Performance evaluation of antigen test (iFlash-2019-nCoV Antigen®) for detection of SARS-CoV-2 virus in serum samples

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Abstract

Molecular assays from nasopharyngeal swabs are the current reference method to diagnose COVID-19. As an alternative, we evaluated the performance of the iFlash-2019-nCoV Antigen® (YHLO, Shenzhen, China), developed for SARS-CoV-2 N-antigen detection in serum samples. Specificity, determined on 50 pre-pandemic samples, was 100%. Overall sensitivity, evaluated on 40 sera from patients with RT-PCR confirmed infection, was 67.5%. However, sensitivity reached 73% in symptomatic patients, 80% in patients with high and medium nasopharyngeal (NP) viral loads (samples with Ct[?]33) and, 90% in samples collected within the first week after symptoms onset. These sera were further analyzed with the COV-QUANTO(r) ELISA and COVID-VIRO(r) LFIA assays (AAZ, Boulogne-Billancourt, France). EIA Ag assays from Yhlo and AAZ had comparable performances, and both were more sensitive than the LFIA. These findings suggest that SARS-CoV-2 N-antigen detection in serum could be an alternative to PCR from NP swabs, at least early after onset of symptoms. Further studies are required to confirm these results.

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Abstract: Molecular assays from nasopharyngeal swabs are the current reference method to diagnose COVID-19. As an alternative, we evaluated the performance of the iFlash-2019-nCoV Antigen® (YHLO, Shenzhen, China), developed for SARS-CoV-2 N-antigen detection in serum samples. Specificity, determined on 50 pre-pandemic samples, was 100%. Overall sensitivity, evaluated on 40 sera from patients with RT-PCR confirmed infection, was 67.5%. However, sensitivity reached 73% in symptomatic patients, 80% in patients with high and medium nasopharyngeal (NP) viral loads (samples with Ct[?]33) and, 90% in samples collected within the first week after symptoms onset. These sera were further analyzed with the COV-QUANTO(r) ELISA and COVID-VIRO(r) LFIA assays (AAZ, Boulogne-Billancourt, France). EIA Ag assays from Yhlo and AAZ had comparable performances, and both were more sensitive than the LFIA. These findings suggest that SARS-CoV-2 N-antigen detection in serum could be an alternative to PCR from NP swabs, at least early after onset of symptoms. Further studies are required to confirm these results.

Keywords: SARS-CoV-2, antigen, serum, COVID-19, diagnostic

Severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) infection has caused a global pandemic since early 2019 and has become a major public health concern all over the world (1, 2). Therefore a specific, sensitive, and rapid SARS-CoV-2 diagnostic method is crucial for reducing the disease spread.

Nucleic Acid testing, primarily by real-time reverse-transcription Polymerase Chain Reaction (RT-PCR), from nasopharyngeal (NP) swabs remain the cornerstone of COVID-19 diagnostic (3). However, RT-PCR tests require experienced laboratories, are expensive and may have relatively long turnaround times (4, 5). False negative rates of SARS-CoV-2 RT-PCR assays up to 30% have been reported and Covid-19 diagnosis in these symptomatic patients is then inferred mostly by typical findings at chest computed tomography (6). Hence, alternative complementary assays such as antigen detection tests could contribute in improving SARS-CoV-2 diagnosis.

In this study, we aimed to evaluate the performance of the iFlash-2019-nCoV Antigen(r) (YHLO, Shenzhen, China), (YHLO Ag), a Chemiluminescent immunoassay, run on the iFlash 1800 analyzer (YHLO, Shenzhen, China) for SARS-CoV-2 N-antigen in serum samples .

Specificity was assessed on 50 pre-pandemic serum samples collected in 2019.

Sensitivity was evaluated on 40 serum samples collected on the same day as the NP sample in patients with a positive RT-PCR in NP sample (range of Ct values: 11-41 with Alinity m SARS-COV-2 assay, Abbott Molecular). Of these, 3 were collected in asymptomatic patients (range Ct values 39-41) and 37 in symptomatic patients (range Ct values 11-40).

All pre-pandemic samples were negative with the YHLO antigen test, the specificity was therefore 100%. Compared to NP RT-PCR, the overall sensitivity of YHLO Ag was 67.5% (27/40). The YHLO Ag assay was able to detect N antigen in the serum of patients with high (Ct<23), medium (23[?]Ct<33) and low (33[?]Ct) NP viral loads with a 85.7% (6/7), 75% (6/8) and 14.3% (1/7) sensitivity, respectively. Antigenic result was negative in all asymptomatic patients (0/3), and positive in 73% (27/37) of the symptomatic ones. In addition, N antigen detection rate by time after onset of symptoms was 90% (18/20) on samples collected before day 7; 66,7% (6/9) on samples collected between day 7 and 14; and 33,3% (2/6) on sera collected after 14 days (table 1). This low antigen sensitivity beyond 14 days has been linked to anti-N IgG seroconversion (5). Indeed, antigen detection rate was 92.9% (13/14) in samples without detectable total anti-N antibodies (Elecsys(r) Anti-SARS-CoV-2 immunoassay, Roche), and only 44.4% (8/18) in patients with detectable antibodies (p=0.004) (Table 2). In addition, samples with positive N-antigenemia exhibited lower anti-N antibody index: mean +/- SD indexes were 4.39 +/- 7.43 and 35.52 +/- 39.50 for samples with positive and negative antigenemia, respectively (p = 0.001).

Serum samples from Covid-19 patients were further analyzed with the microplate ELISA COV-QUANTO immunoassay (r) (AAZ, Boulogne-Billancourt, France) (AAZ ELISA) and the Lateral Flow Immunoassay (LFIA) COVID-VIRO (r) (AAZ, Boulogne-Billancourt, France) (AAZ RDT). Concordance was 92.3% (36/39 samples) between YHLO Ag and AAZ ELISA, and 92% (23/25 samples) between YHLO Ag AAZ RDT (Table 3). YHLO Ag assay's performances were comparable to AAZ ELISA test, and both EIA assays were more sensitive than the LFIA.

In summary, the iFlash-2019-nCoV Antigen(r) (YHLO) had an excellent specificity (100%) and an overall sensitivity of 67.5%, compared to NP RT-PCR. However, sensitivity was 73% in symptomatic patients; 80% in patients with high and medium NP viral loads (12/15 samples with Ct[?]33), a surrogate marker of infectivity (7), and reached 90% in samples collected within 7 days after onset of symptoms. We acknowledge that the number of samples is small and that further studies are needed to confirm our results. Nevertheless, SARS-CoV-2 antigenemia is already emerging as a useful as a complementary test to improve COVID-19 diagnosis, especially in patients with a high clinical suspicion or typical imaging findings for COVID-19 and several PCR-negative NP samples. Additionally, SARS-CoV-2 antigenemia could be an alternative to NP swabs in patients that refuse or have contra-indication of this type of sampling (8).

In conclusion, SARS-CoV-2 antigenemia could be offered as a complementary diagnostic tool for Covid-19. Additional studies are needed to determine its position in the diagnostic arsenal.

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Table 1: Sensitivity of the iFlash-2019-nCoV Antigen(r) according to NP viral load, serum sampling from symptoms' onset and presence of symptoms.

Ag Detection in serum	**NP viral load**	**NP viral load**	**NP viral load**	**NP viral load**	**NP viral load**	**NP viral load**
	High (CT<23)	Medium (23[?]Ct<33)	Medium (23[?]Ct<33)	Low (33[?]Ct)	Low (33[?]Ct)	Overall sensitivity
	85.7% (6/7)	75% (6/8)	75% (6/8)	14.3% (1/7)	14.3% (1/7)	59.1% (13/22)
	Serum sampling from symptoms' onset	**Serum sampling from symptoms' onset**	**Serum sampling from symptoms' onset**	**Serum sampling from symptoms' onset**	**Serum sampling from symptoms' onset**	**Serum sampling from symptoms' onset**
	< 7	Between 7 and 14	Between 7 and 14	> 14	> 14	Overall sensitivity
	90% (18/20)	66.7% (6/9)	66.7% (6/9)	33.3% (2/6)	33.3% (2/6)	74.3% 26/35
	Presence of symptoms	**Presence of symptoms**	**Presence of symptoms**	**Presence of symptoms**	**Presence of symptoms**	**Presence of symptoms**
	yes	yes	no	no	Overall sensitivity	Overall sensitivity
	73% (27/37)	73% (27/37)	0% (0/3)	0% (0/3)	67.5% 27/40	67.5% 27/40

Table 2: SARS-CoV-2 N-antigenemia according to total anti-N total antibody status.

		anti-N total antibodies	anti-N total antibodies
		Positive	Negative
YHLO Ag	Positive	8	**13**
	Negative	**10**	1

Cut-off values: 1.00 COI for iFlash-2019-nCoV Antigen® (YHLO Ag) and 0.80 U/mL for Elecsys® Anti-SARS-CoV-2 immunoassay (Roche Diagnostics)

Table 3: Concordance between YHLO Ag and AAZ Ag Assays (ELISA, RDT).

		AAZ ELISA	AAZ ELISA	AAZ ELISA	AAZ RDT	AAZ RDT
		Positive	Negative	Positive	Positive	Negative
YHLO	Positive	25	**1**[a]	12	12	**2** [b]
	Negative	**2** [c]	11	**0**	**0**	11

[a]: no Ct value available; sample collected 2 days after symptoms onset. [b] Ct values were 17 and 32, samples collected 8 and 13 days after symptoms onset.[c] one sample with no available Ct collected 2 days after symptoms onset, and one with a Ct value of 20, collected 7 days after symptoms onset.