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# Identification of respiratory viruses with a novel point-of-care multianalyte antigen detection test in children with acute respiratory tract infection

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## ABSTRACT

**Background:** Rapid etiological diagnosis of a respiratory virus infection may have impact on antiviral and antibiotic therapy, patient cohorting, and prediction of the clinical course. Most point-of-care tests for detection of respiratory viruses have limitations in diagnostic performance and clinical usability. A novel, multianalyte point-of-care antigen detection test system (mariPOC<sup>®</sup>; ArcDia International Oy Ltd., Turku, Finland) detects eight respiratory viruses (influenza A and B viruses, respiratory syncytial virus (RSV), adenovirus, human metapneumovirus, and parainfluenza type 1, 2, and 3 viruses) from a single nasopharyngeal swab specimen by a fully automated, random-access immunoassay method.

**Objectives:** To evaluate mariPOC<sup>®</sup> point-of-care test system in comparison with reverse transcription polymerase chain reaction (RT-PCR) in a pediatric emergency department setting.

**Study design:** Prospectively collected samples from 158 children (mean age, 1.8 years) with respiratory symptoms and/or fever were analyzed both by mariPOC<sup>®</sup> and by multiplex RT-PCR.

**Results:** The sensitivities and specificities (95% confidence intervals) of the mariPOC<sup>®</sup> test were for influenza A ( $n = 7$ ), 71% (38–100) and 100%; influenza B ( $n = 22$ ), 86% (72–100) and 98% (95–100); RSV ( $n = 35$ ), 89% (78–99) and 100%; adenovirus ( $n = 12$ ), 25% (1–50) and 97% (95–99); and for human metapneumovirus ( $n = 8$ ), 50% (15–85) and 100%, respectively. Parainfluenzaviruses were detected only in five patients.

**Conclusions:** This novel point-of-care test system is a rapid, practical, and specific method for simultaneous detection of eight respiratory viruses. Compared with RT-PCR, its sensitivity is moderately high for detection of RSV and influenza viruses, and low for adenovirus.

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## 1. Background

Children under 7 years of age have every year on average from 4 to 6 respiratory tract infections.<sup>1,2</sup> Acute respiratory tract infections are the most common cause for admitting children to hospital in developed countries.<sup>3</sup> Children's respiratory tract infections are mainly of viral etiology. Common respiratory viruses include influenza A and B viruses, respiratory syncytial virus (RSV), adenovirus, parainfluenza virus (PIV) types 1, 2, and 3, and rhinovirus.<sup>1,4</sup> The clinical importance of more newly discovered human

metapneumovirus (hMPV), coronaviruses OC43/HKU1 and 229E/NL63, and human bocavirus is still only partly understood.

All respiratory viruses cause non-specific local and systemic symptoms such as fever, cough, rhinitis, fatigue, and headache, which makes it impossible to clinically differentiate between illnesses caused by specific pathogens. Even with influenza, which is often considered to have a typical clinical manifestation, the diagnosis based on clinical grounds only is inaccurate.<sup>5</sup> This is particularly true in young children who are unable to express subjective symptoms.

Etiologic diagnosis of respiratory infection would be needed in the setting of pediatric emergency department to target antiviral therapy of influenza and to prevent transmission of viruses in the hospital by means of patient cohorting and optimized infection control measures. Additional benefits of viral detection might include reduction of the use of chest X-rays or laboratory investigations, avoidance of overuse of antibiotics, and better prediction of the clinical course.<sup>6</sup>

**Abbreviations:** hMPV, human metapneumovirus; NPV, negative predictive value; PIV, parainfluenza virus; POC, point-of-care; PPV, positive predictive value; RSV, respiratory syncytial virus; RT-PCR, reverse transcription polymerase chain reaction.

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Polymerase chain reaction (PCR) has become a standard method in respiratory viral diagnostics. This is due to its high sensitivity, good availability, and shorter delay in results in comparison with conventional methods such as viral culture.<sup>7</sup> Multiplex reverse transcription (RT) PCR methods allow detection of several viruses at the same time. However, RT-PCR, or viral antigen detection performed in a laboratory, does not provide information timely for clinical decisions at the emergency department.

Point-of-care (POC) tests are designed to be available directly at the site of patient care, accessible to staff without laboratory training, and provide results quickly enough to have an impact on clinical decision-making.<sup>8</sup> For influenza A and B viruses and RSV, POC testing has been used frequently, although studies evaluating diagnostic performance of these tests have provided varying results.<sup>9–15</sup> The practicality of POC tests in the emergency department may be compromised by the need of hands-on time for performing the test or unclear visualization of the result.

## 2. Objectives

The objective of this study was to evaluate the diagnostic performance and practical usability of a novel multianalyte detection system for eight respiratory viruses, the mariPOC<sup>®</sup> (ArcDia International Oy Ltd., Turku, Finland), in a clinical ambulatory care setting. We compared the results of this rapid antigen detection test performed in the pediatric emergency department with multiplex RT-PCR test performed in laboratory conditions.

## 3. Study design

### 3.1. Participant recruitment

Participants were recruited prospectively between January 11 and March 17, 2011, in the pediatric emergency department of Turku University Hospital, Turku, Finland. The department serves children between 0 and 16 years of age. Patients were eligible for the comparative study between the mariPOC<sup>®</sup> test and RT-PCR if they were hospitalized from the emergency department with respiratory symptoms (cough, rhinitis, wheezing) or fever without a focus, or if they were treated as outpatients for a respiratory infection suspected to be caused by influenza virus, RSV, or adenovirus. Suspicion of these viruses was used as an inclusion criterion because we have earlier used other POC tests routinely for detection of influenza A or B virus, RSV, and adenovirus. Influenza was suspected in patients with high fever and cough, RSV in those with bronchiolitis or other wheezing illness, and adenovirus in those with non-streptococcal tonsillitis.

Patients with two or more visits to the emergency department during the study period were included in the study only at the first visit when eligible. Parents were asked to fill out an inquiry about chronic illnesses of the child, onset of symptoms and the immunization status for seasonal influenza. Other clinical data were derived from the medical records.

The Ethics Committee of the Hospital District of Southwest Finland approved the study protocol. Parents of all participating children gave their informed consent.

### 3.2. Specimen collection

Nasopharyngeal samples were collected by using flocked swabs (Copan, Brescia, Italy). A nasopharyngeal swab was inserted through either nostril and specimen was collected from a depth of at least 7 cm. The swabs were eluted in 1.3 ml of RTI sample buffer (ArcDia International Oy Ltd., Turku, Finland) and inserted to the mariPOC<sup>®</sup> multianalyte antigen detection test system at

the pediatric emergency department immediately after sampling. Emergency department nurses, who collected the samples, were trained to use the test system by the manufacturer (1 h of training). After POC analysis, the remaining sample solution was transferred to the Virus Diagnostic Laboratory, Department of Virology, University of Turku. Samples from 74 children admitted to hospital were analyzed immediately by PCR tests, and samples from 84 children were stored at  $-70^{\circ}\text{C}$  until analyzed. In 11 cases the first sample was not available and RT-PCR was done from a new sample taken <72 h after the first one.

### 3.3. mariPOC<sup>®</sup> test system

mariPOC<sup>®</sup> multianalyte test system provides pathogen specific results for eight respiratory viruses from a single nasopharyngeal swab (influenza A and B viruses, RSV A/B, adenovirus, hMPV, and parainfluenza type 1, 2, and 3 viruses). Nasopharyngeal sample kit includes also a test for *Streptococcus pneumoniae*. The same test system also allows simultaneous detection of adenovirus and group A streptococcus from a throat swab. *S. pneumoniae* or throat swab results were not included in this study.

The test is fully automated and allows random-access analysis. The platform applies proprietary two-photon fluorescence excitation detection technique and antigen detection by immunometric assay principle.<sup>16</sup> The technique is characterized with microvolume analysis and separation-free fluorescent measurement of bioaffinity binding reactions.<sup>17</sup>

The nasopharyngeal swab sample in RTI sample buffer is inserted into the analyzer, capped and bar coded, for automated analysis. The analyzer dispenses sample in 20  $\mu\text{l}$  aliquots to parallel wells of the test plate, one well per pathogen. The wells contain the reagents in the dry state. The test system automatically reports, on to a screen of the user-interface, preliminary results after 20 min. Final results are reported after 2 h. In this study, final mariPOC<sup>®</sup> results were compared with the results provided by the reference methods, with the exception of four cases when the mariPOC<sup>®</sup> system failed to report final results. In these cases preliminary results were compared with the results of reference method.

### 3.4. Reference methods for detection of respiratory viruses

A multiplex PCR assay (Seeplex RV12; Seegene, Seoul, Korea) was used as a reference method for detection of respiratory viruses (influenza A and B viruses, RSV A and B, adenovirus, hMPV, parainfluenza type 1, 2, and 3 viruses, rhinovirus A/B, and coronaviruses OC43/HKU1 and 229E/NL63). cDNA synthesis was performed using random-hexamer primers (Fermentas, York, UK). Multiplex PCR assay was performed according to the manufacturer's instructions. Sensitivity of the multiplex PCR test for influenza B virus from samples stored at  $-70^{\circ}\text{C}$  was found to be lower than that from fresh samples. Therefore, an in house PCR test was used as a reference method for the detection of influenza B virus in stored samples. The PCR was performed as described earlier.<sup>18</sup> The primers and probes have been described earlier.<sup>19</sup> The specimens with contradictory results for influenza B in multiplex and in-house PCR tests were tested also by a time-resolved fluoroimmunoassay method, which gave concordant results with the in-house PCR test.

### 3.5. Data analyses

Data were analyzed by descriptive methods. Sensitivities, specificities, and positive and negative predictive values with 95% confidence intervals were calculated separately for each virus regardless of detection of two viruses in some samples. Days from onset of symptoms to sampling were compared between subgroups

**Table 1**  
Demographic and clinical characteristics of 158 children included in study.

Characteristic	No.	%
<b>Age</b>		
0 to <6 months	33	21
6 months to <1 year	23	15
1 to <3 years	37	23
3 to <7 years	25	16
7–17 years	40	25
<b>Sex</b>		
Male	90	57
Female	68	43
<b>Underlying conditions</b>		
None	120	76
Asthma	12	8
Neurological	8	5
Cardiological	7	4
Malignancy or other immunosuppression	2	1
Other <sup>a</sup>	9	6
<b>Symptoms</b>		
Any respiratory tract symptom	133	84
Wheezing	46	29
Fever (>38 °C)	120	76
<b>Time between symptom onset and sample</b>		
0 to <1 day	8	5
1 to <3 days	56	35
3 to <7 days	63	40
7 days or longer	31	20
<b>Hospitalization</b>		
Outpatients	89	56
Inpatients	69	44

<sup>a</sup> Diabetes mellitus ( $n=1$ ), APECED ( $n=1$ ), oligoarthritis ( $n=1$ ), pulmonary disease other than asthma ( $n=2$ ), anorexia nervosa ( $n=1$ ), prematurity ( $n=1$ ), renal insufficiency ( $n=1$ ), and Crohn's disease ( $n=1$ ).

by *t*-test after Levene's test for equality of variances, with two-sided  $p=0.05$  as the limit of statistical significance.

#### 4. Results

Samples from a total of 158 children were tested both by the mariPOC<sup>®</sup> test system and by RT-PCR. Patient characteristics are listed in Table 1. The median age of the children was 1.8 years (interquartile range, 0.6–7.1 years). Sixty-nine patients (44%) were admitted to hospital.

Clinical personnel of the emergency department regarded the mariPOC<sup>®</sup> test system as easy to use at the point of care. The positive results were available already within 20 min in 76% of patients with a final positive result for at least one virus. In 1% of patients the preliminary result was reported as positive but the final result was negative. The median time between onset of symptoms and sampling was 3 days for the cases positive already in the preliminary report ( $n=54$ ) vs. 5 days in those positive only in the final

**Table 2**  
Diagnostic yield of mariPOC<sup>®</sup> and RT-PCR in nasopharyngeal samples from children with a respiratory tract infection or fever ( $n=158$ ).

	No. (%)	
	mariPOC <sup>®</sup>	RT-PCR
Samples positive	69 (44)	104 (66)
Samples positive for 2 viruses	2 (1)	11 (7)
Samples positive for viruses covered by mariPOC <sup>®</sup>	69 (44)	84 (53)
Samples negative	89 (56)	54 (34)

report ( $n=15$ ,  $p=0.07$ ). The time from onset of symptoms to sampling was not significantly longer in children with false negative result in mariPOC compared to children with positive results in both mariPOC and PCR (median, 3.5 vs. 3 days,  $p=0.36$ ).

mariPOC<sup>®</sup> and RT-PCR were done in separate samples in 11 cases. The time interval between obtaining these samples was less than 24 h in 9 cases, 44 h in 1 case, and 66 h in 1 case. In these latter two cases RSV was detected both by mariPOC<sup>®</sup> and by RT-PCR. Time delay between sampling did therefore not affect results in these cases.

The multiplex RT-PCR method Seplex RV12 detects rhinoviruses and coronaviruses OC43/HKU1 and 229E/NL63, which are not included in the mariPOC<sup>®</sup> test system. The mariPOC<sup>®</sup> system was positive for at least 1 of 8 viruses in 69 of 158 samples (44%). In comparison, the RT-PCR was positive for at least 1 of 8 viruses covered by the mariPOC<sup>®</sup> in 84 (53%), and for at least 1 of the 12 analyzed viruses in 104 (66%) of 158 samples (Table 2). RT-PCR detected rhinovirus in 12 (7.6%) and coronaviruses in 13 (8.2%) children. Eleven samples were positive for 2 viruses by multiplex RT-PCR and 2 samples were positive for 2 viruses by mariPOC<sup>®</sup>. None of the samples were positive for more than 2 viruses.

Sensitivities, specificities, and positive and negative predictive values of the mariPOC<sup>®</sup> system for each virus in comparison with RT-PCR are shown in Table 3. Influenza (A or B) was detected in 29 children by RT-PCR and in 24 of them also by the mariPOC<sup>®</sup> test system (18 (75%) after 20 min in the preliminary report), giving an overall sensitivity of 83% for any influenza. Thirty-five children in the study population were positive for RSV by RT-PCR. mariPOC<sup>®</sup> detected RSV in 31 of these patients (sensitivity 89% and specificity 100%), and 28 (90%) of the positive results were reported already after 20 min. The mariPOC<sup>®</sup> test gave sensitivity of 25% for adenovirus compared with RT-PCR. Nine samples were negative for adenovirus by mariPOC<sup>®</sup> but positive by PCR. Six of these 9 samples were double positive findings by multiplex-PCR; in 3 samples adenovirus was found together with RSV, in 2 with hMPV and in 1 with rhinovirus. HMPV was detected in 8 patients by RT-PCR and in 4 of them also by mariPOC<sup>®</sup>. The small number of parainfluenza virus findings prevented evaluation of the test's diagnostic performance

**Table 3**  
Sensitivities, specificities, and predictive values, according to virus, of mariPOC<sup>®</sup> compared with RT-PCR among 158 children.

Virus	mariPOC <sup>®</sup>	RT-PCR		% (95% confidence interval)			
		Positive	Negative	Sensitivity	Specificity	PPV	NPV
Influenza A virus	Positive	5	0	71 (38–100)	100	100	99 (97–100)
	Negative	2	151				
Influenza B virus	Positive	19	3	86 (72–100)	98 (95–100)	86 (72–100)	98 (95–100)
	Negative	3	133				
Influenza A or B virus	Positive	24	3	83 (69–97)	98 (95–100)	89 (77–100)	96 (93–99)
	Negative	5	126				
Respiratory syncytial virus	Positive	31	0	89 (78–99)	100	100	97 (94–100)
	Negative	4	123				
Adenovirus	Positive	3	4	25 (1–50)	97 (95–99)	43 (6–80)	94 (90–98)
	Negative	9	142				
Human metapneumovirus	Positive	4	0	50 (15–85)	100	100	97 (95–100)
	Negative	4	150				

PPV, positive predictive value; NPV, negative predictive value.

for these viruses, as only two samples were positive for parainfluenza type 2 virus, and three samples for parainfluenza type 3 virus by RT-PCR.

## 5. Discussion

The mariPOC<sup>®</sup> antigen detection system was highly specific and moderately sensitive in the diagnostics of influenza and RSV infections in children. The test system had a sensitivity of 83% for influenza viruses and 89% for RSV, with specificities of 97% or higher for all tested viruses, in comparison with RT-PCR. These test characteristics are satisfactory in clinical settings where rapid reporting of results outweighs the minor limitations in sensitivity.

Since the availability of effective neuraminidase inhibitor drugs for treatment of influenza, there has been a need for rapid influenza tests in ambulatory clinics.<sup>20</sup> This need was highlighted during 2009 influenza A (H1N1) pandemic, but official instructions guided healthcare providers to use PCR tests instead of POC tests because of the poor sensitivity of the latter. Studies have reported sensitivities of 19–27% and specificities of 97–100% for influenza POC tests when compared with RT-PCR.<sup>11,21,22</sup> However, in studies in children, better sensitivities and specificities of POC tests for influenza have been reported.<sup>9,14,23</sup> This may be partly due to higher viral loads in children than in adults.<sup>24,25</sup> In a study of influenza POC test, with immunofluorescence test or viral culture as the reference method instead of more sensitive RT-PCR, sensitivity of 91% and specificity of 97% were reported.<sup>26</sup> Earlier commercially available POC tests might have challenges especially in detection of influenza B virus.<sup>11,14,27</sup> The mariPOC<sup>®</sup> test performed well in detection of both influenza A and B viruses, but the sensitivity for influenza A had a large confidence interval due to the low number of cases.

Yearly RSV epidemics are a major cause for hospitalizations of children. Although antiviral treatment is not available, the benefits of rapid detection of RSV have been demonstrated.<sup>28</sup> In detection of RSV, the performance of mariPOC<sup>®</sup> test appears to be comparable to other rapid tests, which have been documented to be both sensitive and specific.<sup>10,15,29</sup>

The diagnostic yield of the mariPOC<sup>®</sup> test was fairly good with a detection of virus in 44% of samples. Higher diagnostic yield of RT-PCR resulted from better sensitivity and from detection of rhinoviruses and coronaviruses that are not included in the mariPOC<sup>®</sup> system. The yield and possibly also diagnostic performance of the mariPOC<sup>®</sup> test system might be different in another epidemiologic situation. We purposefully conducted our study during a season when influenza viruses and RSV were circulating in the community, because the need for rapid testing appears to be highest for these viruses. From January to March 2011, pandemic-type influenza A (H1N1) and influenza B of Yamagata line were the dominant strains of influenza viruses in Finland. Influenza A dominated the epidemic in January and influenza B in February and March. Thus, the higher number of influenza B than influenza A viruses in our study reflects the epidemiologic situation. The fairly high proportion of samples positive for RSV in our study is in accordance with the epidemiologic occurrence of RSV during winter–spring, 2011, in Finland.

Adenovirus was often detected by PCR simultaneously with another respiratory virus. Possibly in some of these cases adenovirus was an innocent bystander, and the other virus was the pathogen. Indeed, adenovirus has been detected in some studies frequently also from asymptomatic individuals by PCR.<sup>30</sup> These data suggest that multiplex RT-PCR may have in some settings excessively high sensitivity leading to clinically irrelevant findings.

The mariPOC<sup>®</sup> test system was found to be easy to incorporate in the work of clinical personnel in the emergency department. Short hands-on time was regarded as an important advantage over earlier used non-automated POC tests. In 76% of the positive cases

the result was received already in the preliminary report at 20 min. Rapid and clear reporting of the results and automated archiving of the data were considered as important features of the test system by the users. Costs were not evaluated in this study but they can be suggested to be lower by the POC test system compared with multiplex PCR.

Our results demonstrate that the mariPOC<sup>®</sup> test system provides a new option for the detection of multiple respiratory viruses from a single nasopharyngeal swab in a rapid and user-friendly way. The test system had a high specificity for all tested viruses and it had a moderately high sensitivity for detecting influenza viruses and RSV. Further clinical evaluation regarding identification of hMPV, adenovirus and parainfluenza viruses by the test system is needed.

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## Competing interests

None declared.

## Ethical approval

The Ethics Committee of the Hospital District of Southwest Finland approved the study protocol. Parents of all participating children gave their informed consent.

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