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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 2018;24:1055**

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.

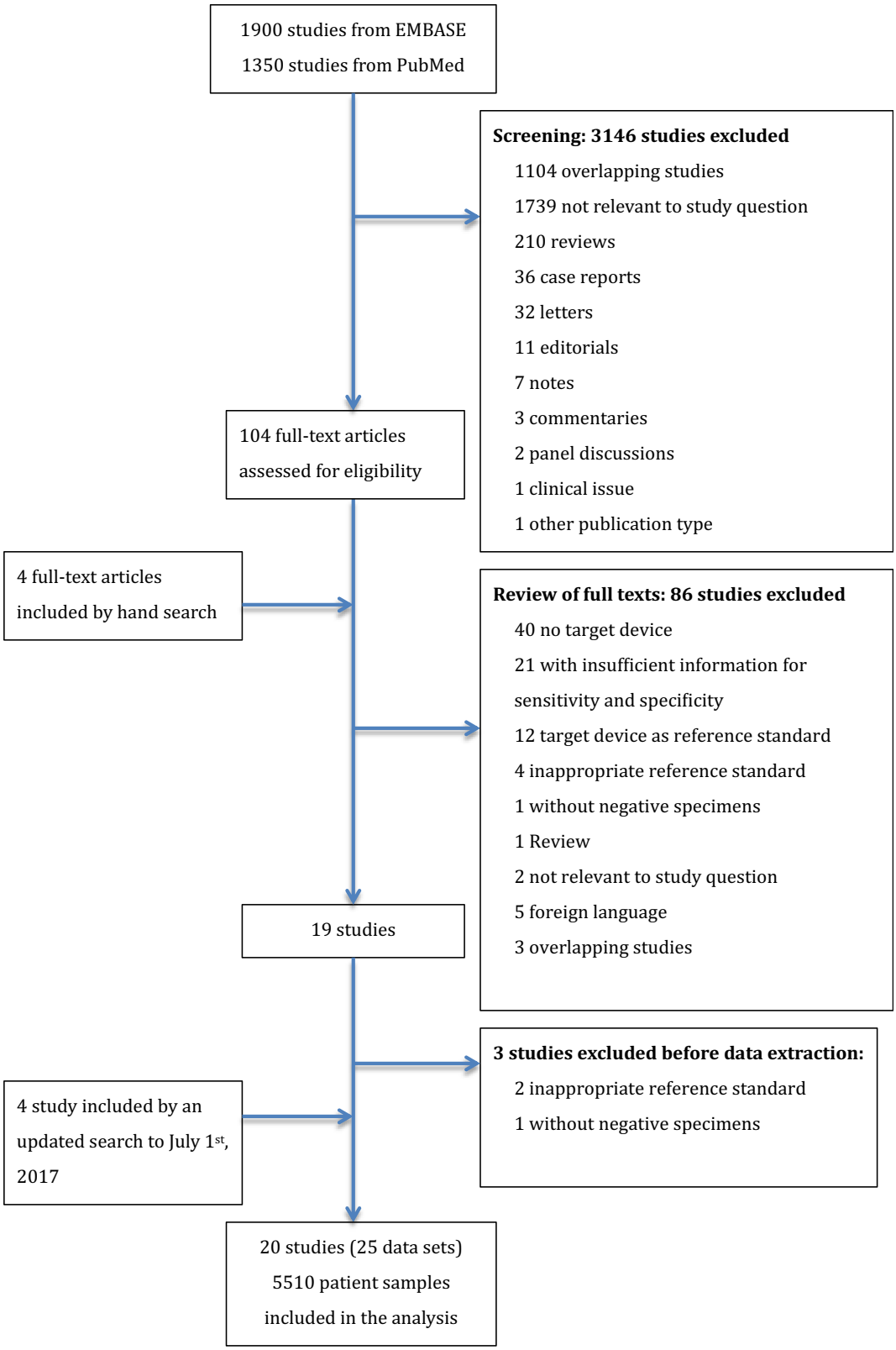

Fig. 1. PRISMA flow diagram for selection of articles for meta-analysis.

specificity. Studies outside of the United States that used non–US FDA–approved versions of the mPCRs were also included. We included conference abstracts, posters and oral presentations only if they provided sufficient information to calculate the sensitivities and specificities of the FDA-approved mPCR systems. Studies were excluded for the following reasons: (a) they only analysed clinical specimens previously determined to be positive (without negative specimens), (b) they used our target mPCR systems as the sole

standard to validate other mPCR systems and (c) they used an mPCR assay not approved by FDA as reference. In addition, we excluded reviews, guidelines, case reports, editorials, panel discussions, letters, notes and comments. For multiple publications, only the latest available publications with complete data of the same patient group were included. Studies that compared more than one FDA-approved mPCR systems with the same reference standard, used different reference methods for different viruses, used different samples for different viruses or reported prospective and retrospective samples independently have separate data sets for each comparison. Three reviewers independently screened the titles and abstracts from PubMed and Embase. Disagreements or uncertainties were resolved by discussion.

Data extraction

A data extraction form was used in 20 included studies by two reviewers before being finalized. Each data set was extracted by two authors independently to avoid bias. The form consisted of the following characteristics: study type, study design, patient age, patient inclusion criteria, specimen type, mPCR systems used, reference standard and 2 × 2 tables. The 2 × 2 tables were further used to calculate sensitivities and specificities of the target assays. For the reference methods, virus culture and direct fluorescent antibody were grouped together because they are universally recognized as the reference standard [28,29]. Reverse transcription (RT) PCR referred to both commercial RT-PCRs and in-house RT-PCRs. Composite reference standard was defined as a standard that used more than one comparator assays. Discrepant analyses encompassed studies that resolved discrepancies between target assay and comparator assay by bidirectional sequencing, RT-PCR, repeated testing or other methods. Studies were also grouped on the basis of whether they incorporated FilmArray, Verigene RV+ and Hologic Gen-Probe Prodesse assays as part of a composite reference standard. Studies that had <5% of the samples taken from the lower respiratory tract were categorized as using upper respiratory specimens. Children were defined as patients younger than 18 years. Studies that included both children and adult population were categorized as mixed.

Quality assessment

The quality of the eligible studies was independently assessed by two reviewers using the Quality Assessment of Diagnostic Accuracy Studies 2 tool (QUADAS-2) [30]. For each diagnostic study, we determined the risk for bias and general applicability in all four domains of QUADAS-2 and reported them separately. Those with low risk of bias or low concern regarding applicability were judged as low. A study would be judged as unclear if there were insufficient data for interpretation.

Data synthesis and statistical analysis

A bivariate model was applied to estimate summary sensitivity and specificity. The positive likelihood ratios (LR+) and negative likelihood ratios (LR−) were then calculated from summary sensitivity and specificity. The bivariate model approach modelled the logit-transformed sensitivity and specificity simultaneously to account for the inherent negative correlation between sensitivity and specificity that may arise due to different thresholds in different studies [30]. In addition, the bivariate model could also account for between-study heterogeneity. All analyses except for the summary receiver operating characteristic curve (ROC) were performed by the 'mada' package in R software (R Foundation for Statistical Computing, Vienna, Austria; http://www.r-project.org/). The summary ROC and area under the ROC was calculated by the 'midas' package in STATA (STATA Inc, College Station, Texas). A two-sided p value of <0.05 indicated statistical significance for all tests.

Results

Identification of studies

Our comprehensive search yielded 1900 studies from Embase and 1350 studies from PubMed. After inclusion and exclusion following the protocol and an updated search to 1 July 2017 (Fig. 1), a total of 20 studies (25 data sets) were eligible for analysis, encompassing a total of 5510 patient samples. ProFlu+ was the only Prodesse assay with enough data sets for quantitative analysis. Parainfluenza virus was not analysed because of insufficient data.

Characteristics of included studies

The laboratory characteristics of FilmArray RP, Verigene RV+ and Prodesse ProFlu+ are illustrated in Table 1. A simplified summary of the characteristics of the included studies is provided in Table 2. Details of the characteristics and key results of each individual study are provided in Supplementary Materials S2. None of the studies specifically recruited adults only, and approximately 30% of the studies obtained their samples from children. Discrepant analysis and composite reference standard were the two most commonly applied reference standards.

Quality assessment

The studies varied in quality. Quality assessment by the QUADAS-2 tool is demonstrated in Fig. 2. For the 'Patient Selection' domain, some studies were not clear about how the patients were recruited. Some studies used refrigerated samples that were pre-collected without specifying their original sources; others did not avoid a case–control design. For the 'Reference Standard' domain, most studies did not specify the use of blinding for reference

Table 2
Characteristics of the 20 included studies (25 data sets)

Characteristic	*n* (data sets) (%)
Age	
Children	8 (32%)
Mixed	10 (40%)
Unclear	7 (28%)
Index test	
FilmArray RP	10 (40%)
Verigene RV+	6 (24%)
Prodesse ProFlu+	9 (36%)
Reference standard	
Virus culture/direct fluorescent antibody	4 (16%)
RT-PCR	4 (16%)
Composite	7 (28%)
Discrepant analysis	10 (40%)
Specimen type	
Upper respiratory specimen	22 (88%)
Mixed upper and lower respiratory specimen	2 (8%)
Unclear	1 (4%)
Study design	
Prospective	11 (44%)
Retrospective	12 (48%)
Mixed	2 (8%)
Country	
United States	18 (72%)
Belgium	3 (12%)
Korea	1 (4%)
Japan	2 (8%)
Taiwan	1 (4%)

RT-PCR, reverse transcription–PCR.

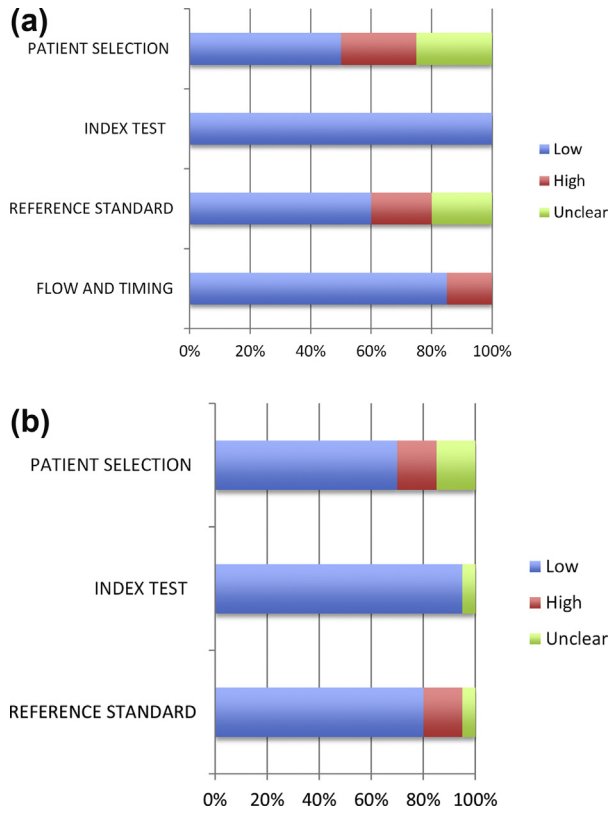

Fig. 2. QUADAS-2 for included studies. (a) QUADAS-2 bias assessment. (b) QUADAS-2 applicability assessment. QUADAS-2, Quality Assessment of Diagnostic Accuracy Studies 2.

standard. Some studies that were of low quality in these regards used the index test as part of the reference method. Others did not use the same reference method on all their samples. However, most studies were identified as high quality for flow and timing. Overall, the majority of the studies had low concern regarding applicability.

Diagnostic accuracy of mPCRs

Table 3 lists the point estimates of sensitivity, specificity, LR+, LR− and area under the plasma concentration vs. time curve value of each mPCR assay for each virus. Multiplex PCRs demonstrated very high area under the receiver operating characteristic curve (AUROC) (≥0.98) for influenza A virus (FluA), influenza B virus (FluB), respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) and high to moderate AUROC for adenovirus. For FluA, sensitivity estimates were acceptable (>0.9) for FilmArray RP and Verigene RV+, and high (>0.95) for Prodesse ProFlu+; specificity estimates were high (>0.98) for all three mPCRs. The overall LR+ were well beyond 10, indicating a high rule-in value. The overall LR− were all below 0.1, which suggested a high rule-out value. Table 3 provides point estimates for FluB, RSV, hMPV and adenovirus. Fig. 3 shows the forest plot of sensitivity and specificity of the mPCRs for each virus. Fig. 4 illustrates the receiver operating characteristic curve and area under the receiver operating characteristic curve for the detection of each virus.

Discussion

Overall, in our analyses including 5510 patient samples, mPCR systems demonstrated high diagnostic accuracy (AUROC ≥0.98) for FluA, FluB, RSV and hMPV, with the exception of adenovirus (AUROC 0.89). FilmArray RP, Verigene RV+ and Prodesse ProFlu+ demonstrated a summary sensitivity for FluA of 0.911 (95% confidence interval (CI), 0.848–0.949), 0.949 (95% CI, 0.882–0.979) and 0.954 (95% CI, 0.871–0.985), respectively. The three mPCRs were comparable in terms of detection of FluA, and FilmArray RP and Verigene RV+ were comparable for RSV detection. Although Prodesse ProFlu+ was found to have a statistically significant higher sensitivity than FilmArray RP for FluB detection, they demonstrated comparable AUROC and thus comparable accuracy.

Of the five respiratory viruses that these systems detect, the diagnosis of influenza virus infection may have the greatest clinical impact [31]. On the basis of our study, overall, the mPCRs exhibited reasonable sensitivity (0.940; 95% CI, 0.902–0.964) and high specificity (0.987; 95% CI, 0.979,0.992) for FluA. In current clinical practice, the immunoassay-based rapid influenza diagnostic test is most widely used for screening influenza infections [8]. Although the rapid influenza diagnostic test can detect FluA and FluB in respiratory specimens in approximately 15 to 30 minutes, its sensitivity is limited, as reported by a previous meta-analysis [32]. This may give false-negative results, which prevents its use as a reliable excluding diagnostic tool in clinical practice. Furthermore, commonly used reference standards such as RT-PCR or virus culture may take several days to yield results and have little value for

Table 3
Accuracy estimates of included studies

Test	Sensitivity (95% confidence interval)	Specificity (95% confidence interval)	LR+ (95% confidence interval)	LR− (95% confidence interval)	AUC (95% confidence interval)
Influenza A virus					
FilmArray	0.911 (0.848, 0.949)	0.995 (0.988, 0.998)	186 (74.9, 368)	0.0928 (0.052, 0.153)	0.99 (0.98, 1)
Verigene	0.949 (0.882, 0.979)	0.982 (0.944, 0.995)	65.2 (15.9, 185)	0.058 (0.0206, 0.122)	0.99 (0.98, 1)
Prodesse	0.954 (0.871, 0.985)	0.983 (0.973, 0.989)	57.65 (40.48, 76.94)	0.053 (0.022, 0.0896)	0.99 (0.99, 1)
Summary	0.940 (0.902, 0.964)	0.987 (0.979, 0.992)	76.9 (42.4, 126)	0.06 (0.03, 0.101)	0.99 (0.98, 1)
Influenza B virus					
FilmArray	0.822 (0.689, 0.905)	0.994 (0.980, 0.998)	167.50 (40.9, 503.00)	0.188 (0.093, 0.313)	0.98 (0.94, 1)
Prodesse	0.963 (0.907, 0.986)	0.992 (0.969, 0.998)	136.73 (30.5, 385)	0.04 (0.014, 0.097)	0.99 (0.99, 1)
Summary	0.932 (0.877, 0.963)	0.993 (0.986, 0.997)	154.4 (66.5, 304)	0.072 (0.034, 0.124)	0.99 (0.99, 1)
RSV					
FilmArray	0.911 (0.821, 0.958)	0.987 (0.971, 0.994)	73.1 (29.4, 150)	0.09 (0.0412, 0.172)	0.98 (0.98, 0.99)
Verigene	0.977 (0.929, 0.993)	0.993 (0.962, 0.999)	219.30 (23.5, 868)	0.027 (0.0076, 0.072)	0.99 (0.98, 1)
Summary	0.942 (0.84, 0.972)	0.991 (0.980, 0.996)	109.45 (47.5, 226)	0.06 (0.03, 0.118)	0.99 (0.99, 1)
Adenovirus					
FilmArray	0.670 (0.516, 0.794)	0.991 (0.961, 0.998)	86.9 (20.3, 273)	0.337 (0.212, 0.494)	0.89 (0.85, 0.91)
hMPV					
FilmArray	0.914 (0.835, 0.956)	0.999 (0.854, 1)	74.5 (29.4, 188.98)	0.167 (0.093, 0.301)	0.98 (0.97, 0.99)

AUC, area under the curve; hMPV, human metapneumovirus; LR−, negative likelihood ratio; LR+, positive likelihood ratio; RSV, respiratory syncytial virus.

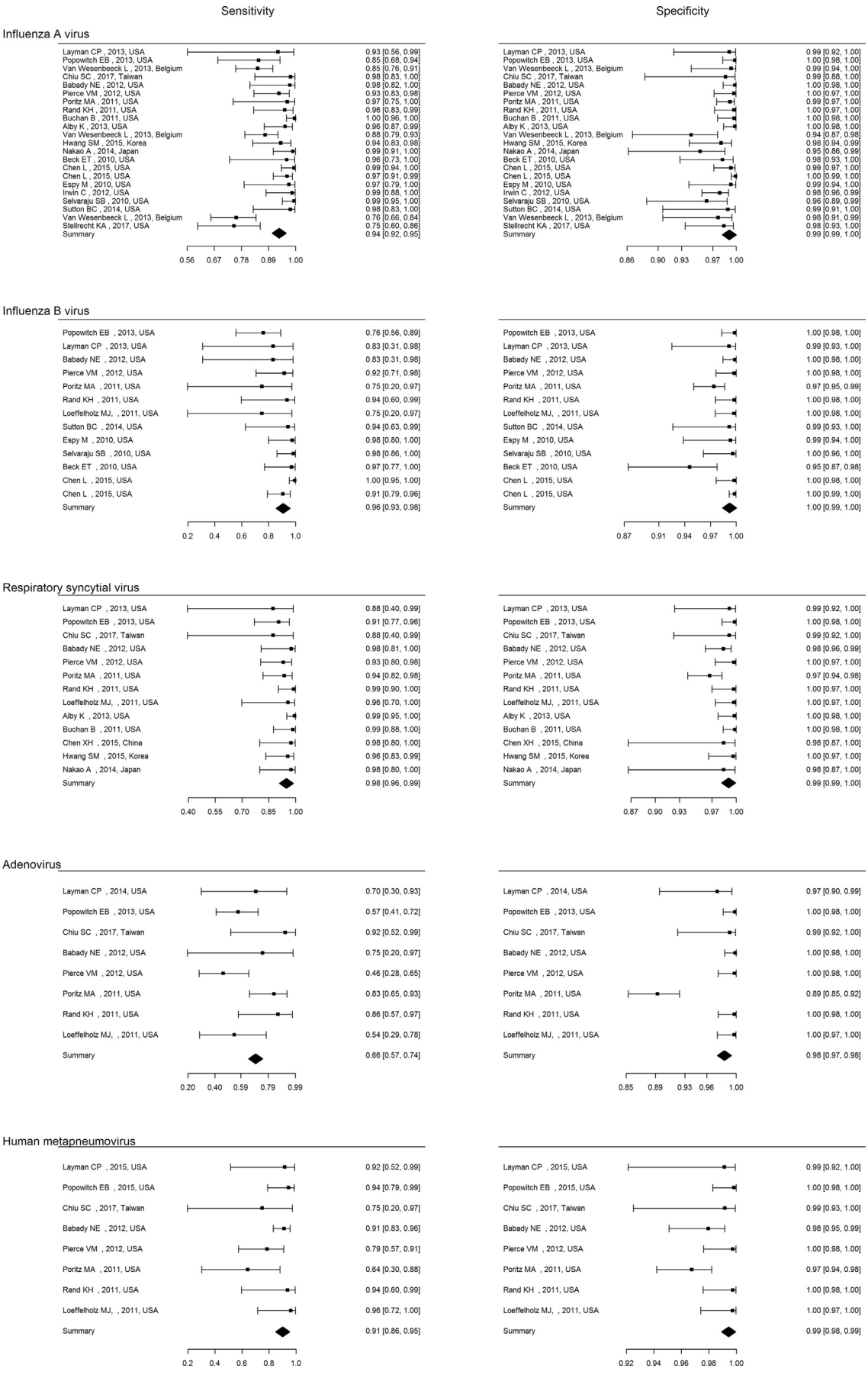

Fig. 3. Forest plot of sensitivity and specificity of multiplex PCRs for diagnosis of each virus.

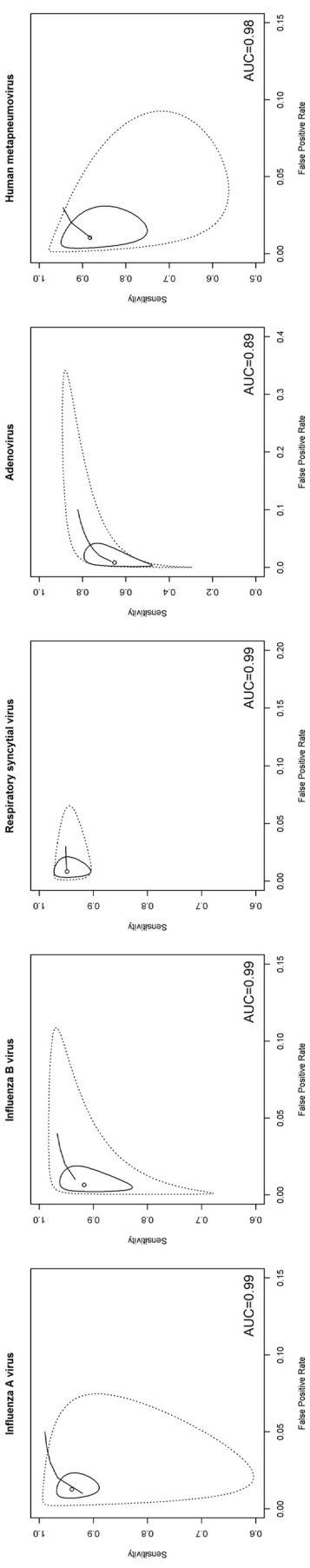

Fig. 4. Receiver operating curve analysis of multiplex PCRs for detection of different virus infections. Solid lines indicate the 95% confidence region, and the dashed lines indicate the 95% prediction region. AUC, area under the curve.

treatment decision. Our meta-analysis showed that mPCRs provide highly accurate results in a clinically relevant time frame and may potentially change the current diagnostic and treatment practice for FluA infection.

As a result of the low sensitivity of the rapid influenza diagnostic tests, the decision of antiviral treatment for FluA infection is largely based on clinical grounds alone [5], which may lead to over-prescription of anti-influenza drugs and increasing drug resistance [33,34]. The high sensitivity and specificity of the mPCRs, as demonstrated, could thus guide the precise use of the antiviral treatment. In a virtual population with a 20% prevalence (pretest probability) of FluA infection, a LR+ of 186 (FilmArray RP), 65.2 (Verigene RV+) and 57.65 (Prodesse ProFlu+) translates into a posttest probability of 97.89%, 94.22% and 93.51%. Similarly, an overall LR+ for FluA of 76.9 translates into a posttest probability of 95.06%. In other words, approximately 19 of 20 patients who tested positive for FluA truly have FluA infection. In the same population with a 20% prevalence of FluA infection, a LR− of 0.0928 (FilmArray RP), 0.058 (Verigene RV+) and 0.053 (Prodesse ProFlu+) translates into a posttest probability of 2.27%, 1.43% and 1.31%, while an overall LR− for FluA of 0.06 translates into a negative predictive value of 1.48%. That is, among 200 patients who tested negative for FluA, only three with FluA infection may be missed. Although influenza virus infection may have dire consequences in certain vulnerable population, such as the elderly or those who are immunocompromised, a negative test result may justify withholding empiric antiviral treatment in apparently healthy young men, pregnant woman or children.

A study by Van Wesenbeeck et al. [25] of 171 clinical samples concluded that FilmArray RP and Prodesse ProFlu+ have better sensitivity for FluA than Verigene RV+. This is contrary to our results, which found the three mPCRs to be comparable for FluA detection. Our meta-analysis synthesized data across different studies encompassing 5510 patient samples and may present a more accurate estimate of the real situation. Although these three systems have comparable accuracy for FluA, FilmArray RP has the shortest hands-on time (2 minutes) and run time (1 hour) among the three mPCRs and includes sample preparation in its panel. Furthermore, its reagents can be stored at room temperature, and its assay detects the largest number of targets. Therefore, FilmArray RP may be the best choice in emergency rooms or during the influenza season.

There is less interest in rapid diagnostic tests for RSV, hMPV and adenovirus as a result of a lack of specific treatments available against these viruses and the common practice of supportive care in clinical settings. Nevertheless, RSV is one of the leading causes of ARIs in children [35] and immunocompromised patients, and it may cause severe complications; it therefore requires definitive diagnosis. Our results revealed that mPCRs are highly sensitive and specific for RSV and hMPV, and they offer a more rapid and accurate alternative to traditional methods [7,36]. The current treatment is ribavirin for high-risk infants and young children [37], with several clinical trials for novel RSV treatments underway [38–41]. In contrast, for adenovirus, FilmArray RP had a moderate to low sensitivity (<70%), but its superior specificity (>0.99) makes it a reliable rule-in tool. The literature has demonstrated that commercial FilmArray RP (v1.6) has low sensitivity for adenovirus [24,42–44], but its sensitivity greatly improved in the commercial FilmArray 1.7 [42], developed later. Of note, none of our included studies specifically reported using FilmArray 1.7.

To our knowledge, this is the first report to perform a comparative meta-analysis on different mPCR platforms and different viruses. Not only did we provide detailed comparison on the characteristics of the three systems but we also provided quantitative accuracy measure for the different viruses by different

systems. There are several limitations to our study, however. Firstly, some included studies did not provide sufficient details regarding the version of FilmArray RP used (premarket or commercial). We traced their publication dates and contacted the FilmArray RP manufacturers as well as the authors of the original study at an attempt to confirm the version of FilmArray RP. However, sensitivity analysis showed no clinically significant difference between the sensitivities and specificities of precommercial and FDA-approved commercial assays (data not published). Secondly, some included studies have a retrospective study design; they failed to specify their inclusion criteria, and they used only previously stored samples from patients with unknown characteristics. There is also concern regarding diagnostic review bias, in which the interpretation of the result of reference test is made with knowledge of the index test result [45]. However, a test review bias is highly unlikely because the interpretation of the index tests (mPCRs) was objective. Of note, five of the included studies adopted the case–control design, which tends to give an overestimation of the accuracy of the index test [45]. In addition, results of this study only showed the accuracy of three commercial mPCR systems for diagnosis of respiratory virus infection. Whether they have equal strength in therapeutic guidance remains to be validated. Lastly, our study does not provide an answer to whether mPCR systems improve the outcome of patients with respiratory virus infection compared to clinical diagnosis alone.

Conclusions

Analysis of eligible studies showed that three commercial mPCR systems—FilmArray, Verigene RV+ and GenProbe Prodesse ProFlu+—may provide important diagnostic information to help early identification of influenza virus, respiratory syncytial virus, adenovirus and hMPV. The great improvement of the sensitivity of the rapid diagnosis of influenza may have the potential to change current modes of diagnosis and management. However, not all of these systems are equally useful in terms of inclusion or exclusion diagnosis. Clinicians should interpret the results on the basis of likelihood ratio and pretest probability. The next step would be to assess whether provision of information from these mPCR tests can modify clinical outcomes.

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Transparency declaration

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.cmi.2017.11.018.

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