Inclusivity and Exclusivity of a Highly Multiplexed PCR System for the Detection and Quantification of Lower Respiratory Tract Pathogens

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Background

Identification of etiological agents of lower respiratory tract infections (LRTI) is a diagnostic challenge as multiple bacteria, viruses, and fungi are implicated. Typical bacterial pathogens that can cause LRTIs are often listed as normal flora of the oropharyngeal passage. Approximately 40% of community-acquired pneumonia and 94% of hospital-acquired pneumonia cases can be attributed to these opportunistic bacteria. However, clinical samples such as sputum, endotracheal aspirates (ETA), and bronchoalveolar lavage (BAL) routinely used for LRTI diagnoses are collected from or through the oropharyngeal passage, and may be contaminated. Quantification of these commensal bacteria can help determine whether they may be normal flora or causative agents of the pneumonia. The FilmArray® LRTI Panel (FA-LRTI, BioFire Diagnostics, LLC) is being developed to be rapid (~1 h) and sensitive alternative to culture and traditional methods for identification and relative quantification of common bacterial causes of LRTI as well as qualitative detection of several viruses, fungi, and select antibiotic resistance genes. This study evaluated the inclusivity and exclusivity of a prototype FA-LRTI Panel composed of 37 assays to detect multiple analytes.

Methods

A total of 506 isolates of viruses, fungi, and bacteria (some of which contained antibiotic resistance markers) were tested with a development (RUO) version of the FA-LRTI Panel by multiple users at three different sites. Culturable bacteria and fungi were grown on a McFarland standard of 2 in an appropriate liquid media and diluted to test concentrations. Unculturable bacteria and fungi, as well as viruses, were diluted to appropriate test levels based on the provider’s ‘quantification. Inclusivity (reactivity) for bacteria was assessed at a moderate concentration of 10^4 CFU/mL and exclusivity (specificity) was assessed at a high concentration (~10^6 CFU/mL) for organisms not expected to be detected by the panel.

Inclusivity

All inclusivity strains tested at 10^4 CFU/mL were detected by the panel:
- 99.99% quantitative target analytes (bacteria, fungi, antibiotic resistance markers)
- 99.99% quantitative target analytes (viruses)
- FA-LRTI Panel quantification was within 1 log unit of the test concentration (10^4 CFU/mL) across multiple strains of quantitative analytes.

Inclusions were concordant with titers estimated by other molecular methods.

Exclusivity

- Panel exclusivity was assessed using 209 species:
  - On-panel organisms (no intra-panel cross-reactivity)
  - Phylogenetic-neighbors (species specificity)
  - Representative subset of normal oropharyngeal flora
- 100% specificity observed for 34/37 targets 3/37 assays were found to interact with near-neighbor species
- Smaltophila assay with S. acidominophilus and S. mitribaldicus
- Protease assay with C. mycoploecies
- Ecoli assay with E. hermannii
- Interactions were infrequent, and will only be observed as cross-reactivity at high titers of organisms.

Conclusions

These results indicate that the prototype FA-LRTI Panel can identify numerous pathogens implicated in LRTI with a high degree of specificity. This study suggests that the prototype FA-LRTI Panel can provide accurate and consistent quantification across a variety of commensal bacterial strains that is comparable to traditional microbiological methods.

All data presented were obtained with a development (RUO) version of the panel. The FilmArray LRTI Panel has not been evaluated by the FDA or other regulatory agencies for in vitro Diagnostics use.