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Clinical performance evaluation of the Fluorecare® SARS-CoV-2 & Influenza A/B & RSV rapid antigen combo test in symptomatic individuals

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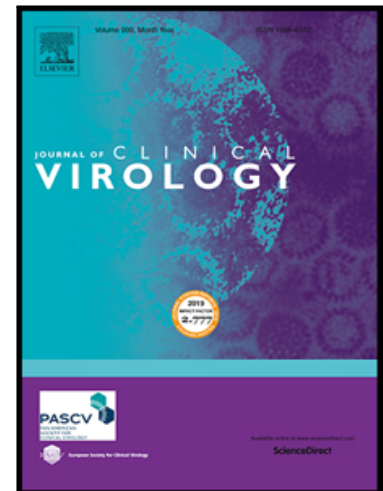
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1 Clinical performance evaluation of the Fluorecare[®] SARS-CoV-2 & Influenza A/B & RSV rapid antigen
2 combo test in symptomatic individuals

3 Short Communication

4 Multiplex antigen test performance

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15

16 Abstract

17 **Background:** A SARS-CoV-2+Flu A/B+RSV Combo Rapid test may be more relevant than Rapid
18 Antigen Diagnostic (RAD) tests targeting only SARS-CoV-2 since we are facing a concurrent circulation of these
19 viruses during the winter season.

20 **Objectives:** To assess the clinical performance of a SARS-CoV-2+Flu A/B+RSV Combo test in
21 comparison to a multiplex RT-qPCR.

22 **Study Design:** Residual nasopharyngeal swabs issued from 178 patients were included. All patients,
23 adults and children, were symptomatic and presented at the emergency department with flu-like
24 symptoms. Characterization of the infectious viral agent was done by RT-qPCR. The viral load was
25 expressed as cycle threshold (Ct). Samples were then tested using the multiplex RAD test Fluorecare[®]
26 SARS-CoV-2 & Influenza A/B & RSV Antigen Combo Test. Data analysis was carried out using
27 descriptive statistics.

28 **Results:** The sensitivity of the test varies according to the virus, with the highest sensitivity observed for
29 Influenza A (80.8.% [95%CI: 67.2 - 94.4]) and the lowest sensitivity observed for RSV (41.5% [95%CI: 26.2 -

30 56.8]). Higher sensitivities were observed for samples with high viral loads ($Ct < 20$) and decrease with low viral
31 loads. The specificity for SARS-CoV-2, RSV and Influenza A and B was $>95\%$.

32 **Conclusions:** The Fluorecare[®] combo antigenic presents satisfying performance in real-life clinical
33 setting for Influenza A and B in samples with high viral load. This could be useful to allow a rapid (self-)isolation
34 as the transmissibility of these viruses increase with the viral load. According to our results, its use to rule-out
35 SARS-CoV-2 and RSV infection is not sufficient.

36

37 Introduction

38 The RNA viruses SARS-CoV-2, influenza A (Flu A), influenza B (Flu B) and the respiratory syncytial virus (RSV)
39 are the most threatening viruses causing acute lower respiratory infections with overlapping clinical
40 manifestations [1]. The early and rapid diagnosis and differentiation between these viruses is essential for clinical
41 management, infection control, and epidemiological surveillance [2]. In parallel to the widely used Reverse
42 Transcriptase – quantitative Polymerase Chain Reaction (RT-qPCR), rapid antigen detection (RAD) tests were
43 developed to allow faster isolation of patients and lower virus spreading [3]. Unlike RT-qPCR, RAD tests do not
44 require laboratory equipment or trained personnel and therefore offer unique advantages from a public health
45 perspective, especially for remote and resource-limited areas, medical emergencies or mass testing purposes [4].
46 In previous studies, we reported that these RAD tests presented good performance to detect SARS-CoV-2 in
47 patients with high viral loads ($Ct < 25$) with sensitivities varying from 91 to 98% [5, 6]. Nevertheless, a SARS-CoV-
48 2 + Flu A/B + RSV Combo Rapid test may be more relevant since we are facing a concurrent circulation of these
49 viruses during the winter season.

50 Objectives

51 This study assesses the clinical performance of a SARS-CoV-2 + Flu A/B + RSV Combo test in comparison to a
52 multiplex RT-qPCR.

53 Study Design

54 Residual nasopharyngeal swabs issued from 178 patients who presented between December 25, 2022, and
55 January 6, 2023, at the Clinique Saint-Pierre (Ottignies, Belgium) were included. All patients were symptomatic
56 and presented at the emergency department with flu-like symptoms. Samples included were issued from both
57 adults and children. Nasopharyngeal swabs were collected in Vacuette[®] Virus Stabilization Tubes (Greiner Bio-
58 One GmbH, Kremsmünster, Austria). Following routine analyses, samples were fully anonymized and frozen at -
59 20°C. Following thawing, RT-qPCR was done with the Allplex[®] SARS-CoV-2/FluA/FluB/RSV kit (Seegene, Arrow
60 Diagnostics, Seoul, Korea). This method identifies SARS-CoV-2 RNA (by targeting three viral genes: *N*, *S* and
61 *RdRP*), Influenza A, Influenza B and RSV RNA and serves as reference in the current study. The viral load was

62 expressed as a cycle threshold (Ct). Samples with Ct values < 35 were selected for the present study. Samples
63 with higher Ct were not included, as these could be false positive results or residual DNA, with less or no risk of
64 transmission. Following RT-qPCR, samples were tested using the multiplex antigenic test Fluorecare[®] SARS-
65 CoV-2 & Influenza A/B & RSV Antigen Combo Test (Microprofit Biotech, Shenzhen, China). Briefly, two drops of
66 the viral transport medium were delivered in the 3 different wells of the device (SARS-CoV-2, Flu A/B and RSV).
67 After 20 minutes, samples for which both the control and test lines were present were considered positive, while
68 samples for which only the control line was present were considered negative, as objectivated by two
69 independent blinded operators.

70 Data analysis was carried out using descriptive statistics. Sensitivity and specificity were calculated with RT-
71 qPCR results as the reference. The comparison between RT-qPCR viral load (Ct) and the antigenic test result
72 was done using an unpaired t-test, which was computed to assess the difference between groups. One-way
73 ANOVA was performed to compare mean Ct values between each virus. The significance threshold was set at p
74 < 0.05. Data analysis was performed using GraphPad Prism[®] software (version 9.0.0, California, USA).

75 Results

76 In the entire cohort, the sensitivity of the test varies according to the virus, with the highest sensitivity observed for
77 Influenza A (80.8% [95%CI: 67.2 - 94.4]) and the lowest sensitivity observed for RSV (41.5% [95%CI: 26.2 -
78 56.8]). As expected, higher sensitivities were observed for samples with high viral loads (Ct < 20) and decrease
79 with low viral loads (Table 1). Samples with RAD negative tests showed lower viral load (higher Ct) compared to
80 RAD positive tests (Figure 1). The specificity for SARS-CoV-2, RSV and Influenza A and B was 100% for SARS-
81 CoV-2 and RSV and 96.0% and 96.9% for influenza A and B, respectively (Table 1). Among the 5 false positive
82 results for Influenza A, one sample showed positive SARS-CoV-2 RT-qPCR (Ct = 13.66), 3 samples were positive
83 for RSV RT-qPCR (Ct = 18.06, 31.80 and 33.15) and one was positive for both SARS-CoV-2 and RSV (Ct =
84 17.80 and 20.52, respectively). Among the 4 false positive cases for Influenza B, all were positive for Influenza A
85 with RT-qPCR (Ct = 18.00, 19.12, 23.31 and 31.74) (Table 1). Of these, only two showed a positive RAD test for
86 Influenza A. All SARS-CoV-2 samples were expected to be Omicron sublineage according to the current
87 epidemiological situation in Belgium.

88 According to our results, this device presents good performance for Influenza A, B and SARS-CoV-2 in patients
89 presenting high viral loads (Ct < 25). Clinical performance was more limited for low viral loads (Ct > 25) and were
90 insufficient for RSV. Similar to our results, Franck *et al.* reported sensitivity below 50% for three distinct RSV RAD
91 tests [7], while Reina *et al.* noted also significantly lower Ct values (\approx 19) in samples positive for RSV RAD test [8].
92 On the other hand, concordant results were observed by Moesker *et al.* with the BinaxNow Influenza AB[®] and
93 BinaxNow RSV[®] [9], which highlighted 30% of false positive Influenza RAD tests, as caused by RSV. As reported
94 by several authors, sensitivity of RSV RAD tests seems higher in pediatric context, due to higher viral loads in this

95 population. Therefore, one limitation of this study is that it includes a heterogeneous population. However, we are
96 confident in our results since our mean RSV Ct value is very close to those reported in studies investigating solely
97 pediatric samples [8].

98 Compared to previous SARS-CoV-2 RAD tests, the Fluorecare[®] combo antigenic test seems less sensitive,
99 although ideally, the comparison should be made on the same selection of samples [5, 6]. False negative
100 samples predominantly displayed high Ct values which reflects the lack of sensitivity of these RAD techniques.

101 Conclusions

102 To conclude, the Fluorecare[®] combo antigenic does not reach WHO's minimum performance requirement of 80%
103 sensitivity for SARS-CoV-2 in our study population. For influenza A and B its performance is limited to samples
104 with high viral load (i.e. Ct values below 25). For RSV, whatever the Ct value, its performance is insufficient and
105 the use of this RAD to rule-out RSV infection should be avoided. Additional studies are needed to assess
106 performance in asymptomatic individuals but we do not expect better performance than in the present cohort
107 based on our previous experience with SARS-CoV-2 RAD tests [6]. In addition, external validation of such RAD
108 tests utilizing an EQA proficiency panel would be important [10]. Such rapid test could nevertheless be useful to
109 allow a rapid (self-) isolation as the transmissibility of these viruses increase with the viral load [11]. However,
110 while its use in the clinical setting is certainly limited due to the easier access RT-qPCR multiplexing platforms, its
111 ambulatory deployment should be accompanied by a statement of its current limited performance in samples with
112 expected low viral load. These samples may turn falsely negative with the current device and in case of any
113 doubt, have to be confirmed by RT-qPCR.

114

115 **Conflicts of interest**

116 The authors have no relevant conflicts of interest to disclose in relation to this article.

117 **Acknowledgments**

118 None

119

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157

158 Table 1. Sensitivity and specificity for each virus detected with the Fluorecare® combo antigenic test
159 across various RT-qPCR Ct value ranges.

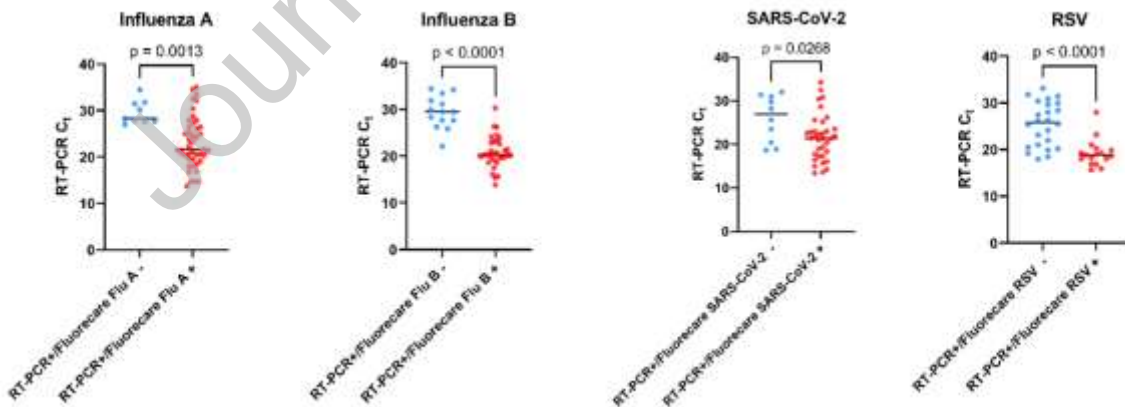
No. of Positive Patients with RAD test (Sensitivity %)				
(Pos. / total)				
Ct (gene M)	Influenza A	Influenza B	SARS-CoV-2	RSV
< 20	12/12 (100%)	12/12 (100%)	13/15 (86.7%)	13/17 (76.5%)
< 25	28/29 (96.5%)	29/31 (93.5%)	27/31 (87.1%)	16/26 (61.5%)

< 30	37/42 (88.1%)	30/41 (73.2%)	31/38 (81.6%)	17/36 (47.2%)
20 – 25	16/17 (94.1%)	17/19 (89.5%)	14/16 (87.5%)	3/9 (33.3%)
25 – 30	8/13 (61.5%)	1/10 (10.0%)	4/7 (57.1%)	1/10 (10%)
30 – 35	6/10 (60%)	1/6 (16.6%)	4/7 (57.1%)	0/5 (0%)
Total (< 35)	42/52 (80.8%)	31/47 (65.9%)	35/45 (77.8%)	17/41 (41.5%)
[95%CI]	[67.2 – 94.4]	[51.6 – 80.2]	[63.2 – 92.4]	[26.2 – 56.8]
Mean Ct	24.26	23.40	22.58	22.88
[95%CI]	[22.68 – 25.84]	[21.87-24.93]	[20.91-24.24]	[21.28-24.48]
No. Of Negative Patients with RAD test (Specificity %)				
(Neg. / total)				
Neg. with RT-qPCR	121/126 (96.0%)	127/131 (96.9%)	133/133 (100%)	135/135 (100%)
[95%CI]	[87.3 – 100]	[88.33 – 100]	[91.5 – 100]	[91.6 – 100]

160

161 Abbreviations: CI, Confidence Interval; Ct, cycle thresholds; RAD, Rapid Antigen Diagnostic; RSV, Respiratory
 162 Syncytial Virus; RT-qPCR, Reverse Transcriptase quantitative Polymerase Chain Reaction

163 **Figure 1: Representation of positive and negative results on the Fluorecare® combo antigenic**
 164 **test as a function of the Ct value in RT-qPCR.**



165

166 Abbreviations: Ct, cycle thresholds; RSV, Respiratory Syncytial Virus; RT-PCR, Reverse Transcriptase
 167 quantitative Polymerase Chain Reaction

168