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Journal of Clinical Virology 142 (2021) 104942

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Paper from the 21st ESCV meeting

Detection of SARS-CoV-2 N protein allelic variants by rapid high-throughput CLEIA antigen assay

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The spreading of SARS-CoV-2 genetic variants may pose challenges in the identification of new positives. In particular, there are concerns with the possibility that SARS-CoV-2 genetic variants could escape antigen detection tests, that are commercially available and widely used [1]. In a recent report it is claimed that some rapid antigenic tests failed to identify rare SARS-CoV-2 variants circulating in Veneto (a region of Italy) [2]. The Authors speculated that the undetected variants contained mutations inside the major N antigen B cell epitope, which negatively affected the test results.

Here, we report the data obtained using an automated chemiluminescence enzyme immunoassay (CLEIA) for rapid antigen detection of SARS-CoV-2 (Lumipulse Fujirebio, Inc., Tokyo, Japan) with a number of variants. The system can detect and quantitatively estimate the presence of SARS-CoV-2 nucleocapsid protein in nasopharyngeal swabs or Saliva. The cut-off for positivity considered in this work was 1.34 pg/ml, as recommended by the Manufacturer.

The system was tested with 18 nasopharyngeal swabs positive for SARS-CoV-2 variants that had been identified by sequencing the whole genome to identify the variant type. The variants included five P.1 (501Y.V3 Brazil), seven B.1.1.7 (501Y.V1 UK), two B.1.351 (501Y.V2 South Africa), one rare variant related to the South African lineage (B.1.1.34), and three B.1 related variants: the B.1.258, the B.1.1.420 and the B.1.177.75. All these variants exhibited aminoacid substitutions inside or close to the functional N antigenic epitope, clustered from position 229 to 374 (Table 1).

In all cases the system returned a positive result (Table 1), suggesting that the protein region identified by Lumipulse is not particularly affected by mutations, allowing the detection of all the variants tested in this study.

In particular, all B.1.1.7 variants, including the one with the P279Q mutation, located around the N functional recognition domain, did not affect the result of the antigen test. The identification of the B.1.177.75 variant carrying the P365S mutation, considered as one of the disruptive amino-acid substitutions negatively influencing the results of the antigen tests [2], confirmed the accuracy of the Lumipulse assay. Similar results were obtained with the other variants, such as P.1, B1.1.34, B1.351 lineages.

For most of the tested samples, we found a correlation between the Ct and the Lumipulse values (Table 1). However, with two samples yielding similar Ct (Ct 23.3 and Ct 23.9), the corresponding Lumipulse readings were 1324.07 and 29.98 pg/ml. This might reflect a possible effect by the different genetic background: in fact, the B.1.1.7 specimen yielding the lower value had an additional mutation (P279Q) in the N protein gene (Table 1). For the remaining two samples with Ct > 30, the effect of genetic background of SARS-CoV-2 on antigen assay could not be assessed, since the Lumipulse readings were < 10 pg/ml. The low amount of protein (2.76 pg/ml) observed with the B.1.258 variant, having the aminoacid substitution P326R, was apparently related with a weak rRT-PCR positivity (Ct 34.5) of this specimen (Table 1), and close to the limit of detection.

The good performances of the test are evident and widely recognized [3,4]. Although we cannot exclude a lower sensitivity of this antigen test with respect to some genetic backgrounds, our experience confirmed that the CLEIA SARS-CoV-2 antigen assay used in this study, unlike many commercially available immunochromatographic tests [5], is a reliable and accurate tool for the detection of several of the new spreading variants and may be a valid platform that could be used for population screening purposes.

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https://doi.org/10.1016/j.jcv.2021.104942

Received 11 May 2021; Received in revised form 2 August 2021; Accepted 4 August 2021

Available online 8 August 2021

Table 1
SARS-CoV-2 variants identified by Lumipulse assay.

LINEAGE	N Ct*	N mutations#	GISAID	Lumipulse assay (pg/ml)
P.1	16.7	P80R, R203K, G204R	EPI_ISL_1,416,319	>5000
P.1	18.5	P80R, R203K, G204R	EPI_ISL_1,416,317	>5000
P.1	23.3	P80R, R203K, G204R,	EPI_ISL_1,416,312	1324.07
P.1	20.8	P80R, R203K, G204R	EPI_ISL_1,169,910	>5000
P.1	14.4	P80R, R203K, G204R,	EPI_ISL_1,163,691	>5000
B.1.1.7	20.4	D3L, R203K, G204R, S235F	EPI_ISL_1,416,315	>5000
B.1.1.7	20.3	D3L, R203K, G204R, S235F	EPI_ISL_1,416,320	>5000
B.1.1.7	17.9	D3L, R203K, G204R, S235F	EPI_ISL_1,169,911	4807.03
B.1.1.7	16.82	D3L, R203K, G204R, S235F	EPI_ISL_1,163,692	>5000
B.1.1.7	16.8	D3L, A156S, R203K, G204R, S235F	EPI_ISL_1,169,905	>5000
B.1.1.7	18.9	D3L, A156S, R203K, G204R, S235F	EPI_ISL_1,169,906	>5000
B.1.1.7	23.9	D3L, R203K, G204R, S235F, P279Q	EPI_ISL_983,097	29.98
B.1.258	34.5	P326R	EPI_ISL_911,527	2.76
B.1.1.420	17	P13S, A152S, S197T, R203K, G204R	EPI_ISL_1,195,961	>5000
B.1.177.75	15	A220V, P365S	EPI_ISL_1,195,962	>5000
B.1.351	22.7	T205I	EPI_ISL_1,163,689	4120.2
B.1.351	24.6	T205I	EPI_ISL_1,408,885	3663.38
B.1.1.34	32.2	R203K, G204R	EPI_ISL_1,408,886	7.61

*as determined by conventional rRT PCR testing with Allplex SARS-CoV-2 Assay (Seegene, Korea); #referred to the genomic sequence reported in GISAID.

Acknowledgments

This research received no specific grant from any funding agency in the public, commercial, or no-for-profit sectors. We declare no competing interests.

References

[1] FIND (Foundation for Innovative New Diagnostics). SARS-CoV-2 diagnostic pipeline. (continuously updated). https://www.finddx.org/covid-19/pipeline.
[2] C. Del Vecchio, G. Brancaccio, A.R. Brazzale, E. Lavezzo, F. Onelia, E. Franchin, L. Manuto, F. Bianca, V. Cianci, A. Cattelan, S. Toppo, A. Crisanti, Emergence of N antigen SARS-COV-2 genetic variants escaping detection of antigenic tests. PREPRINT, medRxiv (2021), https://doi.org/10.1101/2021.03.25.21253802.
[3] A. Gili, R. Paggi, C. Russo, E. Cenci, D. Pietrella, A. Graziani, F. Stracci, A. Mencacci, 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. 105 (2021) 391–396.
[4] T. Ishii, M. Sasaki, K. Yamada, D. Kato, H. Osuka, K. Aoki, T. Morita, Y. Ishii, K. Tateda, Immunochromatography and chemiluminescent enzyme immunoassay for COVID-19 diagnosis, J. Infect. Chemother. 27 (6) (2021) 915–918.
[5] S. Jungnick, B. Hobmaier, L. Mautner, M. Hoyos, M. Haase, A. Baiker, et al., Detection of the new SARS-CoV-2 variants of concern B.1.1.7 and B.1.351 in five SARS-CoV-2 rapid antigen tests (RATs), Germany, March 2021, Euro Surveill. 26 (16) (2021), 2100413.