ABSTRACT

Introduction: We have previously described the FilmArray platform (previous posters at FilmArray.com) for performing nested multiplex PCR (nmPCR) in a fully automated, closed system and its application to the detection of respiratory pathogens. In the past year we have focused on preparing the system for analytical and clinical studies, the latter to start in November 2009. This has included surveys and consultations to determine the label target panel for the Respiratory Pathogens Panel. The effort that went into designing a diagnostic platform and the hurdles to bring changes is important to choose the panel cautiously.

Methods: In order to design a reporter panel that meets the needs and expectations of the clinical laboratory we conducted a web survey of clinical lab directors and physicians regarding which respiratory pathogens they would like to see included in the panel. In addition we conducted in depth interviews with several survey respondents and consulted with laboratory experts and infection disease physicians.

Results: Although there were consensus on a core set of organisms there were marked differences on the relevance of some newly recognized viruses and on the importance of several bacteria. In this report we chose to include them in this panel so as to include the increasing number of unknown viruses whose clinical relevance is likely important but not fully understood. There was significant interest in several bacteria, including H. influenzae and S. pneumoniae which are common causes of bacterial pneumonia. We did not include them as they are known to cause the most number of healthy individuals. We did not include S. pneumoniae, S. pyogenes, M. tuberculosis, and Legionella species due to regulatory or assay development considerations. The final FilmArray Respiratory Pathogens Panel has assays for Adenovirus, Bocavirus, Coronavirus (229E, HKU1, OC43, NL63), Influenza A (pan MA, pan NS, H1, H3), Influenza B, Metapneumovirus, Parainfluenza (1, 2, 3, 4), RSV, Rhinovirus, S. aureus, S. pneumoniae, Bordetella pertussis, C. pneumoniae, L. pneumophila.

The survey also asked for physicians who currently diagnose respiratory infection. Based on our data two thirds of respiratory infections in the US are diagnosed based on observation. Physicians cited the following top three reasons for the lack of additional testing 1) it takes long to get results, 2) current tests do not detect all relevant respiratory pathogens, 3) most respiratory infections are viral and can’t be treated empirically.

Conclusions: With a one hour time to result and a full panel of respiratory pathogens tests, the FilmArray addresses two of the main concerns of clinicians. Use of the FilmArray for such composite panels will be defined by the composite panel of pathogens present in respiratory diseases and their clinical significance. In the long run this will drive the development of the vaccines and antibiotic/medications that improve patient outcomes.

MOLECULAR DETECTION OF RESPIRATORY PATHOGENS

Figure 1. The FilmArray Instrument

Figure 2. FilmArray in use at PCMC microbiology laboratory, Salt Lake City, UT

Figure 3. Schematic of Nested Multiplex PCR

Figure 4. The FilmArray Pouch

Figure 5. FilmArray RP Software Display at the end of a Run

Figure 6. FilmArray RP analysis of an NPA sample Detected BOCAvirus and Influenza A

Figure 7. FilmArray amplification of 506 RNA transcripts

Figure 8. Final panel of Respiratory Pathogens Adenovirus Bocavirus Coronavirus (229E) Coronavirus (HKU1) Coronavirus (OC43) Coronavirus (NL63) Coronavirus (SARS) Influenza A Influenza B Influenza C Mycoplasma pneumoniae Mycoplasma suis Pneumocystis jirovecii Rhinovirus Bordetella pertussis Chlamydophila pneumoniae Legionella species (all) Herpes simplex virus (HSV) Metapneumovirus Parainfluenza 1 Parainfluenza 2 Parainfluenza 3 Influenza B parainfluenza 4 Respiratory Syncytium Virus Strepococcus pneumoniae Staphylococcus pneumoniae

CONCLUSION

The Table 2 indicates that for an upper respiratory pathogen panel include a) newly discovered organisms of unknown significance; b) less frequently observed organisms which are difficult to test in clinical trials and c) organisms of unknown clinical significance. We have tested these various combinations with comprehensive platform there will be debate as to the best set of organisms for this kind of panel. The respiratory pathogen panel. This panel will define the composite panel of pathogens present in respiratory disease and their clinical significance. In the long run this will drive the development of the vaccines and antibiotic/medications that improve patient outcomes.