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Review

Clinical and economical impact of multiplex respiratory virus assays ☆

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ABSTRACT

During the last decade, a variety of molecular assays targeting respiratory viruses have been developed and commercialized. Therefore, multiplex PCR are increasingly used in everyday clinical practice. This improves our understanding of respiratory virus epidemiology and enhances our concerns about their clinical impact in specific patient populations. However, questions remain regarding cost-effectiveness of performing these diagnostic tests in routine and their real impact on patient care. This article will review available data and highlight unresolved questions about cost-effectiveness, infection control, clinical utility and public health impact of multiplex respiratory virus assays.

1. Introduction

Respiratory viruses (RV) are ubiquitous and cause a large variety of clinical symptoms. For many years, procedures for diagnosis of respiratory virus infections have included culture and serology, which are time consuming, labor intensive and insensitive. Direct immunofluorescence assays (DFA) improved the turnaround time, but slightly compromised the sensitivity compared to culture. More recently, molecular assays have been developed and progressively multiplexed in order to diagnose a large number of respiratory viruses in single assays. New viruses that could not be detected by conventional virology have been discovered. Various commercial multiplex respiratory virus assays are now accessible to many clinical laboratories, although their impact remains unclear.

Diagnosis of RV is frequent in children. Evidence of viral infection is present in up to 43–67% of pediatric community-acquired pneumonia using molecular diagnostics (Ruuskanen et al., 2011). Routine diagnosis of respiratory virus infection in adult populations is more recent since respiratory viruses were considered benign for a very long time. Respiratory viruses have been detected in 15–56% of adult community-acquired pneumonia (Ruuskanen et al., 2011). Impact of respiratory virus diagnosis in specific pediatric and adult populations (eg, neonates, patients with cystic fibrosis, neutropenic patients or patients with chronic obstructive pulmonary disease [COPD]) is still incompletely understood. This article will review available data and highlight unresolved questions about cost-effectiveness, infection control, clinical utility, and public health impact of multiplex respiratory virus assays.

2. Multiplex respiratory virus assay technologies

Many multiplex respiratory virus assays have been published and marketed in the last years. Different nucleic acid based amplification technologies have been used to detect respiratory viruses including polymerase chain reaction (PCR), nucleic acid sequence-based amplification, transcription mediated amplification, strand displacement amplification, loop mediated isothermal amplification, rolling circle amplification, helicase-dependant amplification, and multiplex ligation-dependent probe amplification. However, only a few of these methods are appropriate for multiplexing. PCR has emerged as the easiest technology for multiplexing a large number of targets. The first multiplex respiratory virus assays used gel electrophoresis as a detection method, but it was long, labour intensive and necessitated manipulation of ethidium bromide. Some assays using real-time PCR were designed with moderately multiplexed reactions (e.g., influenza

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A, influenza B, and influenza H1N1; influenza A, influenza B and RSV; parainfluenza 1, 2, and 3) and performed in combination to cover a larger range of viruses (e.g. Prodesse, Simplexa, Respiratory pathogens Fast-track diagnostics, Altona Diagnostics). This strategy can be adapted for quantitative results if samples are collected appropriately and standard curves amplified with the reaction. However, no quantitative commercial assay is available yet. By limiting the multiplex reaction to 3 or 4 targets, it is possible in theory to get better sensitivity by eliminating primer dimer and competition between multiple targets. Other assays used highly multiplexed reactions including all targets (over 20 targets) in the same reaction. These assays need new detection methods in order to identify easily and rapidly each target present in the specimen. These detection technologies include microsphere hybridization associated with flow cytometer detection, LED camera detection or barcode detection (e.g., xTAG RVP, Resplex II, MultiCode-PLX), microcapillary electrophoresis (e.g., Seeplex RV12 and RV15, Respifinder, Iceplex), electrospray ionization mass spectrometry (e.g., PLEX-ID), nested-PCR with melting curve analysis (e.g., FilmArray), and solid phase hybridization microarrays (e.g., Infiniti respiratory virus panel, NGEN Respiratory Virus Analye-specific reagent, Verigen respiratory Virus Plus Nucleic Acid test, ICubate, eSensor genmark). Only a few of the numerous commercial multiplex respiratory virus assays are US Food and Drug Administration approved (xTAG RVP and RVP fast, Prodesse assays, Verigen respiratory Virus Plus Nucleic Acid test and FilmArray Respiratory panel). We have seen in the last years an increasing number of studies comparing commercial and laboratory developed assays as well as studies comparing commercial assays with each other. These studies are difficult to perform because of the high cost of reagents and the large number of targets to validate. The best way to perform comparison studies is to compare head to head two or three methods with prospectively collected samples. However, because viruses have changing epidemiology, it can be difficult to collect enough samples to validate every target. Overall, most multiplex respiratory virus assays have comparable performance, but each assay has small differences in performance among different targets depending on circulating strains. Problems that have been encountered are mainly lack of sensitivity for specific subtypes of adenovirus and inability to differentiate rhinovirus from enterovirus (Bibby et al., 2011; Chandrasekaran et al., 2012; Gharabaghi et al., 2011; Hayden et al., 2012; Mahony et al., 2007; Renaud et al., 2012). The principal differences among the multiplex respiratory virus assays concern the throughput, turnaround time, ease of use, automation, versatility, use of a closed system to reduce contamination and cost. The number of analyses to perform and the expected turnaround time dictate the best assay for the clinical laboratory. Some contamination issues have been reported with open platforms that need manipulation of amplification products. The ideal multiplex respiratory virus assay would be an assay that is a closed system with high throughput and a short turnaround time. Although many would consider quantitative results very useful to differentiate shedding from symptomatic infection and to follow immunocompromised patients with anti-viral treatment, the literature has been inconsistent about the correlation between viral loads and symptoms (Campbell et al., 2010; Franz et al., 2010; Jansen et al., 2010; Martin et al., 2008).

It is important to mention that multiplex PCR will detect only the targets included in the reaction and as users' dependence on molecular assays increase, the necessity for constant review of the targets will be essential. This process is not always easy when using commercial platforms that do not publicise their targets. Mutant viruses can emerge and give false negative results with molecular assays. This limitation of multiplex PCR is important and will have to be considered in any high risk population or setting.

3. Cost-effectiveness

Even though multiplex PCR assays can detect several different viruses simultaneously and rapidly, their advantages in terms of cost reduction over other rapid diagnostic assays (DFA, antigen detection) are still unclear. When rapid antigenic diagnostic tests for viral infections became widely used, several studies demonstrated their clinical utility in reducing length of hospital stay, performance of ancillary diagnostic tests and antibiotic consumption among pediatric (Bonner et al., 2003; Esposito et al., 2003; Sharma, 2002; Woo et al., 1997) (Abanses et al., 2006; Benito-Fernández et al., 2006; Byington et al., 2002; Ferronato et al., 2012; Iyer et al., 2006; Noyola & Demmler, 2000) and adult (Barenfanger et al., 2000; D'Heilly et al., 2008; Falsey et al., 2007) populations. Their cost-effectiveness was also shown with both populations (Barenfanger et al., 2000; Woo et al., 1997). However, In the specific setting of the emergency department (ER), a recent Cochrane analysis did not show a statistically significant difference in antibiotic prescription and ER length of stay in young children presenting with acute febrile respiratory illness tested with rapid antigenic viral diagnostic assays in the ER compared to those not tested (Doan et al., 2012). This difference between hospitalized patients and ER patients illustrates that diagnostic tools must be used in specific settings in order to provide most benefits.

Since the advent of molecular diagnosis, different authors have tried to demonstrate that multiplex PCR could be cost-effective compared to conventional rapid diagnostic assays. Despite their reduced turnaround time, higher sensitivity and specificity and capacity to detect an extended range of viruses, clinical and financial gains afforded by PCR seem modest. Garcia-Garcia et al. showed that, compared with conventional virology, diagnosis using respiratory virus PCR resulted in a reduction in antibiotic prescriptions (Garcia-Garcia et al., 2012). Oosterheert et al. performed a randomized controlled trial to evaluate the clinical and economic impact of real-time PCR for detection of respiratory viruses and atypical pathogens among hospitalized adults. Despite a notable increase in etiologic diagnostic yield from 21% to 43%, this study failed to demonstrate any statistically significant reduction in antibiotic use, additional diagnostic tests ordered, antibiotic cost and length of hospital stay (Oosterheert et al., 2005). Similar results were obtained by Wishaupt et al. who performed a multicenter, controlled clinical trial among pediatric patients in Netherlands. They concluded that even if reverse transcriptase PCR (RT-PCR) yielded more viral diagnoses, it did not have a significant influence on patient care (Wishaupt et al., 2011). In contrast, Mahony et al. generated a cost analysis study to determine if multiplex PCR testing was more or less costly than conventional virology assays. They used decision tree analytic modeling techniques to compare the costs of four diagnostic strategies. In their cost calculation, they took into account the viral assay cost and the entire cost of the hospital stay, adjusted according to the test outcome (true or false positive or negative). Their results showed that performing the Luminex xTAG RVP alone was the least costly approach (Mahony et al., 2009). Because of the paucity of data available and heterogeneity of assays studied, it is difficult to conclude that molecular diagnosis is a cost-effective approach in routine use compared to conventional tests. However, it is probable that cost calculations including financial impact of molecular assays on hospital stay, antibiotic use and infection control would be favourable. More studies will be needed to determine populations or situations in which multiplex PCRs would be the most useful in order to optimize their clinical and financial impact.

From a strictly laboratory point of view, establishment of multiplex PCR could be efficient and cost-effective. Dundas et al. demonstrated that in their laboratory, Luminex xTAG RVP was slightly more expensive than conventional techniques but increased laboratory efficiency by decreasing the hands-on time and operational steps. Moreover, it offered the possibility to standardize workflow for all respiratory specimens, an attractive strategy to conform to lean methodology (Dundas et al., 2011). Also, in an Australian study evaluating the performance of a laboratory developed PCR compared to DFA and viral culture, the authors concluded that molecular