Full paper

Performance Assessment of the LIAISON® SARS-CoV-2 Antigen Assay On Nasopharyngeal Swabs

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SUMMARY

The SARS-CoV-2 pandemic is ongoing worldwide, causing prolonged pressure on molecular diagnostics. Viral antigen (Ag) assays have several advantages, ranging from lower cost to shorter turnaround time to detection. Given the rare occurrence of low-load viremia, antigen assays for SARS-CoV-2 have focused on nasopharyngeal swab and saliva as biological matrices, but their effectiveness must be validated. We assayed here the performances of the novel quantitative Liaison® SARS-CoV-2 Ag assay on 119 nasopharyngeal swabs and obtained results were compared with Hologic Panther and Abbott m2000 RT-qPCR. The Ag assay demonstrated a good correlation with viral load, shorter turnaround time, and favorable economics. The best performance was obtained in the acute phase of disease.

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INTRODUCTION

The ongoing COVID-19 pandemic has caused 248 million cases and 5 million deaths worldwide as of November 3, 2021. The pandemic has been a challenge for both wards and laboratories, with great pressure exerted on molecular diagnostics, namely SARS-CoV-2 RT-PCR. The supply of PCR reagents has often suffered shortages during the pandemic, and alternative direct or indirect diagnosis has been implemented. Antigen (Ag) assays have a timeframe of usability like that of RT-PCR, and have several advantages over molecular methods, ranging from stability to cheaper cost and shorter turnaround time. Both point-of-care (lateral-flow immunochromatographic assays (LFIA)) and laboratory setting (high-throughput instrument-based) Ag tests for SARS-CoV-2 have been released, with the former category, unfortunately, offering poor performance in terms of assay precision (Houston et al., 2021; Gremmels et al., 2021; James et al., 2021; Schildgen et al., 2021; Möckel et al., 2021; Favresse et al., 2021; Osterman et al., 2021; Young et al., 2020; Yamayoshi et al., 2020).

We report here our experience with the use of the Liaison® SARS-CoV-2 Ag assay (DiaSorin, Saluggia, Italy).

MATERIALS AND METHODS

Nasopharyngeal swab specimens

Residual diagnostic samples from 119 consecutive nasopharyngeal swabs (NPS), placed into sterile universal transport medium, were used for the study.

Samples were randomly chosen from patients hospitalized in COVID-dedicated wards, with a mean age of 78 (28-95), a clinical picture spanning from mild to critical, and in a period ranging from 0 to 95 days after the diagnosis of infection.

Molecular tests confirmed 92 samples as SARS-CoV-2 positive and 27 as virus-negative. After routine testing, aliquots of specimens were taken and stored at -80°C until use.

SARS CoV-2 genome detection

NPS had previously been tested for SARS-CoV-2 RNA using two different molecular platforms. The real-time SARS-CoV-2 assay (Abbott Molecular, Des Plaines, IL) is a qualitative test performed on the Abbott m2000 platform and targets the N and RNA dependent RNA polymerase (RdRp) genes of the viral genome.

The Aptima assay (Hologic, San Diego, CA) uses transcription-mediated amplification technology for SARS-CoV-2 RNA amplification. It targets two regions within the ORF1ab gene of the viral genome and is performed on the Panther instrument.

SARS CoV-2 Ag detection

On NPS samples previously analyzed by molecular methods, the LIAISON® SARS-CoV-2 Ag (DiaSorin, Saluggia, Italy) was performed according to the manufacturer's recommendations. The assay is a direct two-step sandwich chemiluminescence immunoassay, and quantitatively detects SARS-CoV-2 nucleocapsid protein Ag in NPS by using the LIAISON® XL analyzer. The analyzer automatically calculates SARS-CoV-2 Ag concentrations from 22 to 10^5 $TCID_{50}$/mL.

Statistical analysis

SPSS software version 23 (IBM, Chicago, IL, USA) was used for statistical analysis. Transformed Ag load in Log format was used for analysis. Chi-square test and Fisher's exact test were applied to evaluate the heterogeneity of contingency tables. The coefficient of determination R^2 (Spearman rho coefficient) was used to measure the overall correlation between methods. To test the repeatability of the Ag method, four samples were tested in quadruplicate, and the coefficient of variation (CV) was calculated. All p values presented are based on two-tailed tests, and $p < 0.05$ was considered statistically significant.

RESULTS

The 119 randomly selected NPS were tested both by molecular and antigenic methods. Considering the 92 molecular-positive specimens, 84% (77) were detected as positive when the LIAISON® SARS-CoV-2 Ag was applied, whereas 100% (27) of samples molecularly detected as virus negative also tested negative by the Ag assay. Overall, the Ag assay concordance was 87% (104 of 119 samples), with good agreement.

Interesting, a reverse correlation (r = -0.74) between the Liaison® SARS-CoV-2 Ag assay and the cycle threshold in Abbott m2000 RT-qPCR was observed (Figure 1A). Of note, the sensitivity of Ag assay was 84% overall when compared to Abbott m2000, but 100% for samples with Ct <22 (Table 1).

Instead, a lack of correlation was noted between Liaison® SARS-CoV-2 Ag assay and the RLU readings from Hologic Panther (Figure 1B and Table 2). This is not unexpected given the lack of a precise correlation between RLU and viral loads.

Of note, 94.4% sensitivity was observed when patients tested within 10 days post onset of symptoms, but dropped to 82% when all patients were considered, in a period ranging from 0 to 95 days after diagnosis of infection.

Finally, intra precision and reproducibility of the Ag assay were investigated by testing, in four independent experiments, a panel of 4 samples having different Ct values by Abbott platform. The differences between measured $TCID_{50}$/ml values were small and the mean CV variation was 0.0185 (Table 3).

DISCUSSION

Given the transient and low-grade viremia, antigen tests for SARS-CoV-2 have mainly focused on NPS and saliva as the biological matrix. Nasal and nasopharyngeal samples usually harbor higher viral loads because of the higher number of replication-competent cells, and most assays should be used within 10 days from the onset of symptoms. Assays can be classified according to the setting for usage (laboratory vs. point-of-care testing (POCT)) or according to the detected antigen (spike vs. nucleocapsid protein).

Many POCT based on immunochromatographic LFIA have entered the market (e.g., Abbott's BinaxNOW™ COVID-19 Ag Card (James et al., 2021, Young et al., 2020, Okoye et al., 2021, Kashiwagi et al., 2021) or Roche's Panbio COVID-19 Ag Rapid Test Device (Gremmels et al., 2021, Mak GCK et al.,2021, Linares et al., 2020, Fenollar et al., 2021, Fund 2021), but they generally perform worse than instrument-based antigen assays (sensitivities and specificities in the 70-80% range vs. >90%, respectively). Table 4 summarizes the currently marketed high-throughput, laboratory setting SARS-CoV-2 Ag assays for use with nasal, oropharyngeal or nasopharyngeal swab (Kashiwagi et al., 2021, Chen et al., 2021).

To date, few studies have compared the performance of Ag versus molecular assays in nasopharyngeal swabs, but none of them tested the effectiveness of Ag assays on SARS CoV-2 detection. The LIAISON® SARS-CoV-2 Ag arises as an alternative solution to identify acute COVID-19 infection, with a declared 94.6% sensitivity on nasopharyngeal swabs. Our study represents the first validation of the test against two robust RT-qPCR and TMA platforms, suggesting that the Ag assay can be used reliably in the laboratory as an alternative to molecular testing whenever high viral loads are suspected and in the acute phase of the disease. In these situations, the LIAISON® SARS-CoV-2 Ag test supplies quick and reliable results, contributing to contain the virus spread.

Declaration of Conflicting Interests

We declare we have no conflicts of interest related to this manuscript.

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Table 1. Percentage of positive Liaison® SARS-CoV-2 Ag assay readings according to different cycle threshold ranks in Abbott m2000

Cycle threshold value (Abbott m2000)	No. examined	Liaison® SARS CoV-2 Ag	
		Positive (%)	$TCID_{50}$ per ml (mean ± standard error)
< 22	35	35 (100)	17039 ± 5649
22 - 26	25	16 (64)	3902 ± 2681
> 26	7	3 (43)	30 ± 3
Total	*67*	*54 (81)*	*12051 ± 3776*

Table 2. Percentage of positive Liaison® SARS-CoV-2 Ag assay readings according to different RLU ranks in Hologic Panther

RLU (Hologic, Panther)	No. examined	Liaison® SARS CoV-2 Ag	
		Positive (%)	$TCID_{50}$ per ml (mean ± standard error)
< 1246	13	12 (92)	19833 ± 11097
≥ 1246	12	11 (92)	15678 ± 9579
Total	*25*	*23 (92)*	*17846 ± 7230*

Table 3. Reproducibility of Liaison® SARS-CoV-2 Ag assay readings

Sample / Ct value (Abbott m2000)	Liaison® SARS-CoV-2 Ag assay determination ($TCID_{50}$/ml)				CV value
	A	B	C	D	
1 / 22	1523	1458	1500	1484	0.018
2 / 24	371	363	362	372	0.014
3 / 25	208	213	203	201	0.026
4 / 26	186	184	188	181	0.016

Table 4. Summary of high-throughput, laboratory setting SARS-CoV-2 Ag assays for use with nasal swabs, nasopharyngeal swabs, or oropharyngeal swab. N: nucleocapsid. S: spike.

Vendor	Instrument	Kit brand	Ag	Method	TAT (min)	Ref
Roche	Cobas e411 analyzer, cobas e601/e602 modules, cobas e801 module	Elecsys® SARS-CoV-2 Antigen	N	One-step double Ab sandwich IA	18	n.a. https://diagnostics.roche.com/global/en/products/params/elecsys-sars-cov-2-antigen-test.html
DiaSorin	LIAISON® XL, LIAISON® XS and LIAISON®	Liaison® SARS-CoV-2 Ag	N	Sandwich IA	42	Lefever et al., 2021, Häuser et al., 2021, Hartard et al., 2021 https://www.diasorin.com/sites/default/files/allegati_prodotti/liaisonr_sars_cov-2_antigen_pag_sing_m0870004372_a_digital_lr.pdf
BBB Inc. (by Celltrion USA, Inc.)	SAMPINUTE™ Analyzer	Sampinute COVID-19 Antigen MIA	RBD	Magnetic Electrochemical Sandwich IA (MESIA)	10	n.a. https://www.celltrion.com/en-us/kit/sampinute

Quidel Corporation	Sofia and Sofia 2 instrument	Sofia SARS Antigen FIA	N	Sandwich IFA	15	(Young et al., 2020, Pray et al., 2021, Beck et al., 2021) https://www.quidel.com/sites/default/files/product/documents/EF1438903EN00.pdf
Becton, Dickinson and Company	BD Veritor™ Plus Analyzer	BD Veritor System for Rapid Detection of SARS-CoV-2	N		15	(Young et al., 2020) https://www.bd.com/documents/guides/directions-for-use/IDS_BD-Veritor-Plus-SARS-CoV-2-500048916_DF_EN.pdf
Meso Scale Discovery	MSD	e S-PLEX ®		Electrochemiluminescence (ECL)		(Pollock et al., 2021)
Quanterix Corporation	Simoa HD-X Analyzer	Simoa® SARS-COV-2 N Protein Antigen Test	N			n.a.
OrthoClinical Diagnostics	VITROS 3600 Immunodiagnostic System and the VITROS 5600/XT 7600 Integrated Systems	VITROS Immunodiagnostic Products SARS-CoV-2 Antigen Reagent Pack	N			n.a.
Fujirebio	LUMIPULSE G1200	Lumipulse® G SARS-CoV-2 Ag	N		35’	(Aoki et al 2020, Hirotsu et al., 2020)
Luminostics, Inc	Clip Analyzer	Clip COVID Rapid Antigen Test	N			n.a.

LumiraDx UK Ltd. SARS-CoV-2	LumiraDx Platform	LumiraDx SARS-CoV-2 Ag Test	N	Microfluidic immunofluorescence assay	(Kohmer et al., 2021)

Figure 1. A) Correlation of Liaison® SARS-CoV-2 Ag assay readings (logTCID$_{50}$/ml) with cycle threshold values on Abbott m2000 RT-qPCR. B) Correlation of Liaison® SARS-CoV-2 Ag assay readings (logTCID$_{50}$/ml) with RLU values on Hologic Panther TMA.