Lumipulse® *G*

Literature Overview

Lumipulse *G* SARS-CoV-2 Ag. Nucleocapsid protein antigen assay.

EXCELLENT PERFORMANCE OF THE LUMIPULSE *G* SARS-COV-2 Ag ASSAY

- Excellent sensitivity and specificity
- Good correlation with RT-PCR
- Using nasopharyngeal swab and saliva samples
- High association with infectiousness

Lumipulse *G* SARS-CoV-2 Ag* was the first high-sensitive nucleocapsid protein antigen (Ag) assay launched on a fully automated chemiluminescent platform. It has been used by Japanese authorities since August 2020 for quarantine screening of arriving travelers in major international Japanese airports.[11,12,13, 14]

Fujirebio's advanced technology supports also multiple European test centers to set-up easily accessible, reliable and rapid COVID-19 testing e.g. testing in Germany's largest airports and screening in Italy for specific target communities such as schools, residential care homes, prisons to control and monitor the spread of the SARS-CoV-2 virus.[3,8,11]

The excellent performance, high quality and accessibility of the Lumipulse *G* SARS-CoV-2 Ag testing solution has been evaluated in multiple published studies.

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Lumipulse *G* SARS-CoV-2 Ag. Nucleocapsid protein antigen assay.

Excellent performance of the Lumipulse *G* SARS-CoV-2 Ag assay. Validated in multiple studies.

Ref.	Key word	Key word	Title	Reference
1	Evaluation Validation	Association with infectiousness	Prospective study of 1308 nasopharyngeal swabs from 1033 patients using the Lumipulse SARS-CoV-2 antigen test: Comparison with RT-qPCR	Hirotsu Y. *et al*. Int J Infect Dis. 2021;105:7-14
2		Association with infectiousness	Lumipulse G SARS-CoV-2 Ag assay evaluation using clinical samples from different testing groups.	Menchinelli G. *et al*. Clin Chem Lab Med. 2021;59(8):1468-1476
3		High NPV in both low and high prevalence setting	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.	Gili A. *et al*. Int J Infect Dis. 2021;105:391-396
4		Sensitive quantitative test	Clinical validation of quantitative SARS-CoV-2 antigen assays to estimate SARS-CoV-2 viral loads in nasopharyngeal swabs	Aoki K. *et al*. J Infect Chemother. 2021;27(4):613-616
5		Large scale study in low prevalence setting, including variants	Comparative analysis of antigen and molecular tests for the detection of SARS-CoV-2 and related variants: a study on 4266 samples	Caputo V. *et al*. Int J Infect Dis. 2021;108:187-189
6		Compared to other (automated) Ag assays	Comparison of Roche and Lumipulse quantitative SARS-CoV-2 antigen test performance using automated systems for the diagnosis of COVID-19.	Hirotsu Y. *et al*. Int J Infect Dis. 2021;108:263-269
7			Comparison of four commercial, automated antigen tests to detect SARS-CoV-2 variants of concern.	Osterman A. *et al*. Med Microbiol Immunol. 2021; 1-13
8		Rapid clinical diagnosis of patients in emergency department (ED)	The Challenge of Using an Antigen Test as a Screening Tool for SARS-CoV-2 Infection in an Emergency Department: Experience of a Tertiary Care Hospital in Southern Italy	Loconsole D. *et al. Biomed Res Int*. 2021; Volume 2021, Article ID 3893733, 7 pages
9	Nasopharyngeal and saliva samples	High detection performance	Immunochromatography and chemiluminescent enzyme immunoassay for COVID-19 diagnosis	Ishii T. *et al*. J Infect Chemother. 2021;27(6):915-918
10	Paired nasopharyngeal and saliva samples	Early symptomatic patients	Performance of qualitative and quantitative antigen tests for SARS-CoV-2 in early symptomatic patients using saliva	Yokota I. *et al*. Infect. Dis. Rep. 2021, 13(3), 742-747;

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Ref.	Key word	Key word	Title	Reference
11	Paired nasopharyngeal and saliva samples	Agreement associated with time since onset of symptoms	Salivary SARS-CoV-2 antigen rapid detection: A prospective cohort study	Basso D. *et al*. Clin Chim Acta. 2021;517:54-59
12	Self-collected saliva	Large scale screening	A Novel Strategy for SARS-CoV-2 Mass-Screening Using Quantitative Antigen Testing of Saliva	Yokota I. *et al*. http://dx.doi.org/10.2139/ssrn.3719066
13			Logistic advantage of two-step screening strategy for SARS-CoV-2 at airport quarantine	Norizuki M. *et al.* Travel Med Infect Dis. 2021;43:102127
14			Effective screening strategies for detection of asymptomatic COVID-19 travelers at airport quarantine stations: Exploratory findings in Japan	Norizuki M. *et al.* Glob Health Med.. 2021;3(2):107-111
15		Fresh versus frozen saliva samples	Saliva is a valid alternative to nasopharyngeal swab in chemiluminescence-based assay for detection of SARS-CoV-2 antigen	Amendola A. *et al*. J Clin Med. 2021;10(7):1471
16		Screening in primary schools using Salivette collection device	Effective screening strategy against SARS-CoV-2 on self-collected saliva samples in primary school setting: A pilot project.	Bordi L. *et al*. J Infect. 2021;83(1):e8-e10
17	Nasopharyngeal swabs	Analytical performance and set-up of diagnostic algorithm	SARS-CoV-2 Diagnostic Tests: Algorithm and Field Evaluation From the Near Patient Testing to the Automated Diagnostic Platform	Yin N. *et al.*Front Med. 2021 https://doi.org/10.3389/fmed.2021.650581

Lumipulse *G* SARS-CoV-2 Ag. Nucleocapsid protein antigen assay.

Excellent clinical performance has been shown in multiple studies

High sensitivity and specificity of the Lumipulse *G* SARS-CoV-2 Ag assay has been demonstrated in multiple studies for different target groups including both symptomatic and asymptomatic individuals, selected cohorts and unselected real-life cohorts such as schools, hospital health care workers, airport travellers, etc. showing comparable performance for different SARS-CoV-2 variants.[1,2,3,4,5]

The excellent clinical performance of the Lumipulse *G* SARS-CoV-2 Ag assay offers optimization of diagnostic, surveillance, and screening strategies in both small and large communities and in the general population.

Ref.	Target population	Number of samples	Clinical sensitivity	Clinical specificity	Overall concordance with RT-PCR
1	Hirotsu Y. *et al*. Int J Infect Dis. 2021;105:7-14				
	• Symptomatic group Asymptomatic high-risk contact group • Asymptomatic screening group	1033 nasopharyngeal swabs	92.5% (37/40)	100.0% (989/989)	99.7% (1026/1029)
2	Menchinelli G. *et al*. Clin Chem Lab Med. 2021;59(8):1468-1476				
	• Diagnostic group: symptomatic & asymptomatic-exposed • Monitoring-recovering Screening: asymptomatic without known exposure	594 nasopharyngeal swabs	79.9% (155/194) for Ct ≤40 97.6% (124/127) for Ct <30 100.0% (87/87) for Ct < 25	99.3% (397/400)	92.9% (552/594)
3	Gili A. *et al*. Int. J. Infect. Dis. 2021;105:391-396				
	Selected cohort (symptomatic and asymptomatic high-risk contact)	226 nasopharyngeal swabs	Standard cut-off (1.34pg/mL)		
			90.5% (86/95)	91.6% (120/131)	91.2 % (206/226)
			Optimized cut-off (1.24 pg/mL)		
			92.6% (88/95)	90.8% (119/131)	91.6% (207/226)
	Real-life unselected screening cohort (schools, hospital healthcare workers, etc)*	1738 nasopharyngeal swabs	Standard cut-off (1.34 pg/mL)		
			100.0% (90/90)	92.1% (1518/1648)	92.5% (1608/1738)
			Optimized cut-off (1.645 pg/mL)		
			100.0% (90/90)	94.8% (1562/1648)	95.1% (1652/1738)
4	Aoki K. *et al*. J Infect Chemother. 2021;27(4):613-616				
	Hospitalized patients with COVID-19 or suspected COVID-19	548 nasopharyngeal swabs	91.7% (22/24) overall 100.0% (12/12) for Ct ≤30	98.5% (516/524)	98.2% (538/548)

5	Caputo V. *et al*. Int. J. Infect. Dis. 2021;108 :187-189				
	Individuals which were enrolled in IRCSS reference center*	4266 naso-oropharyngeal swabs	86.6% (436/503) Overall	97.3% (3661/3763)	96.0% (4097/4266)
			100.0% (260/260) for viral load > 5.4log^{10} cp/mL		
8	Loconsole D. *et al*. Biomed Res Int. 2021 https://doi.org/10.1155/2021/3893733				
	Patients admitted to the emergency department of a tertiary care hospital, without previous history of SARS-CoV-2 infection	911 nasopharyngeal swab in universal transport medium (UTM)	All patients enrolled		
			94.9% 8/11 CLEIA + samples with Ct-value ≥30	97.4% (677/695)	96.8% (882/911)
			Asymptomatic		
			91.8%	**97.8%**	97.3% (535/550)
			Symptomatic		
			95.8%	**96.4%**	96.1% (347/361)
			≤7 days postsymptom		
			97.3%	**97.1%**	97.2% (242/249)
			>7 days postsymptom		
			93.6%	**89.5%**	92.4% (61/66)

*including samples containing SARS-CoV-2 variants

Compared to other automated Ag testing[6,7]

Hirotsu Y. *et al*. compared two Ag quantification tests (Elecsys and Lumipulse) and concluded that both tests accurately detect SARS-CoV-2 Ag in RT-qPCR-positive samples with high viral loads showing high diagnostic accuracy up to nine days after the onset of symptoms.

In addition, Ag levels correlated with viral loads and Ct values determined by RT-qPCR. Furthermore, it is mentioned that both the Roche and Lumipulse Ag tests can process large numbers of specimens using automated systems and compared to RT-qPCR the advantage of automated assays is their low cost, scalability, rapid turnaround time, low hands-on-time requirements, and lower error rates.[6]

Osterman A. *et al* compared four different automated SARS-CoV-2 Ag assays (Lumipulse, LIAISON, Euroimmun ELISA and Elecsys) and concluded that the available automated Ag tests for SARS-CoV-2 show variable performance with marked differences in analytical sensitivity and are not all necessarily superior to a rapid test. However, the Lumipulse *G* SARS-CoV-2 Ag assay showed superior sensitivity, higher than that of

the rapid antigen test. A good specificity of 97% was obtained, which is line with the Roche Elecsys assay (97.69%) however lower than the other Ag assays evaluated (99.67%-100%). Lumipulse SARS-CoV-2 Ag gave the best overall performance (AUC of 0.873) meaning that it offers the best combination of optimal sensitivity and specificity.[7]

Similar performance for SARS-CoV-2 virus variants

The genetic diversification of SARS-CoV-2 and the emergence of Variants of Concern (VOCs), which carry mutations not only in the spike region but also in the nucleocapsid region, raises concerns whether the SARS-CoV-2 tests are able to detect also these variants.
The currently designed Variants for Concern are Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2) and Omicron (B.1.1.529) according to WHO variant webpage (Tracking SARS-CoV-2 variants (who.int) update November 2021)
The Lumipulse *G* SARS-CoV-2 Ag assay detects the nucleocapsid protein (NP) of the SARS-CoV-2 virus in nasopharyngeal swabs and saliva samples using multiple monoclonal antibodies against the nucleocapsid protein region for detection. The epitopes used by these monoclonal antibodies do not include those mutated regions by currently known variants including the Omicron variant newly classified as VOC. The Lumipulse *G* SARS-CoV-2 Ag assay was tested with recombinant SARS-CoV-2 nucleocapsid protein containing the amino acid mutations associated with the known VOCs and found to be reactive for all mutations tested. Fujirebio is planning to evaluate the Lumipulse SARS-CoV-2 Ag assay by using the recombinant nucleocapsid protein of the SARS-CoV-2 and clinical samples of Omicron variant. As soon as new information becomes available, this will be communicated (Fujirebio Quality Statement date 6th of December 2021).

Preliminary genome sequencing results of some SARS-CoV-2 positive samples included in the screening cohort in the evaluation study of Gili A. *et al*. showed that Lumipulse *G* SARS-CoV-2 Ag assay detected both Gamma VOC (P.1) and Alpha VOC (B.1.1.7)[3].

The study conducted by Caputo V. *et al* on samples collected between December 2020 and February 2021 included 138 samples with the H69-V70 deletion within the S-gene, which was identified as suggestive for the Alpha variant. All samples were analyzed using the Lumipulse *G* SARS-CoV-2 Ag assay with a similar accuracy as other positive non-variant samples[5].

In Italy VOC202012/01-B1.1.7 lineage is circulating widely. In the Apulia region, the Alpha VOC was first detected at end of December 2020 and the estimated prevalence in March 2021 reached 93%. In this region, the antigen assays continue to be used successfully to detect SARS-CoV-2.[8]

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Lumipulse *G* SARS-CoV-2 Ag assay

Can be applied on both Nasopharyngeal swabs and Saliva samples

Early reliable identification of SARS-CoV-2 infection is key to reduce community transmission. Saliva testing for SARS-CoV-2 is one of the strategies for COVID-19 diagnosis and monitoring.

Saliva testing has significant advantages over nasopharyngeal swab testing. It is faster, less demanding for health care resources and it is non-invasive, allowing a more comfortable sample collection for the person to be tested.

Although Real Time-PCR (RT-PCR) methods are highly accurate and reliable, they are time-consuming, require dedicated laboratory equipment and experienced personnel, and they are more expensive. CLEIA (Chemiluminescent Enzyme Immunoassay) antigen testing requires simple laboratory instrumentation, usable by minimally trained personnel, easy implementation, and results are obtained within 30-35 minutes processing up to 120 samples per hour.[11,12]

However, none of these advantages support the use of saliva testing if results are not reliable.

A combination of CLEIA and NAAT (Nucleic Acid Amplification Test) in a two-step testing approach using self-collected saliva is evaluated as the most suitable strategy for mass screening of SARS-CoV-2. This strategy has greatly facilitated efficient managing of international passenger's flow at Japanese airports and it may benefit mass screening at other large venues[12,16] Testing on saliva is also advocated for screening asymptomatic individuals in order to rapidly detect and isolate infected individuals and their contacts, thus limiting viral spread and containing further waves of the pandemic.

Lumipulse *G* SARS-CoV-2 Ag testing has been demonstrated to be reliable and accurate also in saliva, with highest sensitivity achieved within the first week of onset of symptoms and/or high viral load (Ct-values <25).[9,10,11,15]

The importance of using freshly collected saliva has been demonstrated in the study of Amendola A. *et al.* as freezing and thawing saliva samples can have an impact on the stability and the structure of protein(s) which could lead to a suboptimal performance of the Ag detection assay.[15]

Lumipulse *G* SARS-CoV-2 Ag. Nucleocapsid protein antigen assay.

Excellent clinical performance using saliva samples has been shown in multiple studies

Ref.	Target population	Number of samples	Clinical sensitivity	Clinical specificity	Overall concordance with RT-PCR
9	Ishii T. *et al*. J Infect Chemother. 2021;27(6):915-918				
	• COVID-19 patients • non-COVID-19 patients	486 nasopharyngeal swabs	91.7% (22/24)	99.6% (460/462)	99.2% (482/486)
		136 saliva samples	88.9% (8/9)	96.9% (123/127)	96.3% (131/136)
10	Yokota I. *et al*. Infect Dis Rep. 2021;13(3):742-747				
	• Symptomatic COVID-19 patients • Asymptomatic patients COVID-19 negative persons in screening	17 paired nasopharyngeal swabs	100.0% (17/17)	99.3% (305/307)	Overall: 98.5% (336/341) Saliva: 98.5% (319/324)
		17 paired saliva, 307 negative saliva samples	82.4% (14/17)* *3 CLEIA neg. samples had Ct values ≥32		
11	Basso D. et al. Clin Chim Acta. 2021;517:54-59				
	• Symptomatic - confirmed COVID-19 cases • Asymptomatic - exposed (contact cases)	234 paired Nasopharyngeal swabs	Ct ≤ 40: 81.6%	93.8%	Not available
			Ct <26: 100.0%		
		234 paired Saliva samples	Ct ≤ 40: 71.8%* *optimal cut-off, sampling within 7 days from symptom onset	96.8%* *using optimal cut-off and for samples witing 7 days from symptom onset	Not available
			Ct <26: 100.0%		
12	Yokota I. *et al.* http://dx.doi.org/10.2139/ssrn.3719066				
	• Symptomatic: Covid-19 patient • Asymptomatic-exposed (high risk case contact) • Asymptomatic-screening (airport quarantine)	1924 saliva samples asymptomatic	72.9% (35/48)	99.3% (1863/1876)	98.6% (1898/1924)
		132 saliva samples symptomatic	80.5% (33/41)	97.8% (89/91)	92.4% (122/132)
		2056 saliva samples In total	76.4% (68/89)	99.2% (1952/1967)	98.2% (2020/2056)
13	Amendola A. *et al.* J Clin Med. 2021;10(7):1471				
	Fresh saliva samples • Positive • Negative	127 fresh saliva samples (85 negative samples of which 40 samples were from recovered COVID19 patients)	Ct<25: 92.8% (13/14)	94.1% (80/85)	Not available
			Ct<30: 75.0% (21/28)	100.0% (45/45)	Not available

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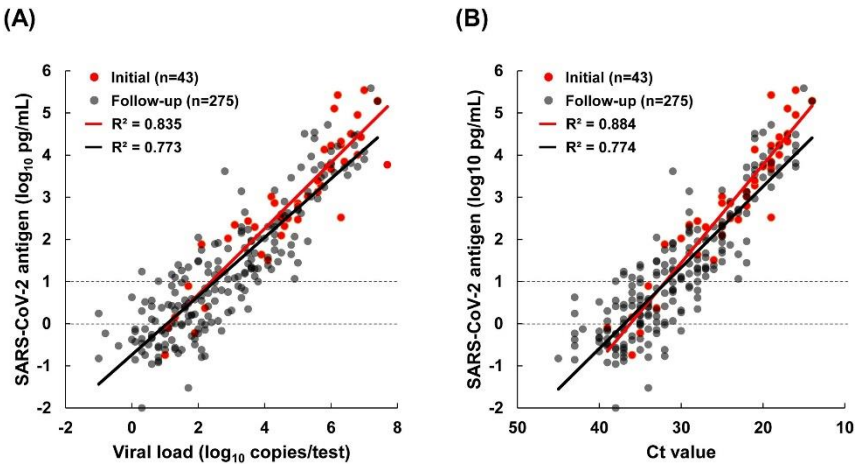
Quantitative measurement

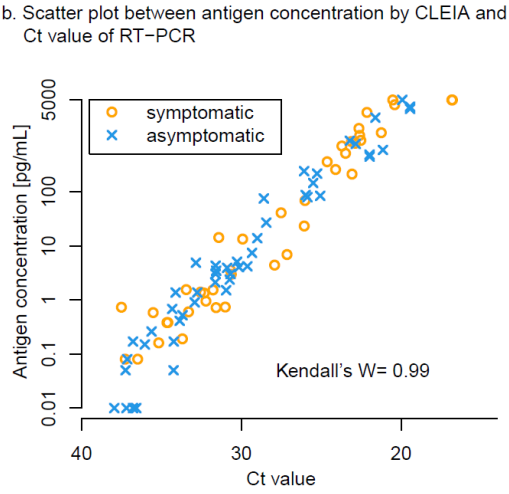
Measuring antigen concentrations by CLEIA as a novel approach for viral detection with equivalent utility compared to RT-PCR

High correlation between Lumipulse Ag values and RNA viral load values by RT-PCR has been shown in multiple studies.[1,2,5, 7,12]

For initial samples a high correlation between Ct value and antigen level was observed (R^2=0.835) while the follow-up samples showed a lower correlation (R^2=0.774)., variability showed an increase in follow-up samples which included lower viral load samples collected from hospitalized patients in late phase of infection or recovery.[1]



Comparison of the viral load between RT-PCR and Lumipulse *G* SARS-CoV-2 Ag [12]
Antigen concentration and Ct values determined by the PCR test using saliva are plotted as orange circles (symptomatic) and blue crosses (asymptomatic). A high correlation is indicated by Kendall's coefficient of concordance (0.99). Data were plotted with positive PCR tests.



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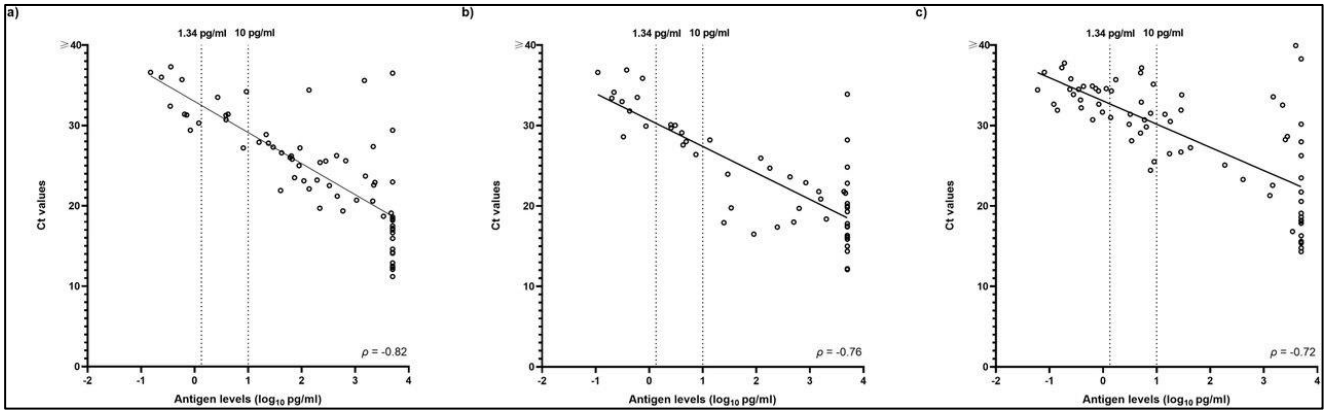
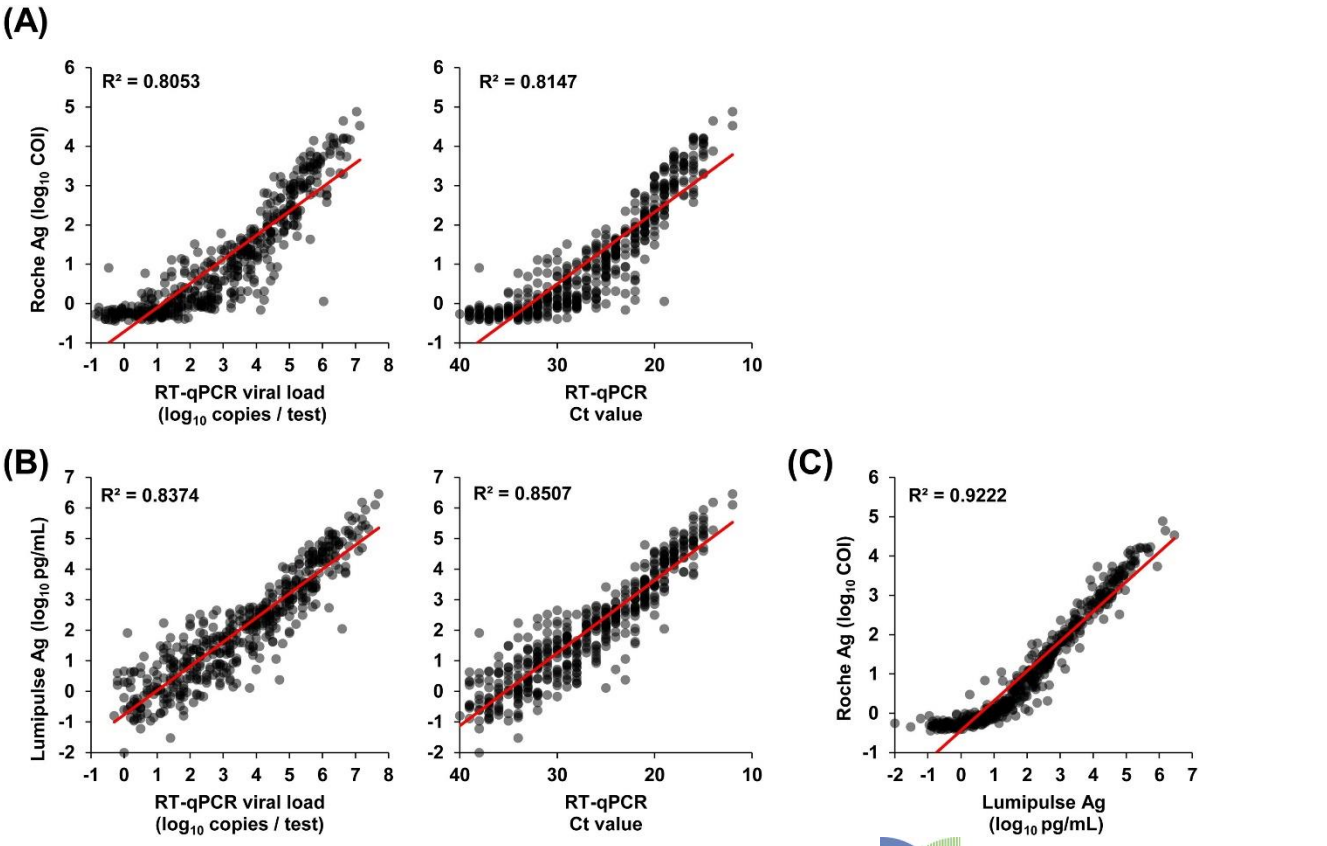
Significant association between antigen levels and Ct-values in either diagnostic (a), monitoring (b) or screening group (c).[2]



Hirotsu Y. *et al*. compared two automated SARS-CoV-2 Ag assays (Elecsys and Lumipulse) showing Ag levels correlated with viral loads and Ct values determined by RT-qPCR. A slightly better positive correlation was observed for the Lumipulse *G* SARS-CoV-2 Ag assay (B) versus the Elecsys assay (A): respectively R^2 =0.837 and 0.851 versus R^2 =0.805 and 0.815. The Lumipulse Ag levels and Elecsys COI values were highly correlated (C).[6]



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Lumipulse *G* SARS-CoV-2 Ag. Nucleocapsid protein antigen assay.

High association with infectivity - Detection of infectious people rather than infected people

Results highly suggest that the samples "missed" by Lumipulse *G* SARS-CoV-2 Ag are not containing active replicating virus.

Standard RT-PCR assays targeting SARS-CoV-2 genomic RNA are used as an indicator of viral presence. RT-PCR for subgenomic RNA was recently proposed as an indicator of active viral replication.

From 194 RT-PCR positive samples, 101 samples generated also subgenomic RNA positive result. All these 101 samples also generated a Lumipulse *G* SARS-CoV-2 Ag positive result.
The remaining 93 RT-PCR positive samples were negative for the presence of subgenomic RNA. From these 93 samples, 39 also gave a negative result for SARS-CoV-2 antigen with Lumipulse *G* SARS-CoV-2, whereas only 54 of these 93 samples gave a Lumipulse *G* SARS-CoV-2 Ag positive result. (Table 3 – publication by Menchinelli G. *et al*.)[2]

The drop in antigen levels appears to mark the point when levels of infectious viral particles diminish.[1]

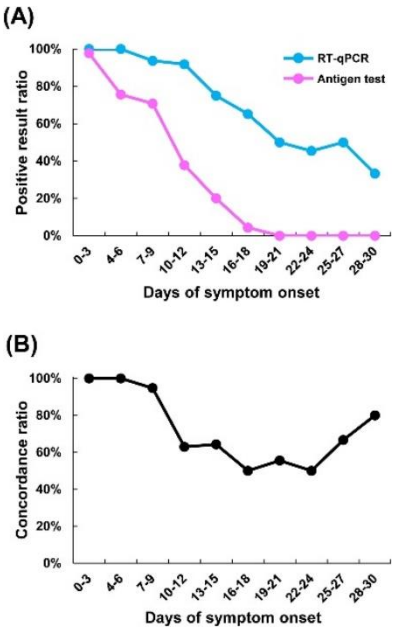


Figure A and B show the effect of the timing of sample collection since symptom onset on test results.
In total, 250 samples were collected from 36 symptomatic patients with COVID-19 until 30 days after symptom onset, and these samples were subjected to both the Lumipulse *G* SARS-CoV-2 Ag assay and quantitative RT-PCR (A). Positive result ratio was calculated by the number of positive samples on RT-qPCR or antigen test during the period since symptom onset divided by the total number of samples tested in that period.
(B) The concordance ratio indicates the percentage of samples with equivalent results from the antigen test and RT-qPCR when excluding samples with inconclusive antigen test results.

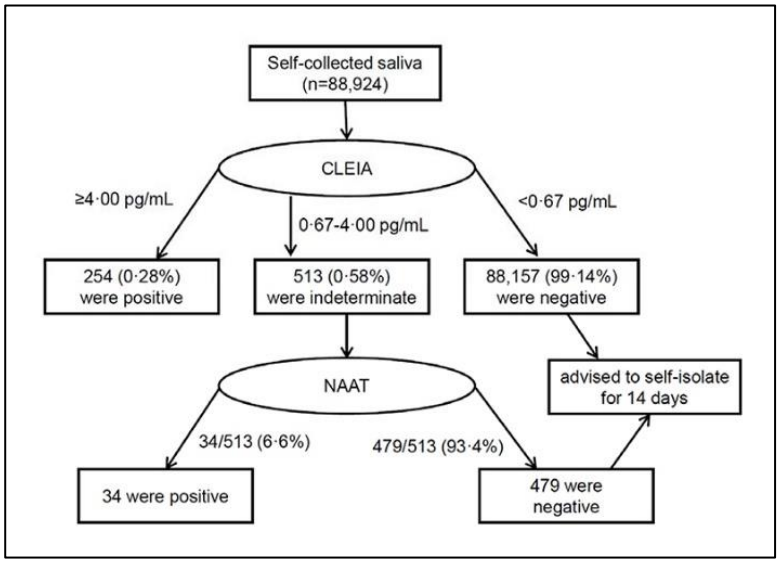
Discordant results in samples collected from persistent viral-shedding patients have been observed. These results suggested that viral load was low and protein translation was likely to be attenuated in host cells.
RT-PCR can reflect the presence of non-infectious viral "debris" in samples collected several weeks after symptom onset or recovery. RT-PCR is not able to directly indicate the presence of viable and infectious SARS-CoV-2 virus. Studies revealed that infectiousness of SARS-CoV-2 is maintained in clinical samples for only 8-10 days approx. after onset of symptoms.

Allows optimization of cut-offs according to target population

Lumipulse *G* SARS-CoV-2 Ag cut-offs can be adapted and chosen depending on the local epidemiology and objectives of the screening.

The wide dynamic range of the Lumipulse *G* SARS-CoV-2 Ag assay allows adjustment of the cut-off depending on the requirements of the specific laboratory performing the assay.[7]

A two-step testing algorithm with initial CLEIA combined with the use of a defined grey zone identifying samples needing confirmatory testing by NAAT, has been proven to be a highly reliable approach for improving diagnostic accuracy. Using the Lumipulse *G* SARS-CoV-2 Ag assay for first line screening in a two-step testing strategy would save 80-90% of RT-PCR tests needed.[12,17]



Flow chart of mass screening of international arrivals by the two-step strategy.[13]

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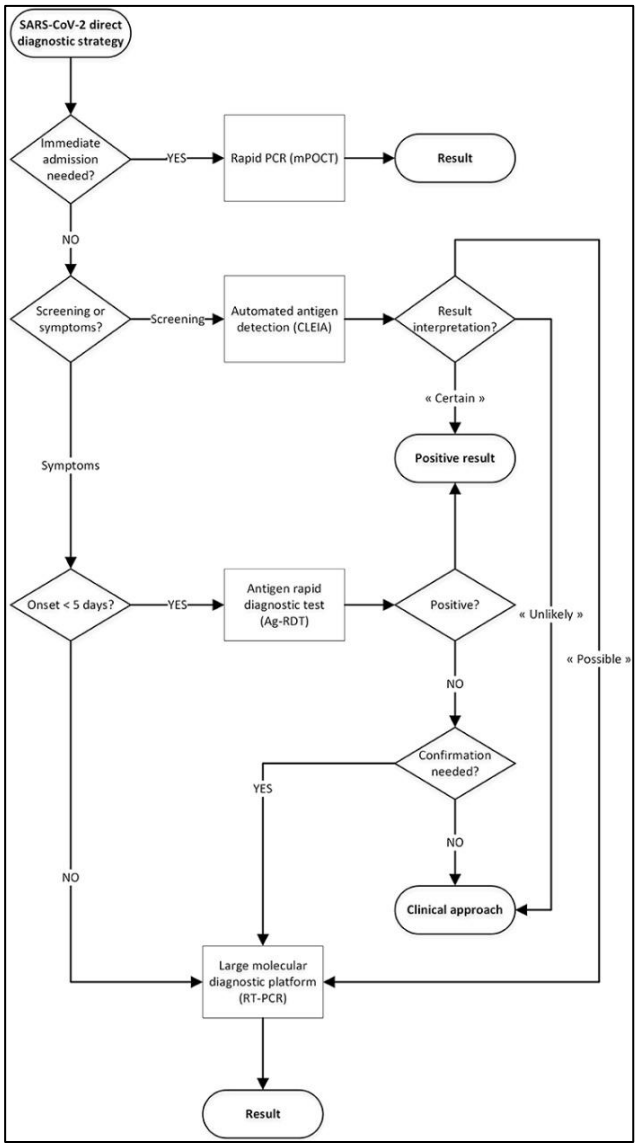
Proposal for a SARS-CoV-2 direct diagnostic decision algorithm[17]

The proposed hospital diagnostic algorithm is based on combined rapid molecular testing, automated Ag testing, rapid Ag testing and conventional RT-PCR molecular testing and four defined clinical situations (screening of asymptomatic patients, patients requiring immediate admission, symptomatic outpatients with symptoms lasting for either less or for more than 5 days).

Lumipulse *G* SARS-CoV-2 Ag cut-offs were adapted and chosen depending on the local epidemiology and objectives of the screening.

Using the Lumipulse *G* SARS-CoV-2 Ag assay for pre-admission screening of 93 asymptomatic patients would have saved 87 RT-PCR tests (93.5%) versus one missed low-positive (Ct value=26.04).



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Lumipulse *G* SARS-CoV-2 Ag assay

- Short time to result
- High throughput
- No need for specialized (molecular) laboratory equipment and technicians
- Lower cost versus molecular testing

Because of the short time to obtain the result (30 minutes), Lumipulse *G* SARS-CoV-2 Ag is particularly useful in facilities where it is difficult for many people to stay for a long time, such as airports.[4]

Reduction in analytical time allows speed-up testing while maximizing the number of tested individuals.[6,11]

Lumipulse *G* SARS-CoV-2 Ag is an interesting intermediary tool because of its higher throughput and sensitivity versus rapid Ag tests and faster time-to-result than RT-PCR.[6,17]