



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



A rapid and cost-effective diagnostic algorithm for the detection of SARS-CoV-2 infection in the emergency area by combining highly sensitive antigenic test and RT-PCR

Sara Salvetti^{a,*}, Federica Lavinia^a, Nico Rosi^b, Simone Vanni^b, Luca Masotti^c, Roberto Tarquini^d, Silvia Guarducci^e, Gian Maria Rossolini^{f,g}, Iolanda Montenora^a

^a Microbiology Unit, San Giuseppe Hospital, Empoli, FI, Italy

^b Emergency Department, San Giuseppe Hospital, Empoli, FI, Italy

^c Internal Medicine II, San Giuseppe Hospital, Empoli, FI, Italy

^d Internal Medicine I, San Giuseppe Hospital, Empoli, FI, Italy

^e Management Unit, San Giuseppe Hospital, Empoli, FI, Italy

^f Department of Experimental and Clinical Medicine, University of Florence, FI, Italy

^g Microbiology and Virology Unit, Careggi University Hospital, FI, Italy

ARTICLE INFO

Article history:

Received 14 March 2022

Revised in revised form 12 May 2022

Accepted 16 May 2022

Available online 23 May 2022

Keywords:

SARS-CoV-2

COVID-19

turn-around time

antigen detection

Lumipulse assay

RT-PCR

ABSTRACT

A diagnostic algorithm for SARS-CoV-2 infection in patients admitted to the emergency area, based on a combination of rapid antigen and molecular testing, has been evaluated with 3070 nasopharyngeal swabs. Compared to molecular test alone, the proposed algorithm allowed to significantly reduce costs and average time to results.

© 2022 Elsevier Inc. All rights reserved.

The COVID-19 pandemic has been a major stressor for the healthcare system worldwide [1,2]. A prompt and accurate diagnosis is crucial to identify infected individuals and limit the spread of the virus in healthcare settings, particularly with patients admitted to the emergency department (ED). In fact, in this setting a delay in releasing results may negatively reflect on time-dependent interventions and impair the efficient turn-around of patients, while the test accuracy is a matter of major concern since false-positive or -negative results can lead to wrong admission of patients to the COVID-19 or non-COVID-19 settings and expose healthy patients to a risk of nosocomial infection.

Viral RNA detection by molecular techniques (e.g., RT-PCR) is the gold standard for the diagnosis of SARS-CoV-2 infection [3,4]. However, it is expensive and usually has a time to results (TTR) of several hours. Rapid molecular tests, such as the Xpert[®] Xpress SARS-CoV-2 (GeneXpert[®], Cepheid, USA) have been released, which may be very

useful for COVID-19 diagnosis in the ED setting. Nevertheless, these platforms are very expensive and affected by a low processivity.

Recently, a quantitative and fully automated antigen test based on chemiluminescence enzyme-immunoassay has been launched on the market [5]. This assay, named Lumipulse[®]G SARS-CoV-2 Ag (LPG) (Fujirebio, Japan), is an accurate diagnostic tool for SARS-CoV-2 [6–9]. Nevertheless, using this test, a variable percentage of false-positive results has been reported [10,11], and a grey zone (between 1.34 and 10.0 pg/mL) has been suggested by the Manufacturer.

The present study was aimed at developing a diagnostic algorithm for the detection of SARS-CoV-2 infection in patients admitted to the ED, to rapidly assign them to COVID or non-COVID settings.

From April 26 to June 22, 2021, a total of 3070 patients were admitted to the ED of San Giuseppe Hospital (Azienda USL Toscana Centro, Italy). These patients were consecutively triaged to assess the need for time-dependent intervention and the presence of COVID-19-related symptoms. A nasopharyngeal (NP) sample was obtained from each patient using swabs collected in 3 mL of PBS (VACUETTE[®] Virus Stabilization Tubes, Greiner Bio-One, Austria). Of the 3070 samples, 411 were immediately analyzed using a molecular-fast (MF)

* Corresponding author. Tel.: +39.0571.706552; fax: +39.0571.706611.

E-mail address: sara.salvetti@uslcentro.toscana.it (S. Salvetti).

Table 1
Summary of the results from testing NP samples from symptomatic (A) and asymptomatic (B) patients. Nonreactive: Ct values ≥ 40 for both RdRp and Orf8 targets; Reactive: Ct value of at least 1 target < 35 ; Reactive, low viral load: Ct values between 35 and 40.

Antigenic test result (A)	N	RT-PCR		
		Nonreactive	Reactive	Reactive, low viral load
<1.0 pg/mL	498	488	3	7
1.0 pg/mL \leq Ag < 50.0 pg/mL	75	54	20	1
≥ 50.0 pg/mL	63	0	63	0
Total	636	542	86	8

Antigenic test result (B)	N	RT-PCR		
		Nonreactive	Reactive	Reactive, low viral load
<1.0 pg/mL	1879	1879	0	0
1.0 pg/mL \leq Ag < 50.0 pg/mL	136	122	12	2
≥ 50.0 pg/mL	8	0	8	0
Total	2023	2001	20	2

assay (i.e., GeneXpert®) as patients were judged to require a time-dependent intervention following triage. The remaining 2659 samples, including 636 from symptomatic and 2023 from asymptomatic patients, were analyzed with the LPG antigen test, using the Lumipulse 600II automated system, after centrifugation at 5000 g for 5' [9], and were also tested with the SARS-CoV-2 ELITE MGB® RT-PCR assay (ELITechGroup, France). Statistical analyses were carried out using the RT-PCR as the reference test for the diagnosis of SARS-CoV-2 infection (<http://vassarstats.net>).

The antigen test detected 71 specimens with a value of ≥ 50.0 pg/mL, 211 specimens with a value between 1.0 and < 50.0 pg/mL, and 2377 specimens with a value < 1.0 pg/mL (these 2 cut-off values were formally suggested by the Health Authority of the Tuscan regional Government [12] for interpretation of results of the LPG antigen test, according to the results of a large multi-center regional survey). Confirmation by molecular testing yielded variable results for the different categories (Table 1).

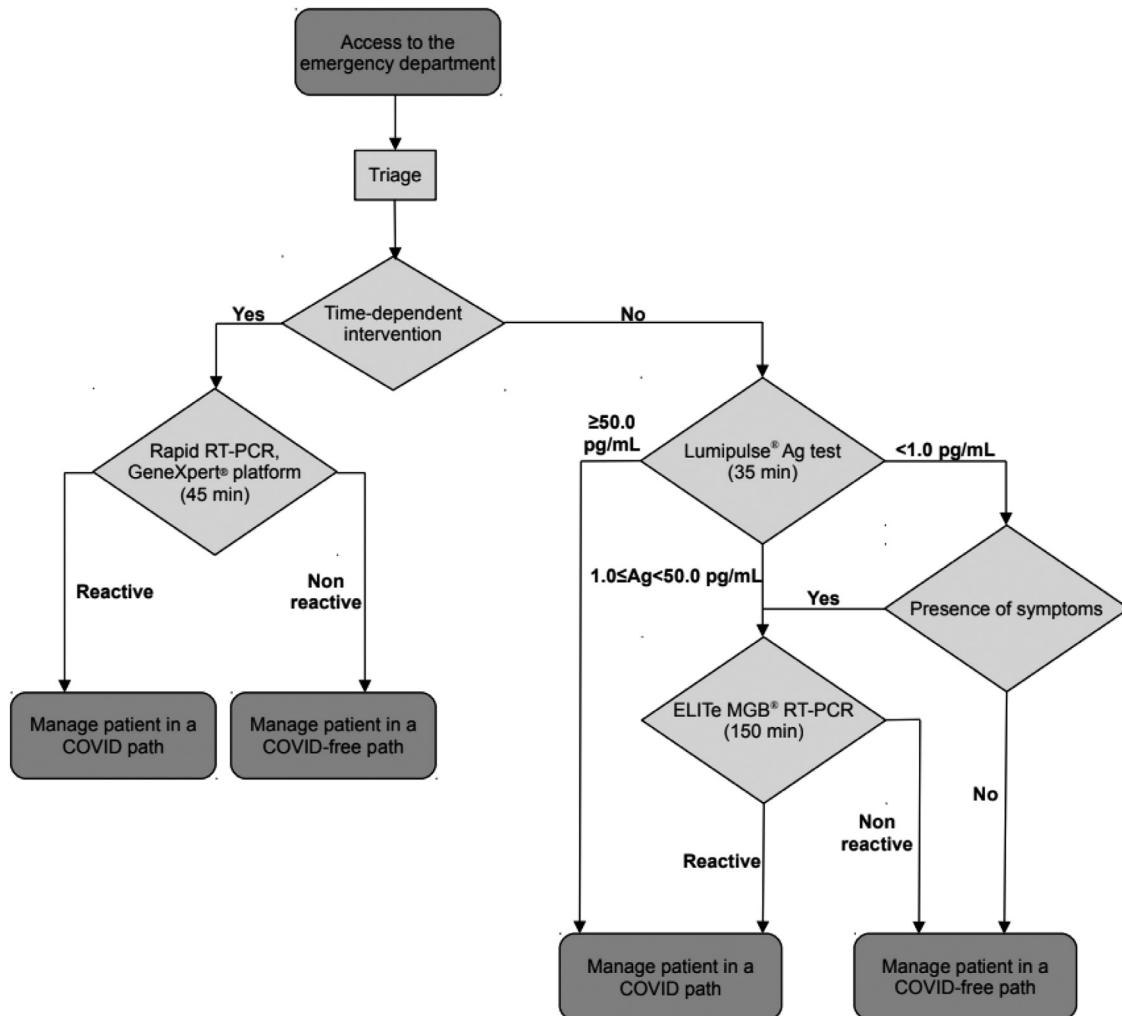


Fig. 1. Proposed algorithm for the management of patients in the emergency area. In brackets is the turn-around time after test started on the analyzer.

Considering 1.0 pg/mL as a cut-off to discriminate samples positive for SARS-CoV-2 antigen from negative samples, the overall data showed a sensitivity of 91.4% (106/116; 95% confidence interval [CI]: 84.3%–95.6%) and a specificity of 93.1% (2367/2543; CI: 92.0%–94.0%). The RT-PCR and antigen test overall agreement was 93.0% (2614/2659 samples). The positive predictive value (PPV) and negative predictive value (NPV) of LPG resulted 37.6% (106/282; CI: 32.0%–43.6%) and 99.6% (2367/2377; CI: 99.2%–99.8%), respectively.

When data were analyzed separately for symptomatic or non-symptomatic patients, we observed a PPV and NPV value of 60.9% (84/138; CI: 52.2%–69.0%) and 98.0% (488/498; CI: 96.2%–99.0%), respectively, for symptomatic patients, and of 15.3% (22/144; CI: 10.0%–22.4%) and 100.0% (1879/1879; CI: 99.7%–100.0%), respectively, for asymptomatic patients.

When data were analyzed considering an antigen concentration ≥ 50.0 pg/mL for positivity to increase test specificity, PPV reached 100.0% both in symptomatic (63/63; CI: 93.0%–100.0%) and asymptomatic (8/8; CI: 60.0%–100.0%) patients. By contrast, false negative results slightly increased, particularly in symptomatic patients: NPV was 95.0% (542/573; CI: 92.3%–96.2%) and 99.3% (2001/2015; CI: 98.8%–99.6%) in symptomatic and asymptomatic patients, respectively (Table 1).

Altogether, these data suggested that: (1) LPG is a valid diagnostic assay showing overall high sensitivity and specificity; (2) shifting the cut-off of the antigen test up to 50.0 pg/mL, might avoid the occurrence of false positive results; (3) a grey zone for samples with an antigen concentration between 1.0 and < 50.0 pg/mL should be considered, confirming them by RT-PCR; (4) the slight decrease of NPV (95.0% vs 98.0%) observed in symptomatic patients when the cut-off of the antigen test was 50.0 pg/mL, could be by-passed confirming negative swabs from these patients by RT-PCR.

In conclusion, since LPG demonstrated good performance compared to RT-PCR, we propose a renewed algorithm for the diagnosis of SARS-CoV-2 infections of patients from the ED (Fig. 1), which is intended for patients who do not require time-dependent intervention (the latter are always tested with the MF assay). According to this algorithm, the quantitative LPG antigen test is initially performed, and when the sample shows a result ≥ 50.0 pg/mL, the patient is managed in a COVID path. When sample shows a result between 1.0 and < 50.0 pg/mL (grey zone), the SARS-CoV-2 ELITE MGB[®] RT-PCR assay is used as a reflex test and the patient is managed in a COVID or COVID-free paths based on the molecular test result. Finally, when sample shows a result < 1.0 pg/mL, the asymptomatic patient is directly managed in a COVID-free path, while the sample from a symptomatic patient is subjected to confirmatory RT-PCR assay.

Supposing to apply the proposed algorithm to the 2659 ED patients not requiring a time-dependent intervention, in the absence of test failures, 73.0% would have received SARS-CoV-2 test results in $\sim 45'$ while 27.0% in $\sim 210'$ due to the need for a reflex RT-PCR test (Supplementary Table 1). With the aim to reduce TTR, we might suppose the use of the MF instead of ELITE MGB[®] RT-PCR assay as the reflex test; although hypothesized algorithm would allow significant savings ($\sim 50.0\%$) compared to MF alone, costs would increase of $\sim 30.0\%$ in comparison to the proposed algorithm (Supplementary Table 1). Moreover, MF platforms are affected by a low processivity unless several modules are available, thus inducing an increase in TTR due to samples waiting to be analyzed.

Acknowledgments

We would like to thank all the technical staff of the Microbiology Unit, San Giuseppe Hospital, for the excellent assistance during the study.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethics

The study was approved by the Institutional Review Board of the Emergency Department of the Azienda USL Toscana Centro (n° 05220), and informed consent was obtained from all subjects.

Declaration of competing interest

This study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. All the authors declare the absence of any dual or conflicting interest.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:[10.1016/j.diagmicrobio.2022.115727](https://doi.org/10.1016/j.diagmicrobio.2022.115727).

References

- [1] Sohrabi C, Alsafi Z, O'Neill N, Khan M, Kerwan A, Al-Jabir A, et al. World Health Organization declares global emergency: a review of the 2019 novel coronavirus (COVID-19). *Int J Surg* 2020;76:71–6. doi:[10.1016/j.ijssu.2020.02.034](https://doi.org/10.1016/j.ijssu.2020.02.034).
- [2] Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med* 2020;382:727–33. doi:[10.1056/NEJMoa2001017](https://doi.org/10.1056/NEJMoa2001017).
- [3] World Health Organization. Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases: interim guidance. 2020. Available at: <https://apps.who.int/iris/handle/10665/331329>.
- [4] Ciotti M, Benedetti F, Zella D, Angeletti S, Ciccozzi M, Bernardini S. SARS-CoV-2 infection and the COVID-19 pandemic emergency: the importance of diagnostic methods. *Chemotherapy* 2021;66:17–23. doi:[10.1159/000515343](https://doi.org/10.1159/000515343).
- [5] Fujirebio. Fujirebio Europe announces CE Marking of the fully automated Lumipulse[®] G SARS-CoV-2 antigen assay and the rapid antigen device ESPLINE[®] SARS-CoV-2. 2022. Available at: <https://www.fujirebio.com/en/news-events/fujirebio-europe-announces-ce-marking-of-the-fully-automated-lumipulser-g-sarscov2>.
- [6] Hirotsu Y, Maejima M, Shibusawa M, Nagakubo Y, Hosaka K, Amemiya K, et al. Comparison of automated SARS-CoV-2 antigen test for COVID-19 infection with quantitative RT-PCR using 313 nasopharyngeal swabs including from seven serially followed patients. *Int J Infect Dis* 2020;99:397–402. doi:[10.1016/j.ijid.2020.08.029](https://doi.org/10.1016/j.ijid.2020.08.029).
- [7] Hirotsu Y, Maejima M, Shibusawa M, Amemiya K, Nagakubo Y, Hosaka K, et al. Prospective study of 1308 nasopharyngeal swabs from 1033 patients using the LUMIPULSE SARS-CoV-2 antigen test: comparison with RT-qPCR. *Int J Infect Dis* 2021;105:7–14. doi:[10.1016/j.ijid.2021.02.005](https://doi.org/10.1016/j.ijid.2021.02.005).
- [8] Gili A, Paggi R, Russo C, Cenci E, Pietrella D, Graziani A, et al. Evaluation of Lumipulse[®] G SARS-CoV-2 antigen assay automated test for detecting SARS-CoV-2 nucleocapsid protein (NP) in nasopharyngeal swabs for community and population screening. *Int J Infect Dis* 2021;105:391–6. doi:[10.1016/j.ijid.2021.02.098](https://doi.org/10.1016/j.ijid.2021.02.098).
- [9] Baccani I, Morecchiato F, Chilleri C, Cervini C, Gori E, Matarrese D, et al. Evaluation of three immunoassays for the rapid detection of SARS-CoV-2 antigens. *Diagn Microbiol Infect Dis* 2021;101:115434. doi:[10.1016/j.diagmicrobio.2021.115434](https://doi.org/10.1016/j.diagmicrobio.2021.115434).
- [10] Ogawa T, Fukumori T, Nishihara Y, Sekine T, Okuda N, Nishimura T. Another false-positive problem for a SARS-CoV-2 antigen test in Japan. *J Clin Virol* 2020;131. doi:[10.1016/j.jcv.2020.104612](https://doi.org/10.1016/j.jcv.2020.104612).
- [11] Kobayashi R, Murai R, Moriai M, Nirasawa S, Yonezawa H, Kondoh T, et al. Evaluation of false positives in the SARS-CoV-2 quantitative antigen test. *J Infect Chemother* 2021;27:1477–81. doi:[10.1016/j.jiac.2021.06.019](https://doi.org/10.1016/j.jiac.2021.06.019).
- [12] Regione Toscana. Emergenza COVID-19. Linee di indirizzo per la gestione del prelievo e delle analisi dei test molecolari, dei test antigenici e dei test sierologici nell'ambito delle infezioni da SARS-CoV-2. Sostituzione Allegato A della DGRT 1371/2020. Regione Toscana: Firenze, Italy; 2021. Available at: <http://www301.regione.toscana.it/bancadati/atti/DetttaglioAttiG.xml?codprat=2021DC00000000109>.