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Abstract (poster session)

Comparison of in-house real-time polymerase chain reaction (RT-PCR) assays with a multiplexed, automated system for detection of respiratory tract pathogens in clinical samples

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Objectives: PCR methods have been shown to be more sensitive than culture and antigen detection methods for diagnosis of respiratory virus infections. We developed a panel of semi-automated in house real-time PCR (rtPCR) assays for routine high-throughput detection of respiratory pathogens. In this study we compared its performance with the the fully automated FilmArray® multiplexed respiratory panel (Idaho Technology Inc., Salt Lake City, UT). Methods: Comparative analysis of the two diagnostic systems in 200 retrospective clinical specimens collected from the upper and lower respiratory tract and in serial dilutions of different respiratory virus isolates. For the in house rtPCR method, nucleic acids were purified in a NucliSens easyMAG instrument (BioMerieux). PCR reactions were set up by a liquid handler (Perkin-Elmer) and amplified in ABI-Prism HT7900 Systems (Life Technologies). The turnaround time was 5-6 h and up to 22 samples could be handled simultaneously. The FilmArray® system detected respiratory pathogens from unprocessed samples in about 1 hour, but analyzed only one sample per run. For this study, pathogens detected by both methods were considered (influenza A subtypes, influenza B, RSV A-B, hMPV A-B, coronaviruses NL63, OC43, 229E, and HKU1, adenovirus, PIV 1-3, bocavirus, rhinovirus, enterovirus, B. pertussis, M. pneumoniae, and C. pneumoniae). Results: There was a complete agreement between the two assays in the detection and typing of all viruses, with the exception of adenoviruses, for which the in house rt-PCR method was more sensitive. Perfect agreement was demonstrated in genotyping and subtyping influenza viruses, hPIVs, and coronaviruses, while both assays demonstrated some cross-reactivity between rhinoviruses and enteroviruses. Application of both tests in parallel in the diagnostic routine demonstrated that the FilmArray® system was useful for rapid testing of specimens collected from critical patients with results early available to support real time clinical decisions. Conclusion: The two methods showed a very good agreement in pathogen detection and typing. The in house rtPCR method allowed a large number of samples to be analyzed in a few hours and was more suitable to rapidly manage many routine samples during the winter season, while the FilmArray® system demonstrated a significantly reduced run time and hands-on time, suitable for rapid testing of critical patients and, potentially, for point-of-care testing.