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DIAGNOSTIC PERFORMANCE OF SARS-CoV-2 BY ANTIGEN DETECTION ENZYME IMMUNOSORBENT ASSAYS

Medical Microbiology

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ABSTRACT

Background: Quick identification and isolation of COVID-19 cases is critical in curtailing the pandemic. Rapid immunochromatographic antigen tests (RAT) were introduced for the same purpose but were unable to achieve the expected impact on pandemic control because of its low sensitivity. Enzyme Linked Immunosorbent Assay (ELISA) is more sensitive than immunochromatography test. Hence, the recently introduced COVID-19-Antigen-ELISA (J. Mitra & Co. Pvt. Ltd COVID-19 Ag MICROLISA) is believed to be more sensitive than RAT tests available for the rapid diagnosis of COVID-19. Further, COVID-19-Antigen-ELISA (COVID-Ag-ELISA) can also be performed on sample collected in Viral Transport Medium (VTM) that has been collected for gold-standard Real-time, Reverse transcriptase polymerase chain reaction (RT-PCR) used for diagnosis of COVID-19. Therefore, the present study was undertaken to assess performance of COVID-A g-ELISA with RAT and gold standard RT-PCR test. **Method:** This study was done on VTM samples that were collected for RT-PCR test and stored after RT-PCR test results at -80℃ or -20℃. COVID-19-Antigen-ELISA test was performed as per kit literature provided by the manufacturer. The sensitivity, specificity, PPV and NPV were calculated to evaluate the performance of ELISA. **Result:** In this study, percentage positivity of COVID-A g-ELISA was higher than RAT but lower than RT-PCR. Also, ELISA was able to pick up 27 RAT negative samples. Sensitivity of ELISA for samples with Ct value ≤ 25 (62.5) was almost similar to samples with Ct value ≥ 25 (64.5). Specificity of test was 100%. In this study, percentage positivity of COVID-A g-ELISA test was found inversely proportional to duration of stored VTM samples. **Conclusion:** COVID-A g-ELISA can be used as a screening test alternative to RAT.

KEYWORDS

COVID-19, Antigen ELISA, RT-PCR, RAT test.

INTRODUCTION:

Corona virus disease (COVID-19) is an infectious disease caused by the SARS-CoV-2 virus. It was first identified in December 2019 from the cases of unexplained pneumonia in Wuhan, China. From there SARS-CoV-2 virus has spread globally. In March 2020, the World Health Organization (WHO) has declared COVID-19 as a pandemic. Even after two years of pandemic, cases are being reported from various parts of the world (1,2).

Screening, testing and contact tracing of COVID-19 cases plays a pivotal role in control of the COVID-19 pandemic (3). RT-PCR is considered as the powerful diagnostic test for SARS-CoV-2 infection and is accepted as gold standard test for diagnosis of COVID-19 disease (4). However, unavailability of RT-PCR testing facility became the limiting factor for controlling the pandemic. Also lack of RT-PCR laboratory setup, skilled laboratory personnel and high-cost RT-PCR test became economic burden for the low or mid-income underdeveloped and developing countries (5). Though RT-PCR test is considered highly sensitive, it has long turn-around time (6-8 hours). This delay in testing and reporting increases the spread of infection. To overcome this, the World Health Organization (WHO) had consented to implement antigen detecting rapid diagnostic tests (Ag-RDTs) (6). However, rapid antigen test has low sensitivity (7). So, it was unable to fulfill expectations of controlling the spread of infection. Therefore, there is a need for a rapid, technically less demanding, cost-effective diagnostic test in addition to the gold standard RT-PCR which can be performed on a large number of samples. Such a test will help in identifying and breaking the chain of infection and there by helping in curtailing the pandemic.

Like any other infectious diseases, antigen detection by ELISA test may be a more sensitive early diagnostic tool for COVID-19 disease in its acute stage (8). Therefore, the present study was undertaken to assess the utility of commercially available antigen ELISA for diagnosis of COVID-19 disease.

AIMS & OBJECTIVES:

1. To assess the performance of COVID-Ag-ELISA for diagnosis of COVID-19 disease

2. To compare results of COVID-Ag-ELISA with Ct value of RT-PCR test results

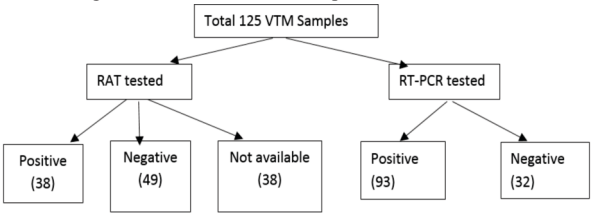
3. To assess the effect of storage duration on COVID-Ag-ELISA test

MATERIALS AND METHOD:

The present study was conducted in a tertiary care hospital after getting ethical approval from the Ethical Committee of this hospital.

Type Of Study: Diagnostic test evaluation

Clinical Specimens: Nasopharyngeal samples received in VTM for COVID-19 RT-PCR tests were stored at refrigerated temperature (-80℃ or -20℃) after testing. In the present retrospective study, stored 125 RT-PCR tested samples were used for evaluating performance of COVID-Ag-ELISA. Details of 125 samples:



Kit used: J. Mitra & Co. Pvt. Ltd COVID-19 Ag MICROLISA Kit. It is Microwell ELISA test for the qualitative detection of COVID-19 (SARS-CoV-2) nucleocapsid antigen in human nasopharyngeal swab, nasal swab and VTM samples.

Principle Of The Test: COVID-19 Ag MICROLISA test is an Enzyme Immunosorbent Assay based on "Sandwich ELISA".

Procedure: COVID-Ag-ELISA was performed on stored VTM samples as per the instructions given by the manufacturer. The 300 µl VTM samples were taken in each sample collection tube provided by manufacturer to which 50 µl lysis buffer was added. Then these samples were vortexed for 5 minutes and further these VTM samples were incubated for 10 minutes. Out of these treated VTM samples, 200 µl of each sample was taken on microtiter well plate that were coated with anti-COVID-19 antibodies specific to nucleocapsid antigen. The COVID-19 antigen, if present in the specimen, binds to the anti-COVID-19 antibodies adsorbed onto the surface of the wells. The

enzyme substrate reaction was read by EIA reader for absorbance at a wavelength of 450 nm and 630 nm. If the sample did not contain COVID-19 antigen, then enzyme conjugate will not bind and the solution in the wells will be colourless or will show a faint background colour. The amount of antigen detected in the sample was calculated in terms of COVID-19 antigen Units. Interpretation of results are as follows:

- If Antigen Units are < 9, sample was interpreted as **Negative** for COVID-19
- If Antigen units are between 9-11, sample was interpreted as **Equivocal** for COVID-19
- If Antigen units are >11, sample was interpreted as **Positive** for COVID-19

RESULTS:

This study was conducted on samples collected from June 2020 to February 2022. A total of 125 randomly selected samples collected in all three waves of the pandemic were studied. These samples were stored for different durations, i.e., one month, two month, three months, six months, 12 months, 18 months, and 27 months were tested.

Table No 1: Comparison of COVID-Ag-ELISA with Rapid antigen test

ELISA	RAT (n = 87)	
	Positive	Negative
Positive	22	21
Negative	16	28
Total	38	49

Out of 87 RAT tested samples, 43 samples were tested positive by COVID-Ag-ELISA. When COVID-Ag-ELISA compared with RAT, sensitivity of ELISA was 57.89%.

Table No 2: Comparison of COVID-Ag-ELISA results with Ct value of RT-PCR

	RT-PCR (n= 125)			
ELISA	Ct ≤25	Ct ≥25	Negative	Total
Positive	23	27	7*	57
Negative	15	22	22	59
Equivocal	2*	4*	3*	9*
Total	40	53	32	125

*Equivocal was included as positive

Out of 125 RT-PCR tested samples, 65 were tested positive by COVID-Ag-ELISA. Surprisingly, 7 samples which were reported negative because of their high Ct value (≥35) as per ICMR guidelines, came positive by a COVID-Ag-ELISA test. Confirmed RT-PCR negative samples (22) were also negative by COVID-Ag-ELISA.

Table No 3: Variation in Sensitivity and Specificity of COVID-Ag-ELISA based on Ct value

	Sensitivity	Specificity	PPV	+NPV
Ct value >25	(25/40) 62.5%	100%	100%	59.45%
Ct value ≥ 25	(31/53) 64.51%	100%	100%	50.00%

Sensitivity of COVID-Ag-ELISA (with RT-PCR as gold standard) on samples with Ct value ≤ 25 (62.5%) was similar to samples with Ct value ≥25 (64.51%) and specificity was 100%.

Table No 4: Effect of storage on performance of COVID-Ag_ELISA

Duration	Positive	Equivocal	Negative	Total
Up to 3 months	43 (60.46%) (7 with high Ct value)	9*(3reported negative)	34 (22 were known negative)	86
6 months	6 (60%)	0	4	10
12 months	5 (62.5%)	0	3	8
18 months	2 (13.33%)	0	13	15
24 months	1 (16.66%)	0	5	6
Total	57	9	59	125

*Equivocal was included as positive

Percentage positivity of COVID-Ag-ELISA decreases from 60% to 16% when duration of sample storage increases (i.e., 3 months to 24 months).

DISCUSSION:

In literature, few in-house developed, SARS-CoV-2 antigen enzyme-linked immunosorbent assay (ELISA) systems have been tested for diagnosis of COVID-19 disease. (4) But there is no study regarding commercially available COVID-Ag-ELISA kits. To fulfill the requirement of testing many samples daily, cost-effective, high-throughput test with higher sensitivity and specificity is necessary [4]. COVID-Ag-ELISA can fill this gap.

In the present study, COVID-Ag-ELISA was performed on 93 RT-PCR positive and 32 RT-PCR negative samples. Out of these samples, RAT results were available for 87 samples.

In the present study, as per table no. 1, the percentage positivity of COVID-Ag-ELISA was 57.89% when compared with RAT. COVID-Ag-ELISA could not pick up all RAT Positive samples, this may be due to the reason that COVID-Ag-ELISA was not performed on the sequential sample after RAT positive result. As per ICMR guidelines, suspected Influenza like illness (ILI) case, who tests negative for COVID-19 by RAT should be tested sequentially by RT-PCR to rule out COVID-19 infection whereas, a positive RAT result should be considered as true positive and does not need reconfirmation by RT-PCR test (9). In this study, out of 49 RAT negative samples, 21 samples were tested positive by COVID-Ag-ELISA, which suggests that it gives less false negative result. All 21 samples that tested positive with COVID-Ag-ELISA and negative by RAT were positive by the gold-standard RT-PCR test.

As per table no 2, total positivity of COVID-Ag-ELISA was 60.21%. In a study done by Pandey et al., (10), sensitivity of RAT was lower (53.6%). While in a study done by Wölfl-Duchek et al., (11), sensitivity of RAT was higher (73.3%). This may be due to the difference in the antigen kits used, stage of the disease at which patients were tested and load of virus particles present in the respiratory tract.

In this study as per table no 3, sensitivity of COVID-Ag-ELISA for RT-PCR positive samples with Ct value ≤ 25 (62.5%) was equivalent to samples with Ct value ≥ 25 (64.51%). While Adnan et al., (4) in their study on in house COVID-Ag-ELISA, reported sensitivity of 69.6% for sample with Ct value ≤ 35 and 75.4% sensitivity for samples having Ct value ≤ 30 in RTPCR test, which is contradictory to this study. In this study, specificity of COVID-Ag-ELISA was 100% for samples with both Ct value ≤ 25 as well as ≥ 25, which is comparable to the study of Adnan et al., where specificity was 99.9%.

In this study, 125 samples were tested to check the effect of storage on results of COVID-Ag-ELISA. As per the table no 4. there was a significant decrease in positivity from 60.46% to 16.7% as the storage duration of sample increased (from 3 months to 24 months). Dzung et al., (12) has studied the effect of prolonged unfrozen storage and repetitive freeze thawing of SARS-CoV-2 VTM where they did not find any significant impact on cyclic threshold value of RT-PCR result. However, they studied this effect up to 21 days only. In the present study the storage duration of samples studied ranged from 3 months to 24 months. Further study, using large number of samples may be required to confirm the effect of duration on COVID-Ag-ELISA test.

To date, there are no reports on commercially available SARS-CoV-2 antigen detection ELISA in literature. This is the first study of its kind. Present study shows that COVID-19 antigen ELISA has a potential to make significant contribution in controlling pandemic by screening large number of samples at a time to identify infectious spreaders. COVID-Ag-ELISA will also have impact on diagnosing carriers, especially in areas that lack laboratory facility of RT-PCR testing and areas where RT-PCR results take more than 24-48 hours. COVID-Ag-ELISA has a few more advantages such as two tests (RT-PCR and COVID-Ag-ELISA) can be performed with a single swab, thus it is minimally invasive for patients and environment friendly as less biomedical waste is generated.

CONCLUSION:

COVID-Ag-ELISA is a better, cost-effective, and sensitive screening test that has a short turn-around-time. It can be performed on a large number of samples, so it may even be an alternative test for RT-PCR in future.

LIMITATION OF STUDY:

Study was done on stored samples and not on fresh samples.

Dissimilarities in the number of samples stored for different duration. Further studies using fresh samples are required to assess its utility.

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CONFLICT OF INTEREST: None

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