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Performance evaluation of automated chemiluminescence immunoassay based antigen detection – Moving towards more reliable ways to predict SARS-CoV-2 infection

Diptanu Paul, Akshita Gupta, Sheetalnath Rooge, Ekta Gupta * 

Department of Clinical Virology, Institute of Liver & Biliary Sciences, New Delhi, India

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ABSTRACT

Real-time reverse transcription-polymerase chain reaction (RT-PCR) has been the most reliable armoury for the diagnosis of COVID-19, considered to be the reference standard but fails to reproduce the correct predictability about the infectivity of the disease every time. Antigen detection however puts foothold in this aspect even though lacks in sensitivity, especially conventional Rapid Antigen Tests (RATs). Recently developed Chemiluminescence Immunoassay (CLIA) based antigen detection tests are promising and displayed better sensitivity. In the current study we have evaluated VITROS® SARS-CoV-2 Ag Test CLIA Kit, which was tested on 148 patient’s samples attended to a tertiary care centre for testing of SARS-CoV-2. The performance of the kit was evaluated in comparison to RT-PCR and RAT and found to be a good test for antigen detection, best within the first few days of infection. The test has shown sensitivity of 94.3 % and specificity of 100 % in samples with corresponding Ct values of \( \leq 25 \) by RT-PCR, which corresponds to high viral load and can predict ability of spreading the disease by the patients. With the results being semiquantitative along with improved sensitivity it can replace RATs for antigen detection for screening, provided good laboratory set up is included under consideration.

1. Introduction

Real-time reverse transcription-polymerase chain reaction (RT-PCR) has a pivotal role in detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. It is considered to be the reference standard test for diagnosis of SARS-CoV-2 but to maintain its analysis throughput sufficient manpower, skilled operators, costly machines with consumables and strict biosafety measures are required. Moreover, in spite of having high sensitivity and specificity RT-PCR do not always reflect the true infectivity of the patients, as the RT-PCR test can detect too low levels of nucleic acid (RNA of SARS-CoV-2) of the virus, suggesting low viral load, and hence are very unlikely to cause infection in others. On the other hand, antigen based tests are used more often as a screening test despite their variable sensitivity and specificity. Rapid Antigen Tests (RATs) are faster, easy to interpret, less expensive, minimal training is required to adopt its use and it can pick up active ongoing infections too. Currently there are approximately 175 RAT kits available (FIND, 2021), but the average sensitivity of RATs is only 56.20 % (95 % CI 29.50–79.80%), according to a recently published Cochrane study (Dinnes et al., 2021). If the antigen detection is done on a better platform like Chemiluminescence Immunoassay (CLIA), the sensitivity increases up to 96.40 % (Favresse et al., 2021), which helps in better detection of SARS-CoV-2 antigens during the phase of early infection. Moreover, to perform RAT and RT-PCR together, two separate samples are required. First dry nasopharyngeal (NP) swab is required for RAT and then separate nasopharyngeal (NP) and oropharyngeal (OP) swabs are required to take for RT-PCR. This double sample collection can be difficult and inconvenient for patients, which is not required for antigen detection by CLIA as single NP and OP swab sampling is sufficient for performing both CLIA and RT-PCR.

Therefore, in this current study we have evaluated the performance of one newly developed automated CLIA based antigen detection test –“VITROS® SARS-CoV-2 Ag Test CLIA Kit” (VITROS). We also evaluated the performance of SARS-CoV-2 antigen detection methods; CLIA based VITROS and lateral flow assay based RAT in comparison to the reference standard RT-PCR test and also compared the performance of VITROS

* Corresponding author.

E-mail addresses: dr.dip.dp@gmail.com (D. Paul), akshitagupta1412@gmail.com (A. Gupta), sheetalnath.rooge@gmail.com (S. Rooge), ektagaurisha@gmail.com (E. Gupta).

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2. Materials and methods

2.1. Study design & study population

All consecutive patients with clinical sign and symptoms suggestive for COVID-19 infection who visited the Outpatient Department of the Institute, as per the National guidelines (ICMR, 2021) for testing were included after obtaining informed consent for the study.

2.2. Ethical approval

The study was approved by the Institutional Review Board of the Institute of Liver and Biliary Sciences and conducted in accordance with the Declaration of Helsinki.

2.3. Sample collection

Two NP swabs and one OP swab were collected from each patient simultaneously. One NP swab was used for RAT. The remaining NP swab and OP swab were transferred into a tube of viral transport medium (VTM). This VTM tube was then sent to our lab for VITROS and RT-PCR. All samples were collected as per the available guidelines (COVID, 2021), with due biosafety precautions. The specimens were properly packaged and maintained in cold chain during transportation. Clinical and demographic data of all the patients were also collected and evaluated.

2.4. Analytical procedures

A total of 148 samples were tested for SARS-CoV-2 Antigen by RAT and VITROS and confirmation of COVID-19 was done by viral RNA detection by RT-PCR. RAT was performed at the point of collection and in the lab two aliquots were prepared from collected VTM tube fluids. One aliquot was used for VITROS and another aliquot was used for RT-PCR on the same day of collection. Aliquots were stored at -80 °C for future use after performing all the tests.

2.5. RAT

RAT was performed using Angcard® COVID-19 rapid Antigen Test kit (Angstrom® Biotech Pvt. Ltd., Alwar, Rajasthan, India), following manufacturer’s instructions.

2.6. VITROS

Automated antigen detection was performed using VITROS® SARS-CoV-2 Ag Test CLIA Kit (Ortho Clinical Diagnostics, Mumbai, Maharashtra, India) on VITROS® 3600 Immunodiagnostic System (Ortho Clinical Diagnostics, Raritan, NJ, USA). Tests were performed as per the instructions mentioned in the test kit literature. A signal to cut off (S/CO) value of ≥ 1 was considered to be positive test result.

2.7. RT-PCR

RT-PCR was performed using RealStar® SARS-CoV-2 RT-PCR kit 1.0 (Altona® Diagnostics, Germany) following all manufacturer’s instructions and a Ct value of ≤ 35 for E and S gene were considered to be Positive.

2.8. Statistical analysis

All the data was first entered in Microsoft Excel spreadsheet and then analysed with the help of MedCalc® statistical software (MedCalc Software Ltd, Acacialaan 22 8400 Ostend Belgium), GraphPad Prism® software (version 9.0.0, California, CA, USA) and Microsoft Excel® (version 2016) (Microsoft corporation, Redmond, Washington, USA).

3. Results

Overall in all 148 patients all the three tests could be performed. Out of them COVID-19 infection as confirmed by RT-PCR positivity was seen in 98 (66.20 %) patients. Baseline characteristics of the study population are described in Table 1.

3.1. Performance of VITROS and RAT vs RT-PCR

On comparing the performance of VITROS with RT-PCR, VITROS could detect 72 RT-PCR positive patients, so the antigen detection assay demonstrated a sensitivity of 73.47 % (95 % CI: 63.59%–81.88%) with a specificity of 100 % (95 % CI: 92.89%–100.00%).

Whereas, RAT could detect only 51 RT-PCR positive patients, so sensitivity and specificity of RAT were 52.04 % (95 % CI: 41.71%–62.24%) and 100.00 % (95 % CI: 92.89%–100.00%) respectively.

Overall, VITROS could detect 21 (21.42 %) more number of patients than RAT in terms of antigen detection. In ROC curve analysis of VITROS and RAT in comparison to RT-PCR, VITROS showed AUC of 0.87 (P < 0.001) and RAT showed AUC of 0.76 (P < 0.001) (Fig. 1).

3.2. Comparison of RT-PCR cycle threshold (Ct) value with VITROS and RAT results

Ct values indirectly signify the amount of virus in the respiratory sample. The Ct values obtained on RT-PCR were compared to the positivity of VITROS, the highest Ct value corresponding with positive VITROS was 30.33 (confirmatory S gene) but the highest Ct value corresponding with positive RAT result was 23.16.

In our study we have considered Ct value of ≤ 35 on RT-PCR as positive. However, with Ct values of ≤ 25 which corresponds to high viral load, conventional card based antigen detection tests (RAT) shows better sensitivity (Thommes et al., 2021; Pickering et al., 2021).

Therefore, we also evaluated the performance of VITROS, and RAT based on Ct value of ≤ 25. Out of 98 RT-PCR positive samples 70 (71.40 %) samples were of ≤ 25 Ct value and 28 (28.60 %) were of > 25 Ct value. VITROS could detect 66 out of 70 (94.30 %) samples of ≤ 25 Ct value whereas, RAT could detect 51 out of 70 (72.80 %) samples of the same Ct value. Therefore, the sensitivity of VITROS for samples with Ct values ≤ 25 got enhanced to 94.30 % from overall sensitivity of 73.47 %.

(Fig. 2).

Antigen positivity of the VITROS can be semi-quantitated as s/co. A good correlation between Log_{10} value of antigen s/co and RT-PCR Ct values were seen (R^2 = 0.79) as depicted in Fig. 3.

| Characteristics                  | COVID-19 confirmed cases (n = 98, 66.20 %) | COVID-19 negative cases (n = 50, 33.80 %) |
|----------------------------------|-------------------------------------------|-------------------------------------------|
| Male: Female                     | 62.36 (1.72:1)                            | 34.16 (2.12:1)                            |
| Median age (IQR) years           | 35 (12–87)                                | 35 (15–77)                                |
| Symptomatic patients n(%)        | 73 (74.49 %)                              | 12 (24 %)                                 |
| Fever n(%)                       | 71 (97.20 %)                              | 11 (91.60 %)                              |
| Asymptomatic cases n(%)          | 25 (25.51 %)                              | 38 (76 %)                                 |
| RAT Positive n(%)                | 51 (52 %)                                 | 0                                         |
| RAT negative n(%)                | 47 (47.90 %)                              | 50 (100 %)                                |
| VITROS Positive n(%)             | 72 (73.50 %)                              | 0                                         |
| VITROS Negative n(%)             | 26 (26.50 %)                              | 50 (100 %)                                |
3.3. Comparison of duration of illness till which the SARS-CoV-2 antigen is detectable by VITROS and RAT

The median duration of illness until RAT gave positive results was 2 days (IQR: 0–6 days) and for VITROS also it was 2 days (IQR: 0–7 days). Maximum number of positive RAT (n = 19, 37.30 %) and positive VITROS (n = 28, 38.90 %) samples were from patients on their 3rd day of illness. However, 12 (16.70 %) VITROS positive and eight (15.70 %) RAT positive samples were collected from asymptomatic patients. Semiquantitative data of VITROS as s/co was compared with days of sample collection after onset of illness, which is depicted in Fig. 4.

3.4. Comparison of performance of VITROS vs RAT

On comparing VITROS with RT-PCR an overall agreement was found to be 83.34 % (Cohen’s k: 0.67) whereas, with same criteria RAT shows an overall agreement of 44 % (Cohen’s k:0.53).

Other statistical parameters comparing both antigen detection test methods are summarised in Table 2.

4. Discussion

The present study evaluated a newly developed antigen detection assay (VITROS) which is based on CLIA. Antigen detection for diagnosis of COVID-19 by RAT kit lacks sensitivity and chances of missing the cases are always high, if used alone as a diagnostic tool. Several studies have evaluated the performance of RAT before and found its wide ranges of sensitivity (Favresse et al., 2021; Yamayoshi et al., 2020; Lai and Lam, 2021), the average sensitivity being 56.20 % (Dinnes et al., 2021).

VITROS has shown potential to be a better tool for antigen detection as excellent correlation ($R^2 = 0.94$) was found between antigen signal to cut off (s/co) and Ct values of RT-PCR by Favresse et al. and they also demonstrated sensitivity and specificity of the test to be 100 % till Ct value $\leq 33$ (Favresse et al., 2021). Another study by Hirotsu et al. evaluated LUMIPULSE SARS-CoV-2 Ag kit (Fujirebio, Tokyo, Japan) based on CLIA, which showed 55.20 % sensitivity and 99.60 % specificity, with a 91.40 % overall agreement rate and good correlation with RT-PCR ($R^2 = 0.77$) (Hirotsu et al., 2020). In our study we have found good overall agreement (83.34 %), good correlation ($R^2 = 0.79$) and sensitivity of 94.29 % with corresponding RT-PCR Ct values $\leq 25$, which is similar to findings of available literature (Favresse et al., 2021; Porte et al., 2020). Only four samples with RT-PCR Ct values $\leq 25$ were antigen negative by VITROS, the reason for which can be attributed to the low levels of SARS-CoV-2 antigen at the time of their presentation for testing (>5 days after onset of illness).

Fig. 1. ROC curve analysis of antigen detection tests in comparison to RT-PCR.

Fig. 2. Depicting results of both the antigen testing methods (CLIA – VITROS and RAT) in comparison to Ct values of RT-PCR results of the same number of samples.

Fig. 3. Correlation between Ct values of RT-PCR positive samples and Log 10 value of s/co of antigen test by VITROS.

Fig. 4. Showing distribution of antigen positive samples by VITROS (n = 72) depicted as Log$_{10}$ value of s/co against the day on which patient presented for sample collection after onset of illness. Asymptomatic cases were defined as day 0 (Zero). The declining trend of antigen detection can be seen with progression of days after onset of illness.
Table 2
Performance of antigen detection tests against RT-PCR result.

| Performance values | VITROS Performance | RAT Performance |
|--------------------|--------------------|-----------------|
| Sensitivity         | 97.47 % (95 % CI; 63.59%–81.88%) | 52.04 % (95 % CI; 41.71%–62.24%) |
| Specificity         | 100.00 % (95 % CI; 92.89%–100.00%) | 100.00 % (95 % CI; 92.89%–100.00%) |
| Negative Likelihood Ratio | 0.27 (95 % CI; 0.19 to 0.37) | 0.48 (95 % CI; 0.39 to 0.59) |
| Positive Predictive Value | 100.00 % | 100.00 % |
| Negative Predictive Value | 99.81 % (95 % CI; 99.74%–99.73%) | 99.66 % (95 % CI; 99.59%–99.89%) |
| Accuracy            | 99.81 % (95 % CI; 97.17%–100.00%) | 99.66 % (95 % CI; 96.89%–100.00%) |

In this study we also found the performance of CLIA based VITROS® SARS-CoV-2 Ag Test CLIA Kit is better than RAT for antigen detection of COVID-19, considering its sensitivity (73.47 %), specificity (100 %), PPV (100 %), NPV (99.81 %) and accuracy (99.81 %); which makes it more reliable as a screening tool in COVID-19 diagnostics. VITROS is a high-throughput automated assay which can perform 150 tests per hour and can be very useful for screening of large numbers of patients who may be likely to transmit the infection. However, in comparison to RAT, VITROS needs special set up and automated specialised CLIA instrument, which can restrict its use to specialised laboratories only. But VITROS is definitely more adaptable than RT-PCR to less well equipped facilities, so it does have a place for the local health system if they can afford the instrument, as a wide range of serological assays also can be performed with the instrument.

As a limitation of this study we can consider small study population, lack of data on disease prevalence in India and the use of single RAT kit to compare the results of antigen detection. In future, multiple platforms for antigen detection with different kits on a larger population can be done for further confirmation of our data in favour of the newly developed automated antigen detection kit and evaluation of its performance.

5. Conclusion

VITROS for COVID-19 antigen detection has shown very good results when tested within the first week of infection and correlates well with the Ct values of ≤ 25 which can be considered as surrogate of high viral load and contagiousness. It also can replace RAT as a screening tool in a proper set up and together with RT-PCR it can solve the purpose of diagnosing all COVID-19 positive cases along with added ability to predict contagiousness of the cases.

Author contribution

Diptanu Paul: Conceptualization, Methodology, Writing- Original draft preparation, Software. Akshita Gupta: Data curation, Software, Editing. Sheetalnath Rooge: Software, Editing. Ektak Gupta: Conceptualization, Validation, Supervision, Reviewing, Final Editing. All patient details have been de-identified and anonymised as per institutional ethical committee instructions for clinical studies.

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Declaration of Competing Interest

The authors report no declarations of interest.

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