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Performance of a rapid multi-analyte 2-photon excitation assay in children with acute respiratory infection☆,☆☆,★

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ABSTRACT

The purpose of this study is to evaluate the diagnostic performance of the novel 2-photon excitation-based mariPOC© Assay (ArcDia Laboratories, Turku, Finland) for antigen detection of respiratory viruses versus real-time polymerase chain reaction (PCR). The mariPOC Assay and 2 multiplex real-time PCR techniques were performed on nasopharyngeal samples from pediatric patients with suspicion of acute respiratory infection admitted to a children's hospital in Spain during October 2011 to January 2013. A total of 233 samples were studied. Sensitivities and specificities (95% confidence interval) of the mariPOC Assay were for respiratory syncytial virus (RSV), 78.4% (69.7–85.6) and 99.2% (96.3–100.0); influenza virus (IFV) A, 66.7% (26.2–94.0) and 99.6% (97.9–100.0); IFV-B, 63.6% (33.6–87.2) and 100.0% (98.7–100.0); human metapneumovirus (hMPV), 60.0% (34.5–81.9) and 100.0% (98.6–100.0); adenovirus (ADV), 12.5% (0.6–48.0) and 100.0% (98.7–100.0), respectively. The mariPOC Assay is a highly specific method for simultaneous detection of 8 respiratory viruses but has sensitivities that range from moderately high for RSV to moderate for IFV and hMPV and low for ADV.

1. Introduction

Acute respiratory infections, in particular those confined in the lower respiratory tract, are the leading cause of childhood mortality and morbidity worldwide (Madhi and Klugman, 2006; WHO, 2005). The disease burden from respiratory infections in the young is associated with substantial consumption of healthcare resources in developed countries: lower respiratory tract infections have been described as the most common disease in children admitted to hospital, whereas highly recurrent infections in the upper respiratory tract trigger a large number of visits to pediatric practices and emergency departments (Tregoning and Schwarze, 2010).

Frequently detected viruses during acute respiratory infections include adenovirus (ADV), bocavirus (BoV), coronavirus (CoV), enterovirus (EV), human metapneumovirus (hMPV), influenza virus (IFV), parainfluenza virus (PIV), rhinovirus (RV), and respiratory syncytial virus (RSV). Regardless of the causative agent, symptoms of respiratory infections due to viruses are not only similar between different viruses but also overlap with those of bacterial respiratory infections, which makes etiologic diagnosis even more challenging. Because the majority of respiratory infections in children have a viral origin, empirical antibiotic treatment only on the basis of clinical suspicion is imprecise. It may also have been 1 of the causes of the global emergence of resistant pathogens (Jacobs, 2003). Early and accurate detection of the viral or bacterial agent can orientate timely and specific treatment as well as reduce antibiotic misuse and spread of hospital-acquired respiratory infections. Benefits would not only derive from improved clinical outcomes but also from a more effective utilization of healthcare resources (Barenfanger et al., 2000; Bonner et al., 2003; Doan et al., 2012).

Current methods for early detection of respiratory viruses include rapid antigen detection tests, particularly using immunochromatography, and molecular techniques such as those based on the polymerase chain reaction (PCR). Rapid antigen detection tests have been described as simple specific methods that are especially suitable for viral detection at the point of care (Takahashi et al., 2010). Nonetheless, these tests have modest and highly variable sensitivities and are only available for a limited number of common viral targets. In contrast, PCR has become the reference method for diagnosing viral respiratory infections due to its high sensitivity and high specificity (Mahoney, 2010). Multi-testing capabilities of a wide range of viruses and high throughput are other advantageous features of PCR. However, techniques based on PCR still require stringent conditions of fixed and bulky equipment, expert technicians, and dedicated

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separate laboratory rooms to prevent crossed contaminations. As a consequence, performance of PCR typically remains centralized in reference laboratories, which, in most cases, may not be easily accessible for prompt diagnosis.

The mariPOC© Assay (ArcDia Laboratories, Turku, Finland) has been presented as an antigen detection test for rapid multiple diagnostics of 8 targeted respiratory viruses and *Streptococcus pneumoniae* from a single nasopharyngeal aspirate sample or swab at the point of care. The test is based on fluorescence detection as a result of the formation of immunocomplexes of MAb-Ag-MAb* (monoclonal antibody–antigen–labeled monoclonal antibody) on polystyrene microparticles (used as solid phase). Fluorescence is detected by a proprietary separation-free 2-photon excitation technique and is proportional to the analyte concentration. The mariPOC Assay provides random-access, fully automated sample processing and multi-analysis capabilities with minimal hands-on operation and high throughput (Hänninen et al. 2000; Koskinen et al., 2007).

The objective of the present study was to determine the performance characteristics of the mariPOC Assay versus 2 multiplex real-time PCR techniques (Luminex xTag RVP Fast Assay; Abbot Laboratories, Wiesbaden, Germany, and Anyplex II RV16; Seegene, Inc., Seoul, Korea), which were considered the reference diagnostic methodology, in the clinical laboratory of a referral children's hospital. This information could be of interest to assess accuracy of the mariPOC Assay for the simultaneous identification of common respiratory viruses at an early stage of infection.

2. Materials and methods

The study included excess nasopharyngeal aspirate samples available at the Molecular Microbiology Laboratory of the Children's Hospital Sant Joan de Déu after routine analyses of a multiple viral profile between October 2011 and January 2013. A first subset of fresh samples was prospectively analyzed by the mariPOC Assay and by the Anyplex II RV16 Assay, while a second subset of fresh samples was analyzed by the Luminex xTag RVP Fast Assay prospectively, frozen at −80 °C for storage and then defrosted for retrospective analysis by the mariPOC Assay. Data of results of these PCR assays were prospectively registered and linked with sample identification numbers. No demographic and epidemiological data were linked with each sample.

Specimens were collected from children/adolescents <18 years admitted to the hospital with non-specific suspicion of acute respiratory infection. The setting is a 345-bed size, tertiary-care children's hospital located in the metropolitan area of Barcelona (Spain), which provides healthcare coverage to a pediatric population of ~200,000 children.

2.1. Microbiological methods

Search for pathogens covered respiratory viruses that could be detectable by all 3 techniques (ADV; hMPV; IFV-A and IFV-B; PIV-1, PIV-2, and PIV-3; and RSV) and other specific viruses only targeted by the PCR techniques (CoV, EV, RV, BoV, and PIV-4). Of note, since the Luminex xTag RVP Fast Assay could not detect EV and RV separately, positive EV/RV samples by this technique were retested by the Anyplex II RV16 Assay to identify the causative virus.

Performance by the mariPOC Assay was carried out according to the manufacturer's instructions through the following steps: transfer of 0.3 mL of aspirate sample and 1.3 mL of buffer to the sample tube; 30-second vortex operation; 2- to 10-minute wait; 10- to 15-second vortex operation; 5-minute centrifugation; insertion of the sample tube in the mariPOC analyzer and automated analysis. Molecular amplification was preceded by RNA/DNA virus extraction in a MagNA Pure compact instrument (Roche Laboratories, Basel, Switzerland) using a 200-μL sample eluted in 50 μL, of which 10 μL was utilized for the RVP Fast Assay in a 96-well plate format and 8 μL for the Anyplex II RV16 Assay according in each case to the manufacturers' instructions. The 2 PCR tests included internal controls to check potential inhibitions by substances present in each specimen that could invalidate the results. In addition, the mariPOC assay also included an internal control to assess the integrity of all steps.

2.2. Statistical analysis

Statistical analysis was conducted using Statistical Package for Social Sciences software (SPSS, version 19; IBM Corp., Chicago, IL, USA). Performance characteristics were determined utilizing 2-by-2 contingency tables and entering categorical variables of positive and negative results by the mariPOC and the PCR tests, for each type of virus and globally both for the set of viruses included in the mariPOC viral panel and for the extended PCR viral panel. Chi-square test or Fisher's exact test (2-tailed) were used to compare the categorical variables. Confidence intervals (CI) were set at 95% and significance at a 2-sided *P*-value of <0.05 for all statistical analysis.

3. Results

A total of 241 excess nasopharyngeal aspirate samples were available for the study. Eight of them were excluded, as the mariPOC Assay inhibited in 3 samples and the PCR tests in 5 samples. The final study comprised 233 samples. A total of 139 (59.7%) samples had positive results, and 94 (40.3%), negative results by the PCR tests for the set of viruses of the mariPOC panel. Of the 139 samples with positive results by PCR, 102 (73.4%) also tested positive by the mariPOC Assay, and 37 (26.6%) tested negative. A number of 250 viral agents were identified by PCR. Viruses most frequently detected among 233 samples were RSV (n = 102, 43.8%) and RV (n = 61, 26.2%), whereas hMPV (n = 15, 6.4%), CoV (n = 13, 5.6%), BoV (n = 13, 5.6%), IFV-B (n = 11, 4.7%), and PIV-4 (n = 10, 4.3%) were found in lower rates. Presence of ADV (n = 8, 3.4%); EV (n = 8, 3.4%); IFV-A (n = 6; 2.6%); and PIV-1, PIV-2, and PIV-3 (n = 3, 1.3%) was minor in the sample collection. Viral co-infections were detected in 47 (20.2%) samples, and RSV was present in all co-infected samples. Table 1 records the distribution of all respiratory viruses detected by the PCR techniques.

Overall sensitivity of the mariPOC Assay for the viruses included in its panel was 73.4% (95% CI 65.6–80.2). The assay had sensitivity of 78.4% for detection of RSV, but lower values were found for IFV-A (66.7%); IFV-B (63.6%); hMPV (60.0%); and, particularly, for ADV (12.5%). Overall specificity of the assay was 97.9% (95% CI 93.2–99.6)

Table 1
Respiratory viruses detected by PCR among 233 samples.

Virus by PCR assay	No. specimens positive by PCR	No. specimens negative by PCR	(%) of specimens positive by PCR
RSV	102	131	(43.8)
RV	61	172	(26.2)
Metapneumovirus	15	218	(6.4)
BoV	13	220	(5.6)
CoV	13	220	(5.6)
IFV-B	11	222	(4.7)
Parainfluenza-4 virus	10	223	(4.3)
ADV	8	225	(3.4)
EV	8	225	(3.4)
IFV-A	6	227	(2.6)
Parainfluenza-1/-2/-3 virus	3	230	(1.3)
Total specimens (n = 233)	188[a]	45	(80.7)

[a] Forty-seven samples were positive to more than 1 virus. The total number of virus detected was 250.

Table 2
mariPOC performance characteristics (in relation to PCR reference methodology).

Virus	No. of specimens with results					% sensitivity (95% CI)	% specificity (95% CI)	% positive predictive value (95% CI)	% negative predictive value (95% CI)
	Total PCR positive	mariPOC positive PCR positive	mariPOC negative PCR positive	mariPOC positive PCR negative	mariPOC negative PCR negative				
RSV	102	80	22	1	130	78.4 (69.7–85.6)	99.2 (96.3–100.0)	98.8 (94.1–99.9)	85.5 (79.3–90.5)
hMPV	15	9	6	0	218	60.0 (34.5–81.9)	100.0 (98.6–100.0)	100.0 (71.7–100.0)	97.3 (94.5–98.9)
IFV-B	11	7	4	0	222	63.6 (33.6–87.2)	100.0 (98.7–100.0)	100.0 (65.2–100.0)	98.2 (95.8–99.4)
IFV-A	6	4	2	1	226	66.7 (26.2–94.0)	99.6 (97.9–100.0)	80.0 (33.4–99.0)	99.1 (97.1–99.9)
ADV	8	1	7	0	225	12.5 (0.6–48.0)	100.0 (98.7–100.0)	100.0 (5.0–100.0)	97.0 (94.1–98.7)
Parainfluenza-1/-2/-3 virus	3	1	2	2	228	-	-	-	-
Total for mariPOC viral panel	139	102	37	2	92	73.4 (65.6–80.2)	97.9 (93.2–99.6)	98.1 (93.8–99.7)	71.3 (63.1–78.6)
Total for PCR panel	188	103	85	1	44	54.8 (47.6–61.8)	97.8 (89.5–99.9)	99.0 (95.4–100.0)	34.1 (26.3–42.6)

and showed homogeneous values for all viruses of the panel. Positive and negative predictive values of 98.1% (95% CI 93.8–99.7) and 71.3% (95% CI 63.1–78.6) were obtained, respectively. When extending evaluation to all viruses detectable by the PCR reference techniques, overall sensitivity of the mariPOC Assay declined to 54.8% (95% CI 47.6–61.8), while overall specificity maintained a value of 97.8% (95% CI 89.5–99.9). Measures of the mariPOC performance characteristics are detailed in Table 2.

Comparative analysis of the 2 subsets of specimens processed prospectively and retrospectively by the mariPOC Assay resulted in an overall sensitivity of 68.6% for samples analyzed prospectively versus 76.1% for those analyzed retrospectively ($P = 0.20$), while overall specificities for each subset were 97.5% and 98.1%, respectively ($P = 0.62$).

4. Discussion

Results of this study suggest that the mariPOC Assay was highly specific for the viruses covered by the test but showed variable sensitivities that ranged from moderately high for RSV to worryingly low for ADV. Thus, confirmation of negative results by means of more sensitive methods such as PCR would be a recommendable practice when using the mariPOC Assay in a clinical laboratory environment. High specificities found concurred with those reported in a previous evaluation of the assay versus PCR in a pediatric emergency department setting (Ivaska et al., 2013). However, we observed comparably higher sensitivity for hMPV and lower sensitivities for RSV, IFV-A, IFV-B, and ADV in our study. We hypothesize that differences in sensitivities between the 2 evaluations could derive from differences in factors that have an impact on positivity such as virus microbiological characteristics, course of infection, children age, and reference PCR techniques used (Ohmit and Monto, 2006). It is to be noted that sensitivity found for RSV was similar to sensitivities of 72.0–79.8% documented for rapid RSV tests versus PCR in other studies in children (Aslanzadeh et al., 2008; Miernyk et al., 2011; Munjal et al., 2011). Similarly, sensitivities of the assay for IFV were not discordant with the variable values in a range of 45.0–83.0% of rapid IFV tests in children described elsewhere (Alexander et al., 2005; Cruz et al., 2010; Grijalva et al., 2007; Sandora et al., 2010). In contrast, a recent study comparing the mariPOC test against the direct antibody assay (Tuuminen et al., 2013) reported higher sensitivities both for RSV (100%) and IFV-A (85.7%). The fact that this study was performed during the seasonal winter epidemics, when severity of infections is more likely to increase positive results, may explain the improved values of sensitivity described in the study for both pathogens. Sensitivities for hMPV were considerably lower than values in a range of 70.6–82.3% previously reported for other rapid hMPV tests compared to PCR (Kikuta et al., 2008; Matsuzaki et al., 2009). Likewise, sensitivity for ADV was under the value of 55% documented for an ADV immunochromatographic test (Levent et al., 2009). Interestingly, despite low sensitivity for detection of PIV, coverage of this virus could be a distinctive feature of the mariPOC Assay since, to our knowledge, no other rapid antigen test targeting PIV has reached the market so far. In turn, uncovered detections of CoV, EV, RV, BoV, and PIV-4 could be considered drawbacks of the assay in comparison with multiplex PCR techniques, which usually offer coverage of a wider range of respiratory agents. The mariPOC Assay proved to be robust as few samples inhibited compared to the number of inhibitions by the PCR techniques. Since there were not significant differences in sensitivities and specificities delivered by the assay for the subset of samples that had been frozen for storage until testing versus the subset of fresh samples analyzed prospectively, preprocessing activities do not seem to have influenced results.

Our results should be interpreted in light of the potential limitation of the low number of samples that tested positive for IFV-A, IFV-B, ADV, and PIV. During the pandemic influenza season, specific PCR techniques targeting IFV were performed instead of non-specific multiplex PCR to confirm presence of suspected influenza. This cost-effective practice followed in our clinical laboratory would explain that few IFV samples were identified as positive during the study. In the case of ADV and PIV, the low rates of positives found in relation to these viruses may reflect their less important role in the epidemiology of viral respiratory infections in our geographical area and are in line with positive rates reported for these viruses in other local studies (García-García et al., 2012; Piñero Fernández et al., 2012). It is also important to note that the quantity and ability to detect viruses by antigen or PCR assays vary with age. Since this study was conducted from the laboratory approach, no demographic data of patients were registered. This fact does not affect comparison of results by the 2 techniques in the same sample, but it could be a potential limitation for comparing our results with those from other studies.

In conclusion, the mariPOC Assay proved to be highly specific for all respiratory viruses included in its panel but only appeared to show moderately high sensitivities for RSV. Negative results by the assay for the rest of the viral panel should be confirmed by performance of more sensitive methods when available before clinical decision making. Further operational performance and cost-effectiveness evaluations would help to assess utility of the mariPOC Assay to diagnose viral respiratory infections against current multiplex PCR techniques and rapid antigen detection tests.

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