Analytical Performances of the Point-of-Care *BIOSYNEX COVID-19 Ag BSS* for the Detection of SARS-CoV-2 Nucleocapsid Protein in Nasopharyngeal Swabs: A Prospective Field Evaluation During the COVID-19 Third Wave in France

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Research

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Abstract

Background: Evaluating the accuracy and reliability of rapid diagnostic testing kits is crucial for surveillance and diagnosis of SARS-CoV-2 infections in general population. The aim of the study was to assess the analytical performances of the antigen-rapid diagnosis test (Ag-RDT) *BIOSYNEX COVID-19 Ag BSS* (Biosynex Swiss SA, Freiburg, Switzerland), targeting the SARS-CoV-2 N nucleocapsid protein, for the diagnosis of COVID-19, by reference to real-time RT-PCR (rtRT-PCR).

Methods: A total 967 adults living in Paris region were prospectively included during the third wave of the COVID-19 epidemic in France. Paired nasopharyngeal flocked swabs were collected at the same timepoint from persons aged ≥18 years receiving testing for SARS-CoV-2, at two private laboratories.

Results: Overall, the Ag-RDT showed high sensitivity, specificity, PPV and NPV of 81.8%, 99.6%, 96.6% and 97.5%, respectively, as well as high or almost perfect agreement (97.0%), reliability assessed by Cohen's κ coefficient (0.87), and accuracy assessed by Youden's J index (81.6%) to detect SARS-CoV-2. The analytical performances of the Ag-RDT remained high in the event of significant viral excretion (*i.e.*, N gene C_t values ≤ 33 by reference rtRT-PCR), while the sensitivity of the Ag-RDT dropped to 55.2% with low or very low viral shedding (C_t> 33).

Conclusions: The Ag-RDT *BIOSYNEX COVID-19 Ag BSS* showed high specificity and sufficient sensitivity for the detection of SARS-CoV-2. This test is a promising potential easy diagnostic tool, especially in situations of symptomatic COVID-19 and/or proven contagiousness.

Introduction

The Coronavirus disease 2019 (COVID-19) pandemic continues to spread across the world. The effective isolation and early treatment of patients infected by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) require rapid, simple and accurate diagnostic tools.

While currently-recommended nucleic acid amplification tests (NAAT), such as real-time reverse transcription polymerase chain reaction (rtRT-PCR) assays, remain the gold standard cornerstone for the diagnosis of SARS-CoV-2 infection [1, 2], immunological methods can also be used to detect viral antigens [2–4]. Indeed, performing rtRT-PCR is expensive, time-consuming, and requires special equipment and qualified operators. Faster, cheaper, and easier to use alternative tools could be represented by novel point-of-care antigen-detecting rapid diagnostic tests (Ag-RDT) [3]. Ag-RDT relies on direct detection of SARS-CoV-2 viral proteins produced by replicating virus in nasal swabs and other respiratory secretions, often the virus N nucleocapsid protein, preferred because of its relative abundance and conserved structure, or other viral proteins such as the spike protein [4]. Most Ag-RDTs use sandwich catching by anti-SARS-CoV-2 monoclonal antibodies to detect viral antigens in the simple-to-use lateral flow immunoassay format allowing results in < 30 minutes. Around 180 Ag-RDTs for SARS-CoV-2 infection are currently commercially available or in development [5]. However, there is significant variability reported with respect to their diagnostic performances and a lack of external validation for many of the available tests, which still require clinical validation [6–9].

Our study aimed to evaluate in the field the Ag-RDT *BIOSYNEX COVID-19 Ag BSS* (Biosynex Swiss SA, Freiburg, Switzerland; reference SW40006) for the diagnosis of COVID-19 using prospectively collected samples from adults living in the Paris region during the third wave of the COVID-19 epidemic in France. The results of this test were compared with qualitative and quantitative results obtained in parallel using rtRT-PCR as a reference test.

Material And Methods

Rapid antigen test. The Ag-RDT *BIOSYNEX COVID-19 Ag BSS* is a rapid qualitative membrane-based immunochromatographic test that uses highly sensitive monoclonal antibodies to detect the SARS-CoV-2 N nucleocapsid protein in nasopharyngeal secretions sample. The test includes a reaction membrane, and three buffers (sample, reagent and absorbent). The reagent buffer contains colloidal gold particles conjugated to monoclonal antibodies directed against the SARS-CoV-2 N protein; the reaction membrane also contains secondary antibodies directed against the N protein. The test strip is placed inside a plastic cassette. The test was performed according to manufacturer instruction by mixing nasopharyngeal secretions with 300 µL of dilution buffer in a tube. After, one minute, 4 drops were added in the appropriate well. When the nasopharyngeal secretions are added to the sample well, crossing the strip, the dry conjugates of the reagent buffer are solubilized, allowing the conjugate to migrate with the sample and to react with the anti-SARS-CoV-2 antibodies immobilized on the membrane. If SARS-CoV-2 antigens are present in the sample, the complexes between the anti-SARS-CoV-2 conjugate and the virus are captured by anti-SARS-CoV-2 monoclonal antibodies specific to the test line area (T). The absence of a T line suggests a negative result. To serve as a procedural control, a red line appears in the control line area (C), indicating that the correct volume of sample has been added and that the membrane has played its role. Visual interpretation of results is performed 15 min after.

Study population and procedures. Paired nasopharyngeal flocked swabs were collected at the same timepoint from persons aged ≥ 18 years receiving testing for SARS-CoV-2, at two private laboratories (site A: *Centre Cardiologique du Nord*, Saint-Denis, France; site B: *Laboratoire Paris XV*, Paris, France) during the third wave of the COVID-19 epidemic (March and April 2021). The sites offered SARS-CoV-2 testing to anyone in the community who wanted testing for suspected COVID-19, travel, as a pre-operative assessment, as a contact-case exposure of an individual infected with SARS-CoV-2 or as control of SARS-CoV-2 infection in the 30 days preceding. A questionnaire capturing demographic information (sex and age), reasons for testing and current and past–14-day symptoms for symptomatic patients was administered to all participants. Suggestive symptoms of COVID-19 were headache, fatigue, fever, or upper or lower respiratory symptoms. Asymptomatic individuals were defined as those not reporting any of these symptoms. Inclusion criteria were aged more than 18 years and agreement to undergo two concurrent nasopharyngeal swabs for rtRT-PCR and Ag-RDT. Thus, at both sites, a health care professional first collected nasopharyngeal secretions in one nostril, using the swab provided in the *BIOSYNEX COVID-19 Ag BSS* kit, immediately followed by a nasopharyngeal swab for rtRT-PCR. Covid-19 antigen rapid testing was immediately carried out on-site using the Ag-RDT according to the manufacturer's instructions. The other nasopharyngeal swabs were stored in physiological serum (NaCl 0.9%) (1000 µL) at + 4°C and analyzed within 24–48 hours by the reference rtRT-PCR.

Reference multiplex molecular detection of SARS-CoV-2. Nucleic acid extraction was performed from 300 µL elution volume of nasopharyngeal flocked swab sample, using EX3600 extractor (Liferiver & Shanghai ZJ Bio-Tech Co.), according to the manufacturer's instructions, and finally eluted in 50 µL (final volume). SARS CoV-2 was detected in 5 µL of extracted RNA using the multiplex real-time PCR Novel Coronavirus (2019-nCoV) Real-Time Multiplex RT-PCR Kit (Detection for 3 Genes) (Liferiver & Shanghai ZJ Bio-Tech Co., Ltd, Shanghai, China), which constituted the reference multiplex rtRT-PCR for SARS-CoV-2 RNA detection. This assay can simultaneously detect 3 coronavirus target genes, including the SARS-like (including SARS-CoV-2, SARS-CoV, bat SARS-like coronavirus) conserved region of envelope protein gene (E), RNA-dependent RNA polymerase gene (ORF1ab of RdRP gene) and nucleocapsid protein gene (N), using reverse transcription followed by real-time PCR, providing individual cycle threshold (C_t) values for each target gene. Real-time PCR was carried out with CFX96™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA), according to the manufacturer's instructions. The experiment and result interpretation were carried out according to the manufacturer's protocol. According to the manufacturer's instructions, samples showing an exponential growth curve and Ct value ≤ 41 were considered positive. A unique C_t value > 41 was considered negative.

Statistical analyses. Data were entered into an Excel database and analyzed using IBM® SPSS® Statistics 20 software (IBM, SPSS Inc, Armonk, New York, USA). Medians were calculated for quantitative variables. The results were presented along with their 95% confidence interval (CI) using the Wilson score bounds for categorical variables [10]. Comparisons of frequencies between positive and negative results of Ag-RDT and rtRT-PCR testing between sites and all other variables were computed using the Pearson's Chi-square test or Fisher's exact test according to their validity conditions. The results of SARS-CoV-2 RNA detection by the multiplex rtRT-PCR were used as the reference standard to estimate the sensitivity and specificity of the study Ag-RDT, with corresponding 95% CI. The concordance between study Ag-RDT and multiplex molecular detection of SARS-CoV-2 RNA was assessed by percent agreement corresponding to the observed proportion of identical results between Ag-RDT compared to rtRT-PCR detection. The reliability between the study Ag-RDT and the multiplex molecular detection of SARS-CoV-2 RNA was estimated by Cohen's κ coefficient [11], and the degree of agreement was determined as ranked by Landlis and Koch [12]. The accuracy of the study Ag-RDT to correctly diagnose SARS-CoV-2 infection was estimated by Youden's J index (J = sensitivity + specificity − 1) [13]. Positive predive values (PPV) and negative predictive values (NPV) were calculated according to Bayes's formulae, by considering the official reported prevalence of SARS-CoV-2-RNA positivity in symptomatic patients in Paris's area, France, on 12th April 2021, *e.g.* around the peak of the third wave epidemic in France (Santé publique France 2021; https://www.santepubliquefrance.fr/).

Ethics statement. The study was used as a clinical evaluation of the continuous quality improvement program and COVID-19 management measures performance evaluation, according to the national law on the accreditation of medical biology laboratories [14]. The dataset was completely anonymous and did not contain any identifiable personal health information.

Results

Paired nasopharyngeal respiratory swabs were collected from 967 persons, including 741 from site A and 226 from site B (Table 1). Participants ranged in age from 18 to 95 years (median = 34 years). The sex ratio of the study population was 0.94. The main reasons for testing were air travel (35.6%), contact-case exposure of an individual infected with SARS-CoV-2 (35.1%), suspected COVID-19 (n=212, 21.9%), pre-operative assessment (4.4%) and control of SARS-CoV-2 infection in the 30 days preceding (3.0%). At the time of testing, the majority (722, 74.7%) of participants were asymptomatic. A total of 245 (25.3%) participants reported at least one COVID-19–compatible symptom, including 212 suspected COVID-19 cases, 29 (8.5%) contact-cases, 3 (0.9%) travelers, and 1 (3.0%) patient with recent past-history of COVID-19. Among symptomatic patients, the median time of symptom duration before sampling was 4 days (range, 0-20 days). All comparisons between positive and negative results of Ag-RDT and rtRT-PCR testing between sites and all other variables did not reach statistical significance (not shown).

The vast majority [(114/124 (91.9%)] of positive results appeared within the first five minutes, and frequently [(31/124 (25.0%%)] within one minute. Test results and main performances characteristics of the Ag-RDT *BIOSYNEX COVID-19 Ag BSS* compared with the reference rtRT-PCR in the study population according to COVID-19–compatible symptoms are depicted in Table 2. Using rtRT-PCR as the standard, three false-positive *BIOSYNEX COVID-19 Ag BSS* test results occurred, among specimens from asymptomatic (n=2) or symptomatic (n=1) participants. Among 148 rtRT-PCR positive results, 27 (18.2%) were false-negative *BIOSYNEX COVID-19 Ag BSS* test results (23 in specimens from asymptomatic persons and 4 in specimens from asymptomatic persons). Overall, the Ag-RDT *BIOSYNEX COVID-19 Ag BSS* showed high sensitivity, specificity, PPV and NPV of 81.8%, 99.6%, 96.6% and 97.5%, respectively. Testing among asymptomatic participants indicated the following for the Ag-RDT *BIOSYNEX COVID-19 Ag BSS* (with rtRT-PCR as the standard): sensitivity, 79.4%; specificity, 99.7%; PPV, 97.3%; and NPV, 97.2% (Table 2); among symptomatic persons, sensitivity was 95.0%; specificity, 99.4%; PPV, 95.6%; and NPV, 96.3%. For participants who were within 7 days of symptom onset, the Ag-RDT *BIOSYNEX COVID-19 Ag BSS* sensitivity was 96.6%, specificity, 99.4%, PPV, 95.7%, and NPV, 99.4%.

The analytical results according to the level of viral excretion assessed by the N gene C_t values by the reference rtRT-PCR are shown in Table 3. Overall, the Ag-RDT *BIOSYNEX COVID-19 Ag BSS* showed high or almost perfect agreement (97.0%), reliability assessed by Cohen's κ coefficient (0.87), and accuracy assessed by Youden's J index (81.6%) to detect SARS-CoV-2. These analytical performances were further stratified according to the cycle threshold (C_t) values of the N gene detected by reference rtRT-PCR considering C_t-related criteria of very high (C_t≤20) and high (C_t≤33) SARS-CoV-2 RNA excretion. Indeed, viral loads with C_t> 33 are considered to be low and correspond to moderate or very low viral excretion [15-18]. Conversely, samples with C_t ≤33 have a significant SARS-CoV-2 viral load, as in individuals symptomatic for COVID-19 or contagious. C_t values ≤ 20 indicate very high viral shedding [16-18]. There were two distinct situations. In the event of significant viral loads (high or very high) in real-time PCR (C_t≤33), the Ag-RDT *S BIOSYNEX COVID-19 Ag BSS* showed excellent analytical performances, with sensitivities between 83.3% and 100.0%, specificities of 99.8%, PPV between 98.3% and 98.6% and NPV between 97.7% and 100.0%. In the event of low or very low viral loads (C_t> 33), the sensitivity of the Ag-RDT *BIOSYNEX COVID-19 Ag BSS* showed reduced analytical performances with 55.2% sensitivity, while its specificity remained high (98.8%).

Discussion

We herein evaluated the analytical performances of the novel point-of-care Ag-RDT *BIOSYNEX COVID-19 Ag BSS* by reference to multiplex rtRT-PCR for SARS-CoV-2 RNA detection as the gold standard in a real-life community setting. In this evaluation, the sensitivity of the Ag-RDT *BIOSYNEX COVID-19 Ag BSS* was lower among specimens from asymptomatic persons (79.4%) than among specimens from symptomatic persons (95.0%). Specificity (> 99.0%) was high in specimens from both asymptomatic and symptomatic groups. The prevalence of having SARS-CoV-2 RNA-positive rt-RT-PCR results in this population was relatively high (12.8% overall; 6.5% for asymptomatic participants and 31.4% for symptomatic participants), and the estimated PPVs and NPVs of the Ag-RDT *BIOSYNEX COVID-19 Ag BSS* were elevated in all groups of participants. However, administering the Ag-RDT in lower prevalence settings will likely result in lower predictive values. In the event of significant viral excretion (*i.e.*, N gene C_t values below 33 by reference rtRT-PCR), the Ag-RDT RDT *BIOSYNEX COVID-19 Ag BSS* showed high sensitivity (from 83.3–100.0%) and specificity (> 99.0%) for SARS-CoV-2 RNA detection, with excellent concordance, reliability and accuracy with the reference multiplex rtRT-PCR, and PPVs and NPVs above 97.0%. The sensitivity of the study Ag-RDT dropped however to 55.2% with low or very low viral shedding (C_t> 33). Taken together, these observations demonstrate that the Ag-RDT *BIOSYNEX COVID-19 Ag BSS* harbored high analytical performances, which makes it suitable to be used as point-of-care Ag-RDT in various hospital and non-hospital settings where a rapid diagnosis of SARS-CoV-2 is necessary. Although less sensitive than RT-PCR, the Ag-RDT *BIOSYNEX COVID-19 Ag BSS* could be beneficial due to its rapid results, ease of use, and independence from existing laboratory structures. Testing criteria focusing on patients with typical symptoms in their early symptomatic period onset could further increase its diagnostic value.

In the present series, the sensitivity of the Ag-RDT *BIOSYNEX COVID-19 Ag BSS* was 81.8% overall, and the positive detection rate was comparable to the rtRT-PCR in the majority (88.2%) of patients with $C_t \leq 33$. Twelve of 14 (85.7%) false-negative subjects with significant viral excretion ($C_t \leq 33$) were asymptomatic, although conflicting evidence exists regarding the relationship between symptom severity and viral shedding [19]. In the present large series, false-positive test results were rarely observed, providing 99.6%-specificity in our study, which exceeded the performance recommended by the World Health Organization (WHO) [20]. Some false-positive results have been reported in other antigen tests [21–23]. While definitive proof is lacking, possible causes for the false-positives include the high viscosity of specimens and interference of human antibodies [24].

Finally, the Ag-RDT RDT *BIOSYNEX COVID-19 Ag BSS* fulfilled the current WHO's recommendations for a screening Ag-RTD stating that, at minimum, Ag-RDTs would need to correctly identify significantly more cases than they would miss (sensitivity ≥ 80%) and would have very high specificity (≥ 97–100%) [20]. Furthermore, analytical performances of comparable order as those of our study Ag-RDT were previously reported for some Ag-RDTs in lateral flow immunoassay format [7, 9, 21, 25–35], while several studies have reported much lower sensitivity levels contrasting with always high specificity [3, 36–41]. For example, a comparable Ag-RDT such as the novel COVID-VIRO® from AAZ (Boulogne Billancourt, France) showed a sensitivity of 96.7% and a specificity of 100% in a real-life community setting [31]. In addition, the Ag-RDT *RDT BIOSYNEX COVID-19 Ag BSS* fulfilled also the current recommendations of the French High Authority of Health (*Haute Autorité de santé*, Saint-Denis, France) for a screening Ag-RTD stating that, at minimum, Ag-RDTs would need to correctly identify significant proportions of symptomatic patients (sensitivity ≥ 80%) as well as asymptomatic individuals (sensitivity ≥ 50%) and would have very high specificity (≥ 90%) [42].

We analyzed our results according to the estimated viral load in SARS-CoV-2 in the samples. There is an ongoing debate regarding the C_t value corresponding to the threshold of infectivity (*i.e.*, patient considered as contagious) [16]. Indeed, there is a trend to a natural gradual decrease of the SARS-CoV-2 RNA load in the nasopharyngeal samples overtime during the course of infection, at the origin of varying levels of contagiousness [43]. La Scola et al. found that patients with C_t value > 33 are not contagious because of the low number of positive cultures [44]. This is consistent with the Centers for Disease Control and Prevention (CDC) recommendations, which propose a C_t value of 33 as a surrogate of contagiousness [15], with C_t values ≤ 20 indicating very high viral shedding [16–18]. In our series, we have stratified the nasopharyngeal samples according to the level of viral excretion, indirectly evaluated by the value of the C_t of the N gene according to the reference rtRT-PCR, in order to calculate the performance of the study Ag-RDT at different proposed cut-offs for contagiousness.

Our results clearly show that the analytical performances of the Ag-RDT *BIOSYNEX COVID-19 Ag BSS* were much better in the event of a high viral load, *i.e.*, in the case of significant viral excretion. These observations demonstrate the interest of the Ag-RDT *BIOSYNEX COVID-19 Ag BSS* as a rapid rule-in test for COVID-19 with samples at high viral load, in symptomatic patients for example, and point caution with its use as a singular rule-out test especially in the setting of samples with lower viral loads.

The SARS-CoV-2 RNA positive subpopulation of our clinical samples collection was characterized by a wide range of C_t-values with medium and low C_t-values dominating. This allowed the calculation of sensitivity and specificity values with higher relevance for clinical practice. The C_t-dependent evaluation showed very good sensitivity for highly and moderately SARS-CoV-2 positive samples ($C_t \leq 33$). In contrast, the sensitivity of the assay with specimens containing only a limited viral load was lower. Thus, COVID-19 infection would not be detected in patients in the very early or late phase of the infection typically associated with a low viral load. However, differentiation between contagious and non-contagious individuals may be possible with this assay. Samples with C_t-values > 33 usually do not allow culturing of the virus indicating low infectivity [16, 44]. Such individuals may be regarded as non-contagious despite carrying low virus loads. This differentiation of individuals may be of particular importance for the decision on access to susceptible individuals, for example in nursing homes or in many other medical circumstances. Similar observations of dramatic decrease of sensitivity of Ag-RDT for SARS-CoV-2 antigen detection at C_t thresholds around 25–33 were previously reported [7, 38, 45, 46], confirming that Ag-RDTs were most effective to identify RT-PCR positive symptomatic patients or asymptomatic subjects with high viral loads in their respiratory secretions (*i.e.*, C_t values ≤ 33).

In our study, the accuracy of the Ag-RDT *BIOSYNEX COVID-19 Ag BSS* was estimated by the percent positive agreement and not sensitivity. Since the agreement is measured relative to an RT-PCR test, which may be imperfect itself [47]. Compounding this uncertainty, we have largely exceeded the minimum

sample size of 30 positive cases that is required to apply for evaluation [8], which made it possible to restrict the confidence intervals of the evaluated variables.

Our study has several strengths. All samples were collected from one nasopharynx with flocked swabs, which is optimal for the evaluation of Ag-RDT clinical performances in our study. The Ag-RDT was performed in parallel to RT-PCR. The study population included a variety of situations outside the hospital setting with a majority of young adults without comorbidities, who mostly had typical and mild COVID-19 symptoms when being symptomatic. This currently describes the majority of SARS-CoV-2 infected individuals, and an important group for limiting community transmission. The findings in this investigation are also subject to limitations. Participants might have inadvertently reported common nonspecific symptoms as COVID-19–compatible symptoms. This investigation evaluated the *BIOSYNEX COVID-19 Ag BSS* antigen test, and the results presented here cannot be generalized to other agencies-authorized SARS-CoV-2 antigen tests. Finally, the *BIOSYNEX COVID-19 Ag BSS* antigen test characteristics might be different depending on whether an individual had been previously tested positive.

Conclusion

The Ag-RDT *BIOSYNEX COVID-19 Ag BSS* showed very high specificity and sufficient sensitivity for the detection of SARS-CoV-2. Given the simple procedures and shorter turnaround time involved with this test, it is a promising option as an alternative diagnostic modality especially in situations of symptomatic COVID-19 and/or proven contagiousness. The test can also be offered to test asymptomatic individuals in many situations of potential exposure to SARS-CoV-2 and in mass screening at the population level.

Abbreviations

Ag-RDT: Antigen-detecting rapid diagnostic tests

CDC: Centers for Disease Control and Prevention

COVID-19: Coronavirus disease 2019

NAAT: nucleic acid amplification test

rtRT-PCR: real-time reverse transcription polymerase chain reaction

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2

WHO: World Health Organization

Declarations

Ethics approval and consent to participate. The study has been approved by the local scientific committee of Parc de l'Innovation, Strasbourg, France, and informed participants consent was obtained.

Consent for publication. All authors approved the submission of the manuscript for publication.

Availability of data and materials. The data is available and can be used for the academic or research purposes.

Competing interest. The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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Contributions. FF, RD and LB have conceived and designed the research; FF, NA and RD performed the experiments and STW the statistical analyses; RD, STW and LB analyzed the results and drafted the manuscript.

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Tables

Table 1. Characteristics of persons providing paired upper respiratory swab (n=967) for real-time reverse transcription-polymerase chain reaction (rRT-PCR) testing and *BIOSYNEX COVID-19 Ag BSS* rapid diagnostic testing for SARS-CoV-2 at two private laboratory sites, by test results, Paris, France, spring 2021.

Characteristics	To number of persons (column %)	Number of persons (row%)			
		rRT-PCR-negative	rRT-PCR-positive	Antigen-negative	Antigen-positive
Total	967 (100)	819 (84.7)	148 (15.3)	844 (87.3)	123 (12.7)
Testing site					
A	741 (74.6)	633 (85.4)	108 (14.6)	651 (87.9)	90 (12.1)
B	226 (25.4)	186 (82.4)	40 (17.6)	193 (85.4)	33 (14.6)
Sex					
Female	498 (51.5)	429 (86.2)	69 (13.8)	438 (88.0)	60 (12.0)
Male	469 (48.5)	390 (83.2)	79 (16.8)	406 (86.6)	63 (13.4)
Age group, years					
18-49	740 (76.4)	629 (85.0)	111 (15.0)	647 (87.4)	93 (12.6)
50-64	157 (16.3)	129 (82.2)	28 (17.8)	133 (84.7)	24 (15.3)
≥65	70 (7.3)	61 (87.1)	9 (12.9)	64 (91.4)	6 (8.6)
Median age (range)	34 (18-83)	34 (18-83)	32 (18-82)	34 (18-83)	37 (18-82)
Current symptoms					
≥1	245 (25.3)	165 (67.4)	80 (32.6)	168 (68.6)	77 (31.4)
None	722 (74.7)	654 (90.6)	68 (9.4)	676 (93.6)	46 (6.4)
Days from the onset					
0-3	107 (43.3)	38 (35.5)	69 (64.5)	41(38.3)	66 (61.7)
4-7	122 (49.8)	48 (39.4)	74 (60.6)	69 (56.6)	53 (43.4)
>7	16 (6.9)	11 (68.7)	5 (31.3)	12 (75.0)	4 (25.0)
≤ 7	229 (93.1)	86 (37.6)	143 (62.4)	110 (48.0)	119 (52.0)
Median (range)	4 (0-20)	4 (0-20)	3 (0-15)	4 (0-20)	3 (0-10)
Positive test results in past 30 days					
Yes	29 (3.0)	20 (68.9)	9 (31.1)	21 (72.4)	8 (27.6)
No/Unknown	938 (97.0)	799 (85.2)	139 (14.8)	823 (87.7)	115 (12.3)
Exposure to a diagnosed COVID-19 case (case-contact)					
Yes	340 (35.1)	282 (82.9)	58 (17.1)	293 (86.2)	47 (13.8)
No/Unknown	627 (64.9)	537 (85.7)	90 (14.3)	551 (87.9)	76 (12.1)
Travel					
Yes	344 (35.6)	330 (95.9)	14 (4.1)	337 (98.0)	7 (2.0)
No	623 (64.4)	489 (78.5)	134 (21.5)	507 (81.4)	116 (18.6)
Preoperative assessment					
Yes	42 (4.4)	38 (90.5)	4 (9.5)	40 (95.2)	2 (2.8)
No	925 (95.6)	781 (84.4)	144 (15.6)	804 (86.9)	121 (13.1)

Table 2. Test results and performances characteristics of the *BIOSYNEX COVID-19 Ag BSS* rapid diagnostic test compared with real-time reverse transcription-polymerase chain reaction (rRT-PCR) for SARS-CoV-2 testing among asymptomatic and symptomatic persons at two private laboratory sites, by test results, Paris, France, spring 2021.

Results and performances	rRT-PCR (number of test, %) Negative	Positive	Total
***BIOSYNEX COVID-19 Ag BSS* results**			
All participants (n=967)			
Positive	3 (0.3)	121 (12.5)	124 (12.8)
Negative	816 (84.4)	27 (2.8)	843 (87.2)
Total	819 (78.8)	148 (21.2)	967 (100)
Asymptomatic (n=722)			
Positive	2 (0.3)	45 (4.2)	47 (6.5)
Negative	652 (90.3)	23 (5.2)	675 (95.5)
Total	654 (90.6)	68 (9.4)	722 (100)
Symptomatic (≥1 symptom) (n=245)			
Positive	1 (0.4)	76 (31.0)	77 (31.4)
Negative	164 (67.0)	4 (1.6)	168 (68.6)
Total	165 (67.4)	80 (32.6)	245 (100)
Symptomatic (≤ 7 days from symptom onset) (n=229)			
Positive	1 (0.5)	72 (31.4)	73 (31.9)
Negative	153 (66.8)	3 (1.3)	156 (68.1)
Total	154 (67.3)	75 (32.7)	229 (100)
***BIOSYNEX COVID-19 Ag BSS* performances** (%, 95%CI)			
All participants			
Sensitivity		81.8 (79.2 – 84.1)	
Specificity		99.6 (98.9 – 99.8)	
PPV£		96.6 (95.3 – 97.6)	
NPV£		97.5 (96.3 – 98.3)	
Asymptomatic			
Sensitivity		79.4 (76.3 – 82.2)	
Specificity		99.7 (98.9 – 99.9)	
PPV		97.3 (95.8 – 98.2)	
NPV		97.2 (95.7 – 98.2)	
Symptomatic			
Sensitivity		95.0 (91.5 – 97.1)	
Specificity		99.4 (97.4 – 99.9)	
PPV		95.6 (92.2 – 97.5)	
NPV		99.3 (97.2 – 99.8)	
Symptomatic (≤ 7 days from onset)			
Sensitivity		96.0 (92.6 – 97.9)	
Specificity		99.4 (97.3 – 99.9)	
PPV		95.7 (92.2 – 97.7)	
NPV		99.4 (97.3 – 99.9)	

£ PPV and NPV were calculated according to the Bayes's formulae, by taking into account the official reported prevalence of SARS-CoV-2-RNA positivity in COVID-19-suspected patients in Paris's area, France, of 12.2% on 12th April 2021 [Santé publique France 2021; https://www.santepubliquefrance.fr/].

CI: Confidence interval; NVP: Negative predictive value; PPV: Positive predictive value

Table 3. Analytical performances of the *BIOSYNEX COVID-19 Ag BSS* rapid diagnostic test for the qualitative detection of the N protein of SARS-CoV-2 using 967 prospectively collected nasopharyngeal swab samples by reference rRT-PCR#, according to their N gene C_t values.

		BIOSYNEX COVID-19 Ag BSS[§]												
		N gene C_t *(median; range)*	**N**	**TN** *(n)*	**FN** *(n)*	**TP** *(n)*	**FP** *(n)*	**Sensitivity** *(% [95% CI])*[μ]	**Specificity** *(% [95% CI])*	**Agreement**[a]	**Concordance**[b]	**Youden's J index**[c]	**PPV**[d] *(% [95% CI])*	**NP** *(% [95* *CI])*
Detectable N gene C_t[£] **by rRT-PCR**[#]	**≤ 20**	17.9 (13.9-20.0)	35	NA	0	35	NA	100 (99.6 – 100)	99.6 (98.9 – 99.8)	99.6 (98.9 – 99.8)	0.95 (0.93 – 0.96)	99.6 (98.9 – 99.8))	97.2 (95.9 – 98.1)	100 (99 – 100
	21 – 33	27.2 (20.1-33.0)	84	NA	14	70	NA	83.3 (80.7 – 85.6)	99.6 (98.9 – 99.8)	98.1 (97.0 – 98.8)	0.65 (0.62 – 0.68)	82.9 (80.3 – 85.2)	96.7 (95.3 – 97.7)	97. (96 – 98.
	> 33 – 41	35.9 (34.0-39.2)	29	NA	13	16	NA	55.2 (51.8 – 58.5)	99.6 (98.9 – 99.8)	98.1 (96.9 – 98.8)	0.65 (0.62 – 0.68)	54.8 (51.4 – 58.1)	95.0 (93.3 – 96.3)	94. (92 – 95.
	All positive C_t values	26.9 (13.9-39.2)	148	NA	27	121	NA	81.8 (79.2 – 84.1)	99.6 (98.9 – 99.8)	96.9 (95.9 – 97.8)	0.87 (0.85 – 0.89)	81.4 (78.8 – 83.7)	96.6 (95.3 – 97.6)	97. (96 – 98.
Undetectable N gene (C_t > 41)			819	816	0	0	3	NA	NA	NA	NA	NA	NA	NA

§ Paired nasopharyngeal samples in each nostril were collected with a flocked swab for each volunteer patients by trained healthcare personnel (nurses, doctors or biologists). The collection of the two simultaneous samples was always carried out by the same operator. Molecular testing as well COVID-19 antigen detection were carried out on fresh samples;

[a] Agreement = TP + TN / TP+FP+TN+FN, expressed in percentage;

[b] The Cohen's k coefficient calculation was used to estimate the concordance [11] and interpreted according the Landis and Koch scale [12], as follows: < 0 as indicating no agreement, 0–0.20 as slight, 0.21–0.40 as fair, 0.41–0.60 as moderate, 0.61–0.80 as substantial, and 0.81–1 as almost perfect concordance;

[c] The accuracy of the test *BIOSYNEX COVID-19 Ag BSS* to correctly diagnose SARS-CoV-2 infection was estimated by Youden's J index (J = sensitivity + specificity − 1) [13];

[d] PPV and NPV were calculated according to the Bayes's formulae, by taking into account the official reported prevalence of SARS-CoV-2-RNA positivity in COVID-19-suspected patients in Paris's area, France, of 12.2% on 12th April 2021 [Santé publique France 2021; https://www.santepubliquefrance.fr/];

[μ] 95% confidence intervals in brackets were calculated by using the Wilson score bounds;

[£] The C_t values of N gene detection by the reference Liferiver rRT-PCR were used to classify nasopharyngeal samples according to their level of SARS-CoV-2 RNA excretion; C_t of 20 and 33 were taken as thresholds of very high and high SARS-CoV-2 RNA excretion, respectively, as previously stated [15-18];

[#] The CE IVD-marked Novel Coronavirus (2019-nCoV) Real Time Multiplex RT-PCR Kit (Detection for 3 Genes) (Liferiver & Shanghai ZJ Bio-Tech Co., Ltd, Shanghai, China) constituted the reference multiplex rRT-PCR for SARS-CoV-2 RNA detection. This assay detects three target genes of SARS-CoV-2 (E, RdRP and N genes).

C_t: Cycle threshold; FN: False negative; FP: False positive; NA: Not attributable; NPV: Negative predictive value; PPV: Positive predictive value; rRT-PCR: real-time reverse transcription-polymerase chain reaction; TP: True positive; TN: True negative