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Evaluation of diagnostic utility and performance of rapid SARS-CoV-2 antigen detection assay in comparison with Real-Time RT-PCR in Kolkata, India

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Abstract

Background: COVID-19 has so far affected millions of people in India. The present study was undertaken to find out the performance and reliability of rapid antigen test (RAT) in compared to reverse transcription polymerase chain reaction (RT-PCR).

Methods: The pre and existing medical conditions and clinical signs and symptoms were noted. The nasopharyngeal swab samples were taken for RAT, while both nasopharyngeal and oropharyngeal swab samples were mixed in a sterile viral transported medium (VTM) for RT-PCR. All patients were examined by RAT, while symptomatic negative in RAT were re-examined by RT-PCR.

Results: Total 18,965 samples were examined by RAT and 3,998 samples by RT-PCR. Among them, only 5,753 patients (30.3%) were symptomatic and 1,757 patients (9.2%) were symptomatic positive. RAT showed overall 15.2% positive cases. Only 3.7% samples exhibited false negative results in RAT, which were found positive in RT-PCR. Interestingly, Ct (cycle threshold) values were >30 in all these samples.

Conclusion: Hence, specific antigen-based rapid diagnostic test (RDT) will be most useful and reliable among any other qualitative tests for screening purpose.

Keywords: ARDS, COVID-19, Rapid antigen, RT-PCR, SARS-CoV-2

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Évaluation de l'utilité diagnostique et des performances du test de détection rapide de l'antigène sars-cov-2 par rapport à la rt-pcr en temps réel dans l'hôpital de soins tertiaires covid-19 à Kolkata, en Inde

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Résumé

Contexte général de l'étude: Le COVID-19 a jusqu'à présent touché des millions de personnes en Inde. La présente étude a été entreprise pour déterminer les performances et la fiabilité du test antigénique rapide (RAT) par rapport à la réaction en chaîne par polymérase de transcription inverse (RT-PCR).

Méthode de l'étude : Les conditions médicales préexistantes et existantes ainsi que les signes et symptômes cliniques ont été notés. Les échantillons d'écouvillonnage nasopharyngé ont été prélevés pour la RAT, tandis que les échantillons d'écouvillonnage nasopharyngé et oropharyngé ont été mélangés dans un milieu transporté viral stérile (VTM) pour la RT-PCR. Tous les patients ont été examinés par RAT, tandis que les négatifs symptomatiques en RAT ont été réexaminés par RT-PCR.

Résultats de l'étude : 18 965 échantillons au total ont été examinés par RAT et 3 998 échantillons par RT-PCR. Parmi eux, seuls 5 753 patients (30,3 %) étaient symptomatiques et 1 757 patients (9,2 %) étaient symptomatiques positifs. Le RAT a montré un total de 15,2 % de cas positifs. Seuls 3,7 % des échantillons ont présenté des résultats faussement négatifs en RAT, qui ont été trouvés positifs en RT-PCR. Fait intéressant, les valeurs de Ct (seuil de cycle) étaient > 30 dans tous ces échantillons.

Conclusion : Par conséquent, le test de diagnostic rapide (TDR) basé sur un antigène spécifique sera le plus utile et le plus fiable parmi tous les autres tests qualitatifs à des fins de dépistage.

Mots-clés : SDRA, COVID-19, antigène rapide, RT-PCR, SARS-CoV-2

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INTRODUCTION

COVID-19 has affected over 313 million people in worldwide with 5.5 million deaths. In India, till now 35.9 million people affected and 0.48 million demises were recorded (1). SARS-CoV-2 is a highly contagious enveloped virus with a positive stranded RNA and primarily responsible for upper respiratory tract infections in human. Investigations revealed that the genome of SARS-CoV-2 contains ten open reading frames (ORFs). Other than that, one-third of SARS-CoV-2 genome encode with four structural proteins like, spike (S), envelope (E), nucleocapsid (N) and membrane (M) protein (2-3). S glycoprotein directly binds to the angiotensin-converting enzyme 2 (ACE2) receptor and act as a significant determinant of virus entry into host cells. ACE2 receptors are predominant in alveolar cells in lungs and also seen in the kidney, heart and colon (4-5).

The clinical manifestations of COVID-19 disease are fever, cough, respiratory distress, sore throat, anosmia, headache, myalgia, fatigue, abdominal discomfort etc (6). The finding of chest CT scan revealed the presence of pneumonia leading to acute respiratory distress syndrome (ARDS) (7). ARDS is the main death cause of COVID-19. Actually, one of the key mechanisms for ARDS is the cytokine storm, which may trigger a vicious attack through the immune coordination to the body and cause multiple organ failure, and finally lead to death in severe cases of SARS-CoV-2 infection (8-9). In symptomatic COVID-19 infected individuals viral transmission is maximal the day before the development of symptoms (10). Although, nearly 80.0% of infected were asymptomatic, or develop mild to transient symptoms (11). Early detection, isolation and treatment of COVID-19 positive cases and their contacts are still considered most challenging to facilitate restrict this unprecedented pandemic.

Taqman probe based fluorescent detection focusing on ORF 1ab and N gene region of SARS-CoV-2 genome were established on the primer sets and probes in RT-PCR and considered the gold standard for detection of SARS-CoV-2 (12). Nevertheless, RT-PCR tests required accredited medical laboratories, advanced analytical instruments, trained personnel, expensive reagents and plenty of time. The enormous gap between the large number of test samples and the accredited laboratory capacities to perform RT-PCR in a timely manner is the key restraint of the current pandemic (13). Therefore, there is a critical demand for the development of easy to perform rapid diagnostic tests (RDTs) for SARS-CoV-2 detection. Several pharmaceutical companies developed lateral flow immunochromatography based SARS-CoV-2 specific rapid antigen test (RAT) kits (14-16). RATs are less expensive, do not require precise instruments, simple to perform and interpret by minimally trained health workers and proved quick results, although the sensitivity, specificity and reliability are need to be validate (17-18). June, 2020 advisory committee of Department of Health, Govt. of India approved the use of point-of-care rapid antigen test for the detection of COVID-19 (19). The main advantage of RAT is not only to scale up the diagnostic tests but also to identify and isolate the COVID-19 infected patient immediately, which otherwise may be delayed by 2-3 days for RT-PCR reports. Moreover in hospital set up, particularly in any emergency condition or in operation theatre RAT is exceptionally supportive for treating the non-COVID-19 patients. However, published evidence of performance of RAT is limited. The ASSURED criteria (affordable, sensitive, specific, user friendly, rapid and robust, equipment-free, and deliverable to those who need it) of COVID-19 RAT kits were doubtful (20). Therefore, the objective of the present study was to assess and evaluate the diagnostic accuracy, utility and performance of rapid chromatographic immunoassay-based antigen tests and compared the findings with RT-PCR of person presenting in the community or in a tertiary care hospital in Kolkata, India.

MATERIALS AND METHODS

Viral RNA extraction kit (HiPurA, Himedia), RT-PCR kit (AngPCR, Angstrom Biotech), RAT kits (Biosensor, AngCard, LabCare and Oscar), molecular grade RNAase free water (Himedia) and alcohol (Himedia) and other consumables were used in this study. Biosafety cabinet (BSL-II, BioVanguard 4), Real time PCR (BIORAD-C1000 Thermal Cycler), cold micro-centrifuge (Eppendrof), -80^{0}C and -20^{0}C refrigerator (Eppendrof), Vortex shaker (Tarsons) and other minor equipments were used.

A single-centre study was performed in a tertiary care, referral hospital in West Bengal, India, following guidelines of ICMR for rapid antigen test (RAT) and RT-PCR for detection of COVID-19. The RAT study was conducted at the "Fever Clinic" (Influenza like fever) for COVID-19 and RT-PCR at the MRU (ICMR), R.G. Kar Medical College, Kolkata, India between August 1, 2020 and May 31, 2021. The patients were

selected from the out-patient department, emergency department, hospitalized patients, pre-operational patients and local communities who were suspected for COVID-19 infections and referred by the physicians of the hospital.

The patient categories, clinical signs and symptoms, pre-existing medical conditions, hospitalization details, personal contacts etc. was thoroughly noted before taking the specimen samples in the buffer or VTM. Carefully, nasopharyngeal swab were taken using nylon flocked swab sticks and transferred to buffer (0.5 ml) for RAT, while nasopharyngeal-oropharyngeal swab were taken and transferred to VTM (3 ml) for RT-PCR. Initially, RAT tests were performed for all patients who were willing to tests for the detection of COVID-19. It needs fifteen to thirty minutes for confirmation of COVID-19 detection. But, if RAT test results showed negative in patients having signs and symptoms similar to SARS-CoV-2 infection, then compulsorily RT-PCR tests were done. For RT-PCR test, nasopharyngeal and Oropharyngeal samples were collected for second time of the same patient (20). RAT was performed immediately in all the patients as per the manufacturer's instructions. The RAT kits consisted of a sterile swab, viral extraction tubes with buffer, tube nozzles / droppers and a COVID-19 antigen test device. Four separate validated RAT kits were used: (i) Standard Q (SD Biosensor, Haryana), (ii) Angcard (Angstrom Biotech, Rajasthan), (iii) LabCare (Lab-Care Diagnostics, Gujarat) and (iv) OS Kit (Oscar Medicare, New Delhi). The test results were read after 15-30 minutes according to kit protocol. The nasopharyngeal and oropharyngeal swabs in VTM were collected from the symptomatic negative patients in RAT and stored at -80°C (Eppendrof) until use. Total nucleic acid was extracted and purified from the samples using the Viral RNA Purification Kit (HiPurA, Himedia) under the Biosafety cabinet (BioVanguard 4) as per the manufacturer's instructions. Thereafter, commercial RT-PCR kit (AngPCR, Angstrom Biotech) was used to detect SARS-CoV-2 ORF and N gene in Real time PCR (Biorad-C1000, Thermal Cycler CFX 96 IVD, USA). The limit of detection (LOD) of the kit was 100 copies/ml.

Data Management

Demographic data were represented descriptive manner and percentile. Positive and negative predictive values of the test were also computed for both overall and various levels of pre-test probabilities. The agreement between the antigen test ant RT-PCR techniques was evaluated using the Cohen's weighted kappa index. Diagnostic characteristics such as sensitivity and specificity of the test with RT-PCR as reference were calculated.

RESULTS

In this pandemic condition, from August 1, 2020 and May 31, 2021 nearly 18,965 subjects were examined by RAT. Among them 54.8% was male and 45.6% was female (Fig.1A). The average age was 38.1 years (Fig.1B). Out of 18,965 samples tested for RAT, only 2,887 or 15.2% patients exhibited COVID-19 positive, where male was 8.6% and female was 6.6% (Fig.1C). Fig.1D exhibited in these samples, 13212 patients (69.6%) was asymptomatic and 3998 patients (21.1%) was symptomatic negative and 1130 patients (5.9%) was asymptomatic positive. Hence, 21.1% samples were further evaluated by RT-PCR. The month wise distribution of all positive, symptomatic-asymptomatic and asymptomatic positive were represented in Fig.2. A significant surge of COVID-19 positive (34.6% of all positive samples) was noted on the month of April, 2021 and least number of positive samples was found in February, 2021. The maximum patients tested for RAT was comparatively young, *i.e.,* in the age group of 21-40 years. But maximum COVID-19 positive was noted in the age group of 41-60 years (37.2%). The results were also compared in monthly asymptomatic and asymptomatic positive COVID-19 population. A positive correlation (r^2=0.375) was noted in all asymptomatic and asymptomatic positive COVID-19 detection in RAT (Fig.3). Out of 409 patents (33.5%) showed COVID-19 positive. Furthermore, it has been observed that complete vaccination (2 doses) showed more protection than incomplete or single short of vaccination (Fig. 4). Though out the study, four different commercial RAT kits were used: Biosensor, Angcard, Labcare and Oscar. Maximum test (38.1%) was performed by Angcard RAT kits (Fig. 5). Cohen's weighted kappa index showed the reliability between the RAT kits. Out of total 3998 symptomatic negative patients only 148 or 3.7% samples exhibited false negative results in RAT, which were found positive in RT-PCR. The average cycle threshold (Ct) value of RT-PCR positive samples was 33 (Fig. 6).

DISCUSSION

Antigen tests are immunoassays that

detect the presence of a specific viral antigen, which implies current COVID-19 viral infection. Rapid antigen tests are currently authorized to be performed on nasopharyngeal or nasal swab specimens placed directly into the assay's extraction buffer or reagent (21). Although, point to care diagnostic tests (POCTs) for the rapid detection of SARS-CoV-2 antigens are quite promising, but the principal concerns are the false negative rate due to low viral loads (22). The sensitivity of rapid antigen tests depends on its viral load. Earlier studies reported that sensitivity of antigen-based rapid diagnostic test was nearly 80-90% in the first 5-7 days compared to PCR (23-27). The major limitation of these reports was small number of sample size, which was less than thousand. To overcome this hindrance, we examined a large number of samples for RAT i.e., 18,965. Hence, current study analyses the routine use of rapid antigen test in a cohort of symptomatic and asymptomatic patients of all age groups and the performance characteristics of RAT for detecting of COVID-19 virus in nasopharyngeal samples was further compared the results with RT-PCR as the gold standard in case of asymptomatic negative by RAT in hospital set up. In this study, RAT test showed overall 2,887 positive cases (15.2%) in which 1,757 (60.8%) cases were symptomatic positive and 1,130 (39.1%) cases were asymptomatic positive. Most of the clinical symptoms of SARS-CoV-2 infection were reported fever (86.8%). Other than that sore throat (38.5%), cough (31.3%), body ache (26.2%), breathlessness (18.6%), loss of taste and smell (13.1%) and diarrhoea (5.7%) was common.

A large number of asymptomatic patients (13,212) were also tested by RAT and among them 13.3% showed COVID-19 positive with no clinical manifestation. It has been reported elsewhere that 80% of covid-19 infected were asymptomatic, or develop mild to transient symptoms (11). We also assumed that viral load of these COVID-19 positive asymptomatic patients was not sufficient for developing the clinical symptoms.

RT-PCR tests were compulsorily performed on symptomatic negative results in RAT. In the present study, a comparatively large number of symptomatic negative (3998; 21.0%) report was found by RAT. Hence, all these 3998 samples were further examined by RT-PCR and only 148 samples (3.7%) showed positive. In other way, only 3.7% samples showed false negative in RAT. The average Ct values of these samples were 33. The sensitivity, specificity, reliability of rapid antigen tests are depends on the quality of tests kits. Four sensitive, validated and commercially available RAT kits of Biosensor, Angcard, LabCare and Oscar were used throughout the study. However, the existing data indicate that rapid antigen test kits have been reliable up to the viral load in nasopharyngeal samples that showed Ct value 30 when examined by RT-PCR (28-30). Hence, a relatively large number of data supported that rapid antigen tests are relatively sensitive, specific, user friendly, rapid and can be used at the point of care in all age groups. As the diagnostic target of most antigen tests is the nucleocapsid protein and most of the mutations of the variants of concern are on the spike protein, countries should not hesitate to roll out antigen testing. But, it should be keeping in mind that RAT can be less accurate when someone has a lower viral load and could lead to false negative test results. Unfortunately at present even no COVID-19 vaccine assured us to complete protection against SARS-CoV-2, but in near future it should be possible.

CONCLUSION

Although, rapid antigen test for COVID-19 are generally less sensitive than RT-PCR, but main advantage is it can be used for screening testing in high-risk gathering, emergency hospital settings in which repeat testing could quickly identify persons with a COVID-19 infection, thus preventing transmission.

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Conflict of interest: The authors report no declarations of interest. The authors alone are responsible for the content and writing of this article.

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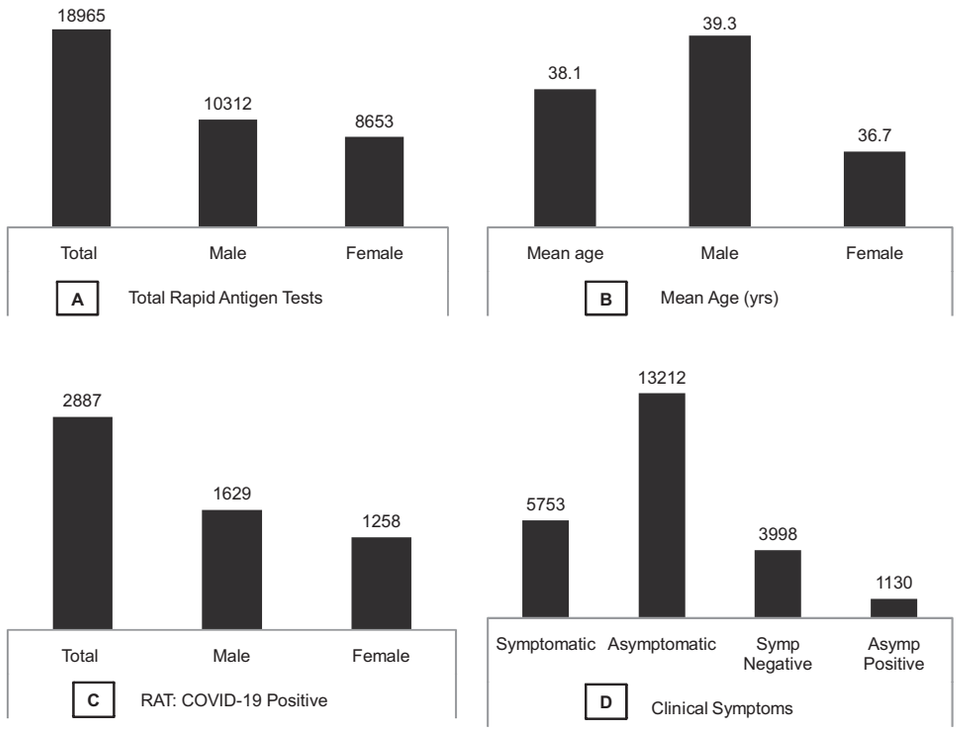

Figure 1 Demographic details of patients
[A] total RAT; [B] mean age; [C] RAT positive; [D] Clinical symptomatic patients

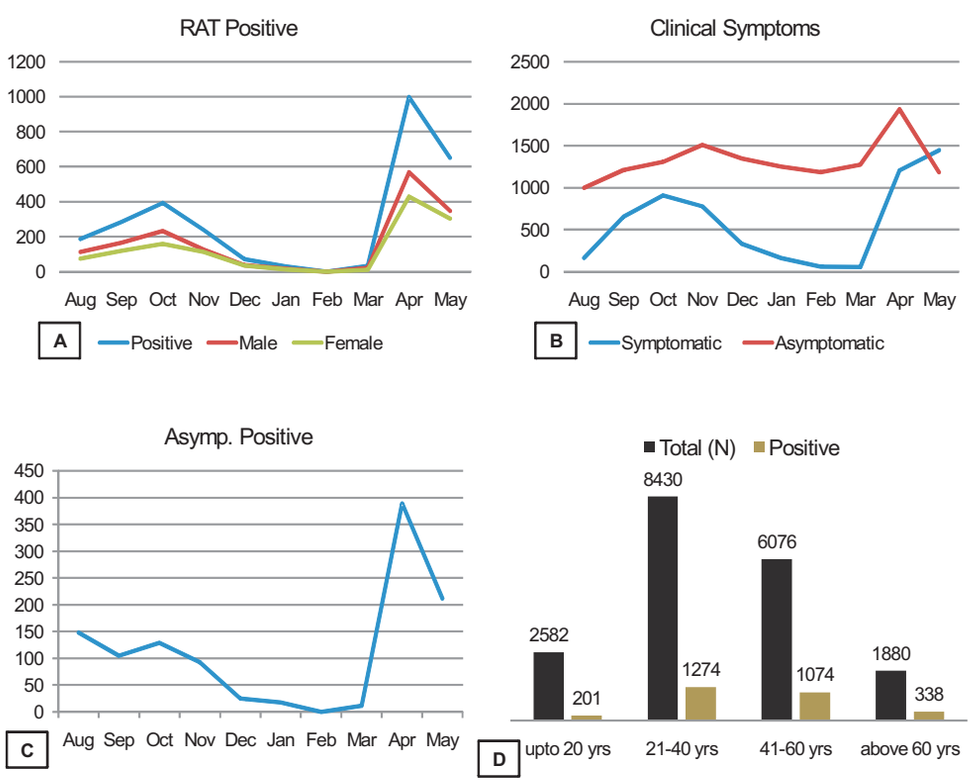

Figure 2 COVID-19 positive in RAT
[A] Infected male and female in different months; [B] clinical symptoms in different months; [C] asymptomatic positive cases in different months; [D] positive cases in different age groups

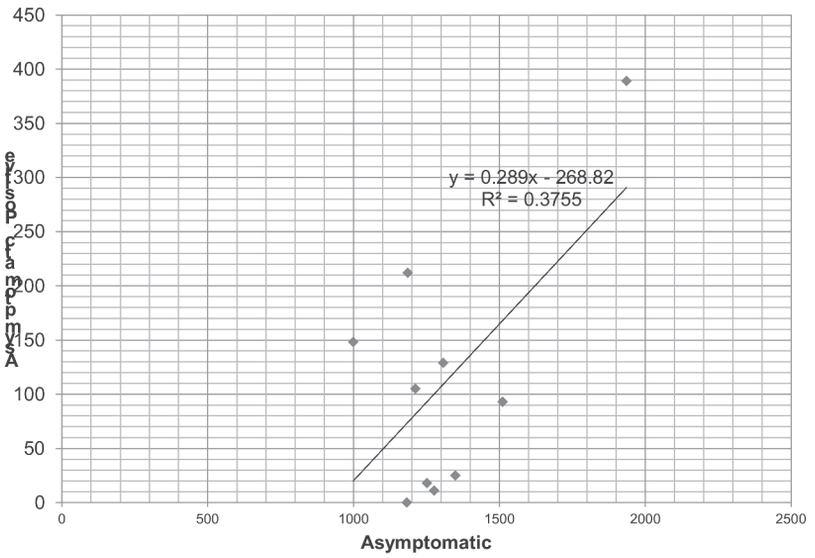

Figure 3 Correlation between asymptomatic and asymptomatic COVID-19 positive RAT [r^2=0.376]

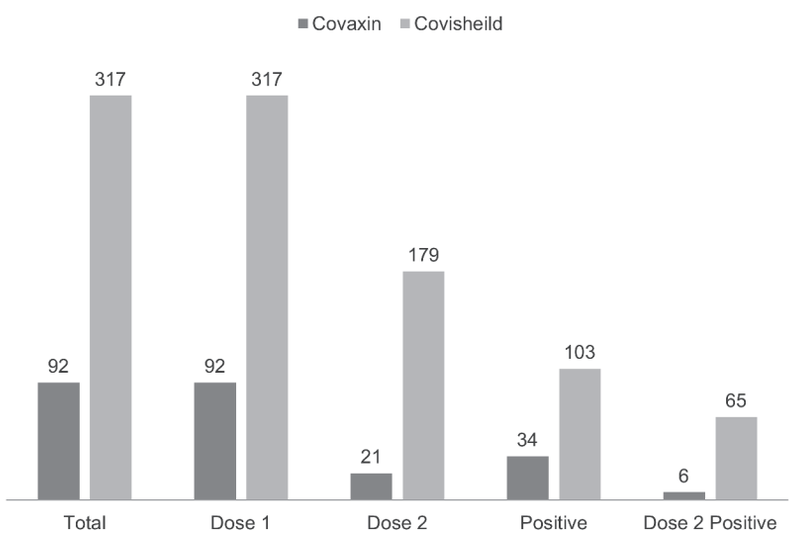

Figure 4 Positive cases by RAT after COVID-19 vaccination

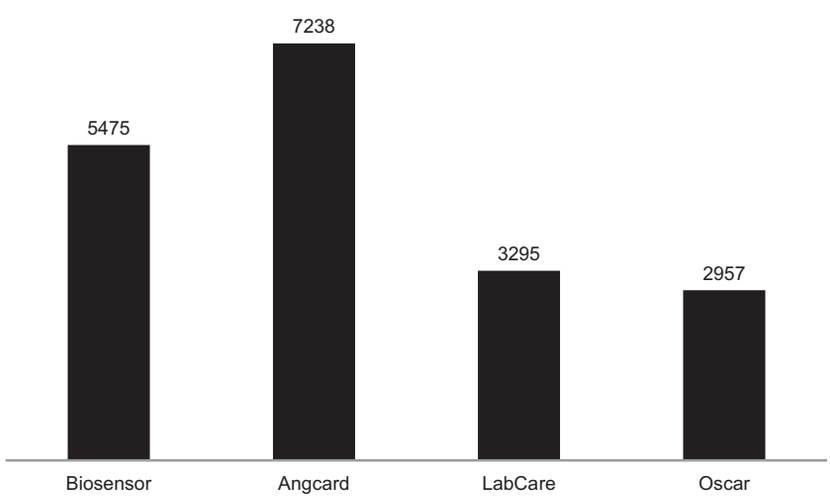

Figure 5 Commercially available COVID-19 antigen kits used

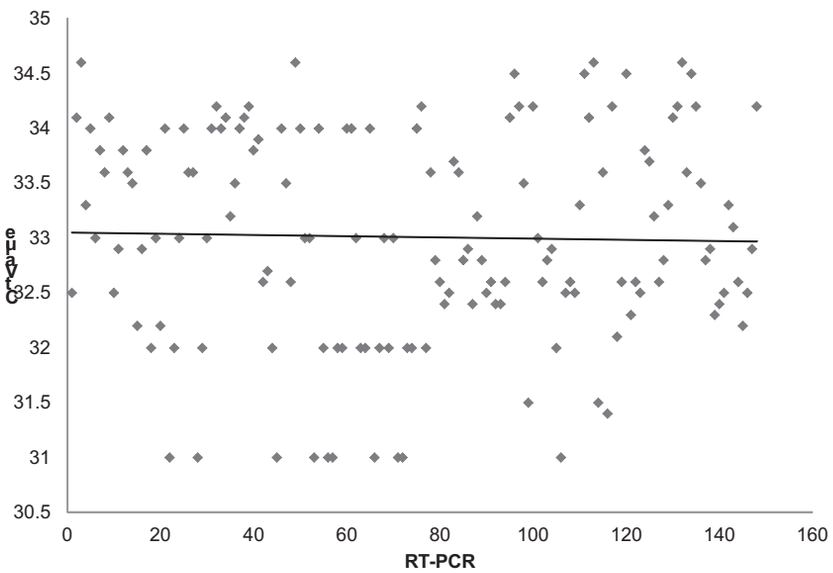

Figure 6 Ct value of RT-PCR in false negative RAT samples