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Analytical performance of the automated multianalyte point-of-care mariPOC® for the detection of respiratory viruses

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The analytical performance of mariPOC® respi test (ArcDia® Laboratories, Turku, Finland) was evaluated using nucleic acid amplification techniques (NAATs) as the gold standard. The mariPOC assay allows automated detection of antigens from 8 respiratory viruses: influenza A and B viruses, respiratory syncytial virus, adenovirus, human metapneumovirus, and parainfluenza viruses 1–3. Positive results from samples with high viral load are available in 20 min. Nasopharyngeal aspirates ($n = 192$) from patients with acute respiratory infection and from previously positive samples were analyzed by mariPOC and NAATs (Simplexa™ FluA/FluB & RSV kit [$n = 118$] and Luminex® Respiratory virus panel xTAG® RVP FAST [$n = 74$]). Sensitivity, specificity, positive predictive value, and negative predictive value of mariPOC were 85.4%, 99.2%, 95.9%, and 97%, respectively, and 84.6% of positive results were reported in 20 min. The good analytical performance and extended portfolio of mariPOC show this rapid assay as a good alternative for the etiological diagnosis of acute respiratory infection in laboratories that are not equipped with molecular assays.

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1. Introduction

Acute respiratory infection (ARI) of viral etiology is a leading cause of medical demand in all age groups representing a significant overload to healthcare systems. Although the majority of these infections are self-limited and mild, severe infections can occur mainly in young children, elderly, immunosupressed individuals, and patients with underlying diseases.

Influenza (Flu) A and B viruses, respiratory syncytial virus (RSV), parainfluenzaviruses (PIV), adenovirus (ADV), rhinovirus (RhV), enterovirus (EV), and human coronavirus (CoV) OC43 and 229E are classical respiratory pathogens associated with ARI (Atmar et al., 2012; Creer et al., 2006; Dimopoulos et al., 2012; Henderson et al., 1979; Jennings et al., 2008; Ruuskanen et al., 2011; Yun et al., 1995) as well as other currently described viruses such as human metapneumovirus (hMPV) (Van den Hoogen et al., 2004), SARS-CoV (Ksiazek et al., 2003), CoV NL63 (Van der Hoek et al., 2004) and HKU1 (Woo et al., 2005), MERS-CoV (Zaki et al., 2012), human bocavirus (BoV) (Allander et al., 2005), and others (Ruuskanen et al., 2011).

Clinical manifestations of ARI are nonspecific. Although seasonal patterns of virus activity could orientate the etiological diagnosis, several viruses can be cocirculating at the same time throughout the year (Ruuskanen et al., 2011). Rapid etiological diagnosis of ARI improves patients' management, as it allows for appropriate and on-time antiviral therapy, which has demonstrated a reduction of complications, duration of symptoms, and hospital stay (D'Heilly et al., 2008; Fiore et al., 2011). Indeed, rapid diagnosis avoids the use of unnecessary antibiotics and ancillary diagnostic studies (Ferronato et al., 2012; Benito-Fernández et al., 2006).

The mariPOC® respi test (mariPOC; ArcDia International Oy Ltd, Turku, Finland) allows automated antigen detection and differentiation of 8 respiratory viruses (Flu A, Flu B, RSV, ADV, hMPV, PIV 1, PIV 2, and PIV 3) and *Streptococcus pneumoniae*. Antigens are sandwiched by capture antibodies and fluorescently labeled antibodies conjugate onto polymer microspheres, forming immunocomplexes in proportion to the concentration of antigen in the sample. The fluorescence signal from individual microspheres is measured by a separation-free assay method based on 2-photon excitation fluorescence detection technology (ArcDia) (Koskinen et al., 2007). The system automatically reports preliminary results in 20 min for samples with high viral load and final results in 2 hours. The instrument allows semicontinuous loading every 5 min, which is the time that it takes for each sample processing. Up to 6 samples can be loaded in the instrument in the same run.

The aim of this study was to evaluate the analytical performance of mariPOC for the detection of respiratory viruses in clinical samples, compared with other rapid antigen detection tests (RDTs) and nucleic acid amplification techniques (NAATs).

2. Materials and methods

2.1. Samples

Nasopharyngeal aspirates (NPAs) from patients with clinical suspicion of ARI of viral etiology attending the University Hospital Virgen

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de las Nieves were prospectively collected and analyzed in parallel by mariPOC and routine methods from January to April, 2013. Samples were separated into 3 aliquots, one for routine viral testing, another for mariPOC analysis, and a third one refrigerated at 4 °C (≤48 hours) or frozen at −80 °C (>48 hours), for further analysis if necessary.

In addition, NPAs (stored at −80 °C), previously positive to respiratory viruses included and not included in mariPOC were retrospectively analyzed for the calculation of sensitivity, specificity, and predictive values.

2.2. Analysis of samples with mariPOC® respi test

Tubes with 1.3 mL of respiratory tract infection (RTI) sample buffer, supplied with the kit (ArcDia International Oy Ltd), were inoculated with 300 μL of fresh NPA (prospective study), vortexed twice during 10–15 sec, centrifuged 5 min at 1000 × g, and inserted into the mariPOC system.

A concentration step was applied to frozen samples (retrospective study) in order to minimize the deleterious effect of freezing/thawing on the sensitivity, as follows: 0.6 mL of the sample was centrifuged for 5 min at 14000 rpm, 300 μL of the supernatant was discarded, and the pellet was added to the tube containing RTI sample buffer.

2.3. Detection methods for respiratory viruses

RDTs for detection of Flu and RSV were carried out by lateral flow immunochromatography: SD Bioline Influenza A/B/A(H1N1) (Alere Healthcare SLU, Barcelona, Spain) for detection of Flu A, Flu B, and Flu A(H1N1)pdm09 and Alere BinaxNOW® RSV (Alere HealthCare SLU) for RSV. NAATs used were Simplexa™ FluA/FluB & RSV kit (Simplexa) (Focus Diagnostics, Cypress, CA, USA) for Flu A, Flu B, and RSV detection and Luminex® Respiratory virus panel xTAG® RVP FAST v2 kit (Luminex) (Luminex®, Austin, TX, USA) for the detection of 19 respiratory viruses.

RDTs and Simplexa were performed on samples from patients who attended emergency units, and NAATs (Simplexa and/or Luminex) were carried out on samples from hospitalized individuals in whom RDTs were negative or not done.

For the evaluation of the analytical performance of mariPOC, NAATs were used as reference techniques.

2.4. Viral strain titration by 50% tissue culture infectious dose ($TCID_{50}$) assay

Viral strains, obtained from clinical isolates, were propagated in appropriate cell lines: Flu A (H1N1)pdm09 and B in MDCK-SIAT1; RSV and ADV in Hep-2, PIV1, PIV2, and PIV3 and hMPV in LLC-MK2 cells. After incubation at 37 °C during 10 days or when cytopathic effect (CPE) was observed, cell monolayers were harvested, aliquoted, and preserved in liquid nitrogen for further use. $TCID_{50}$ assay was performed for each virus as described (Hsiung, 1994). The appearance of CPE was examined daily for 10 days postinfection, and $TCID_{50}$ was calculated using the Spearman–Kärber method (Hamilton et al., 1977).

2.5. Calculation of the limit of detection (LoD) of mariPOC® respi test

The LoD of mariPOC was separately calculated for each of the 8 viruses included in the panel. Ten-fold serial dilutions down to 10^{-6} of each titrated strain (by $TCID_{50}$) were prepared, in quadruplicate, in physiological saline sterile solution. The mariPOC assay was carried out with 100 μL of each dilution added to 1.5 mL of RTI sample buffer as described above. The LoD was defined as the lowest dilution (expressed in $TCID_{50}$) that gave a positive result in 4 repetitions.

2.6. Ethics statements

The study protocol was carried out in accordance with the Declaration of Helsinki. This was a noninterventional study with no additional investigation to routine procedures. Biological material was only used for standard viral diagnostics following physicians' prescriptions. No additional sampling or modification of the routine sampling protocol was performed. Data analyses were carried out using an anonymous database.

2.7. Statistical analysis

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) and their 95% confidence intervals (95% CI) of mariPOC were separately calculated for each virus, regardless of the detection of more than 1 virus in the same sample.

The Cohen's *kappa* coefficient of agreement (SPSS 15.0 software; SPSS, Chicago, IL, USA) between mariPOC and both RDTs (for Flu A, Flu B, and RSV) and NAATs was calculated.

3. Results

A total of 192 NPAs, 160 (83.3%) from pediatric and 32 (16.7%) from adult patients, were analyzed. Pediatric individuals were aged between 0 and 13 years old (mean ± SD = 2 ± 2; median = 1). Patients over 14 years old were aged between 15 and 86 years old (mean ± SD = 49 ± 18; median = 44).

S. pneumoniae was detected by mariPOC in 62% and 15.6% of samples from children and adults, respectively. At least 1 respiratory virus was detected in 67% and 66.7% of the *S. pneumoniae*–positive and *S. pneumoniae*–negative samples, respectively. The mariPOC manufacturer does not recommend reporting this result in patients younger than 7 years in whom a high percentage of colonization by this microorganism could be expected. In our study, 83.3% of the samples were from pediatric patients, most of them younger than 3 years old. Thus, *S. pneumoniae* was not included in data analysis.

The prospective study was carried out in 108 (56.25%) fresh samples from 87 (80.6%) children and 21 (19.4%) adults. The retrospective study included 84 (43.75%) NPAs from 73 (86.9%) children and 11 (13.1%) adults.

Overall, 122 viruses from 115 samples were detected by mariPOC. Two viruses were detected in 7 samples. From the remaining 77 (40.1%) mariPOC-negative specimens, NAATs yielded 59 (76.6%) negative results to viruses included in mariPOC from which 42 were negative and 17 were positive to other viruses not included in the mariPOC panel (RhV/EV, CoV, and BoV).

Luminex and Simplexa were carried out in a subset of 74 and 118 samples, respectively. The results from both methods were considered together as NAATs results for comparison with mariPOC. Overall sensitivity, specificity, PPV, and NPV of mariPOC were 85.4%, 99.2%, 95.9%, and 97%, respectively (Table 1).

The mariPOC assay failed to detect 20 viruses: Flu A (n = 2), Flu B (n = 5), RSV (n = 7), ADV (n = 1), hMPV (n = 2), and PIV 1–3 (n = 3). Indeed, false-positive results (ADV [n = 1], PIV 1 [n = 3], and RSV [n = 1]) were detected by mariPOC in 5 samples. In 3 of these samples, 2 viruses were codetected by mariPOC. and only 1 of the 2 viruses was identified by the reference method (Flu B [n = 2], PIV 3 [n = 1]). No false-positive results were reported preliminarily, and 99 out of the 117 (84.6%) true-positive results were available in 20 min. Codetections of 2 viruses (of those included in the mariPOC panel) was observed in 6 samples by reference methods. In 2 of these samples, the mariPOC panel detected only 1 of the 2 viruses. The lowest sensitivity of mariPOC was obtained for hMPV, ADV, and PIV 1–3.

RDTs for Flu and RSV were performed on 122 and 131 samples, respectively (Table 2). Discordant results between mariPOC and RDTs were resolved by NAATs that showed 6 and 4 false-negative results with mariPOC and RDTs, respectively. The mariPOC assay failed in detecting 2 Flu A and 4 RSV, whereas RDTs did not detect 3 Flu B and 1 RSV.

Excellent agreements of mariPOC were obtained when compared with RDTs (*k* index: 0.924 [95% CI: 0.88–0.968]) and NAATs (*k* index: 0.885 [95% CI: 0.84–0.929]).

Table 1
Analytical performance of mariPOC in 192 nasopharyngeal samples from patients with acute respiratory infection.

Virus	Definitive report results, n mariPOC result/NAATs result					% Sensitivity (95% CI)	% Specificity (95% CI)	% PPV (95% CI)	% NPV (95% CI)
	n	Positive/positive	Negative/positive	Positive/negative	Negative/negative				
Flu A	192	24	2	0	166	92.3 (75.9–97.9)	100 (97.7–100)	100 (86.2–100)	98.8 (95.8–99.7)
Flu B	192	35	5	0	152	87.5 (73.9–94.5)	100 (97.5–100)	100 (90.1–100)	96.8 (92.8–98.6)
RSV	192	38	7	1	146	84.4 (71.2–92.2)	99.3 (96.2–99.9)	97.4 (86.8–99.5)	95.4 (90.9–97.8)
ADV	74	7	1	1	65	87.5 (52.9–97.8)	98.5 (91.9–99.7)	87.5 (52.9–97.8)	98.5 (91.9–99.7)
hMPV	74	7	2	0	65	77.8 (45.3–93.7)	100 (94.4–100)	100 (64.6–100)	97 (89.8–99.2)
PIV (1–3)	74	6	3	3	62	66.7 (35.4–87.9)	95.4 (87.3–98.4)	66.7 (35.4–87.9)	95.4 (87.3–98.4)
Global	798	117	20	5	656	85.4 (78.5–90.35)	99.2 (98.2–99.7)	95.9 (90.8–98.2)	97 (95.5–98.1)

n = number of viral determinations.

The lowest LoD of mariPOC was obtained for hMPV, followed by ADV and PIV-1, Flu B and RSV, and Flu A and PIV-3, which yielded the highest LoD as assessed by $TCID_{50}$ (Table 3).

4. Discussion

NAATs have become the gold standard for the etiological diagnosis of ARI since they are more sensitive than viral culture or antigen detection assays. Currently, many commercial multiplex molecular assays have been developed that allow partial or complete automation of the procedure (Mahony, 2008). However, their use in all clinical settings as point-of-care methods is still limited, in part due to higher costs and/or hands-on time (Wishaupt et al., 2011; Vallières and Renaud, 2013).

RDTs have proved their utility as referral point-of-care diagnostic assays, mainly for RSV and Flu, which have the greatest impact on health systems. However, sensitivity may vary depending on the method and/or type of patient and sample (CDC, 2009; Lieberman et al., 2009; Utokaparch et al., 2011). These assays have also been used for the detection of other respiratory viruses, as hMPV (Matsuzaki et al., 2009) and ADV (Fujimoto et al., 2004; Romero-Gómez et al., 2014), with variable sensitivities. Thus, the routine investigation of these latter viruses by RDTs has not been considered a cost-effective point-of-care approach, since it leads to a significant increase in costs and a more cumbersome processing of respiratory samples in emergency settings.

The mariPOC assay represents the only multiplex antigen detection method that would serve as a point-of-care assay, since highly positive results are available in 20 min, with minimal hands-on time processing.

In the present study, the sensitivity of mariPOC compared with NAATs was 84.4%, 92.3%, and 87.5% for RSV, Flu A, and Flu B, respectively. Furthermore, mariPOC detected Flu B in 3 samples and RSV in 1 sample (confirmed with the reference method) that tested negative with other RDTs.

Previous studies that evaluated mariPOC have reported similar results to ours for RSV and Flu detection (Brotons et al., 2014; Ivaska et al., 2013; Tuuminen et al., 2013). We obtained a higher sensitivity for Flu A (92.3%) than previous studies that reported values ranging from 66.7% to 85.7% (Brotons et al., 2014; Ivaska et al., 2013; Tuuminen et al., 2013).

Table 2
Comparison of mariPOC with other rapid tests (RDTs) for antigen detection of influenza and respiratory syncytial virus.

Virus	Definitive report results, n mariPOC results/RDTs[a] results				mariPOC false results[b]	
	Positive/positive	Negative/positive	Positive/negative	Negative/negative	Negative	Positive
Flu A	24	2	0	96	2	0
Flu B	32	0	3	87	0	0
RSV	37	4	2	88	4	1
Global	93	6	5	271	6	1

n = number of viral determinations.
[a] SD Bioline Influenza A/B/A (H1N1) and Alere Binax Now RSV.
[b] Discordant results between mariPOC and RDTs were confirmed by NAATs.

Several parameters such as detection methods used for comparison, sample type, and/or study population might have influenced in these differences. In our study, most samples were from children under 5 years old (68.8%, data not shown). In the work by Tuuminen et al. (2013), 43.8% were children and another antigen detection method was used for comparison. As previously reported (Chartrand et al., 2012), antigen detection methods are less sensitive for Flu in adults. Indeed, we used NPAs and other studies were conducted on nasopharyngeal swabs (Ivaska et al., 2013; Tuuminen et al., 2013). Previous reports have assessed that the detection rate of respiratory viruses is greater in NPAs than in nasopharyngeal swabs (Meerhoff et al., 2010).

Antigen detection tests for ADV based on immunochromatography have shown variable analytical results. Romero-Gómez et al. (2014) reported moderate sensitivity (80%) and low specificity (60.9%) compared to viral culture and lower sensitivity compared to NAATs (77.9%), whereas Fujimoto et al. (2004) found 100% specificity and sensitivities of 95% and 91% compared with viral culture and NAATs, respectively. In our study, mariPOC yielded a sensitivity of 87.5% and a specificity of 98.5% for ADV detection compared with NAATs.

The evaluation of mariPOC for the detection of ADV, hMPV, and PIV was hampered by the low number of positives in this series. Matsuzaki et al. (2009) obtained a sensitivity of 82.3% and a specificity of 93.8% for a hMPV antigen detection test when compared with real-time reverse transcriptase-polymerase chain reaction similar to the results reported in our study. The advantage of mariPOC over other RDTs for hMPV, ADV, and PIV detection is the multiplexing that also allows the simultaneous detection of the viruses that have a major impact on health systems, i.e., Flu and RSV.

A high specificity of mariPOC was observed for all viruses analyzed (95.4–100%), since only 5 false-positive results were obtained. Furthermore, in 3 of these samples, another respiratory virus was correctly identified in the preliminary report. Thus, only 2 patients would have been wrongly classified as having a viral respiratory infection.

Although molecular assays represent the gold standard for diagnosing viral ARI, nucleic acids of respiratory viruses in asymptomatic individuals have been demonstrated (Jansen et al., 2011). Furthermore, as NAATs are usually associated with longer response time and higher costs, the use of point-of-care antigen detection methods in healthcare settings have demonstrated clinical benefits (Benito-Fernández et al., 2006; D'Heilly et al., 2008; Ferronato et al., 2012). Rapid detection of viral respiratory pathogens is also important for taking appropriate

Table 3
Limits of detection of mariPOC® respi test expressed in $TCID_{50}$.

Virus	LoD (expressed in $TCID_{50}$)
Parainfluenza virus 1	10^2
Parainfluenza virus 2	$1.78 \cdot 10^5$
Parainfluenza virus 3	$1.78 \cdot 10^6$
Influenza A virus	$5.62 \cdot 10^4$
Influenza B virus	10^3
Respiratory syncytial virus	10^3
Human metapneumovirus	5.62
Adenovirus	10^2

isolation measures of confirmed cases in order to prevent nosocomial outbreaks, especially during Flu and RSV epidemic.

We calculated the LoD of mariPOC for each virus in $TCID_{50}$, parameter that indicates viral infectivity.

The LoD of mariPOC for Flu A was $5.62 \cdot 10^4$ $TCID_{50}$/100 μL. Viral load in respiratory secretions reaches its maximum, up to 10^7 TCID50/mL, in 2–3 days postinfection (Yezli and Otter, 2011). As mariPOC uses 300 μL of sample, the LoD for Flu A virus corresponds to $1.87 \cdot 10^4$ $TCID_{50}$ (one third of the $TCID_{50}$ calculated for 100 μL). This amount might be expected to be present in respiratory samples from infected individuals.

Mean titers of $4.14 \cdot 10^4$ $TCID_{50}$/mL of RSV have been reported in infected infants at the time of hospital admission (Hall et al., 1976). The LoD of mariPOC for this virus was 10^3 $TCID_{50}$/100 μL. Thus, a 300-μL sample should contain approximately 333 $TCID_{50}$, which yields a good sensitivity of this assay for RSV detection.

We obtained the lowest LoD of mariPOC for hMPV. This virus grows slightly in viral culture (Deffrasnes et al., 2005).Thus, calculated $TCID_{50}$ could not accurately indicate virus amount in cell culture and, consequently, in a respiratory sample.

It has been reported that low doses (in the range of 5–100 $TCID_{50}$) of ADV are necessary to cause infection (Yezli and Otter, 2011). By the results obtained in this work, a minimum of 33 $TCID_{50}$ of ADV should be present in a respiratory sample to give a positive mariPOC result. This amount is within the range of virus titer present in respiratory samples from infected individuals.

The highest LoD and the lowest sensitivities were demonstrated for PIV 1–3. We did not find any data on the amount of PIV in the respiratory tract following infection. Indeed, the low number of samples tested does not allow further conclusion. However, a 67% of sensitivity of mariPOC for PIV 1–3 and the optimal sensitivities obtained for the other viruses show that this assay may be an optimal point-of-care approach for the diagnosis of viral ARI. This is probably the best single method for laboratories not equipped with molecular assays.

One limitation of this study may be that viral strains were diluted in saline solution and not in virus-spiked-negative NPAs, which would have taken into account possible deleterious effects of the sample on the LoD value. Except for certain respiratory tract components that are not present in dilutions of the viral strains, a similar matrix could be expected for many NPAs, sampled with a variable amount of saline solution. In any case, the results obtained confirm the good performance of mariPOC with viral infectious doses in clinical specimens.

The minimum turnaround time of virological methods is 20 min for RDTs, 1–4 h for NAATs, and 48 h for viral culture. The mariPOC system reported 84.6% of true-positive results in 20 min.

5. Conclusion

The mariPOC assay is a rapid, easy, and specific 1-step method for the multiplex detection of the main respiratory viruses involved in ARI. The good analytical performance and extended portfolio of mariPOC show this rapid assay as a good alternative for the etiological diagnosis of acute respiratory infection in laboratories that are not equipped with molecular assays.

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