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Systematic review

Multiplex PCR system for the rapid diagnosis of respiratory virus infection: systematic review and meta-analysis

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ABSTRACT

Objectives: To provide a summary of evidence for the diagnostic accuracies of three multiplex PCR systems (mPCRs)—BioFire FilmArray RP (FilmArray), Nanosphere Verigene RV+ test (Verigene RV+) and Hologic Gen-Probe Prodesse assays—on the detection of viral respiratory infections.
Methods: A comprehensive search up to 1 July 2017 was conducted on Medline and Embase for studies that utilized FilmArray, Verigene RV+ and Prodesse for diagnosis of viral respiratory infections. A summary of diagnostic accuracies for the following five viruses were calculated: influenza A virus (FluA), influenza B virus, respiratory syncytial virus, human metapneumovirus and adenovirus. Hierarchical summary receiver operating curves were used for estimating the viral detection performance per assay.
Results: Twenty studies of 5510 patient samples were eligible for analysis. Multiplex PCRs demonstrated high diagnostic accuracy, with area under the receiver operating characteristic curve (AUROC) equal to or more than 0.98 for all the above viruses except for adenovirus (AUROC 0.89). FilmArray, Verigene RV+ and ProFlu+ (the only Prodesse assay with enough data) demonstrated a summary sensitivity for FluA of 0.911 (95% confidence interval, 0.848–0.949), 0.949 (95% confidence interval, 0.882–0.979) and 0.954 (95% confidence interval, 0.871–0.985), respectively. The three mPCRs were comparable in terms of detection of FluA.
Conclusions: Point estimates calculated from eligible studies showed that the three mPCRs (FilmArray, Verigene RV+ and ProFlu+) are highly accurate and may provide important diagnostic information for early identification of respiratory virus infections. In patients with low pretest probability for FluA, these three mPCRs can predict a low possibility of infection and may justify withholding empirical antiviral treatments. **H.-S. Huang, Clin Microbiol Infect 2017;▪:1**

Introduction

Acute respiratory tract infections (ARI) cause high morbidity and mortality [1]. Among them, viral ARIs are one of the leading causes for paediatric and geriatric hospitalization and clinic visits [2,3]. Each year, seasonal influenza causes >200 000 hospitalizations and more than $10 billion direct medical costs in the United States. In specific populations (e.g. immunocompromised patients, neonates, and chronic pulmonary disease patients), the high complication and mortality rates from viral ARIs is a major concern [4]. Moreover, empirical antibiotics are commonly prescribed to patients with viral ARIs because of the lack of rapid and sensitive diagnostic methods and nonspecific symptoms, which delay proper treatments and precipitate antibiotic resistance [4–6].

Traditional diagnostic techniques (e.g. virus culture, haemagglutination inhibition assay, enzyme immunoassay and direct fluorescent antibody) were once the mainstays for pathogen detection. However, these methods were either insensitive, time

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consuming, labor intensive or operator dependent [7–9]. New technologies have emerged as a result of massive clinical demands, such as melting curve analysis, microfluidic device and nucleic acid amplification technologies [5,6,10–13]. These molecular diagnostic tools have shorter turnaround times and higher sensitivity for viral pathogens [14,15]. In addition, they allow for detection of a broader panel of viruses and coinfection [15,16], and they thus have become more widely used than the conventional virologic assays [8,17–19]. In particular, multiplex PCR (mPCR) is a validated strategy for the rapid detection and precise identification of a large number of respiratory viruses [19–22] by incorporating several primers within one reaction tube to amplify genomic fragments of many pathogens [22,23]. With the use of a mPCR panel, one study demonstrated a 30% to 50% increase in the diagnostic yield of respiratory viruses compared to direct fluorescent antibody and culture [24].

There are a number of US Food and Drug Association (FDA)-cleared mPCRs available today for detecting respiratory pathogens, each with pros and cons. The characteristics of the three FDA-approved mPCR systems included in our study are listed in Table 1. The BioFire FilmArray RP (FilmArray) respiratory panel [24], which utilizes melting curve analysis, is a random-access molecular test using principles of real-time PCR. The Verigene RV+ test is based on gold nanoparticle technology and silver signal amplification. Lastly, Hologic Gen-Probe Prodesse launches several assays with variable run sizes that also utilize melting curve analysis but with limited multiplexing ability. Although each Prodesse assay can only detect two to three viruses at a time, the Prodesse assays are still viewed as mPCR [25]. These three mPCRs were chosen because they have shorter turnaround times and have more available studies for analysis among a number of FDA-approved mPCRs. There is also one original study that provided direct comparison of these three mPCRs [25].

To gain insight into the optimal diagnostic tool for routine clinical use, we here provide a summary of evidence comparing the diagnostic accuracies of FilmArray, Verigene RV+ and Hologic Gen-Probe Prodesse assays for the detection of viral respiratory infections.

Methods

The protocol of our study was based on the PRISMA (Preferred Reporting Items for Systematic Review and Meta-analysis) statement [26] and the standard guideline for systematic reviews of diagnostic tests by the Cochrane Collaboration [27].

Search strategy

A comprehensive search of literature was conducted using two databases: PubMed (from inception to April 2015) and Embase (from inception to April 2015). The search term combination was: (multiplex AND pcr OR (multiplex AND polymerase AND chain AND reaction) OR filmarray OR verigene OR prodesse OR proflu OR profast OR proadeno OR proparaflu OR (pro hmpv)) AND ((respiratory AND tract AND infection) OR (respiratory AND infection) OR (respiratory AND virus) OR (respiratory AND tract AND disease) OR (respiratory AND disease) OR (common AND cold) OR influenza OR pneumonia OR bronchitis OR bronchiolitis OR rhinosinusitis OR pharyngitis OR laryngitis OR (otitis AND media) OR tonsillitis OR asthma OR copd OR (chronic AND obstructive AND lung AND disease)). The detailed search strategy is provided in Supplementary Materials S1. No language restrictions were applied to the search. The search was then supplemented by bibliographies of retrieved full-text articles and the latest narrative reviews. We also contacted the authors of publications that did not provide required data. An updated search to 1 July 2017 was performed before starting the statistical analysis.

Study selection

Studies that evaluated the performance of FDA-approved mPCR systems for the detection of viral respiratory infection were included, as follow: (a) they assessed the accuracy of one or more the following systems: FilmArray, Nanosphere Verigene RV+ and Hologic Gen-Probe Prodesse assays (ProFlu+, ProFAST, ProParaflu+, ProAdeno+ and Pro hMPV+) against reference standards and (b) they provided sufficient information to calculate sensitivity and

Table 1
Characteristics of BioFire FilmArray RP, Nanosphere Verigene RV+ Test and Hologic Gen-Probe Prodesse assays

Name	BioFire FilmArray	Verigene	GenProbe Prodesse
Technology	Melting curve analysis	Gold nanoparticles with silver signal amplification	Melting curve analysis
Assays	Respiratory panel	Respiratory virus plus test	ProFlu+, ProFAST+, ProAdeno+, ProParaflu+, Pro hMPV+
Targets	• Adenovirus • Coronavirus HKU1 • Coronavirus NL63 • Coronavirus 229E • Coronavirus OC43 • hMPV • Human Rhinovirus/enterovirus • FluA • FluA/H1 • FluA/H3 • FluA/H1–2009 • Influenza B • Parainfluenza virus 1 • Parainfluenza virus 2 • Parainfluenza virus 3 • Parainfluenza virus 4 • RSV	• FluA-H1 • FluA-2009 H1N1 • FluA-H3 • FluA • Influenza B • RSV A • RSV B	• ProFlu+: FluA, influenza B, RSV • ProFAST+: Seasonal FluA/H1, seasonal FluA/H3, 2009 H1N1 influenza • ProAdeno+: Adenovirus • ProParaflu+: Parainfluenza 1, parainfluenza 2, parainfluenza 3 • Pro hMPV+: hMPV
Throughput	1 sample per instrument	1 sample per processor	14 samples per run
Run time (hours)	1	<2.5	4–5
Hands-on time	2 minutes	5 minutes	1.5 hours
Sample preparation included?	Yes	Yes	No
Reagent storage conditions	Room temperature	2–8°C and −20°C	−70°C

FluA, influenza A virus; hMPV, human metapneumovirus; RSV, respiratory syncytial virus.

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