Rapid multiplex PCR assays in patients with respiratory viral infections: is semi-quantitative data useful? A pilot study

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Abstract
Viral respiratory rapid multiplex PCR assays FilmArray® (FA) and ePlex® (eP) provide qualitative results which may not reflect clinical relevance. In a pilot study, we report retrospectively whether the semi-quantitative PCR assay R-GENE® would have facilitated clinical interpretation. Forty-four patients were hospitalized for various respiratory manifestations; all of them have benefited from a respiratory sample during acute symptoms. Among the 44 patients, FA detected 23 positive samples including 31 viruses, 26 of them gave high or moderate R-GENE® scores (cycle threshold < 35), and all but one were consistent with clinical history. Semi-quantitative scores would have allowed for critical interpretation of the results; those are a key additional element for an optimal exploitation of the rapid multiplex PCR assays power.

Keywords Respiratory tract · Multiplex PCR · Semi-quantitative results · Viral respiratory panel

Introduction
Rapid multiplex PCR assays (RMPA) have recently been developed to detect viral respiratory pathogens in a very short time with high sensitivity and specificity [1]. Detection strategies that allow multiple agents to be simultaneously detected with a reduced laboratory turnaround time may have a significant impact on infectious disease management [2].

Analytical performances of RMPA FilmArray® Respiratory Panel 2 Plus (RP2+) (FA) (BioFire-bioMérieux, Marcy-l’Étoile, France) and ePlex® (eP) (GenMark Diagnostics, Carlsbad, USA) for the rapid simultaneous detection of 20 pathogens have already been evaluated, showing equivalent performances except for rhinovirus/enteroviruses and common human coronaviruses (types 229E, NL63, OC43, and HKU1) [3]. However, most RMPA provide qualitative results only and a semi-quantitative result would provide more useful information, for example, helping to monitor the viral infection or to discriminate a clinically significant viral load [4].

In this retrospective descriptive pilot study, viral results obtained with FA and eP were supplemented by semi-quantitative data obtained from our local routinely used real-time duplex PCRs which detect 14 pathogens (R-GENE®, bioMérieux, Marcy-l’Étoile, France). The study’s objective was to determine whether complementing FA and eP with a semi-quantitative assay could have improved clinical relevance of test results and hence patient outcome.
Materials and methods

The study included all patients for whom both eP and FA assays had been performed on the same respiratory sample between January and March 2018 at the virology laboratory of Brest University Hospital, France. As the number of RMPA tests available in the laboratory was limited for this period, this work is a pilot study, in preparation for a larger and prospective study. Respiratory samples had been collected using either a nasopharyngeal (NP) flocked swab placed in universal transport medium (FLOQSwabs™,Copan, Brescia, Italy), NP aspiration, or bronchoalveolar lavage (Table 1) and were tested by viral and/or bacterial screening with RMPA FA and eP. Residual volumes of respiratory samples were stored at – 80 °C for retrospective analyses with semi-quantitative specific duplex real-time PCR assays including influenza A virus and influenza B virus, human metapneumovirus and respiratory syncytial virus, human parainfluenza viruses including types 1, 2, 3, and 4 and common human coronaviruses including types 229E, NL63, OC43, and HKU1, human rhinoviruses and/or enteroviruses, and one simplex real-time PCR for adenoviruses (R-GENE®, bioMérieux, Marcy-l’Étoile, France). Cell control was also performed with a semi-quantitative specific real-time PCR assay (R-GENE®, bioMérieux, Marcy-l’Étoile, France). A semi-quantitative scoring system of the viral load determination could help to determine the viruses which potentially contributed to the disease [7].

A total of 44 patients were included in the present study. Population characteristics are summarized in Table 1. Sample quality was checked by amplifying the hypoxanthine phosphoribosyltransferase (HPRT1) gene with the real-time PCR assay R-GENE®; all of the 44 respiratory samples of high quality for PCR (Ct value of HPRT1 < 30). Unfortunately, such a target is not yet available in RMPA.

Virus-positive samples (23 for FA with 31 viruses, including 6 viral coinfections; 19 for eP with 23 viruses, including 3 viral coinfections) were retrospectively analyzed with a semi-quantitative method (R-GENE®) (Table 2). Concordant positive screening results between the two RMPA corresponded to detected 23 detected viruses. All the 21 negative samples with FA were also negative with eP. One sample which tested positive in FA failed to be identified using eP, but re-testing was not attempted. Of note, no bacterial infections have been detected in the 44 samples tested with FA and eP. Overall the results of the present study give a positive percent agreement (PPA) between FA and eP of 76.6% (57.7–90.1), a negative percent agreement (NPA) of 100% (83.9–100), and an overall percent agreement of 86.3% (73.7–94.3) for virus detection, with a 95% confidence interval. Discordant screening results between the two RMPA corresponded to detected viruses, presence/absence, type, and/or number (Table 2) and concerned six respiratory samples from six patients. FA detected seven more viruses than eP. In three samples, discrepancies were linked with multiple virus detections, with FA demonstrating one or two more viruses than eP (P2, P18, and P39; Table 2). One virus was detected with only FA in each of the other three samples (P4, P10, and P30; Table 2).

Among these three single virus detection discrepancies, eP did not detect one influenza A virus, one rhinovirus/enterovirus, and one influenza B virus, with the influenza A virus positive (moderate) by real-time RT-PCR and the latter
|                      | Overall (n = 44) | Patients with at least one positive result (n = 23) | Patients < 15 yo with at least one positive result (n = 19) | Patients with both RMP\textsuperscript{a} negative results (n = 21) | Patients < 15 yo with both RMP\textsuperscript{a} negative results (n = 4) |
|----------------------|-----------------|-----------------------------------------------|-----------------------------------------------|------------------------------------------------|-----------------------------------------------|
| Children (% < 15 yo) | 23 (0.5)        | 19 (82.6)                                    | –                                             | 4 (19)                                         | –                                             |
|                   | 15 (0.3–63)     | 0.4 (0.1–12)                                 | 0.25 (0.1–0.6)                                | 57 (21.5–68.5)                                 | 2 (0.6–9)                                    |
| Sex (male) (%)     | 29 (65)         | 17 (73.9)                                    | 13 (68.4)                                     | 12 (57)                                       | 3 (75)                                        |
| Sampling technique |                 |                                              |                                               |                                               |                                               |
| Swab (%)           | 22 (50)         | 7 (30)                                       | 5 (26.3)                                      | 15 (71.4)                                     | 2 (50)                                        |
| Bronchoalveolar lavage (%) | 5 (11.4)        | 2 (8.7)                                      | 0 (0)                                         | 3 (14.3)                                      | 0 (0)                                         |
| Nasopharyngeal aspiration (%) | 17 (38.6)     | 14 (60.8)                                    | 14 (73.7)                                     | 3 (14.3)                                      | 2 (50)                                        |
| Delay between result and end of hospitalization (days) (med, Q1–Q3) | 2.5 (1–8)      | 1 (1–6)                                      | 1 (1–5.3)                                     | 7 (1–10)                                      | 6.5 (2–9.5)                                   |
| Hospitalization unit |               |                                              |                                               |                                               |                                               |
| Conventional care unit | 17 (38.6)   | 11 (47.8)                                    | 12 (63.2)                                     | 6 (28.5)                                      | 3 (75)                                        |
| Onco/hematology      | 6 (13.6)        | 1 (4.3)                                      | 0 (0)                                         | 5 (23.8)                                      | 0 (0)                                         |
| Intensive care unit  | 21 (47.7)       | 11 (47.8)                                    | 7 (36.8)                                      | 10 (47.6)                                     | 1 (25)                                        |
| Clinical presentation and management |               |                                              |                                               |                                               |                                               |
| Intensive care unit required during hospitalization (%) | 22 (50)        | 10 (43.5)                                    | 7 (36.8)                                      | 12 (57.1)                                     | 2 (50)                                        |
| Immuno compromised (%) | 9 (20.5)       | 1 (4.3)                                      | 0 (0)                                         | 8 (38)                                        | 0 (0)                                         |
| Respiratory condition history (%) | 7 (15.9)     | 2 (8.7)                                      | 1 (5.3)                                       | 5 (23.8)                                      | 1 (25)                                        |
| Initial nasosinusal symptoms (%) | 15 (34.1)   | 13 (56.5)                                    | 12 (63.2)                                     | 2 (9.5)                                       | 0 (0)                                         |
| Respiratory symptoms at testing (%) | 34 (77.3) | 19 (82.6)                                    | 16 (84.2)                                     | 15 (71.4)                                     | 4 (100)                                       |
| Respiratory distress syndrome (%) | 30 (68.2)    | 12 (52.2)                                    | 7 (36.8)                                      | 11 (52.4)                                     | 2 (50)                                        |
| Oxygen support required (%) | 25 (56.8)    | 13 (56.5)                                    | 10 (52.6)                                     | 12 (57.1)                                     | 3 (75)                                        |
| Fever at time of testing (%) | 20 (45.5)    | 10 (43.5)                                    | 7 (36.8)                                      | 10 (47.6)                                     | 2 (0.5)                                       |
| Normal chest X-rays (%) | 15/34 (44.1) | 10/17 (58.8)                                 | 9/13 (62.2)                                   | 5/17 (29.4)                                   | 0/2 (0)                                       |
| Clinical diagnosis |               |                                              |                                               |                                               |                                               |
| Isolated viral infection (bronchiolitis, viral pneumonia) (%) | 18 (40.9)     | 16 (69.6)                                    | 15 (78.9)                                     | 2 (9.5)                                       | 1 (25)                                        |
| Incidence of chronic respiratory disease (%) | 3 (6.8)         | 1 (4.3)                                      | 1 (5.3)                                       | 2 (9.5)                                       | 0 (0)                                         |
| Bacterial pneumonia (confirmed or not) (%) | 11 (25)      | 2 (8.7)                                      | 0 (0)                                         | 9 (42.9)                                      | 2 (50)                                        |
| Acute distress respiratory syndrome (%) | 2 (4.5)        | 1 (4.3)                                      | 0 (0)                                         | 1 (4.8)                                       | 0 (0)                                         |

\textsuperscript{a} RMP: Reverse Transcription Polymerase Chain Reaction.
two not detected by either real-time PCR. The viral loads of these targets could be at the limit of sensitivity of the assays. The possibility of false positive results with FA related to contamination should also be considered. In multiple virus detection discrepancies, most of the discordant targets had a moderate or even a high signal, which suggests that eP might not correctly detect some viruses, particularly some coronaviruses or rhinovirus/enteroviruses, as was previously reported [3].

Diagnoses of clinicians, who had the FA results, showed that they always considered the detected viruses to be the cause of acute respiratory issues or organ failure (Table 2). Among the 23 virus infected patients, 16 (69.9%) had respiratory symptoms related to an isolated viral infection (Table 1). The median length of hospital stay after the RMPA result (Table 1) was not significantly shorter for the patients who were positive for at least one virus than for negative patients (1 day [1–6] versus 7 [1–10], p = 0.082). Interestingly, only 25% of adults who had a positive result had their antibiotics continued versus 47% among those with a negative result. However, this difference did not reach significance (p = 0.056). It is noteworthy that all of the patients with a positive result and normal chest X-rays were no longer receiving antibiotics after 48 h. Most of the positive cases had been associated with an antibiotic discontinuation after 48 h (time of antibiotherapy reevaluation) (17/23) and were all pediatric cases. A reduction of antibiotic prescription following a positive test result has already been reported in adults [9].

Of the 17 positive samples with a single viral target with FA, all but five had a high or very high RT-PCR signal, which is indicative of an active virus replication and likely cause of patients symptoms (Tables 1 and 2). Indeed, among the five remaining cases, three were serious influenza A virus or parainfluenza virus 3 infection, whose course had begun at least a week earlier, which might explain the moderate or low signals [10]. The influenza B virus detected only with FA (P30) from a child in ICU is more questionable, as the course was not typical of a flu infection. Moreover, influenza B virus viral load has been described as higher in ICU patients than in those in ambulatory settings [11]. This is an example of situation where a complementary semi-quantitative PCR could have been of significant value in aiding clinician decision and overall management of the patient health. The rhinovirus/enterovirus detection (P10) is likely a real positive case with a very low signal because the clinical context was evocative of a viral bronchiolitis, and the next sampling (10 days later) showed a very high signal (data not shown). A semi-quantitative result would have indicated the start of the infection. Of note, the single freeze–thaw cycle to which the samples were subjected is not likely to be the cause of these five remaining lower viral loads, which are consistent with clinical data.

In patients with multiple virus detections, the viruses associated with higher qPCR scores were likely involved in active infection according to the clinical contexts (Table 2) [7]. The other ones may be associated with ending infections or carriage and would not confer increased severity. Influenza virus, metapneumovirus, respiratory syncytial virus, and parainfluenza virus 3 are often presented as “true pathogens,” detected with high signals in samples with or without coinfection, whereas adenovirus, rhinovirus, or coronavirus have often been detected in association with lower signals in symptomatic persons [7, 12]. In our study, not all viral codetections implicated a “true pathogen” (P29 and P39), and in other cases, the “true pathogen” did not always have the highest signal (P7 and P18). None of these

### Table 1 (continued)

|                           | Overall (n = 44) | Patients with at least one positive result (n = 23) | Patients < 15 yo with at least one positive result (n = 19) | Patients with both RMPA<sup>a</sup> negative results (n = 21) | Patients < 15 yo with both RMPA<sup>a</sup> negative results (n = 4) |
|---------------------------|-----------------|-----------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| Isolated fever or ENT disease (%) | 8 (18.2) | 3 (13) | 3 (15.8) | 5 (23.8) | 1 (25) |
| Other (%) | 2 (4.5) | 0 (0) | 0 (0) | 2 (9.5) | 0 (0) |
| Death (n = 19) | 7 (15.9) | 0 (0) | 0 (0) | 7 (33.3) | 1 (25) |
| Directly related to infection (%) | 2 (4.5) | 0 (0) | 0 (0) | 2 (9.5) | 0 (0) |
| Indirectly related to infection (%) | 5 (11.3) | 0 (0) | 0 (0) | 4 (19) | 1 (25) |
| Unrelated to infection (%) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |

Data are expressed as median (interquartile range)
<sup>a</sup>FilmArray® Respiratory Panel 2 Plus and ePlex® Respiratory Pathogen panel

ENT ear, nose, and throat

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| Patient number | Clinician diagnosis                                                                 | FilmArray®          | ePlex®            | Semi-quantitative R-GENE® score |
|---------------|-------------------------------------------------------------------------------------|---------------------|-------------------|-------------------------------|
| P1            | Children Viral bronchiolitis, NAR                                                  | Adenovirus          | Adenovirus        | High                          |
|               |                                                                                     | Coronavirus NL63    | Coronavirus NL63  | High                          |
|               |                                                                                     | Parainfluenza virus 2 | Parainfluenza virus 2 | Very high                  |
| P2            | Children Viral bronchiolitis, NAR, recovery                                           | Coronavirus HKU1    | Negative           | High                          |
|               |                                                                                     | Metapneumovirus     | Metapneumovirus   | High                          |
| P4            | Adult Acute distress respiratory syndrome associated with influenza A virus          | Influenza A virus   | Negative           | Moderate                      |
|               | Antibiotics added without documentation, recovery                                    |                     |                   |                               |
| P7            | Children Viral bronchiolitis, NAR                                                  | Rhinovirus/enterovirus | Rhinovirus/enterovirus | Very high                  |
| P10           | Children Viral bronchiolitis associated with decompensated chronic cardiac condition, NAR, recovery | Respiratory syncytial virus | Respiratory syncytial virus | Moderate                  |
| P11           | Children Viral infection due to RSV                                                 | Respiratory syncytial virus | Respiratory syncytial virus | Very high                  |
| P12           | Adult Bacterial pneumonia associated with influenza A virus                         | Influenza A virus   | Influenza A virus  | Moderate                      |
| P14           | Children ENT symptoms, NAR                                                          | Rhinovirus/enterovirus | Invalid           | Very high                    |
| P18           | Children Respiratory infection due to 3 viruses, NAR                                | Coronavirus NL63 and OC43 | Coronavirus NL63 and OC43 | Very high                  |
| P19           | Children Viral infection due to RSV, NAR                                             | Respiratory syncytial virus | Respiratory syncytial virus | High                      |
| P21           | Children Viral infection complicated with bacterial pneumonia                        | Respiratory syncytial virus | Respiratory syncytial virus | Very high                  |
| P22           | Children Viral bronchiolitis, NAR                                                  | Rhinovirus/enterovirus | Rhinovirus/enterovirus | High                          |
| P23           | Children Viral bronchiolitis, NAR                                                  | Rhinovirus/enterovirus | Rhinovirus/enterovirus | Very high                    |
| P25           | Children Viral bronchiolitis, NAR                                                  | Rhinovirus/enterovirus | Rhinovirus/enterovirus | High                          |
| P29           | Children Viral bronchiolitis, NAR                                                  | Coronavirus OC43     | Coronavirus OC43   | Very high                    |
| P30           | Children Influenza B virus associated with bacterial pneumonia, recovery             | Rhinovirus/enterovirus | Rhinovirus/enterovirus | Low                         |
| P31           | Children Viral bronchiolitis, NAR                                                  | Rhinovirus/enterovirus | Rhinovirus/enterovirus | High                          |
| P32           | Children Atypical viral infection associated with cutaneous rash                    | Rhinovirus/enterovirus | Rhinovirus/enterovirus | Very high                    |
| P34           | Adult Viral infection complicated with acute pulmonary edema                        | Parainfluenza virus 3 | Parainfluenza virus 3 | Low                         |
| P35           | Children ENT symptoms, NAR                                                          | Rhinovirus/enterovirus | Rhinovirus/enterovirus | Very high                    |
| P38           | Viral infection complicated with bacterial pneumonia                                 | Respiratory syncytial virus | Respiratory syncytial virus | Very high                  |
In conclusion, this work points out the high consideration clinicians have of positive RMPA results regardless of the number of pathogens or the atypical clinical presentation. Semi-quantitative data allows for more critical biological interpretation of the results and thus an appropriate use of them in clinic. In our study, these data could have warned physicians of a mismatch between result and clinical situation, or helped them to monitor some viral infections. The combination of qualitative multiplex testing and semi-quantitative real-time PCR in routine use for positive samples is not feasible because it would negate the benefits related to the rapidity of multiplex testing and would generate a significant additional cost. The ideal solution seems to be the development of RMPA tests that also can produce semi-quantitative results. This is the case of the QIAstat Respiratory Panel (QIAstat RP, Qiagen, Hilden, Germany) which has been recently commercialized.

### Author contribution
Each of the authors acknowledges that he or she participated sufficiently in the work to take public responsibility for its content and agrees to the contents of the manuscript in its submitted form.

### Data availability
Available.

### Code availability
Available.

### Declarations

#### Ethics approval
This study (29BRC18.0233) was approved by the Brest University Hospital ethics committee no. 2018CE.48.

#### Consent to participate/consent for publication
An information letter was sent to each patient so they could have opposed inclusion or publication if they wished. No opposition was registered.

#### Competing interests
The authors declare no competing interests.

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