES® generates PCR amplification and melt curves for the target, with resulting Ct values calculated for both the sample processing control and the target.

RESULTS

Analytical Sensitivity: *S.pneumoniae* organisms were serially diluted ten-fold from 1 x 10^4 to 1 x 10^{-1} CFU/mL for these experiments. Results are from three replicate, independent experiments. The different sample types LOD for *S.pneumoniae* are shown in the following table.

Sample type	LOD (CFU/ml)	lytA Ct value Avg(SD)
BAL	1 X 10^2	35.23 (0.50)
WB	1 X 10^2	36.00 (0.70)
CSF	1 X 10^2	33.97 (0.47)
Urine	1 X 10^1	37.13(1.63)

Precision: Reproducibility of the resultant Ct value did not change over the course of the different testing period by different operators. The standard deviation (SD) for lytA Ct values were less than 2.5, on all specimen types.

Accuracy: Ten previous lytA positive were like wise positive with the assay and Ten negative samples for all different specimen types were also negative using lyt A assay, giving a 100 % accuracy for all sample types

Analytical stability: Spiked positive specimens were stored in refrigerator for 8 days. All specimens appeared stable at 4ºC temperature for up to 8 days.

DISCUSSION AND CONCLUSIONS

- The significance of this method validation is to provide a rapid and sensitive test for assessing *S. pneumoniae* infection using four common specimen types.
- A CLIA-validated diagnostic test for invasive pneumococcal infections benefits the local healthcare community by providing quality test results within a few hours of specimen receipt.
- In addition, the ARIES® platform minimizes hands on time for laboratory technologists and allows for validation of laboratory developed tests

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