ORIGINAL ARTICLE

Syndromic sample-to-result PCR testing for respiratory infections in adult patients

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ABSTRACT

Background: Syndromic sample-to-result (SS2R) polymerase chain reaction (PCR) can rapidly identify causative pathogens of respiratory tract infections (RTI). We evaluated diagnostic accuracy and applicability of one of the current SS2R diagnostics, the FilmArray® Respiratory Viral Panel.

Methods: We performed a prospective study among adults presenting with symptoms of RTI at the Emergency Department of the University Medical Centre Utrecht (the Netherlands) during the 2016-2017 viral respiratory season. Clinical data were collected. We compared SS2R results on nasopharyngeal swabs to conventional real time PCR, calculated turnaround times (TAT) and explored implementation barriers using questionnaires.

Results: 62 Patients were included (64.5 yr [interquartile range (IQR) 44.3-75.0]). SS2R sensitivity was 82.9% [95% confidence interval (CI) 67.9-92.9] and specificity was 95.2% [95% CI 76.2-99.9] for detection of all present viruses (n = 60). Kappa agreement (0.73 [95% CI 0.56-0.90]) was good (p = 0.000). Median SS2R TAT was 2:06 hours [IQR 1:45-3:17] compared to 32:00 hours [IQR 26:50-40:42] of conventional PCR (n = 49, p = 0.000). Ease-of-use and fast TAT were unanimously reported as benefits, and low test capacity with a single SS2R system as drawback.

Conclusion: SS2R testing for respiratory viruses offers a rapid and reliable diagnostic method which has great potential for more efficient and targeted management in adult patients with RTI.

KEYWORDS

FilmArray, PCR, rapid test, respiratory virus, validation

INTRODUCTION

Respiratory viruses predominate as causative pathogens in patients hospitalized with severe acute respiratory illness (SARI), accounting for up to 50% of microbial etiologies.[1,2] Although antibiotics can be safely withheld in proven viral infections, viral SARI is often treated with antibiotics because viral and bacterial lower respiratory tract infections (RTI) cannot reliably be distinguished based on clinical presentation.[1-3] This leads to unnecessary costs and higher risk of antimicrobial resistance.[4-7] Furthermore, studies have shown reduced mortality when oseltamivir was administered as early as possible (e.g. within two days of symptom onset).[8] Rapid syndromic sample-to-result (SS2R) diagnostics based on multiplex polymerase chain reaction (mPCR), generally generating results within two hours, are promising in accelerating virus and bacterial identification[2] and consequently in targeting antibiotic and antiviral therapy.[9] In addition, rapidly ruling out a viral cause of RTI could lead to more efficient use of in-hospital isolation facilities during the viral respiratory season.

Currently, a wide range of molecular rapid tests is available. Promising commercially available SS2R techniques are Alere I® Influenza A&B Nucleic Acid Amplification Test (Abbott), Cobas® Liat® (Roche Diagnostics), eSensor® Respiratory Viral Panel (Genmark), FilmArray® Respiratory Panel (BioFire Diagnostics), GeneXpert® (Cepheid), and the Luminex® xTAG Respiratory Viral Panel (Luminex).[2]

This paper describes a prospective clinical study evaluating the accuracy, turnaround time (TAT) and logistical barriers for the implementation of one of these SS2R diagnostics, the FilmArray® Respiratory Panel (from now on SS2R), a FDA cleared /CE marked rapid mPCR for a panel of 17 viruses plus three bacteria commonly found in RTI. The choice for this specific test reflects the choice for this SS2R in our hospital. Apart from the analysis of accuracy and implementation issues, the applicability of the SS2R

is evaluated by calculating the potential impact on use of in-hospital isolation facilities and oseltamivir.

MATERIALS AND METHODS

Patient inclusion and microbiological testing

During the peak of the 2016-2017 viral respiratory season (3 January – 4 February 2017),[10] nasopharyngeal swabs were taken from patients (age ≥ 18) with symptoms of a RTI presenting on the Emergency Department (ED) of the University Medical Center Utrecht (UMCU), a 1042 bed tertiary hospital (The Netherlands). Symptoms of RTI were defined as upper or lower respiratory complaints with acute onset. To obtain informed consent from each patient and calculate TATs of the SS2R, patients were only included during lab opening hours (8 am - 5 pm). Two swabs were taken in parallel, one for the regular diagnostic pathway and one for the experimental diagnostic pathway with the SS2R. The first swab was tested for the most common respiratory viruses (*table 2*) using conventional real-time polymerase chain reaction (RT-PCR). Conventional RT-PCR for respiratory viruses was performed in nasopharyngeal swabs, nasal washes or bronchoalveolar lavage. Nucleic acids were extracted using the total nucleic acid protocol with the MagNA Pure LC nucleic acid isolation system (Roche Diagnostics, Basel, Switzerland). For detection of RNA viruses, cDNA was synthesized using MultiScribe RT and random hexamers (Applied Biosystems, Foster City, CA). Detection of viral pathogens was performed in parallel, using laboratory developed RT-PCR assays specific for the following viruses: respiratory syncytial virus A and B; influenza virus A and B; parainfluenza virus 1-4; rhinoviruses; adenoviruses; human coronaviruses OC43, NL63, and 229E; human metapneumovirus. RT-PCR procedures were performed as described in earlier literature.[11] In brief, samples were assayed in duplicate in a 25-µL reaction mixture containing 10 µL of cDNA, 12.5 µL of TaqMan Universal PCR Master Mix (Applied Biosystems), 300-900 nmol/l of the forward and reverse primers, and 75-200 nmol/l of each probe. To monitor for inhibition, a fixed amount of an internal control virus (murine encephalomyocarditis virus [RNA virus] and porcine herpesvirus [DNA virus]) was added before extraction.[12] The cycle of threshold (Ct) gives an impression of the quantity of the viral load (i.e. a semi quantitative value). The cut-off value for a positive RT-PCR result was a Ct value < 45.[13] The second nasopharyngeal swab was tested with the SS2R (FilmArray® Respiratory Panel version 1.7). The FilmArray® contains all needed reagents in a freeze-dried format for extraction, amplification, and detection steps. Respiratory samples are collected in universal transport media. The FilmArray® test was performed according to the manufacturer's instructions. In brief, prior to run 1 ml of hydration solution and 300 µl of respiratory sample was added to the reagent pouch. The pouch was then placed on the FilmArray® instrument and the test performed using the FilmArray® system. After extraction and purification of all nucleic acids from the sample, a nested multiplex PCR is performed followed by an individual singleplex second-stage PCR reactions to detect the products from the first-stage nested PCR. Both the in-house PCR and FilmArray® were performed by the virology laboratory and results were approved by a clinical virologist. Results of the SS2R were not used in routine care.

Data collection and statistical analysis

Standardized collection of clinical and virological data from ED-presentation and, if applicable, from the following hospital admission was performed manually from the electronic patient charts. Results are given as percentages or median with IQR. The accuracy analysis of the SS2R compared to RT-PCR and a comparison of Ct-values with a t-test, were performed with IBM SPSS Statistics® (Version 21.0). Accuracy was calculated per detected virus, e.g. influenza virus, respiratory syncytial virus (RSV), coronavirus, rhinovirus and human metapneumovirus (HMPV), and per sample. Test concordance of the SS2R compared to RT-PCR was presented using sensitivity, specificity and positive and negative predictive values for clinical purposes. Since RT-PCR alone might not be considered a 'gold' standard accuracy (or overall percentage agreement) and a Cohen's Kappa statistic were calculated as well. For the accuracy analysis, final results of both diagnostics were used after retesting in case of invalid results. Samples with discrepant results were retested with both diagnostics, no additional sequencing was done. Ct-values of positive samples were measured by RT-PCR. TATs of both diagnostics were calculated for patients of whom the SS2R could be obtained on the day of sampling, in hours from sampling at the ED until the result was reported to the study team. During the clinical study only one SS2R system was available, precluding the possibility of parallel testing. Separately, potential barriers for implementation, advantages and disadvantages of the SS2R were explored by interviewing laboratory technicians working with the SS2R. Answers to the questions were presented descriptively.

RESULTS

From January 3rd till February 4th, a (differential) diagnosis of upper or lower RTI was made in 148 adults at the ED, in 104 of whom RT-PCR was performed. Of these, 56 presented during lab opening hours, making a SS2R possible. Additionally, nasopharyngeal swabs for both

Table 1. *Baseline characteristics of adult patients with suspicion of an RTI presenting at the ED in whom an in-house PCR and rapid SS2R was performed on a nasopharyngeal swab (n = 62)*

Characteristics	**n = 62**
Age (yr) - *median (IQR)*	64.5 *(44.3-75.0)*
Male sex - *no. (%)*	22 *(35.5%)*
Comorbidities	
Immunocompromised - *no. (%)*	25 *(40.3%)*
Astma or COPD - *no. (%)*	22 *(35.5%)*
Chronic heart failure - *no. (%)*	12 *(19.4%)*
Disease status at presentation	
Time from first symptoms to hospital presentation (days) - *median (IQR)*	3.0 *(2.0-7.0)**
Fever (temperature ≥ 38.0 °C) - *no. (%)*	27 *(43.5%)*
Oxygen suppletion needed (≥ 1L) - *no. (%)*	24 *(38.7%)*
CRP (mg/l) – normal < 10 mg/l - *median (IQR)*	58 *(21-165)*
Infiltrate at radiologic imaging - *no. (%)*	26 *(41.9%)*
(Differential) diagnosis RTI after ED presentation - *no. (%)*	56 *(90.3%)*
Treatment	
Hospital admission - *no. (%)*, of whom: - In aerogenic isolation - *no. (%)* - Intensive or medium care admission directly after presentation - *no. (%)*	39 *(62.9%)* 21 *(53.8%)* 9 *(23.1%)*
Antibiotics started at presentation - *no. (%)*	43 *(69.4%)*
Oseltamivir started at presentation - *no. (%)*	20 *(32.3%)*
Clinical outcomes	
Length of hospital stay, if admitted (days) - *median (IQR)*	6.0 *(3.0-8.0)*/34[†]
Death within hospital stay - *no. (%)*	5 *(8.1%)*

CRP = C-reactive protein; IQR = interquartile range; n = number; no = number; PCR = polymerase chain reaction; RTI = respiratory tract infection; SS2R = syndromic sample to result; yr = year.
*One missing value because in this patient the duration of symptoms was not reported. Value is calculated using complete cases (n = 61).
[†]5/39 patients were admitted at another hospital due to limited capacity for hospitalization, hence the length of hospital stay of these patients was unknown

RT-PCR and SS2R were taken from six patients after leaving the ED, who had been referred there with respiratory complaints, but with a non-RTI working diagnosis, so that eventually 62 patients were included. Median age was 64.5 years (IQR 44.3-75.0). Twenty-five patients (40%) were immunocompromised at the time of presentation. Thirty-nine (63%) were admitted to the hospital, nine of whom to the Intensive Care Unit (*table 1*). Results of the RT-PCR and SS2R showed 58% and 60% samples with one virus detected and 8% and 2% with dual or triple viral pathogens respectively. Most frequented detected viruses were influenza A virus, coronavirus, rhinovirus and RSV (*table 2*). Viral-bacterial coinfection was present in 14 patients (*Streptococcus pneumoniae* (n = 4), *Haemophilus influenza* (n = 3), *Pseudomonas aeruginosa* (n = 4), *Staphylococcus aureus* (n = 2), *Proteus mirabilis* (n = 1)). Five patients (8%) died, in all of whom a virus was detected (influenza A virus (n = 2), RSV (n = 2), coronavirus (n = 1)).

Diagnostic accuracy

Sixty-two patients were included in the diagnostic accuracy analysis (*table 3*). Compared to the reference method, SS2R had a sensitivity of 82.9% [95% CI 67.9-92.9] and specificity of 95.2% [95% CI 76.2-99.9] for complete virus detection. In two samples the SS2R initially gave an invalid result (one sample negative, one with rhinovirus in the RT-PCR). After retesting with the SS2R, the results were similar to the reference standard. Discrepant results were found in nine samples. SS2R missed influenza A virus (n = 3), RSV (n = 3) and coronavirus (n = 3) in seven samples. The median Ct-value of 36.04 (SD ± 4.21)

Table 2. *Virological results of the RT-PCR* and rapid SS2R* (n = 62)*

Virus	RT-PCR (n (%) of samples)	SS2R (n (%) of samples)
1 virus - *no. (%)*	36 *(58.1%)*	37 *(59.7%)*
≥ 2 viruses - *no. (%)*	5 *(8.1%)*†	1 *(1.6%)*†
0 viruses - *no. (%)*	21 *(33.9%)*	24 *(38.7%)*
Pathogens		
Influenza A - *no. (%)*	22 *(35.5%)*	19 *(30.6%)*
RSV - *no. (%)*	8 *(12.9%)*	5 *(8.1%)*
Coronavirus - *no. (%)*	10 *(16.1%)*	7 *(11.3%)*
Rhinovirus - *no. (%)*	6 *(9.7%)*	7 *(11.3%)*
HMPV - *no. (%)*	1 *(1.6%)*	1 *(1.6%)*

HMPV = human metapneumovirus; n = number; no = number; PCR = polymerase chain reaction; RSV = respiratory syncytial virus; SS2R = syndromic sample to result.
*RT-PCR tested: coronavirus, rhinovirus, influenza virus A and B, RSV A and B, human metapneumovirus, adenovirus, bocavirus, enterovirus and parainfluenza virus 1-4. SS2R tested: adenovirus, coronavirus (229E, HKU1, OC43, NL63), human metapneumovirus, rhinovirus, enterovirus, influenza virus A and B, parainfluenza virus 1-4, RSV A and B, and three bacterial pathogens: *Bordetella pertussis, Chlamydophila pneumonia, Mycoplasma pneumonia*.
†RT-PCR detected multiple viral pathogens in 5 patients: influenza virus A and RSV (n = 1); influenza virus A and coronavirus (n = 1); RSV and coronavirus (n = 1); coronavirus and rhinovirus (n = 1); and influenza A virus, RSV and coronavirus (n = 1). SS2R detected multiple viral pathogens in one patient: coronavirus and rhinovirus (same patient in which in-house RT-PCR detected coronavirus and rhinovirus)

Table 3. *Sensitivity, specificity, PPV, NPV, accuracy (overall percentage agreement) and kappa of the SS2R compared to RT-PCR (n = 62); diagnostic accuracy is given per sample and per virus*

Concordant result	Sample result SS2R/PCR				Sensitivity	Specificity	PPV	NPV	Accuracy	Kappa
	+/+	+/-	-/+	-/-	% (95%CI)	% (95%CI)	% (95%CI)	% (95%CI)	% (95%CI)	% (95%CI)
Complete viral panel	34	1	7	20	82.9 (67.9-92.9)	95.2 (76.2-99.9)	97.1 (83.3-99.6)	74.1 (59.1-85.0)	87.1 (76.2-94.3)	0.73 (0.56-0.90)
Influenza A virus	19	0	3	40	86.4 (65.1-97.1)	100 (91.2-100)	100 (79.1-100)	93.0 (82.3-97.5)	95.2 (86.5-99.0)	0.89 (0.77-1.00)
RSV	5	0	3	54	62.5 (24.5-91.5)	100 (93.4-100)	100 (46.3-100)	94.7 (88.0-97.8)	95.2 (86.5-99.0)	0.74 (0.47-1.00)
Coronavirus	7	0	3	52	70.0 (34.8-93.3)	100 (93.2-100)	100 (56.1-100)	94.6 (87.1-97.8)	95.2 (86.5-99.0)	0.80 (0.58-1.00)
Rhinovirus	6	1	0	55	100 (54.1-100)	98.2 (90.5-100)	85.7 (46.2-97.7)	100 (91.9-100)	98.4 (91.3-100)	0.91 (0.75-1.00)
HMPV*	1	0	0	55	100 (2.5-100)	100 (93.5-100)	100 (5.5-100)	100 (91.9-100)	100 (93.6-100)	1.00 (1.00-1.00)

CI = confidence interval; HMPV = human metapneumovirus; kappa = Cohen's kappa coefficient; n = number; no = number; NPV = negative predictive value; PCR = polymerase chain reaction; PPV = positive predictive value; RSV = respiratory syncytial virus; RVP = Respiratory Viral Panel.
*In six patients no RT-PCR result was reported for HMPV

of these discrepant results (n = 9), with one Ct-value > 40 (Influenza A, Ct-value 40.89), was significantly higher than the median Ct-value of 26.03 (SD ± 7.05) of all concordant virus positive results (n = 37), with one Ct-value > 40 (coronavirus, Ct-value 42.23) (p = 0.001). SS2R had a rhinovirus positive result in one sample (RT-PCR sample negative) and an influenza A / influenza B virus equivocal result in one sample (RT-PCR sample influenza A virus, Ct-value 36.04), the latter being considered concordant in the accuracy analysis.

Logistics

The median TAT of the SS2R was 2:06 hours (IQR 1:44-3:16) for patients of whom the SS2R was performed on the day of sampling (n = 46). For 16 of these (35%), results became available during their ED-stay. The median

RT-PCR sample TAT of the 46 patients was 32:00 hours (IQR 26:50-40:42). Based on the questionnaires, laboratory technicians (n = 5) were positive about ease-of-use, short hands-on time (≤ 10 minutes) and fast TAT of the SS2R. A low test capacity, due to the availability of only one SS2R system, resulting in less optimal TATs, was mentioned as a drawback.

DISCUSSION

This prospective clinical study focuses not only on accuracy and TATs of rapid SS2R testing, but also on applicability and implementation strategies. SS2R showed 90% sensitivity and 95% specificity in the detection of respiratory viruses compared to the current gold standard, RT-PCR. The poor diagnostic accuracy for some viruses, for example RSV, is due to a small number of patients and is reflected in the wide confidence intervals around the accuracy estimates. In our hospital, SS2R had a rapid TAT, even when used in a laboratory setting. The SS2R only had two out of 62 invalid initial results. This SS2R therefore has great potential in the improvement of clinical outcomes inpatients, for example in terms of targeted oseltamivir prescription and possibly also reduced antibiotic treatment. It may also benefit hospital management, for example by contributing to more adequate use of in-hospital isolation facilities. It should be noted that all swabs were taken during a month in the viral respiratory season with a high prevalence of influenza virus and RSV in particular. Not only the virological results, but also potential effects on clinical outcomes should be viewed from this perspective. Among published articles on the accuracy of the specific SS2R (FilmArray®)[14-35] used in this study, only a few compared SS2R to RT-PCR,[14-20] with a calculated pooled sensitivity of 87.6% [95%CI 84.6-90.1] and specificity of 91.1% [95%CI 87.5-93.7] (n = 945 samples, *table 4*). Differences between studies, including the current study, and the relatively low overall sensitivity might be explained by genetic variability of viruses, differences in sampling and analyzing methods, patient numbers and/or heterogeneity of the patients involved. It is hard to predict genetic variability but methods of sampling, data analysis and patient inclusion are influential. It is therefore useful to find out how accuracy can be optimized before implementing SS2R. First, the sampling site has a

Table 4. *Overview FilmArray® validation studies with RT-PCR as reference test (n = 7)*

Study	Year	FA version	No. samples	Patients	Country	Swab method	Discordant analysis	Sens (%), CI (95%)	Spec (%), CI (95%)	Ref. no.
Pierce *et al.*	2011	Pre-market version	280	Children 0-22 years	USA	NPA, NPS, EA, BAL, LA	Repeated testing	86.9 (81.4-90.9)	85.1 (73.8-92.2)	12
Hayden *et al.*	2011	Pre-market version	176	Children 0-18 years	USA	NPS, NPW, BAL, EA	x	90.2 (79.1-95.9)	93.0 (86.3-96.7)	13
Renaud *et al.*	2012	FDA cleared v1	34	Unclear	USA	NPW, NPS, BAL, EA, SP	Repeated testing	76.5 (58.4-88.6)	x	14
Hammond *et al.*	2012	Pre-market version	90	Immuno-compro-mised adults	USA	NPA, BAL	Repeated testing	92.9 (75.0-98.8)	93.5 (83.5-97.9)	15
Van Wesenbeeck *et al.*	2013	FDA cleared	165	Adults (presenting at GP)	Belgium	NPS	Sequencing	85.1 (75.4-91.5)	100 (94.2-100)	16
Piralla *et al.*	2014	Pre-market version	72	Neonates < 30 days	Italy	NPA, NPS, BAL, EA	x	100 (92.8-100)	55.6 (22.7-84.7)	17
Andersson *et al.*	2014	FDA cleared v1.6	128	Children and adults	Sweden	NPS, TS, BAL	Repeated testing	84.2 (75.2-90.4)	77.8 (57.3-90.6)	18

BAL = bronchoalveolar lavage; CI = confidence interval; EA = endotracheal aspirate; et. al. = and others; FA = FilmArray®; FDA = Food and Drug Administration; GP = general practitioner; LA = lung autopsy tissue; n = number; no = number; NPA = nasopharyngeal aspirate; NPS = nasopharyngeal swab; NPW = nasopharyngeal wash; PCR = polymerase chain reaction; ref = reference; RT = reverse transcription; RV = respiratory viral; RVP = Respiratory Viral Panel; sens = sensitivity; SP = sputum; spec = specificity; TC = throat swab; USA =United States of America; v = version

significant effect on sensitivity. Although lower respiratory tract samples like bronchoalveolar fluids, have highest sensitivity,[17,28] nasopharyngeal swabs – which were used in the current study – are the most sensitive upper respiratory samples[36,37] and are most feasible in an ED-setting. Second, in analyzing accuracy data for multiple viral pathogens, the number and choice of viruses and the cut-off Ct-value influence accuracy. In this study, accuracy was calculated both per virus and per sample, using initial results and results of repeated testing for invalid results to reflect clinical practice as close as possible. The high cut-off value of > 45 used in this study, leading to a somewhat lower sensitivity of the SS2R due to one influenza A virus (Ct-value of 40.89) that was missed by the SS2R, was chosen to reflect clinical practice, in which higher cut-off values are often used for RNA-viruses. Furthermore, choosing a reference standard, including the performance of a discrepancy analysis, either by repeated testing or by sequencing, greatly influences accuracy. Although RT-PCR is considered the best available reference standard, composite reference standards and discrepancy analyses may lead to higher numbers of agreement. In this study accuracy measures were given in a way the estimates are interpretable by clinicians. From a more epidemiologic point of view, accuracy or percentage agreement (87% [95% CI 76-94]) with Cohen's Kappa statistic (0.73 [95% CI 0.56-0.90]) might be more suitable considering the imperfect reference standard (*table 3*). Third, significant differences have been shown in the sensitivity of PCR assays between different study populations, with higher sensitivities in patient groups with higher viral loads and lower viral clearance rate[38] due to short symptom duration or specific characteristics such as being a child,[39,40] being immunocompromised[40] or having COPD.[41] The current study is underpowered to compare patient groups, but confirms that discordant SS2R negative results had significantly lower viral loads (e.g. higher Ct-values) than concordant results. In daily practice this means that SS2R has reliable results for patients with high viral loads, being the patients at highest risk of complications and respiratory insufficiency.[42]

In our hospital, SS2R has a rapid TAT compared to that of previous studies (average TAT 2:30 hours (n = 5576)).[34,35,43-48] Since patients were only included during lab opening hours, TATs may not be representative for sample testing during evenings, nights and weekends. A proposal to relocate the SS2R system to the ED raised objections among the staff. Objections included lack of laboratory skills, a highly variable workload of the ED-personnel and need for an isolated test room, making SS2R implementation at the ED unfeasible at our hospital. Also, the ISO-certification would have to be extended for point-of-care testing at the ED. However, the current study showed that TAT was rapid even when used in a laboratory setting. Apart from TATs, the effect of SS2R testing is also affected by early availability of results, which was delayed in this research setting due to the necessity of asking informed consent and taking a second swab. When swabs are taken shortly upon a patient's arrival at the ED, it can be assumed that the percentage of available SS2R results during ED-stay will be much higher than 35%. Altogether, SS2R implementation in the laboratory setting has great potential in affecting clinical outcomes, with fewer practical issues to overcome than at the ED. The effect of rapid testing can be optimized by extending laboratory opening hours and using more than one SS2R system for parallel testing.

Even though the results of this study regarding diagnostic accuracy, TATs and applicability are promising with respect to clinical outcomes, no exact estimations can be made. A recent randomized controlled diagnostic intervention trial[49] showed beneficial effects of rapid testing with the same SS2R on both oseltamivir prescription, duration of antibiotic treatment and use of in-hospital isolation facilities, strengthening our hypothesis. In this trial, as compared to conventional diagnostics, SS2R led to a 26% increase in correct oseltamivir prescription in influenza virus positive patients (91% versus 65%, p = 0.003) and an 8% decrease in the number of patients who received antibiotic therapy for > 48 hours.[49] However, the percentage of antibiotic prescriptions was similar in both groups and the use of SS2R increased the use of in-hospital isolation facilities for patients with confirmed viral RTI by 8% (17% versus 9%, p = 0.02).[49] The hypothesis that rapid viral SS2R testing in patients with RTI may also reduce antibiotic prescription is one of the most important issues in the current landscape of increasing antibiotic resistance. Nevertheless, since the decision to prescribe antibiotics is not based on the SS2R results alone, neither observational nor experimental studies have so far been able to show an advantageous effect of rapid SS2R testing on antibiotic prescription. The most plausible explanation for this disappointing effect of SS2R testing on antibiotic prescription is that many patients have already been started on antibiotics before the results of a rapid test become available,[49] underlining the importance of an optimal implementation strategy.

In conclusion, rapid syndromic sample to result PCR like the FilmArray® Respiratory Panel are fast, easy to use and accurate, especially with high-risk patients. Implementation of rapid mPCR diagnostics in routine care, even when put in a laboratory setting, could further improve clinical management of patients suspected of respiratory tract infection presenting at the emergency department.

DISCLOSURES

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REFERENCES

1. Clark TW, Medina MJ, Batham S, Curran MD, Parmar S, Nicholson KG. Adults hospitalised with acute respiratory illness rarely have detectable bacteria in the absence of COPD or pneumonia; viral infection predominates in a large prospective UK sample. J Infect. 2014;69:507-15.
2. Brendish NJ, Schiff HF, Clark TW. Point-of-care testing for respiratory viruses in adults: The current landscape and future potential. J Infect. 2015;71:501-10.
3. Monto AS, Gravenstein S, Elliott M, Colopy M SJ. Clinical signs and symptoms predicting influenza infection. Arch Intern Med. 2000;160:3243-7.
4. Gonzales R, Malone DC, Maselli JH, Sande M a. Excessive Antibiotic Use for Acute Respiratory Infections in the United States. 2001;33:757-62.
5. Smith SM, Fahey T, Smucny J, Becker L a. Antibiotics for acute bronchitis. Cochrane Database Syst Rev. 2014;3:CD000245.
6. McCullers J a. The co-pathogenesis of influenza viruses with bacteria in the lung. Nat Rev Microbiol. 2014;12:252-62.
7. Hawkey PM. The growing burden of antimicrobial resistance. J Antimicrob Chemother. 2008;62 Suppl 1:1-9.
8. Muthuri SG, Venkatesan S, Myles PR, et al. Effectiveness of neuraminidase inhibitors in reducing mortality in patients admitted to hospital with influenza A H1N1pdm09 virus infection: A meta-analysis of individual participant data. Lancet Respir Med. 2014;2:395-404.
9. Busson L, Mahadeb B, De Foor M, Vandenberg O, Hallin M. Contribution of a rapid influenza diagnostic test to manage hospitalized patients with suspected influenza. Diagn Microbiol Infect Dis. 2017;87:238-42.
10. Teirlinck AC, Van Asten L, Brandsema PS, et al. Annual report Surveillance of Influenza and other respiratory infections in the Netherlands: winter 2016/2017. 2017;RIVM Rapport. http://www.rivm.nl.
11. van de Pol AC, Wolfs TFW, Jansen NJG, van Loon AM, Rossen JWA. Diagnostic value of real-time polymerase chain reaction to detect viruses in young children admitted to the paediatric intensive care unit with lower respiratory tract infection. Crit Care. 2006;10:R61.
12. Van Doornum GJJ, Guldemeester J, Osterhaus ADME, Niesters HGM. Diagnosing herpesvirus infections by real-time amplification and rapid culture. J Clin Microbiol. 2003;41:576-80.
13. Do LAH, van Doorn HR, Bryant JE, et al. A sensitive real-time PCR for detection and subgrouping of human respiratory syncytial virus. J Virol Methods. 2012;179:250-5.
14. Pierce VM, Elkan M, Leet M, McGowan KL, Hodinka RL. Comparison of the idaho technology FilmArray system to real-time PCR for detection of respiratory pathogens in children. J Clin Microbiol. 2012;50:364-71.
15. Hayden RT, Gu Z, Rodriguez A, et al. Comparison of two broadly multiplexed PCR systems for viral detection in clinical respiratory tract specimens from immunocompromised children. J Clin Virol. 2012;53:308-13.
16. Renaud C, Crowley J, Jerome KR, Kuypers J. Comparison of FilmArray Respiratory Panel and laboratory-developed real-time reverse transcription-polymerase chain reaction assays for respiratory virus detection. Diagn Microbiol Infect Dis. 2012;74:379-83.
17. Hammond SP, Gagne LS, Stock SR, et al. Respiratory virus detection in immunocompromised patients with FilmArray respiratory panel compared to conventional methods. J Clin Microbiol. 2012;50:3216-21.
18. Van Wesenbeeck L, Meeuws H, Van Immerseel A, et al. Comparison of the FilmArray RP, verigene RV+, and prodesse ProFLU+/FAST+ multiplex platforms for detection of influenza viruses in clinical samples from the 2011-2012 influenza season in Belgium. J Clin Microbiol. 2013;51:2977-85.
19. Piralla A, Lunghi G, Percivalle E, et al. FilmArray® respiratory panel performance in respiratory samples from neonatal care units. Diagn Microbiol Infect Dis. 2014;79:183-6.
20. Andersson ME, Olofsson S, Lindh M. Comparison of the FilmArray assay and in-house real-time PCR for detection of respiratory infection. Scand J Infect Dis. 2014;46:897-901.
21. Rand KH, Rampersaud H, Houck HJ. Comparison of two multiplex methods for detection of respiratory viruses: Filmarray RP and xTAG RVP. J Clin Microbiol. 2011;49:2449-53.
22. Poritz MA, Blaschke AJ, Byington CL, et al. FilmArray, an automated nested multiplex PCR system for multi-pathogen detection: development and application to respiratory tract infection. PLoS One. 2011;6(10):e26047.
23. Loeffelholz MJ, Pong DL, Pyles RB, et al. Comparison of the FilmArray Respiratory Panel and Prodesse real-time PCR assays for detection of respiratory pathogens. J Clin Microbiol. 2011;49:4083-8.
24. Babady NE, Mead P, Stiles J, et al. Comparison of the Luminex xTAG RVP Fast assay and the Idaho Technology FilmArray RP assay for detection of respiratory viruses in pediatric patients at a cancer hospital. J Clin Microbiol. 2012;50:2282-8.
25. Couturier MR, Barney T, Alger G, et al. Evaluation of the FilmArray® Respiratory Panel for Clinical Use in a Large Children's Hospital. J Clin Lab Anal. 2013;27:148-54.
26. Popowitch EB, O'Neill SS, Miller MB. Comparison of the biofire filmarray RP, Genmark eSensor RVP, Luminex xTAG RVPv1, and Luminex xTAG RVP fast multiplex assays for detection of respiratory viruses. J Clin Microbiol. 2013;51:1528-33.
27. Layman CP, Gordon SM, Elegino-Steffens DU, Agee W, Barnhill J, Hsue G. Rapid multiplex PCR assay to identify respiratory viral pathogens: moving forward diagnosing the common cold. Hawaii J Med Public Health. 2013;72:24-6.
28. Ruggiero P, McMillen T, Tang YW, Babady NE. Evaluation of the BioFire FilmArray Respiratory Panel and the GenMark eSensor Respiratory Viral Panel on Lower Respiratory Tract Specimens. J Clin Microbiol. 2014;52:288-90.
29. Marcone DN, Carballal G, Ricarte C. automatizado de PCR múltiples (FilmArra) y su comparación con. 2015;47(1).
30. Jerris RC, Williams SR, MacDonald HJ, Ingebrigtsen DR, Westblade LF, Rogers BB. Testing implications of varying targets for Bordetella pertussis: comparison of the FilmArray Respiratory Panel and the Focus B. pertussis PCR assay. J Clin Pathol. 2015;68:394-6.
31. Song E, Wang H, Salamon D, Jaggi P, Leber A. Performance characteristics of FilmArray respiratory panel v1.7 for detection of adenovirus in a large cohort of pediatric nasopharyngeal samples: One test may not fit all. J Clin Microbiol. 2016;54:1479-86.
32. Chen JHK, Lam H-Y, Yip CCY, et al. Clinical Evaluation of the New High-Throughput Luminex NxTAG Respiratory Pathogen Panel Assay for Multiplex Respiratory Pathogen Detection. J Clin Microbiol. 2016;54:1820-5.
33. Wahrenbrock MG, Matushek S, Boonlayangoor S, Tesic V, Beavis KG, Charnot-Katsikas A. Comparison of cepheid xpert Flu/RSV XC and biofire filmarray for detection of influenza a, influenza b, and respiratory syncytial virus. J Clin Microbiol. 2016;54:1902-3.
34. Doern CD, Lacey D, Huang R, Haag C. Evaluation and implementation of FilmArray version 1.7 for improved detection of adenovirus respiratory tract infection. J Clin Microbiol. 2013;51:4036-9.
35. Butt S a., Maceira VP, McCallen ME, Stellrecht K a. Comparison of three commercial RT-PCR systems for the detection of respiratory viruses. J Clin Virol. 2014;61:406-10.
36. Kim C, Ahmed J a., Eidex RB, et al. Comparison of Nasopharyngeal and Oropharyngeal swabs for the diagnosis of eight respiratory viruses by real-time reverse transcription-PCR assays. PLoS One. 2011;6:2-7.
37. Ngaosuwankul N, Noisumdaeng P, Komolsiri P, et al. Influenza A viral loads in respiratory samples collected from patients infected with pandemic H1N1, seasonal H1N1 and H3N2 viruses. Virol J. 2010;7:75.
38. Lee N, Chan PKS, Hui DSC, et al. Viral Loads and Duration of Viral Shedding in Adult Patients Hospitalized with Influenza. J Infect Dis. 2009;200:492-500.
39. Borg I, Rohde G, Loseke S, et al. Evaluation of a quantitative real-time PCR for the detection of respiratory syncytial virus in pulmonary diseases. Eur Respir J. 2003;21:944-51.

40. Englund JA, Piedra PA, Jewell A, Patel K, Baxter BB, Whimbey E. Rapid diagnosis of respiratory syncytial virus infections in immunocompromised adults. J Clin Microbiol. 1996;34:1649-53.

41. D'Anna SE, Balbi B, Cappello F, Carone M, di Stefano A. Bacterial–viral load and the immune response in stable and exacerbated COPD: Significance and therapeutic prospects. Int J COPD. 2016;11:445-53.

42. Lee N, Chan MCW, Lui GCY, et al. High Viral Load and Respiratory Failure in Adults Hospitalized for Respiratory Syncytial Virus Infections. J Infect Dis. 2015;212:1237-40.

43. Xu M, Qin X, Astion ML, et al. Implementation of FilmArray Respiratory Viral Panel in a Core Laboratory Improves Testing Turnaround Time and Patient Care. Am J Clin Pathol. 2013;139:118-23.

44. Wang J, Simons DB, Adams JL, Jerris RC, Rogers BB. Multiplex Viral Polymerase Chain Reaction Testing Using the FilmArray Device Compared With Direct Fluorescent Antibody Testing. Lab Med. 2014;45(1):62-4.

45. Pettit NN, Matushek S, Charnot-Katsikas A, et al. Comparison of turnaround time and time to oseltamivir discontinuation between two respiratory viral panel testing methodologies. J Med Microbiol. 2015;64:312-3.

46. Gelfer G, Leggett J, Myers J, Wang L, Gilbert DN. The clinical impact of the detection of potential etiologic pathogens of community-acquired pneumonia. Diagn Microbiol Infect Dis. 2015;83:400-6.

47. Rogers VL, Sheffield JS, Roberts SW, et al. Presentation of seasonal influenza A in pregnancy: 2003-2004 influenza season. Obstet Gynecol. 2010;115:924-29.

48. Rappo U, Schuetz AN, Jenkins SG, et al. Impact of early detection of respiratory viruses by multiplex PCR assay on clinical outcomes in adult patients. J Clin Microbiol. 2016;54:2096-103.

49. Brendish NJ, Malachira AK, Armstrong L, et al. Routine molecular point-of-care testing for respiratory viruses in adults presenting to hospital with acute respiratory illness (ResPOC): a pragmatic, open-label, randomised controlled trial. Lancet Respir Med. 2017;5:401-11.

50. Rogers BB, Shankar P, Jerris RC, et al. Impact of a rapid respiratory panel test on patient outcomes. Arch Pathol Lab Med. 2015;139:636-41.