



# Comparison between nucleic acid amplification tests, antigen immunofluorescence assay, and in vitro infectivity in SARS-CoV-2 diagnosis

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## Abstract

The number of SARS-CoV-2 detection tests requested to the laboratories has dramatically increased together with an urgent need to release reliable responses in a very short time. The two options taken into consideration and analyzed in the current study were the point-of-care test (POCT) based on the nucleic acid amplification test (NAAT) and the Antigen (Ag) rapid test. The POCT-NAAT-based assay was compared with a rapid antigen test of nasopharyngeal swab samples. If the specimen tested positive, it was followed by viral load quantification and by the functional assessment of the residual infectivity. When the initial cycle threshold (Ct) was below 20 (100%), and in the range of 20–25 (92%) and of 25–30 (88%), a great concordance between the POCT-NAAT and the Ag test was observed. Moreover, the positivity of the antigen test was well correlated to a successful infection in vitro (78%), with greater concordance when the initial Ct below 20 or above 35 (100%) and in the range 20–25 (83%). Our findings showed that most of the swabs which tested positive using the antigen test were able to infect the cells in vitro, suggesting that probably only these samples hold residual infectivity and therefore an increased risk of virus transmission at the moment of being tested.

**Keywords** SARS-CoV-2 · Nucleic acid amplification test · Antigen test

## Introduction

The nucleic acid amplification test (NAAT) of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA mainly exploiting the reverse transcription-quantitative polymerase reaction (RT-qPCR) is the gold standard for the virus detection in clinical specimens and it is commonly conducted on nasopharyngeal swabs. The increased number of SARS-CoV-2 detection tests requested to the laboratories has dramatically raised the workload of the diagnostic

procedures multiplying their efforts for the release of reliable responses in a very short time [1]. This need is crucial in the hospitals for the emergency room admission, hospitalization, or urgent surgery in order to avoid nosocomial outbreaks in the hospital wards [2]. Therefore, different rapid diagnostic tests have been developed and are actually present in the market. The point of care test (POCT) based on the RT-PCR platform is a valid option to efficiently obtain results in less than 1 h (e.g., GeneXpert Xpress SARS-CoV-2, Cepheid); meanwhile, exploiting the isothermal amplification procedure the data can be available in less than 30 min (e.g., ID NOW COVID-19 Abbott Diagnostics) so greatly accelerating the diagnostic process [3–5]. However, the main limitation of this procedure is related to the constrain of employing instruments just dedicated to one typology of kit and consumables provided by the same company. Moreover, since it is possible to run a few tests at a time, the POCT presents low processivity, thus representing a main limitation for large-scale use [5]. Antigen rapid test is another cheaper alternative to generate results in 10–30 min; nevertheless, this type of test presents a lower performance in terms of sensitivity [2]. Indeed, the medical

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agencies worldwide, European Centre for Disease Prevention and Control (ECDC), Centers for Disease Control and Prevention (CDC), and World Health Organization (WHO), recommended the use of rapid diagnostic tests [6–8] but requiring high performance, for example, ECDC indicates the use of tests showing  $\geq 90\%$  sensitivity and  $\geq 97\%$  specificity. Moreover, it is important to consider that this type of tests present high sensitivity in patients with high viral load (RT-PCR cycle threshold  $< 25$ ) that primarily occurs in pre-symptomatic and early symptomatic phases (up to 5 days from symptoms onset) [8].

Although these types of patients are the leading cause of SARS-CoV-2 transmission to the neighbors, it should not be forgotten that also individuals with low viral load can be a source of infectious virions [9].

The main target of the antigen test, as well as of the RT-PCR-based assays, is the nucleocapsid (N gene) of SARS-CoV-2 since it is the most abundant and conserved protein in coronavirus [10].

The aim of the present study was the comparison of a POCT-NAAT-based assay with a rapid antigen test followed by the viral load quantification and by the functional assessment of the residual infectivity of the nasopharyngeal swab samples analyzed.

## Materials and methods

### Workflow of the experimental settings

Clinical nasopharyngeal swabs which resulted positive for the SARS-CoV-2 ORF1ab and N genes at the novel coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (Sansure Biotech Inc., Changsha, China) on the iPonatic Portable Molecule Workstation (AB Analitica, Padova, Italy) were tested for the viral antigen nucleocapsid, with the STANDARD F COVID-19 Ag FIA, immunofluorescence immunoassay on the Standard FX 2400 analyzer (SD BIOSENSOR, Suwon, Korea).

Then, the same samples were further quantified with a homemade RT-qPCR targeting the N gene with CDC specific primers and probe [11] on a 7500 Real-Time PCR Applied Biosystem platform (Thermo Fisher Scientific, Waltham, MA, USA); then, the residual infectivity of the specimens was evaluated by seeding them on Vero E6 Cells, a permissive cell line, and monitoring the infection for 7 days. All these procedures have been performed in a Biosecurity level 3 (BLS3) laboratory.

### Clinical nasopharyngeal Swab

The clinical specimens were collected from 46 symptomatic coronavirus disease 2019 (COVID-19) individuals at the

emergency ward of San Polo hospital of Monfalcone (University Hospital “Giuliano Isontina” – “ASUGI,” Gorizia, Italy) from 28/12/20 to 04/02/21. The patients represented a selection of the 76 SARS-CoV-2-positive patients admitted to the emergency ward (10.2% on a total of 745 patients). They presented fever  $> 38\text{ }^{\circ}\text{C}$ , cough, and oxygen saturation  $< 95\%$ . Nasopharyngeal swabs were maintained in universal transport medium UTM (Copan, Brescia Italy) and stored at  $4\text{ }^{\circ}\text{C}$  until analyzed.

### 2019-nCoV kit on the iPonatic instrument

The ORF1ab and N genes were tested with the novel coronavirus (2019-nCoV) kit (S3102E, Sansure Biotech Inc.) on the iPonatic Portable Molecule Workstation (AB Analitica) following the manufacturer’s instruction. Briefly,  $50\text{ }\mu\text{l}$  of the samples was loaded in the cassette together with sample release agent ( $20\text{ }\mu\text{l}$ ), PCR mix ( $20\text{ }\mu\text{l}$ ), and enzyme mix ( $2\text{ }\mu\text{l}$ ). After 2 min of thermal extraction, the amplification ( $3\text{ }\mu\text{l}$  of lysates) was carried out in 40 min (the limit of detection is 200 copies/ml).

### STANDARD F COVID-19 Ag FIA

The rapid test STANDARD F COVID-19 Ag FIA (SD BIOSENSOR) was performed accordingly with the manufacturer’s instruction. It is an immunofluorescence lateral flow rapid test, where the samples react with an anti-nucleocapsid SARS-CoV-2 antibody; then, the fluorescence is read with an appropriate analyzer. Briefly,  $350\text{ }\mu\text{l}$  of the sample was mixed with the extraction buffer and 4 drops were applied on the specimen well of the test device, and after 30 min, they were read at the STANDARD F200 analyzer. The cutoff index (COI)  $\geq 1$  was used to interpret the result as positive.

A previously isolated and quantified (in terms of plaque-forming unit-PFU/ml and viral copies/ml) SARS-CoV-2 was used to assess the detection limit of the kit, by performing a serial dilution of the virus (from  $10^{12}$  to  $10^3$  viral copies/ml).

### N gene RT-qPCR on 7500 Fast Real-Time PCR instrument

The viral load was quantified through RT-qPCR.

Firstly,  $15\text{ }\mu\text{l}$  of the UTM swab was mixed with  $45\text{ }\mu\text{l}$  of distilled water and then were subjected to thermolysis ( $98\text{ }^{\circ}\text{C}$  for  $3'$  and  $4\text{ }^{\circ}\text{C}$  for  $5'$ ); then,  $3.75\text{ }\mu\text{l}$  was tested with CDC primers and probe (Eurofins, Luxembourg) for the viral gene N (nucleocapsid, 500 nM forward primer GGG AGC CTT GAA TAC ACC AAA A, 500 nM reverse primer TGT AGC ACG ATT GCA GCA TTG, 125 nM probe FAM-AYC ACA TTG GCA CCC GCA ATC CTG-BHQ1), using the Luna Universal Probe One-Step RT-qPCR Kit (New England Biolabs, Ipswich, MA, USA), on the 7500 Fast Real-Time PCR

instrument (Thermo Fisher Scientific, protocol: 50 °C for 10', 95 °C for 1', 40 cycles at 95 °C for 10", 60° for 30"). A previously quantified nCoV-CDC-Control Plasmid (Eurofins) was used to create the standard curve.

## Infectivity

Vero E6 epithelial cell lines from *Cercopithecus aethiops* kidney (ATCC CRL-1586) were cultured in MEM + 10% fetal bovine serum, 2 mM glutamine, and 100 U/ml penicillin/streptomycin (Euroclone, Pero, Italy).

The day prior to the experiment, cells were seeded at a density of  $4 \times 10^5$  cells/well in 6 multi-well plates.

One milliliter of each swab was filtered (0.22 µm filter) and seeded on the cells. After 1 h of incubation, the supernatant was harvested, the wells were washed in phosphate-buffered saline, and then, 3 ml of medium (MEM + 2% fetal bovine serum, 2 mM glutamine, and 100 U/ml penicillin/streptomycin, 1 × amphotericin B) were added. After 7 days, the viral load was quantified by using the in-house system for RNA quantification previously described.

The cytopathic effect was assessed with the EVOS XL Core Cell Imaging System (Thermo Fisher Scientific).

## Results

Table 1 reports the principal results of our study.

A well concordance was observed in the analysis of the N gene between the 2019-nCoV kit on the iPonatic and the RT-qPCR for the N gene on the 7500 RT-PCR system, with less than 4-cycle thresholds (Ct) of difference. Only for 2 samples, there was a discrepancy of more than 5 Ct. The differences encountered could be ascribed to the different volumes of analysis (both for the extraction and for the PCR).

The cutoff index (COI) positivity at the STANDARD F COVID-19 Ag FIA was shown for the specimens presented a Ct value below 31 (at the diagnostic test with iPonatic system), and a great concordance between the iPonatic system and the Ag test was observed when the initial Ct was below 20 (100%) and in the range of 20–25 (92%) and of 25–30 (88%).

Interestingly, the positivity at the antigen test was well correlated to successful infection in the Vero E6 cell line (78% of the cases), but it showed great concordance when the initial Ct was below 20 or above 35 (100%) and in the range 20–25 (83%) (Table 2).

Intriguingly, when the samples were stratified based on the data of collection, some differences were observed. Indeed, the concordance was higher in the first group of patients (from 28/12/20 to 8/01/21) and lower in the second (10/01/21–22/01/21) and the third group (23/01/21–04/02/21) possibly implying some differences

related to the SARS-CoV-2 variants circulating in that period (supplementary table 1).

The limit of detection of the antigen assay was also checked with previously isolated SARS-CoV-2: the results are presented in Table 3 and corroborate the findings obtained on the clinical specimens. Indeed, only the dilution with a Ct value < 32 was able to infect the cells and achieve a positive COI at the antigen test.

## Discussion

Despite the vaccination campaign has greatly incremented the coverage in the general population, the rise of new variants might impact, depending upon the variant; as an example, the efficacy of the vaccination immunity reaches 5–10% of vaccine effectiveness against Omicron strain after more than 25 weeks from the second dose and 50–60% after 10 weeks from the booster dose with mRNA vaccines BNT162b2-Comirnaty by Pfizer/BioNTech and Fosun Pharma and mRNA-1273—Spikevax, Moderna and National Institute of Allergy and Infectious Diseases [12]. Therefore, the great challenge for the health system is still the early detection of the COVID-19 cases in order to achieve rapid and efficient isolation, especially in health settings where the block of SARS-CoV-2 transmission in the hospital ward is mandatory [1].

In our work, a good concordance was observed between the results from the iPonatic POCT NAAT assay and the in-house quantification system, indicating the effectiveness of the two molecular systems for the detection of the SARS-CoV-2 positive cases, as we can expect. Some differences were observed regarding the concordance when the samples were stratified according to the date range (each group of approximately 10 days), possibly suggesting some influences of the SARS-CoV-2 variants on the efficiency of the tests.

Nevertheless, the more interesting results of our work derived from the antigen test. Indeed, the agreement between the NAAT and the antigen test was particularly evident when the samples presented a low Ct, and therefore, a high viral load, meanwhile, at Ct > 32, the antigen test failed to detect the virus as others previously observed also for assays distributed by other companies [8]. However, the infectivity assessment showed that most of the samples positive at the antigen test were able to establish a persistent infection with virus amplification in vitro on the Vero E6 cells, especially when the initial Ct is lower than 25. This data indicated that the patients from which the swabs were collected presented intact virions and were potential spreaders of the virus. In this work, we decided to test only the subjects positive at the molecular tests since our main aim is related to the assessment of the residual infectious potentiality of the specimens positive to the antigen test.

**Table 1** The comparison of results from the four methodologies employed in the study

Swab	RT-qPCR N gene (CDC)		Infection 7th day		STANDARD F COVID-19 Ag FIA (SD Biosensor)		Novel coronavirus (2019-nCoV) kit (Sansure Biotech) on iPonatic		
	Ct	Viral copies/ml	Viral copies/ml	Positive/negative	Cutoff index	Positive/negative	ORF gene (Ct)	N gene (Ct)	RNase P gene (Ct)
1	26.22	8.42 × 10 <sup>8</sup>	2.23 × 10 <sup>14</sup>	+	31.77	+	36.86	28.13	27.09
2	31.45	2.54 × 10 <sup>6</sup>	2.62 × 10 <sup>11</sup>	+	1.09	+	35.67	28.85	26.32
3	23.63	1.23 × 100 <sup>9</sup>	1.03 × 10 <sup>14</sup>	+	57.08	+	31.75	22.62	28.27
4	26.71	2.60 × 10 <sup>7</sup>	1.21 × 10 <sup>14</sup>	+	9.44	+	36.09	27.38	26.87
5	18.85	4.85 × 10 <sup>11</sup>	9.80 × 10 <sup>13</sup>	+	58.09	+	32.13	20.18	27.72
6	18.58	6.76 × 10 <sup>11</sup>	2.72 × 10 <sup>13</sup>	+	58.01	+	28.76	20.27	22.77
7	24.3	5.26 × 10 <sup>8</sup>	9.35 × 10 <sup>13</sup>	+	14.93	+	33.08	23.25	29.8
8	20.44	6.64 × 10 <sup>10</sup>	6.80 × 10 <sup>13</sup>	+	26.13	+	30.36	19.2	25.09
9	21.92	1.02 × 10 <sup>11</sup>	1.40 × 10 <sup>14</sup>	+	24.83	+	29.09	20.79	28.4
10	25.73	1.55 × 10 <sup>9</sup>	6.16 × 10 <sup>13</sup>	+	15.03	+	27.5	20.42	27.94
11	18.94	2.69 × 10 <sup>12</sup>	1.52 × 10 <sup>14</sup>	+	58	+	24.99	18.52	26.91
12	ND	ND	ND	-	0.37	-	39.48	36.12	33.15
13	28.41	8.20 × 10 <sup>7</sup>	9.21 × 10 <sup>13</sup>	+	1.29	+	33.2	25.4	27.61
14	33.11	4.67 × 10 <sup>5</sup>	4.35 × 10 <sup>13</sup>	+	2.65	+	37.05	30.74	27.46
15	34.37	4.62 × 10 <sup>4</sup>	2.27 × 10 <sup>13</sup>	+	0.39	-	37.44	32.04	29.82
16	22.44	3.22 × 10 <sup>10</sup>	1.74 × 10 <sup>13</sup>	+	56.48	+	32.43	25.59	25.25
17	27.18	1.54 × 10 <sup>8</sup>	ND	-	1.43	+	36.45	28.55	27.21
18	28.37	4.02 × 10 <sup>7</sup>	4.69 × 10 <sup>13</sup>	+	1.9	+	32.72	25.86	28.02
19	19.14	1.34 × 10 <sup>12</sup>	3.54 × 10 <sup>13</sup>	+	58.11	+	23.45	17.34	28.2
20	23.78	7.10 × 10 <sup>9</sup>	2.46 × 10 <sup>13</sup>	+	58.23	+	27.01	18.16	24.36
21	36.87	2.73 × 10 <sup>3</sup>	ND	-	0.84	-	39.24	33.91	26.1
22	31.21	1.78 × 10 <sup>6</sup>	ND	-	0.65	-	40.61	31.39	26.65
23	27.49	1.11 × 10 <sup>8</sup>	ND	-	4.18	+	39.16	27.49	21.86
24	37.61	5.52	ND	-	0.72	-	ND	35.81	29.84
25	ND	ND	ND	-	0.39	-	38.34	32.69	29.14
26	29.69	2.25 × 10 <sup>5</sup>	4.67E × 10 <sup>13</sup>	+	0.43	-	32.71	26.06	28.19
27	18.57	6.64 × 10 <sup>11</sup>	8.41 × 10 <sup>13</sup>	+	51.32	+	22.17	15.48	26.32
28	18.78	5.00 × 10 <sup>11</sup>	2.31 × 10 <sup>13</sup>	+	50.99	+	22.28	14.96	26.54
29	22.5	3.43 × 10 <sup>9</sup>	8.98 × 10 <sup>13</sup>	+	51.22	+	25.59	19.43	29.5
30	37.23	9.18	ND	-	0.85	-	ND	34.25	26.33
31	27.66	3.42 × 10 <sup>6</sup>	1.30 × 10 <sup>14</sup>	+	1.96	+	33.64	27.15	27.07
32	25.54	5.86 × 10 <sup>7</sup>	1.84 × 10 <sup>12</sup>	+	4.09	+	34.69	26.04	28.48
33	26.75	1.15 × 10 <sup>7</sup>	7.43 × 10 <sup>13</sup>	+	2.61	+	31.68	24.24	27.25
34	36.74	4.45 × 10 <sup>2</sup>	2.03 × 10 <sup>14</sup>	+	0.61	-	42.18	34.65	30.34
35	ND	ND	2.79 × 10 <sup>14</sup>	+	0.09	-	40.4	34.92	28.78
36	18.25	4.54 × 10 <sup>12</sup>	1.31 × 10 <sup>14</sup>	+	51.15	+	29.02	20.62	25.86
37	30.49	1.08 × 10 <sup>6</sup>	ND	-	0.97	-	37.43	28.96	28.89
38	21.79	5.54 × 10 <sup>10</sup>	7.84 × 10 <sup>13</sup>	+	50.41	+	29.24	21.03	24.14
39	33.33	3.11 × 10 <sup>4</sup>	ND	-	2.61	+	33.72	27.5	25.24
40	26.86	9.97 × 10 <sup>7</sup>	ND	-	15.5	+	34.2	27.24	25.04
41	24.92	1.12 × 10 <sup>9</sup>	ND	-	12.17	+	29.02	20.57	ND
42	28.01	2.37 × 10 <sup>7</sup>	7.53 × 10 <sup>12</sup>	+	12.46	+	35.46	28.52	26.18
43	27.21	3.07 × 10 <sup>7</sup>	1.70 × 10 <sup>14</sup>	+	42.58	+	30.18	23.65	30.27
44	26.58	7.10 × 10 <sup>7</sup>	7.05 × 10 <sup>13</sup>	+	0.25	-	28.98	22.6	27.28
45	27.77	1.47 × 10 <sup>7</sup>	5.60 × 10 <sup>13</sup>	+	10.25	+	34.2	27.65	28.96
46	25.06	5.29 × 10 <sup>8</sup>	ND	-	51.1	+	40.03	26.82	27.07

In the table are reported the data from

- the custom reverse transcription-quantitative (RT-q) PCR for the SARS-CoV-2 N (nucleocapsid) gene (with CDC primers and probe) on the 7500 Applied Biosystem Real-Time PCR instrument
- the RT-PCR performed by employing the Novel Coronavirus (2019-nCoV) kit (Sansure Biotech) for the ORF1ab and N genes on the iPonatic device
- the antigen rapid immunofluorescence lateral flow test (STANDARD F COVID-19 Ag FIA, SD Biosensor)
- the infection in the in vitro model of Vero E6 cells of the samples analyzed

Ct, cycle threshold; RT-qPCR, reverse transcription PCR; ND, not detected

**Table 2** The comparison of results from the novel coronavirus (2019-nCoV) kit (Sansure Biotech) on iPonatic device, the rapid antigen immunofluorescence lateral flow assay (STANDARD F COVID-

19 Ag FIA), and the infection in the in vitro model of Vero E6 cells based on the Cycle threshold (Ct) data from the iPonatic

Ct	2019-nCoV kit (Sansure Biotech) on iPonatic device	Culture positive	Antigen immunofluorescence test (STANDARD F COVID-19 Ag FIA)	Concordance culture/antigen test	Concordance culture/antigen test	Concordance 2019-nCoV kit (Sansure Biotech) on iPonatic device/antigen test
<20	7	7	7	7	100%	100%
20–25	12	11	11	10	83%	92%
25–30	17	11	15	11	65%	88%
30–35	8	4	1	5	63%	13%
>35	2	0	0	2	100%	0%

Ct, cycle threshold; RT-qPCR, reverse transcription PCR; ND, not detected

All the patients analyzed attended the emergency room with symptoms (cough with oxygen saturation <95% and fever >38 °C) and were suspected of COVID-19 by the clinicians. Although we are aware that it cannot be excluded their contagiousness based on in vitro assay, performed with the evaluation of the infectivity of the sample on a cell line, it is plausible to think that the patients analyzed in the study presented at the moment of the swab collection weak viral colonization and so the risk of SARS-CoV-2 transmissibility may be very low at that time. These results are in line with those reported by La Scola et al. [13] where the cutoff of 33–34 Ct is the separation between infective and not infective samples.

Generally, the immunofluorescence-based antigen test, like those employed in the current study, showed greater performance in terms of sensibility and specificity with respect to the immunochromatographic colorimetric assays [8], although they are both inferior to the gold standard NAAT. Another type of technology based on microfluidic immunofluorescence, recently developed in the COVID-19 antigen rapid test by LumiraDX claimed a 97.6% positive percent agreement and a 96.6% negative percent agreement within 12 days from symptoms onset, with a 100% positive agreement within 3 days or with Ct <33 [14].

Independent of the kit employed, antigen rapid tests can be a valid and rapid option to detect the infection; in fact,

its use has been evolved and increased during the pandemic. Indeed, the guidelines from the ECDC (lastly updated in October 2021) indicated the antigen test for symptomatic individuals within 5 days from symptoms onset and in a setting with COVID-19 prevalence >10%. Moreover, the ECDC recommended its employment for testing possible and probable COVID-19 cases presented to the healthcare system to accelerate the COVID-19 case assessment and for contact tracing or outbreak detection in closed settings, as well as in all the cases where the NAAT are not promptly available. In cases of prevalence <10% positive symptomatic cases should be confirmed by RT-qPCR or by another antigen test from a different producer. For the testing of asymptomatic subjects, the antigen test is recommended when the COVID-19 prevalence was above 10%; otherwise, the RT-qPCR should be preferred. The antigen test could be also employed for early discontinuation of quarantine when resulting negative and the individual is asymptomatic [6, 8].

In Italy, the last guidelines from the Ministry of Health indicated that the antigen test resulted positive does not require a confirmation test for the diagnosis and the isolation of the COVID-19 patients; moreover, a negative test can be employed for the discontinuation of the quarantine. In case of negative test in symptomatic individuals, a second

**Table 3** Limit of detection of the rapid antigen immunofluorescence lateral flow assay (STANDARD F COVID-19 Ag FIA) with previously isolated SARS-CoV-2. The data from the reverse transcription-quantitative (RT-q)—PCR and the plaque-forming unit (PFU) assay are reported

SARS-CoV-2 standard	RT-qPCR N gene (CDC primers and probe)		PFU assay		STANDARD F COVID-19 Ag FIA	
	Ct	Viral copies/ml	Infection in vitro	PFU/ml	Cutoff index (COI)	Positive/negative
Dilution-1	18.98	2.57 × 10 <sup>12</sup>	+	25,000	57.42	+
Dilution-2	22.54	4.94 × 10 <sup>10</sup>	+	2500	56.06	+
Dilution-3	25.25	2.45 × 10 <sup>9</sup>	+	250	7.26	+
Dilution-4	30	1.26 × 10 <sup>7</sup>	+	25	1.13	+
Dilution-5	32.37	9.04 × 10 <sup>5</sup>	-	2	0.62	-
Dilution-6	36.77	6.90 × 10 <sup>3</sup>	-	0.25	0.84	-

Ct, cycle threshold; RT-qPCR, reverse transcription PCR; PFU, plaque-forming unit

antigenic test or a RT-PCR test should be performed after 2–4 days [15, 16].

The main goal of this type of rapid test would be the mitigation of the huge impact that SARS-CoV-2 diagnosis presents on laboratories in terms of consumables and health workers' engagement.

Liotti et al. [17] assessed the performance of SENSOR BD STANDARD F COVID-19 Ag FIA on 359 nasopharyngeal specimens demonstrating an agreement with RT-PCR of 98% for the negative cases (i.e., specificity) and of 47% for the positive cases (i.e., sensibility) leading to negative and positive predictive values of 82% and 92.5% respectively. Similar to our findings, below 25 Ct the concordance with NAAT is high (95%); moreover, we observed a concordance of 92% below 30 Ct and a limit of the detection set at 32 Ct, while Liotti et al. reported a low agreement yet up to 25 Ct (42% Ct between 25 and 35, and 21% Ct > 35).

Instead, Porte et al. [18] analyzing 64 nasopharyngeal samples showed specificity of 96.9% and sensitivity of 90.6%, but the assay reached 100% sensitivity when the Ct was under 25.

On the other hand, the presence on the market of POCT molecular tests able to have close to 100% specificity and sensibility would accelerate the diagnostic iter with more reliable data, although the costs are obviously higher with respect to antigen assay, as well as the processivity.

Our results would give some important cues on the potential use of the antigen rapid tests; indeed, our findings showed that most of the swabs positive at the antigen test were able to infect the Vero E6 cells, indicating that probably only these samples present residual infectivity at the moment of collection. In this view, testing the symptomatic individuals with antigen test, it is reasonable to estimate that above all the patients positive at the antigen test are likely to be at higher risk of virus transmission; meanwhile, the individuals negative at the assay could be not able to spread the virus. Indeed, the antigen test employment has been strongly revised and implemented during the pandemic time, being currently used for rapid isolation of symptomatic positive individuals, quarantine discontinuation, and the entry of travelers into countries [8].

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**Author contribution** L. Z. and L. C. were involved in performing the experiments and writing—original draft. F. F. and M. R. were involved in the supervision of the experiments conducted in the BLS3 facility, writing—review and editing. S. C. was involved in the conceptualization of the study, writing—review and editing, project management.

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**Code availability** Not applicable.

## Declarations

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**Consent for publication** Not applicable.

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