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Variation in Sensitivity Of Commercial Kits Validated At A Center For SARS-CoV-2 An-Tigen Detection

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Abstract

The present study was planned to report variation in sensitivity of antigen detection kits for diagnosis of COVID-19 during their validation.

Total 9 kits were validated and their cumulative sensitivity, specificity, positive and negative predictive values for all the kits along with Cohen kappa coefficient was calculated. Most kits had good sensitivity to detect cases with high viral load (CT Value >20) whereas the sensitivity decreased in samples having low viral load (CT value 30). All kits' specificity was 100% but sensitivity varied from 81% to 0%.

Keywords: Rapid antigen test, SARS-CoV-2, RT-PCR

Introduction

The pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS- CoV-2) has been rapidly emerging since the time it was first reported in December 2019 and has caused massive public health challenges worldwide.[1] Rapid detection of the cases and timely isolation of their contacts is considered important to diminish this unmatched pandemic. Testing for COVID-19 infection mainly depends on reverse transcription polymerase chain reaction (RT- PCR) performed on a nasopharyngeal specimen or oro-pharyngeal swab. It is considered as gold standard for detection of SARS-CoV-2 ribonucleic acid (RNA).[3]. The real-time RT-PCR test requires specialized laboratory facilities in-terms of equipment, biosafety & biosecurity. RT-PCR tests takes a lot of time and there is also a gap between the number of samples to be tested (large) and number of laboratories (few) to perform RT-PCR.[4]

Therefore, there is demand for a substitute assay such as antigen detection tests which can detect presence of virus itself in respiratory samples. Detection of antigen using bed side assay is in use for rapid diagnosis of COVID-19. ICMR/ Government of India have issued an advisory with specific recommendation for use of rapid antigen tests.[5] Many kits are being developed and few are available commercially in the market. There is lot of uncertainty about their performance; therefor their validation is an important pre utilization step.[6]

To supplement RT-PCR testing the process of clinical validation of performance of rapid antigen tests kits has started in India. Validated kits have been used in testing as per national guidelines for the use of rapid antigen tests.

Here, we are reporting variation in sensitivity of antigen detection kits for diagnosis of COVID-19 as reported during their validation.

Methodology: Total 9 commercial kits were validated by Virology Laboratory, King George's Medical University, Lucknow for various performance related issues during second wave of pandemic (May June, 2021). The validation protocol was provided by Indian Council of Medical research

(ICMR), New Delhi. Total 105 cases and equal number of controls were enrolled for validation of each kit. RT-PCR for covid-19 was taken as gold standard for validation of antigen detection assays. The nasopharyngeal/ oro-pharyngeal samples, as per manufacturer's recommendation, were collected from both cases and controls.

Cases were laboratory RT-PCR confirmed (tested within 48-72 hours) cases of COVID -19. representing different groups of high, medium and low viral load cases represented as CT value of 10-19, 20-29 and 30-35 respectively. Controls were patients with respiratory signs and symptoms which tested negative for RT-PCR for covid-19. From each case and control two samples were collected at the time of enrolment. One sample was put in viral transport medium (VTM) and was sent to Microbiology Laboratory for COVID-19 testing by real time PCR. RT-PCR was done by reagents and protocol provided by National Institute of Virology (NIV, Pune). The second sample was taken in the testing kit antigen buffer and was tested as per manufacturer's instruction at the hospital or laboratory. The rapid antigen test was performed and read as per the test kit instructions.

Results were blinded for technologists performing rapid antigen test as well as real-time RT-PCR test. The blinded results were decoded and interpreted by a third party.

Considering real-time RT-PCR as the gold standard test, the sensitivity, specificity, positive and negative predictive value and Cohen kappa coefficient along with confidence interval (CI) of the rapid antigen test was calculated. Cohen kappa coefficient was calculated by Graph pad. This calculator assesses how well two observers, or two methods, classify subjects into groups. The degree of agreement is quantified by kappa. To interpret kappa following scale was used [7]; Kappa < 0: No agreement, Kappa between 0.00 and 0.20: Slight agreement, Kappa between 0.21 and 0.40: Fair agreement, Kappa between 0.41 and 0.60: Moderate agreement, Kappa between 0.61 and 0.80: Substantial agreement, Kappa between 0.81 and 1.00: Almost perfect agreement.

Results:

A total of 9 rapid antigen kits were validated. The cumulative sensitivity, specificity, positive and negative predictive values for all the kits along with Cohen kappa coefficient is tabulated in TABLE 1. The analysis of different groups of cases as per CT values for sensitivity, specificity and Cohen kappa is tabulated in TABLE 2. Results showed that most of the kits performed well when the CT value of the samples was less than 30. At higher CT value (30-35) the sensitivity of the test kit along with the Cohen kappa coefficient agreement decreased. The sensitivity of one kit was as high as 81% while two kits detected none positives. In general most of the kits did not perform up to mark when tested with patients having low viral load, with CT value (30-35).

Discussion:

During the process of validation we found that different varieties of kits have been developed by different companies in India. Some kits were acceptable but most of them had poor sensitivity. Many reasons can be assigned to poor sensitivity like;

1. Antigen detection has to be done at the site of sample collection. Laboratory staff faced challenges due to non-availability of laboratory facilities on site.
2. The sample which was collected for antigen detection was not usable for RT PCR and vice versa due to the requirement of special buffer for antigen detection. Hence a second sample was used for RT PCR.
3. Detection of different analyte by Gold standard (RT PCR) and test under validation (antigen detection): RT-PCR detects virus RNA which is usually abundant in clinical samples in comparison to viral Antigen.
4. High sensitivity of RT PCR in comparison to antigen detection tests: Rapid antigen tests detect the presence of viral antigen without amplification leading to lesser sensitivity compared to RT-PCR which amplifies the virus target sequences. The sensitivity of rapid antigen kits depends upon few factors such as viral load and symptoms.

According to the available evidence the virus can be isolated from the nasopharyngeal samples of symptomatic patients around 5-6 days before and 9-

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11 days after symptom onset. The viral load in respiratory samples peaks around three days before to three days after symptom onset. [11] The effectiveness of these rapid antigen tests is found to be good when the viral load is highest around the above mentioned days of symptom onset.

There are also come some operational drawbacks with the use of the rapid antigen test kits. The most common sample suggested by various manufactures for detection of SARS-CoV-2 in rapid antigen kits is a nasopharyngeal sample which requires a professional health care professional with proper PPE. Other samples like self-collected saliva or nasal swab are not clinically validated for rapid kits. The drawback of rapid antigen test is that samples cannot be transported to higher laboratories for further characterization such as genome sequencing. Few kits require instruments for analysis, some require external pipettes to transfer the sample onto cassette which limit their property of being point of care. Some even require an external ultra violet (UV) torch to read the results. But most of the test kits are performed on to hand held cassette with visual analysis.

Conclusion: Sensitivity of antigen detection kits is a major challenge. If applied it should be used in first few days of illness when viral load is high.

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Table 1: General characteristics, sensitivity and specificity of various kits validated at the centre

Kit number in chronological order	Test principle	Antigen to be detected	Sample to be used	Interpretation	Analysis time	Sensitivity	Specificity	Positive Predictive value	Negative Predictive value	Cohen Kappa co-efficient at 95% CI (Agreement)
KIT 1	Rapid immunochromatographic test	Antigen not specified.	Both NS&TS samples.	Visually by naked eye	15-30minutes	74%	100%	100%	79.3%	0.740 CI: 0.613 to 0.867 (Substantial)
KIT 2	Fluorescence immunochromatography. To be read using a reader/ torch.	SARS-CoV-2 nucleocapsid(N) antigen	Oropharyngeal sample	UV pen flashlight	15 minutes	4%	100%	100%	51.0%	0.040 CI: -.038 to .118 **(No agreement)**
KIT 3	Rapid immunochromatographic test assay.	Antigen not specified	Both NS&TS samples.	Visually by naked eye	15-30minutes	0%	100%	100%	50%	0.0 **(No agreement)**
KIT 4	Rapid anti capture immunochromatographic assay	Viral nucleoprotein	Both NS&TS samples.	Visually by naked eye	20-25minutes	54%	100%	100%	68.5%	0.540 CI 0.436 to .644 (Moderate)

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KIT 5	A qualitative immunochromatographic assay	Nucelocapsid/spike protein of novel Coronavirus	Nasal swab.	Visually by naked eye	15-20 minutes	60%	100%	100%	71.4%	0.60 CI: 0.498 to 0.702 (Moderate)
KIT 6	Lateral flow assay two site sandwich immunochromatography assay	Nucleocapsid antigen	NS &TS swab	Visually by naked eye	15-20 minutes	47%	100%	100%	65.3%	0.470 CI:0.366 to 0.574 (Moderate)
KIT 7	Fluorescence immunoassay	Specific nucleoprotein antigens to SARS-CoV-2 Ag	Nasopharyngeal sample.	Standard F analyser is required	30 minutes	53%	100%	100%	67.7%	0.516 CI: 0.444 to 0.588 (Moderate)
KIT 8	A qualitative immunochromatographic assay	Antigen not specified	Nasopharyngeal sample.	Visually by naked eye	15-20 minutes	81%	100%	100%	77.3%	0.771 CI:0.687-0.855 (Substantial)
KIT 9	Immunochromatograhic test.	Antigen not specified	NS &TS swab	Visually by naked eye	15-20 minutes	58.1%	100%	100%	70.4%	0.581 CI:0.481-0.681 (Moderate)

Table 2: Kit characteristics analyzed for samples with High, moderate and low viral load in-terpreted as CT values

CT-Value	Kit 1	Kit 2	Kit 3	Kit 4	Kit 5	Kit 6	Kit 7	Kit 8	Kit 9

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CT-Value	Kit 1	Kit 2	Kit 3	Kit 4	Kit 5	Kit 6	Kit 7	Kit 8	Kit 9
10-19 **Total samples tested (T)** **Negative samples (N)** **Positive samples (P)**	Total: 25 N: 0 P:25	Total: 25 N: 20 P: 1 Invalid:5	Total: 21 N: 21 P: 0	Total: 36 N: 2 P:34	Total:37 N:3 P:34	Total:27 N:6 P:21	Total:27 N:0 P:27	Total:26 N:0 P:26	Total:37N :7 P:30
Sensitivity & specificity	100% 100%	4.7% 100%	0% 100%	94.4% 100%	91.89% 100%	77.7% 100%	100% 100%	100% 100%	81.08% 100%
Cohen kappa coefficient at 95% CI (agreement)	1 CI:1.00 to .00 (Almost perfect)	0.040 CI:-.038 to .118 (**No agreement**)	0.00 **(No**)	CI: .869 to1 (Almost perfect)	0.579 CI:0.438 to .740 (Moderate)	0.778 CI:0.641 to 0.941 (Almost perfect)	1 CI: 1.00 to 1.00 (Almost perfect)	1 CI: 1.00 to 1.00 (Almost perfect)	0.811 CI: 0.680 to 0.942 (Almost perfect)
20-29 **Total samples tested (T)** **Negative samples (N)** **Positive samples (P)**	Total: 22 N: 11 P:11	NA	Total: 26 N: 26 P:0	Total:48 N: 30 P:18	Total:42 N: 17 P:25	Total:64 N:38 P:26	Total:143 N:68 P:75	Total:62 N: 15 P:47	Total:53 N: 22 P:31

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CT-Value	Kit 1	Kit 2	Kit 3	Kit 4	Kit 5	Kit 6	Kit 7	Kit 8	Kit 9
Sensitivity & specificity	50% 100%	NA	0% 100%	37.5% 100%	59.5% 100%	40.6% 100%	52.44% 100%	75.80% 100%	58.4% 100%
Cohen kappa coefficient at 95% CI (agreement)	0.5 CI: .278 to.722 (Moderate)	NA	0.00 **(No agreement)**	0.375 (CI 0.230 to.520) (Fair)	0.595 CI 0.438 to 0.752 (Moderate)	0.406 CI:0.279 to 0.534 (Modera te)	0.524 CI: 0.438 to 0.611 (Modera te)	0.758 CI: 0.647 to 0.869 (Substanti al)	0.585 CI: 0.44 to 0.72 (Moderate)
30-35 **Total samples tested (T)** **Negative samples (N)** **Positive samples (P)**	Total: 3 N: 2 P:1	NA	Total: 3 N:3 P:0	Total:16 N: 14 P: 2	Total:21 N: 20 P:1	Total:9 N: 9 P:0	Total:41 N: 32 P:7 Invalid:2	Total: 17 N: 9 P:8	Total:15 N: 15 P:0
Sensitivity & specificity	33.33% 100%	NA	0% 100%	12.5% 100%	4.7% 100%	0% 100%	17.07% 100%	47.05% 100%	0% 100%
Cohen kappa coefficient at 95% CI (agreement)	0.33 CI: -.229 to .896 (Fair)	NA	0.00 **(No)**	0.125 CI:.41-.291 (Slight)	0.048 CI :0.044 to 0.140 (No)	0.00 **(No)**	0.178 CI: 0.054 to 0.301 (Slight)	0.471 CI: 0.219- to 0.722 (Moderate)	0.00 **(No)**

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