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COMPARATIVE ANALYSIS OF SIX COMMERCIALLY AVAILABLE REAL TIME PCR KITS FOR DETECTION OF SARS-COV-2

Microbiology

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ABSTRACT

Background: SARS-COV-2 pandemic posed an enormous burden on society and healthcare systems to timely diagnose and control its spread and various measures are being taken for it. Market is flooded with enormous number of commercially available Real time RT PCR kits claiming excellent sensitivity, specificity, Negative Predictive Value (NPV) and Positive Predictive Value (PPV). Most significant step for detection of SARS-CoV-2 is to identify and use RT PCR kits with highest sensitivity and specificity.
Aims And Objectives: This study was conducted to compare the performance of 6 commercially available kits from different manufacturers and evaluate their performances that are widely used.
Methods: This was a laboratory based observational analytical study conducted at the Department of Microbiology, SMS Medical College, Jaipur over a week period in the month,December 2020.
94 SARS-COV-2 RT PCR positive samples (31 samples:Ct ranging 10-20, 31 samples:Ct ranging 21-30, 32 samples:Ct value ranging 30-35) and 30 SARS-CoV-2 RT PCR negative samples were selected. SARS-CoV-2 RTPCR assay kit from Indian Council of Medical Research – National Institute of Virology (ICMR NIV), Pune was taken as standard in the study.
Results: TRUPCR kit showed highest sensitivity and specificity followed by COVIDSURE and QUANTIPLUS. VIRALDTECT-II kit came out to be less sensitive in comparison to other kits.
PPV was found to be 100% with TRUPCR, VIRALDTECT-II, COVISURE and COVIDSURE while GB SARS had 97.8% and QUANTIPLUS had 95.74%. NPV was highest in TRUPCR i.e. 94.74% followed by QUANTIPLUS(90.24%) and COVISURE(90.2%), COVIDSURE(91.11%), GB SARS-COV-2 (88.37%), VIRALDTECT-II(87.67%).
Conclusion: Considering our findings, it is concluded that all of the commercially available RT-PCR kits, included in this study can be used for routine diagnosis of COVID-19 patients, but batch testing of all the kits should be given priority before using new batches in routine diagnosis.

KEYWORDS

Covidsure, GB SARS, Quantiplus, Tru PCR, ,Viral Detect II

INTRODUCTION-

Coronavirus disease (COVID-19) is by far the most popular and recent illness occurred worldwide and still accounting for Severe Acute Respiratory Diseases, Multiple Organ Injury and fatal outcomes.

SARS- COV-2 has emerged among humans during final months of 2019, in Wuhan; China.[1] It's a massive outbreak, spreading all over the world with recurrent peaks and now found in 212 countries. Hence WHO on 11th March 2020 declared it as a pandemic.[2,3] Many countries have taken extreme steps such as lockdown and imposing curfew to curb the spread of pandemic leading to significant impact to the economies, education and lives of individuals.[2]

SARS-COV-2 is an enveloped human Coronavirus single stranded, positive sense RNA of order Nidovirales with the length of 27-32 kb.[1] They express their replication and transcription complex including RNA dependent RNA polymerase (RdRp), from a single, large Open Reading Frame (ORF1ab), along with structural proteins Envelope (E), Nucleocapsid (N), Spike (S) proteins.[4]

SARS-COV-2 pandemic posed an enormous burden on society and healthcare systems to timely diagnose and control its spread and various measures are being taken for it.[3] Many of these measures critically depend on timely and accurate diagnosis of infected individuals to isolate and mitigate transmission.[5]

Amongst the foremost priorities to facilitate public health interventions, is reliable laboratory diagnosis.[5] Real time Reverse transcriptase polymerase chain reaction (RT-PCR) is the most sensitive and specific assay that can provide crucial etiological evidence for COVID-19 diagnosis.[5,6] However, recently the credibility of this test has been questioned due to its low detection rates. Several cases have been detected which came negative even on repeated testing by RT PCR and COVID-19 features were clearly observed on Computed Tomography images.

The duration of illness at the time of collection of sample, type of sample, technique of collection all play a huge role in detection of virus. Sensitivity of RT-PCR test for detection of SARS COV 2 has been claimed to be ranging from 45-75% where some have claimed sensitivity of RT-PCR 45-60%, others shown 65- 75% .[2] The sensitivity of the RT PCR test assay appears to be affected by multiple factors including the type of primer-probe set used and the frequency of repeat samples as repeated testing of consecutive samples from the same patient, increased the positive rates from 70% from the first sample to an additional 24% when testing a second sample.[2]

Market is flooded with enormous number of commercially available Real time RT PCR kits claiming excellent sensitivity, specificity, Negative Predictive Value (NPV) and Positive Predictive Value (PPV). Most significant step for detection of SARS-CoV-2 is to identify and use RT PCR kits with highest sensitivity and specificity. Apart from sample collection, temperature maintenance during storage and transport of samples as well as RT PCR kits, technical errors during sample processing before PCR etc, the sensitivity and specificity of the RT PCR kits are equally important for detection of SARS-CoV-2.

Comparative studies for sensitivity and specificity among different commercially available kits are still limited. Hence this study was conducted to compare the performance of 6 commercially available kits from different manufacturers and evaluate their performances that are widely used in India.

The ORF1ab/RdRp, E, N and RNaseP are the targets most frequently used for SARS-COV-2 detection by RT-PCR. The most crucial being the ORF1ab and N genes according to several studies as ORF1ab is the main reason for the virus replication and transcription and N protein is expressed through the production of the sub genomic messenger RNAs ,the number of end proteins markedly exceeds that of genomics RNAs in several stages of the replication cycles.[3]

MATERIAL AND METHODS-

Study Design And Clinical Specimen-

This was a laboratory based observational analytical study conducted at the Department of Microbiology, SMS Medical College, Jaipur over a week period in the month of December 2020.

94 SARS-COV-2 RT PCR positive samples (31 samples:Ct ranging 10-20, 31 samples:Ct ranging 21-30, 32 samples:Ct value ranging 30-35) and 30 SARS-CoV-2 RT PCR negative samples were selected for the study. SARS-CoV-2 RTPCR assay kit from Indian Council of Medical Research – National Institute of Virology (ICMR NIV), Pune was taken as standard in the study.

This study was conducted on already reported and stored samples.

Exclusion Criteria-

Samples with Ct value > 35 were excluded.

Selection Of Kits-

Commercially available 6 kits already approved by ICMR, from different manufacturers were selected for the study with different detection targets (E, N, RdRp, ORF1ab). All the kits were suitable for Quant-Studio Real Time PCR Instrument system (Applied Biosystems).

RNA EXTRACTION-

Perkins Elmer chemagic 360 instrument was used for nucleic acid extraction and elute was added to respective mastermix of each kit. All the kits were used as per manufacturer's protocol.

RT PCR -

Quant-studio Real time PCR Instrument was used for RT-PCR.

OUTLINE OF KITS INCLUDED IN THIS STUDY-

The **Multiplex Single tube real time PCR, ICMR- NIV,** Pune kit was taken as standard for this study. It contains a set of TaqMan RTPCR assays for the qualitative detection and characterization of SARS-COV-2 RNA. The assay includes three targets E gene, ORF1ab, RdRp of SARS COV2 genes and one housekeeping gene B Actin gene. Assay has one screening and two confirmatory viral genomic regions targets, reducing the risks of false negatives.

For the reactions to take place 7 µl of nucleic acid elute is added to solution of mastermix. The Ct cut off values is ≤ 35.

The six RT PCR kits compared with the standard kits are as following:

1. TRUPCR SARS COV2 RT qPCR kit (v-3.2) (kilpest India Ltd) :-

- Ct cut off: ≤35
- Lowest limit of detection : Not mentioned
- Elute volume:10µl
- Sensitivity: 99.43%
- Specificity: 99.41%

Interpretation:

RNaseP	E gene	RdRp	Result
+/-	+	+	Positive
+/-	-	+	Positive
+/-	-	-	Negative
-	-	-	Invalid

2.VIRALDTECT-II (GENES2ME Pvt Ltd) Multiplex Real time PCR kit for Covid-19 :-

- Ct cut off: ≤37
- Lowest limit of detection : Not mentioned
- Elute volume:11.5µl
- Sensitivity: Not mentioned
- Specificity: Not mentioned

Interpretation:

E gene/ FAM	RdRp/Ngene(TEXAS Red/Cy5)	RNaseP/HEX	Result
+	+	+/-	Positive
+	-	+	Inconclusive, repeat sample
-	-	+	Negative

3. GB SARS COV2 Real time RT PCR (General biological corporation) :-

- Ct cut off:<37 cycles
- Lowest limit of detection:- 500 copies/ ml
- Elute volume:10µl
- Sensitivity: >95%
- Specificity: 100%

Interpretation:

E gene	ORF1ab	Result
-	-	Negative
+	-	Inconclusive, repeat sample
-	+	Inconclusive, repeat sample
+	+	Positive

4. COVISURE Biotech Asia Pvt GENETIX (Genetix Ltd) :-

- Ct cut off: ≤36
- Lowest limit of detection: 10^3 copies / ml
- Elute volume:5µl
- Sensitivity: 100%
- Specificity: 100%

Interpretation:

RNaseP/HEX/IC	E gene /FAM	RdRp/QUASAR 670	Result
+/-	+	+	Positive
+/-	-	+	Positive
+/-	+	-	Inconclusive, repeat sample
+	-	-	Negative
-	-	-	Invalid

5. Quantiplus (Huwel lifesciences Pvt Ltd) Multiplex Covid-19 Detection kit (V2.0) :-

- Ct cut off: ≤36
- Lowest limit of detection:25 copies/ µl
- Elute volume:10µl
- Sensitivity: Not mentioned
- Specificity: Not mentioned

Interpretation:

E gene/FAM	N gene(Yakima yellow)	IC/Cy5	Result
+	+	+	Positive
-	+	+	Positive
-	-	+	Negative

6. COVIDSURE (Labsystems diagnostics)multiplex Real time RT-PCR kits :-

- Ct cut off:<36
- Lowest limit of detection:<5 copies/ml
- Elute volume:5µl
- Sensitivity: 100%
- Specificity: 100%

Interpretation:

E gene/ HEX	ORF1ab /FAM	Rpp30/ROX	Result
+	+	+	Positive
-	+	+	Positive
+	-	+	Inconclusive, repeat sample
-	-	+	Negative

OVERVIEW OF KITS FOR RT-PCR BASED DETECTION OF SARS COV2 USED IN THE STUDY :-

Manufacturer	Catalog no.	RNA added	Storage condition	Target genes
Trupcr	MFG/IVD/2020/000024	10µl	-20˚C	E, N, RNaseP, RdRp
Viral dtect-II	G2M020220	11.5 µl	-10˚to 30˚C	E, N, RNaseP, RdRp
GB sarscov-2	4PCO052E	10 µl	≤-15˚C	E, ORF1ab
Covisure	CovizOne-100	5 µl	-20˚C	E, RNaseP, RdRp
Quantiplus	PL-QLC NM-03	10 µl	-20˚C	E, N, IC
Covid sure	30201282/30201284	5 µl	-20˚C	E, ORF1ab, RPP30-ROX

To avoid RNA degradation, study was planned in such a manner that entire experiment completed in 24 hours. For this set up, we obtained elutes (extraction done by Perkin Elmer) to conduct the experiment for 6 kits with required quantity of RNA to be added to their respective kits mastermix from the known positive and negative samples.

For any invalid or inconclusive results, sample was retested right from extraction.

RESULTS:

In the present study, a comparison of six commercially available RT PCR kits for detection of SARS- CoV-2 in clinical samples is provided, considering ICMR- NIV RT PCR kits as standard. All RT - PCR kits gave satisfactory results with respect to sensitivity.

Table 1 - Overall Diagnostic Efficacy Of Kits Observed :

Kits	Positive (%) (n=94)	Negative (%) (n=30)	False positive (%)	False negative (%)	Kappa agreement
Trupcr	92 (97.8%)	36(29%)	0	2	0.962
Viral dtect-II	85 (90.4%)	64(51.6%)	0	9	0.884
GB SARSCOV-2	89 (94.6%)	38(30.6%)	2	5	0.878
Covisure	89 (94.6%)	46(37%)	0	5	0.921
Quantiplus	90 (95.7%)	37(29.8%)	4	4	0.048
Covid sure	90 (95.7%)	41 (33%)	0	4	0.932

As shown in Table-1, false positives were found with GB SARSCOV-2 and QUANTIPLUS while false negatives found in all the 6 kits.

All the kits showed excellent agreement with the standard kit except VIRAL DTECT II.

Table 2: Comprehensive Assessment Of Diagnostic Value Of Six Methods

Kits	Sensitivity	Specificity	PPV	NPV
TRUPCR	97.8%	100%	100%	94.74%
VIRALDTECT-II	90.4%	100%	100%	87.67%
GB SARS COV2	94.6%	93.7%	97.80%	88.37%
COVISURE	94.6%	100%	100%	90.2%
QUANTIPLUS	95.7%	90.9%	95.74%	90.24%
COVIDSURE	95.7%	100%	100%	91.11%

As shown in Table-2, TRUPCR kit showed highest sensitivity and specificity followed by COVIDSURE and QUANTIPLUS as depicted in Table 2. VIRALDTECT-II kit came out to be less sensitive in comparison to other kits.

PPV was found to be 100% with TRUPCR, VIRALDTECT-II, COVISURE and COVIDSURE while GB SARS had 97.8% and QUANTIPLUS had 95.74%. NPV was highest in TRUPCR i.e. 94.74% followed by QUANTIPLUS(90.24%) and COVISURE (90.2%), COVIDSURE(91.11%), GB SARS-COV-2 (88.37%), VIRALDTECT-II(87.67%).

For the chosen set of samples, the different assays showed almost a similar pattern of Ct values. On an average difference Ct values (ΔCt) was noted as ±1 to ± 5.

DISCUSSION–

COVID-19 spread is a global public health concern.[6] Early detection of the disease, isolating all the patients and active surveillance of all the contacts are crucial to contain the spread of this disease.

Admist at this situation of COVID-19 disease pandemically, WHO has globally emphasized the importance of molecular diagnosis of SARS COV2 to limit the spread as well as to appropriately treat those patients who have a serious illness.[7] WHO further accentuated that an urgent increase in the laboratory testing, isolation and contact tracing should be the backbone of the pandemic control strategy running worldwide.[8] Detection of viral RNA in clinical specimens is the hallmark of diagnosis and the global need for accurate and validated diagnostic PCR assays has increased.[9]

Initially only the laboratory developed tests in specialized centers were being used but in looking at the outrageous spread in response to outbreak, commercial assays have come out to be more helpful and advantageous for rapid implementation in routine hospital laboratories.[10]

The sensitivity and specificity of COVID-19 RT PCR kits is highly dependent on the quality of the kit apart from other factors[2].

In this study, a comparison of six commercially available RT-PCR kits for detection of SARS-CoV2 was done and it was observed that all RT PCR kits performed satisfactorily giving sensitivity ≥ 94% except only one kit VIRAL DTECT manufactured by GENES2ME PVT LTD,Gurgaon Haryana.

VIRAL DETECT is also an ICMR approved kit but it's a well known fact that there can be differences in different batches of the same kit. Although, there might be difference in the raw materials and production lines employed in production of kits, manufacturers should ramp up suspension to ensure product quality from batch to batch which would result in only a slight difference in detection with allowable error range.[9] False negative results may compromise with timely diagnosis, early treatment, prevention of transmission and assessment of the discharge criteria.[11]

All the kits except QUANTIPLUS manufactured by Huwell life sciences and GB SARS manufactured by General Biologicals Corporation gave 100% specificity. As N gene persists longer than others and remains positive in RT PCR during convalescence too as per study done by Zhang X et al.[7] This may be the reason behind the false positive results by QUANTIPLUS that has target genes E and N. A false positive result may have several effects like delay of correct diagnosis in patients with breathing difficulties or other symptoms, administration of inappropriate treatment, wasteful consumption of personal protective equipment, reduction in healthcare-workers leading to uncareful management of critical patients, unnecessary stress in isolated individuals including family members.[11]

In comparison to ORF1ab, RdRp is less sensitive as reported by Corman et al. and that's why RdRp based assay miss 35% SARS-COV2 positive cases.[12] Hence only RdRp gene based kits (like COVISURE) should be used with caution and supported with confirmatory assays as RdRP is located on ORF1b and its expression requires ribosomal frame shifting, implying that it is produced at significantly lower levels which makes it difficult to identify.[13]

CONCLUSION:

Considering our findings, it is concluded that all of the commercially available RT-PCR kits , included in this study can be used for routine diagnosis of COVID-19 patients, but batch testing of all the commercially available kits should be given priority before using new batches in routine diagnosis.[9] In addition to this with increasing number of commercially available kits, researchers should share information regarding performance of these kits.

LIMITATION OF STUDY-

Stored samples were used for assessing 6 kits, also sample size was small, this type of study should be conducted on large number of samples to avoid the errors.

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