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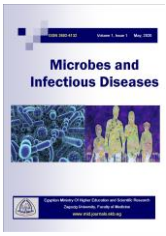

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Original article

Role of SARS-COV2 antigen as a diagnostic test for COVID-19

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ABSTRACT

Background: Successive waves of SARS-CoV-2 infections with increasing transmission rates may burden the laboratories performing molecular diagnostic testing. Alternative diagnostic methods may provide additional diagnostic capacity. Chemiluminescent totally automated antigen detection test for SARS-CoV-2 (Ortho VITROS SARS-CoV-2 antigen test) could be satisfactory replacement for reverse-transcription quantitative polymerase chain reaction (RT-qPCR) for mass screening during outbreaks. **Methods:** RT- qPCR and the VITROS® SARS-CoV-2 antigen were compared. Antigen detection test was assessed using clinical samples (nasopharyngeal swabs in viral transport medium) withdrawn from 668 patients suspected to have SARS-CoV-2 infection. **Results:** From 668 samples, 303 showed SARS-CoV-2 antigens positive and 365 SARS-CoV-2 antigens negative in comparison with RT-qPCR, the sensitivity was 89.11% and the specificity was 100.0% (PPV 100.0 and NPV 91.7). Ct value of 16.0 was the limit of detection of the assay. **Conclusion:** The given results show that VITROS® assay was acceptable for the detection of patients having contagious COVID-19 in the clinical setting. This test showed high sensitivity and specificity in the SARS-CoV-2 detection in samples with a Ct value of 32 or less. Chemiluminescent full automated antigen detection test for SARS-CoV-2 is a feasible substitute to (RT-qPCR) for mass screening.

Introduction

New coronavirus was recognized in 2019 as the cause of a disease outbreak that originated in China. Corona virus disease-19 (COVID-19) gives different clinical presentations, ranges from mild symptoms to severe lung pneumonia that necessitate a ventilator, as well thrombotic strokes, multi-inflammatory syndrome, and others [1].

COVID-19 had developed into a worldwide pandemic and is ongoing to expand, producing considerable impact on economy, patients' morbidity, mortality [2].

The accuracy of epidemiological information has been a milestone in the evolution of effective measures for containment and mitigation of COVID-19, but till now, determination of case definitions has been complicated by the broad clinical picture of COVID-19. COVID-19 diagnosis is mainly done by RT-PCR to detect SARS-CoV-2 viral RNA in respiratory swab specimens, but pre-analytical as well as analytical obstacles had restricted the usage of molecular techniques as a tool for screening of SARS-CoV-2 [3].

Antigen tests could detect the active viral infection, but not the recovery situation. Antigen tests is more reliable than antibody tests as antigens are present before antibodies and are specific to the

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target. Antigen tests can be done on Lateral Flow Immunoassay for quick diagnostic purposes or in ELISA format for highly sensitive and high throughput uses [4].

The plenty of detected molecular material also diminishes with time, and various studies had illustrated that the positive rate of RNA may decrease below 30% by 3 weeks after the start of the symptoms [5]**.** The antigen detection tests usually detect the nucleocapsid proteins of the SARS-CoV-2 that are increased during infection activity. The antigen detection tests could be affected by multiple factors such as collection time, the specimen quality, virus concentration in the collected samples, and reagents quality. The sensitivity of the antigen detection is lower than molecular tests [6], and it is generally positive when there is a very high viral load and the patient is highly contagious, which is observed, in the initial period of infection during the asymptomatic phase (1–2 days) and few days after symptoms onset (5–7days). The antigen test is usually negative when the value of Threshold cycle (Ct) the real-time PCR is above 30, while its sensitivity elevates with Ct values below 25 [7]**.**Therefore, a negative result could not exclude a SARSCoV-2 infection, and could not be used to give guidance for patient discharge decision. however, rapid antigen tests could be beneficial in high prevalence settings, where a positive result most likely gives a true positive predication in the presence of high viral loaded asymptomatic carriers where facilitate contact tracing [8]**.**

Materials and Methods

Six hundred and sixty-eight nasopharyngeal swab specimens were collected from patients suspected of being infected with SARS-CoV-2 at the Armed Forces Laboratories for Medical Research and military hospitals, Cairo, Egypt. Between April 2021 and July 2021.

Asymptomatic carriers were identified as laboratory confirmed COVID-19 patients with no history of clinical signs or symptoms on admission.

SARS COV-2 antigen

SARS COV-2 antigen (VITROS SARS-COV2 Antigen immunoassay- UK) is a chemiluminescent immunoassay for qualitative detection of nucleocapsid protein of SARS COV-2, it was performed using nasopharyngeal swab specimens. The test was done on the VITROS® 3600 automated immunoassay analyzer (Ortho Clinical Diagnostics). The analytical results were reported as signal/cutoff (S/C) values, where ≥1.0 was defined as a positive test result (Reactive) and <1.0 as a negative test result (Non-Reactive) according to manufacturer instruction.

SARS COV-2 Real time PCR

Definitive confirmed diagnosis of COVID-19 patients was done by RT-qPCR according to the nationally recommended protocol, using RNA extracted, RNA extraction was done by (Perkin Elmer –Uk) on (Chemagic d 360-UK), Amplification and detection was done by (V2 Thermofischer – UK) on (QUANTI STUDIO 5 real time PCR-UK) and it was performed according to the manufacturer's instructions.

Results

Total 668 nasopharyngeal swab specimens were collected from patients suspected to be infected with SARS-CoV-2. Three hundred and three (45.36%) were RT-qPCR positive and 365 (54.64%) were found to be negative by RT-qPCR **(Table 1)**.

On other hand, VITROS SARS-COV2 Antigen was done to the same study groups. Two hundred seventy (40.48%) were Reactive while 398 (59.58%) were non-Reactive **(Table 1)**.

Antigens were detected in 270 out of 303 RT-qPCR-positive samples (89.11% sensitivity) (Table1& 2). All RT-qPCR-negative samples (n = 365) gave negative results (100% specificity), (p-Value= <0.001), using SARS COV-2 RNA detection as the reference **(Table1& 2)**.

The correlation between the Ct value obtained by RT-qPCR and the amounts of SARS-CoV-2 antigens determined by the VITROS® SARS-CoV-2 Antigen test within RT-qPCR positive group was negative correlation as Spearman's rho test value was (-0.624) with p-Value <0.001 was significant **(Table 3)**.

The sensitivity of SARS-Cov-2 antigen test using SARS-Cov-2 RNA detection as the reference. In patients with Ct value <=20 sensitivity was 100% (95% CI: 91.78 - 100.0), Ct value >20 - <=25 sensitivity was 95% (95% CI: 83.08 – 99.39), Ct value >25 - <=30 sensitivity was 78.38% (95% CI: 61.79 – 90.17) and Ct value >30 sensitivity was 50% (95% CI: 29.93 – 70.07) **(Table 3) (Figure 3)**.

In patients with Ct value <=30 sensitivity was 91.78% (95% CI: 85.56 – 96.03) and Ct value >30 sensitivity was 59.38% (95% CI: 40.46 – 76.3) **(Table 3) (Figure 3)**.

Table 1. Relation between RT-qPCR and VITROS SARS-COV2 antigen.

Whole group (N= 668)		PCR	
		Negative (N= 365)	**Positive** (N= 303)
		N (%)	N (%)
Ag Reactivity	Non-Reactive (N= 398)	365 (100.0%)	33 (10.9%)
	Reactive (N= 270)	0 (0.0%)	270(89.1%)

Table 2. Sensitivity and specificity for VITROS SARS-COV2 Antigen as a diagnostic test for SARS-Cov-2.

	Sensitivity	Specificity	+PV	-PV	Accuracy	*p*-value
Total Study group (N= 668)	89.11%	100.0%	100	91.7	92.58%	<0.001*

**p*-value significant at (<0.05)

Table 3. Correlation between Ct value obtained by RT-qPCR and the amounts of SARS-CoV-2 antigens.

PCR positive group		**CT value**
Amount of SARS-Cov-2 Ag on VITROS	Spearman's rho	-0.624
	p-value	<0.001
	Sig.	S

	CT	**%**	**Sensitivity (95% CI)**
CT grouping	<= 20	28.4%	100.0% (91.78 - 100.0)
	>20 - <= 25	26.5%	95.0% (83.08 - 99.39)
	>25 - <= 30	24.5%	78.38% (61.79 - 90.17)
	> 30	20.6%	50.0% (29.93 - 70.07)

	CT	**%**	**Sensitivity (95% CI)**
CT grouping	<= 30	79.4%	91.78% (85.56 - 96.03)
	> 30	20.6%	59.38% (40.46 - 76.3)

Figure 1. Relation between RT-qPCR and VITROS SARS-COV2 antigen.

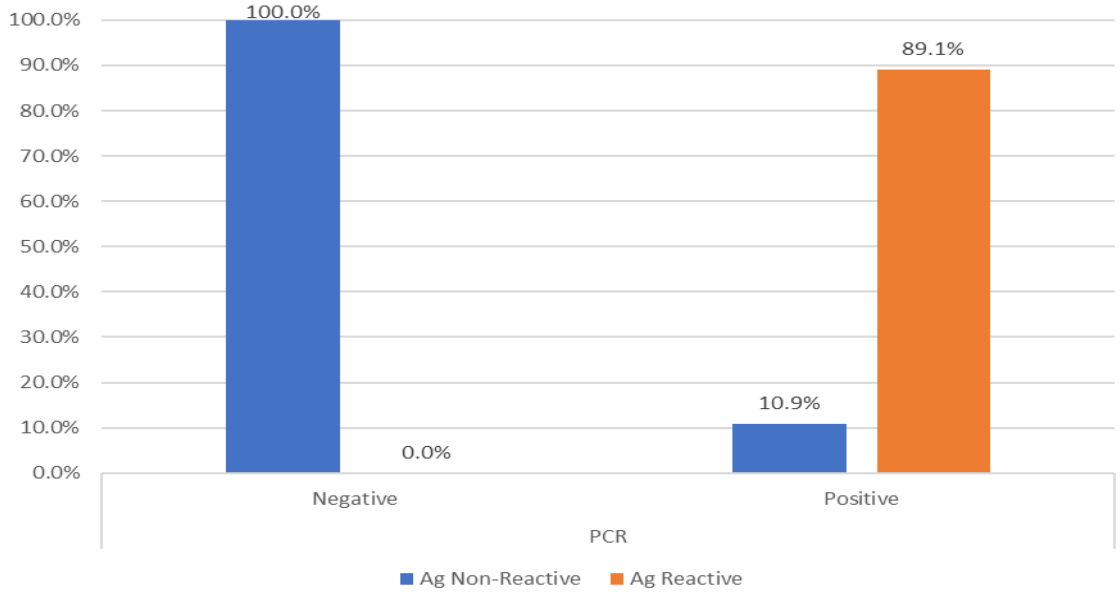
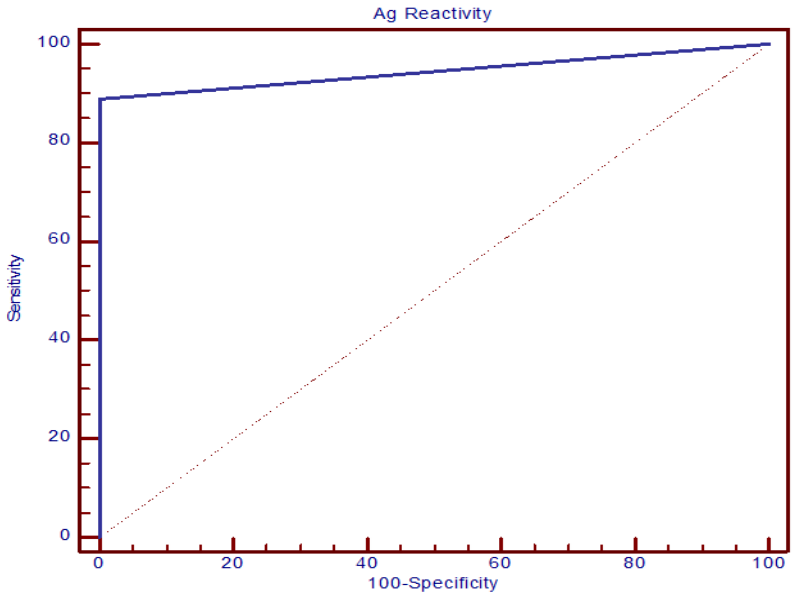


Figure 2. Sensitivity and specificity for VITROS SARS-COV2 antigen as a diagnostic test for SARS-Cov-2.



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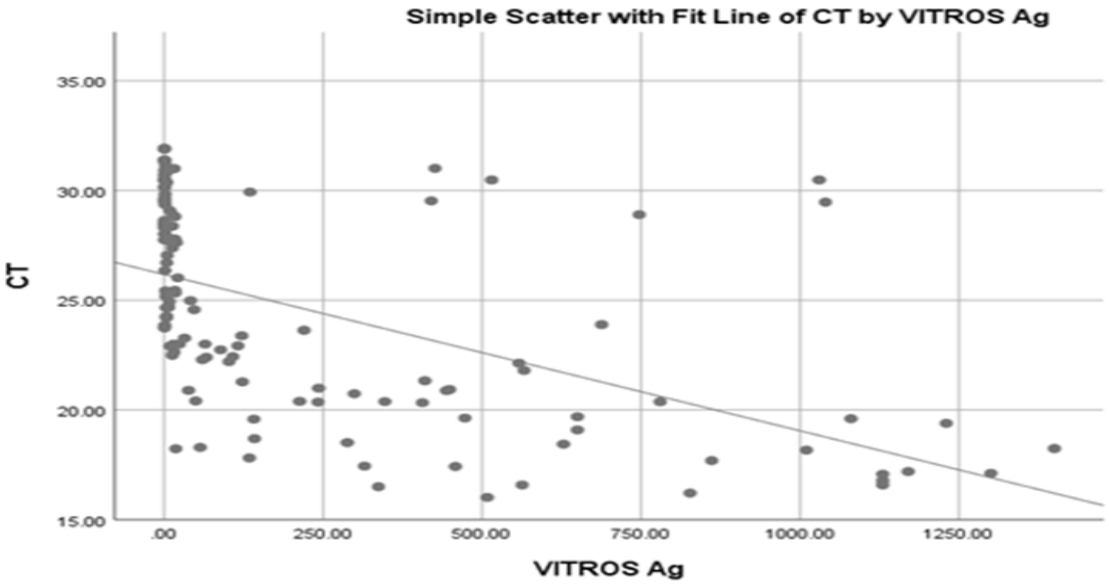

Discussion

Prompt and accurate diagnosis of SARS-CoV-2 has been shown to be valuable during pandemic responses for containment of COVID-19 in clinical settings. In vitro diagnostic real-time reverse transcription–polymerase chain reaction (RT-PCR) might be costly, with proportionally long turnaround time, and need well trained laboratory personnel. Antigen detection tests is rapid and more easily and less costly. The usage of antigen detection tests in comparison with nucleic acid amplification tests, are an area of interest for the rapid diagnosis of SARS-CoV-2 infection [9].

VITROS® SARS-CoV-2 Antigen test can detect SARS-CoV-2 in 48 minutes with high capacity to test around 130 nasopharyngeal swabs per hour. In the present study, it is illustrated that the VITROS® SARS-CoV-2 antigen test sensitivity was 89.11% and specificity was 100% respectively in 668 nasopharyngeal swab specimens and this was compatible with **Matsuzakiet al.** [10], who found that the sensitivity of antigen detection test was 75.5% and specificity was 100% in a study of 128

nasopharyngeal swab at Saitama Medical University Hospital, Saitama, Japan.

In this study, the SARS-CoV-2 Antigen test showed 91.1% sensitivity in Nasopharyngeal swab specimens with Ct values below 30.0 similar to **Matsuzaki et al.** [10], who found sensitivity 100%**.**

In our study, there were 33 samples positive with RT-qPCR but negative for the SARS-CoV-2 Antigen test, all these samples had Ct values above 33.0 and seemed to be in a low viral loads non-contagious recovery phase. In clinical practice, the shedding infectious live virus's detection is not only associated with COVID-19 diagnosis, but also to infection prevention and control precautions in clinical settings, including the termination of patient quarantine [11].

Previous studies showed that a positive Nucleic acid amplification test detects only the viral RNA particles detection and does not always reflect the detection of live virus particles [12]. In another study, **Singanayagam et al.** [13]**,** reported that 8% of samples with Ct values above 35.0 were virus culture positive. In addition, previous studies had also found that high Ct levels were associated with non-infectious SARS-CoV-2.

A further constraint of this study is the use of Ct-values to determine levels of infectivity. Because different RT-qPCR tests may give different Ct values with the similar RNA load. Ct-values can differ considerably, either because of incompatible sampling methods or different RT-PCR methods, which currently lack of the standardized reference materials [14].

Over than 100 commercially COVID-19 PCR tests are qualitative. And the positive results don't distinguish between infective virus and virus fragments. Patient may still show positive PCR long time after recovery. This is confused to patient and healthcare provider. Persistent positive PCR can lead to unnecessary isolation and the inability to return to work and other activities and put great burden on economy [15].

Individuals can experience prolonged shedding of RNA in their swabs. These very low viral loads or viral fragments however don't necessarily indicate infectiousness [16].

These consequences showed that the SARS-CoV-2 antigen test is fruitful in detection of alive viral particles and it could help in selecting infectious COVID-19 patients for applying infection prevention and control precautions. However, it is still necessary to pay attention to false-negative results when using the SARS-CoV-2 antigen test.

Conclusion

The performance of VITROS SARS-COV2 Antigen test was effective to detect the q RT-PCR positive patients with higher viral loads (i.e., CT values <=25). This test also has good specificity and can be used as an alternative or complement to screen and diagnose COVID-19 in asymptomatic or symptomatic patients.

The VITROS® SARS-CoV-2 antigen test realizes high-throughput and quick testing, does not require experienced technicians or multi-step procedures, and can be done using equipment already installed in many laboratories. Thus, this test is a viable alternative to RT-qPCR and is suitable for mass screening during outbreaks.

Conflict of interest

All authors have approved the final article and declared no conflict of interest.

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