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Original Research Article

Diagnosis of COVID 19 (SARS CoV 2) By Two Different Targets RdRp and S GENE-A Comparison Study

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Abstract

Beta-coronavirus, which caused Severe Acute Respiratory Coronavirus-2 Syndrome (SARS-CoV-2), a major respiratory outbreak in Wuhan, China in December 2019, is now prevalent in many countries around the world. Identifying PCR-based viruses is a well-known and relatively stable protocol. So diagnosis is very important for early identification of disease and to prevent further spread. In this study, we evaluated the power of a conventional RT-PCR to detect SARS-CoV-2 by two different genes. *Material and Methods*: We did comparative analysis for RNA dependent RNA Polymerase (Rdrp gene) and Spike (S gene by) RTPCR. We took Two hundered Seventy four (274) Nasopharangeal samples came from different hospitals of Najran zone and run samples in three runs in three different runs and we did comparative analysis of Rdrp gene using Ko gene kit and S gene using Altona kit. *Results:* Analysis was done Qualitatively. We compared Rdrp gene with S gene. We find Sensitivity-100 % and Specificity-96%.In Second run Sensitivity was 100%, Specificity was 98%. In third run Sensitivity and Specificity was 100%.So by comparative analysis of Rdrp and S gene showed that diagnosis by Rdrp gene is more sensitive and specific than S gene. *Conclusion*: Detection of Rdrp gene for SARS CoV2 is more sensitive and specific than Sgene.But diagnosis by Symptoms, Serology, Chest X-Ray and Chest CT Scan gives additional help.

Keywords: SARS CoV2, Rdrp gene, Sgene.

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Introduction

Coronavirus disease 2019 (COVID-19) is a contagious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). On 31 December 2019, the World Health Organization was informed of a cluster of cases of pneumonia of unknown etiology in Wuhan, China. It has since spread worldwide, leading to an ongoing pandemic. Diagnosis by Symptoms, RTPCR, Serology chest Xray and CTScans (Fig 1, 2 is gold standard.

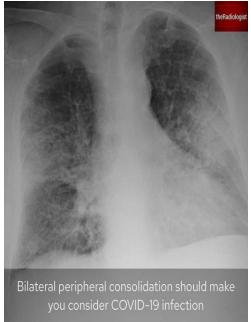


Fig-1

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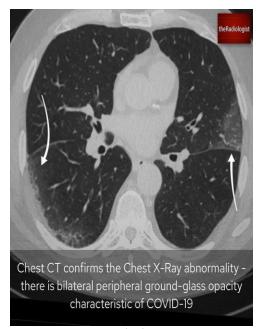


Fig-2

SARS-CoV-2 virus belongs to the B lineage of the β -coronaviruses (β -CoVs); this family comprises an enveloped, non-segmented, positive-sense singlestranded RNA virus genome, with a 5' cap structure and 3' poly-A tail, allowing to perform as an mRNA for translation of the replicase polyproteins [1]. Based on the data of whole-genome analyses, the genome of β-CoVs encodes several non-structural and four structural proteins, including spike (S), envelope (E), membrane (M), and nucleocapsid (N) [2, 3]. Among the known RNA viruses, coronaviruses, which are single-stranded and positive-sense RNA viruses have the largest genome between other RNA viruses, with the GC content ranging from 32% to 43% [4, 5]. The SARS-CoV-2 genomic sequences exhibit a diverse length from 29.8 kb to 29.9 kb with 12 open reading frames (ORFs) encoding 27 proteins [6, 7]. The genomic organization includes 5'- leader sequence- ORF1/ab- S- ORF3a- E-M- ORF6a- ORF7a- ORF7b- ORF8- N- ORF10-3' from left to right and lacks the hemagglutinin-esterase gene which is detected in some β-CoVs. A series of transcription regulatory sequences (TRS) is situated at the junction between each of these ORFs as well as at the 5' end of the genomic RNA downstream of the leader sequence of β-CoVs [8]. About two-thirds of SARS-CoV-2's RNA comprises ORF1a/b region, which with 16 non-structural proteins (nsp1-16) is considered as the largest ORF. The remaining one-third of the genome near the 3'-terminus contains ORFs encode structural and accessory proteins [2].

MATERIAL AND METHODS

Two hundered Seventy four (274) Nasopharangeal samples from suspected COVID symptoms from different hospitals in nejran zone has been taken for study. Samples run at Regional lab.Nejran in three runs by two different kits. Ko gene

kit for Rdrp gene and Altona kit for S gene was used. RNA extraction was done by Bioneer.

The RealStar® (Altona) SARS-CoV-2 RT-PCR Kit 1.0 consists of: Master A , Master B ,Positive Control (B- β CoV, SARS-CoV-2), Internal Control , Water (PCR grade) Master A and Master B contain all components (PCR buffer, reverse transcriptase, DNA polymerase, magnesium salt, primers and probes) to allow reverse transcription, PCR mediated amplification and detection of B- β CoV (target E gene) specific RNA, SARS-CoV-2 (target S gene) specific RNA and Internal Control in one reaction setup.

Procedure

- 1. Pipette 20 μl of the Master Mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube
- 2. Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the controls (Positive or Negative Control).

Reaction Set up

Master mix	20 μl
Sample or Control	10µl
Total Volume	30µl

Reaction mixture set-up for the PowerChekTM 2019-nCoV Real-time PCR (Rdrp gene)

Composition	Volume per reaction
	for Rdrp gene assay
Primer/Probe Mix 2 (RdRP	4μl
gene)	
Primer/Probe Mix (RNA	0.5 µl
Process Control)	
RT PCR PRE Mix	11 μl
Total Volume	15.5µl

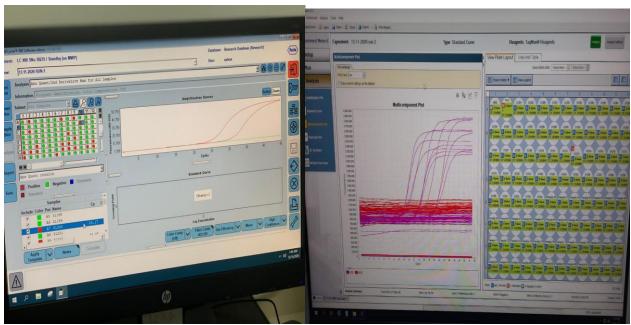
Add the RNA Process Control (RPC) to a patient sample (5 μL of RPC for each sample).

Prepare master mix (15.5 μ L) and add extracted RNA (4.5 μ L) to make 20 μ L total reaction volumes. Perform RT-PCR using the appropriate real-time instrument Analyze data and interpret the results of the patient's sample.

RESULTS

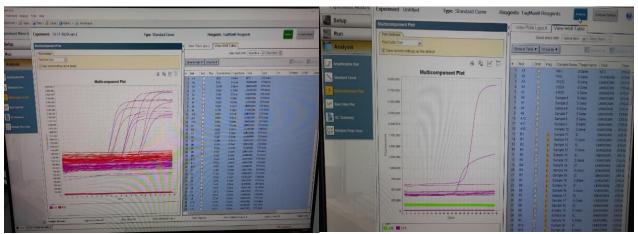
90 samples were taken in run 1. Rdrp gene results were compared with S gene. Analysis was done in qualitative manner. In Run 1, Sensitivity was 100%, Specificity was 96%, True Positive 16, True Negative 71, False Positive 3. In Run 2, 94 samples were taken .Sensitivity was 100%, Specificity was 98%, True Positive-9, True Negative 83, False Positive-2. In Run 3, 94 samples were run, Sensitivity was 100%, Specificity was 100%, True positive-17, True Negative -77.

Total Samples	Sensitivity	Specificity	True Positive	True Negative	False Positive	False Negative
90	100%	96%	16	71	3	0
94	100%	98%	9	83	2	0
94	100%	100%	17	77	0	0



LIGHT CYCLER 480 (Rdrp gene)

REAL TIME PCR 7500 FAST(S GENE)



REAL TIME PCR 7500 (S GENE)

COVID POS.GRAPH

	nod Validation Analyte		PARISON STUE SARS COV2	DOCTOR DR.SMITA SHARMA Comparison Gene S gene				
	rence Gene	Instru	Rdrp gene	Discrepant				
1	Sample ID	Rdrp gene	Sgene	Discrepant Result	Interpretatio	Sensitivity	100%	
2		NON REACTIVE	NON REACTIVE	170	NEGATIVE	Specificity	96%	
3	31197	NON REACTIVE	NON REACTIVE	200	TRUE NEGATIVE	tal Number of sampl	90	
	31198	NON REACTIVE	NON REACTIVE		TRUE			
4	31199	NON REACTIVE	NON REACTIVE	20	NEGATIVE	True Positive	16	
5	31200	REACTIVE	REACTIVE	70	TRUE POSITIVE	True Negative	71	
6	31201	NON REACTIVE	NON REACTIVE	NO	NEGATIVE	False Positive	3	
7 8		REACTIVE	NR	YES	POSITIVE TRUE NEGATIVE	False Negative	0	
9	31203	NON REACTIVE	NON REACTIVE	ZO ZO	TRUE			
10		NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE			
11	31206		NON REACTIVE	NO	TRUE	Note: Any discrepan	t result should b	
12		NON REACTIVE	NON REACTIVE	70	NEGATIVE TRUE NEGATIVE	Note: Any discrepan verified by suitable co before selecting the f	inal interpretation	
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14		NON REACTIVE	NON REACTIVE	70	NEGATIVE			
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17		NON REACTIVE	NON REACTIVE	ИО	TRUE NEGATIVE			
18	31213		NON REACTIVE	9	TRUE NEGATIVE			
19	31214	NON REACTIVE	NON REACTIVE	2	TRUE NEGATIVE			
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30	31224		NON REACTIVE	20 02	TRUE			
30	31225	NON REACTIVE	NON REACTIVE	NO	TRUE			
32		NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE			
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34	31229	NON REACTIVE	NON REACTIVE	NO	NEGATIVE TRUE			
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37	31231		NON REACTIVE	170	TRUE			
38		NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE			
39	31234		NON REACTIVE	9	TRUE NEGATIVE			
40	31235	NON REACTIVE	NON REACTIVE	70	TRUE NEGATIVE TRUE			
41	31236	NON REACTIVE	NON REACTIVE	70	NEGATIVE			
42 43	31237		REACTIVE	70	POSITIVE			
44		NON REACTIVE	NON REACTIVE	70	NEGATIVE TRUE			
45	31239		NON REACTIVE	NO	TRUE NEGATIVE			
46		NON REACTIVE	NON REACTIVE	9	TRUE NEGATIVE TRUE			
47	31242	NON REACTIVE	NON REACTIVE	NO	NEGATIVE TRUE			
48 49	31243		NON REACTIVE	70	NEGATIVE			
50		NON REACTIVE	NON REACTIVE	700	TRUE			
51	31245		NON REACTIVE	NO	TRUE NEGATIVE			
52		NON REACTIVE	NON REACTIVE	20	TRUE NEGATIVE			
53		NON REACTIVE	NON REACTIVE	70	TRUE NEGATIVE TRUE			
54	31249		NON REACTIVE	70	NEGATIVE			
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1		Analyte SARS COV2			I	DOCTOR DR.SMITA SHARMA			
1	Refere	nce Gene	Rdrp gene			Compar	rison Gene Sgene		
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2 28786 NON-REA 2 28786 NON-REA 3 28787 NON-REA 4 28788 NON-REA 5 28780 NON-REA 6 28790 NON-REA 6 28790 NON-REA 8 28792 NON-REA 10 28791 NON-REA 11 28795 NON-REA 11 28796 NON-REA 11 28800 REACT 12 28801 NON-REA 12 28802 NON-REA 13 28802 NON-REA 14 28803 NON-REA 15 28806 NON-REA 16 28800 NON-REA 17 28801 NON-REA 18 28802 NON-REA 19 28803 NON-REA 19 28803 NON-REA 19 28803 NON-REA 19 28804 NON-REA 19 28805 NON-REA 19 28806 NON-REA 19 28806 NON-REA 19 28807 NON-REA 19 28808 NON-REA 19 28809 NON-REA			Rdrp gene	Sgene	Piscrepant Result	Interpretatio	Sensitivity	100%	
28787 NON-REA 28788 NON-REA 28789 NON-REA 28790 NON-REA 28791 NON-REA 28791 NON-REA 28791 NON-REA 28792 NON-REA 28792 NON-REA 28793 NON-REA 28795 NON-REA 28796 NON-REA 28796 NON-REA 28796 NON-REA 28796 NON-REA 28796 NON-REA 28796 NON-REA 28797 NON-REA 28797 NON-REA 28797 NON-REA 28797 NON-REA 28798 NON-REA 28			NON-REACTIVE	NON-REACTIVE	NO	TRUE NEGATIVE	Specificity	98%	
STATE	9	28787	NON-REACTIVE	NON-REACTIVE	NO	TRUE NEGATIVE	Total Number of samples	94	
NON-REA STATE STATE NON-REA STATE NO	-		NON-REACTIVE	NON-REACTIVE	.,0	TRUE			
NON-REAL	4	28788	NON-REACTIVE	NON-REACTIVE	70	NEGATIVE	True Positive	9	
Section	5	28789	NON-REACTIVE	NON-REACTIVE		TRUE NEGATIVE	True Negative	83	
7	6	28790	NON-REACTIVE	NON-REACTIVE	NO	TRUE NEGATIVE	False Positive	2	
9	7	28791	NON-REACTIVE	NON-REACTIVE	NO	TRUE NEGATIVE	False Negative	o	
100 28794 NON.REA 100			NON-REACTIVE	NON-REACTIVE	NO	TRUE NEGATIVE TRUE			
1			NON-REACTIVE	NON-REACTIVE	NO	NEGATIVE			
12 28796 NON-REA			NON-REACTIVE	NON-REACTIVE	70	TRUE NEGATIVE	Note: Any discrepant resul	t should be verified	
131 28797 NON-REA 141 28798 NON-REA 142 28790 NON-REA 143 28790 NON-REA 144 28800 REAC 145 28800 NON-REA 146 28800 NON-REA 147 28800 NON-REA 148 28800 NON-REA 149 28811 NON-REA 149 28812 NON-REA 149 28813 NON-R			NON-REACTIVE	NON-REACTIVE	NO	TRUE	suitable confirmatory meth final interpr	od before selecting	
NON-READER	1.3	28797	NON-REACTIVE	NON-REACTIVE	NO	TRUE NEGATIVE			
Teach Teac			NON-REACTIVE	NON-REACTIVE	70	TRUE NEGATIVE TRUE			
Tensor			REACTIVE	REACTIVE	NO NO	POSITIVE			
NON-REA NON-			REACTIVE	REACTIVE	NO NO	POSITIVE TRUE NEGATIVE	-		
130 28803 NON-REA 221 28806 NON-REA 222 28806 NON-REA 223 28806 NON-REA 224 28806 NON-REA 225 28806 NON-REA 226 28807 NON-REA 227 28810 NON-REA 227 28811 NON-REA 228 28812 NON-REA 239 28813 NON-REA 249 28813 NON-REA 250 28813 NON-REA 251 28815 NON-REA 251 28815 NON-REA 252 28816 NON-REA 253 28816 NON-REA 254 28816 NON-REA 255 28817 NON-REA 256 28817 NON-REA 257 28818 NON-REA 258 28817 NO			NON-REACTIVE	NON-REACTIVE	70	TRUE NEGATIVE			
201 28804 NON.REA 222 28805 NON.REA 223 28805 NON.REA 224 28805 NON.REA 225 28805 NON.REA 226 28805 NON.REA 227 28810 NON.REA 228 28810 NON.REA 229 28811 NON.REA 230 28811 NON.REA 231 28812 NON.REA 231 28813 NON.REA 231 28814 NON.REA 231 28815 NON.REA 231 28815 NON.REA 231 28815 NON.REA 241 28815 NO	19	28803	NON-REACTIVE	NON-REACTIVE	NO	TRUE NEGATIVE	•		
NON-REA NON-	20	28804	NON-REACTIVE	NON-REACTIVE	NO	NEGATIVE			
288 288			NON-REACTIVE	NON-REACTIVE	NO	TRUE NEGATIVE TRUE			
24. 28809 NON-REA 25. 28809 NON-REA 26. 28809 NON-REA 26. 28809 NON-REA 27. 28811 NON-REA 28812 NON-REA 28813 NON-REA 28814 NON-REA 28815 NON-			NON-REACTIVE	NON-REACTIVE	NO	NEGATIVE			
1989 1989			REACTIVE	REACTIVE	70	POSITIVE	-		
262 288130 REACT 27 288131 NON-REACT 288 288131 NON-REACT 289 289 NON-REACT 2			NON-REACTIVE	NON-REACTIVE	NO NO	TRUE			
227 28811 NON-REA 288 28812 NON-REA 289 28813 NON-REA 289 28814 NON-REA 289 28815 NON-REA 289 28816 NON-REA 289 289 289 289 NON-REA 289 289 289 NON-REA 289 NO	26	28810	REACTIVE	REACTIVE	NO	TRUE			
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11 28815 NON-REA 12 28816 NON-REA 13 28817 NON-REA 13 28817 NON-REA 13 28818 NON-REA 13 28818 NON-REA 14 28818 NON-REA 15 28812 NON-REA 16 28812 NON-REA 17 28812 NON-REA 18 28813 NON-REA 18 28814 NON-REA 18 28815 NON-REA 18 288			NON-REACTIVE	NON-REACTIVE	NO	TRUE NEGATIVE TRUE	-		
NON-REA NON-			NON-REACTIVE	NON-REACTIVE	NO	NEGATIVE			
1989 1989			NON-REACTIVE	NON-REACTIVE	NO NO	TRUE			
344 288138 NON-REA 35 288121 NON-REA 36 288221 NON-REA 36 288221 NON-REA 36 288221 NON-REA 36 288222 NON-REA 36 288221 NON-REA 36 288222 NON-REA 36 288221 NON-REA 37 288221 NON-REA 38 288221 N			NON-REACTIVE	NON-REACTIVE	NO	TRUE NEGATIVE			
NON-REA NON-	14	28818		NON-REACTIVE	20	TRUE			
NON-REA NON-	35	28819	NON-REACTIVE	NON-REACTIVE	70	TRUE NEGATIVE			
1989 1989			NON-REACTIVE	NON-REACTIVE	NO	TRUE NEGATIVE TRUE			
NON-REA NON-			NON-REACTIVE	NON-REACTIVE	NO NO	TRUE NEGATIVE TRUE	•		
19824 19824 19824 19824 19824 19825 19824 19825 19824 1982			NON-REACTIVE	NON-REACTIVE	200	NEGATIVE	-		
28822 NON. REA 28822 NON. REA 28823 NON. REA 28824 NON. REA 28824 NON. REA 28825 NON. REA 28825 NON. REA 28826 NON. REA 28827			NON-REACTIVE	NON-REACTIVE	70	TRUE			
28826 NON-REA 28827 NON-REA 28828 NON-REA 28829 NON-REA 28	11	28825	NON-REACTIVE	NON-REACTIVE	NO	TRUE NEGATIVE	•		
100 100			NON-REACTIVE	NON-REACTIVE	70	TRUE NEGATIVE			
28829 NON-REA			NON-REACTIVE	NON-REACTIVE	NO	TRUE NEGATIVE TRUE			
NON-REA NON-REA			NON-REACTIVE	NON-REACTIVE	NO	NEGATIVE	•		
NON-REA NON-			NON-REACTIVE	NON-REACTIVE	70	TRUE			
28832 NON.REA			NON-REACTIVE	NON-REACTIVE	70	TRUE			
28833 NON-REA	18	28832	NON-REACTIVE	NON-REACTIVE	NO	NEGATIVE TRUE NEGATIVE	•		
28835 NON-REA			NON-REACTIVE	NON-REACTIVE	NO	TRUE NEGATIVE			
NON-REA NON-			NON-REACTIVE	NON-REACTIVE	NO	NEGATIVE TRUE			
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28135 NON-REA 28136 NON-REA 28131 NON-REA 28132 REA 28133 NON-REA 28134 NON-REA 28135 NON-REA 28137 NON-REA			NON-REACTIVE	NON-REACTIVE	70	TRUE NEGATIVE			
28130 NON-REA 28130 NON-REA 28131 NON-REA 28	54	28838	NON-REACTIVE	NON-REACTIVE	NO	TRUE	•		
NON-REA NON-REA			NON-REACTIVE	NON-REACTIVE	NO	NEGATIVE TRUE			
SER 28842 NON-REA SER 28843 NON-REA SER 28844 NON-REA SER 28844 NON-REA SER 28844 NON-REA SER 28845 NON-REA SER 28846 NON-REA SER 28846 NON-REA SER 28846 NON-REA SER 28847 NON-REA SER 28857 NON-REA SER 28858 NON-REA SER 28859 REACT SER 28850 NON-REA SER 28850 NON-			NON-REACTIVE	NON-REACTIVE	NO	NEGATIVE TRUE			
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53 28845 NON-REA 52 28846 NON-REA 53 28847 NON-REA 54 28848 NON-REA 56 28850 NON-REA 56 28851 NON-REA 57 28851 NON-REA 57 28854 NON-REA 57 28854 NON-REA 57 28855 NON-REA 57 28855 NON-REA 57 28856 NON-REA 57 28857 NON-REA 57 28857 NON-REA 57 28858 NON-REA 57 28859 NON-REA 57 28850 NON-REA 58 28860 NON-REA 58 288	50	28844	NON-REACTIVE	NON-REACTIVE	NO	TRUE			
BASE STONE STATE S			NON-REACTIVE	NON-REACTIVE	NO	TRUE NEGATIVE			
28845 NON-REA 28851 NON-REA 28852 REACT 28853 NON-REA 28852 NON-REA 28853 NON-REA 28853 NON-REA 28854 NON-REA 28854 NON-REA 28855 NON-REA 28856 NON-REA 28857 NON-REA 28857 NON-REA 28858 NON-REA 28858 NON-REA 28858 NON-REA 28859 NON-REA 28850 NON-REA 2885			NON-REACTIVE	NON-REACTIVE	NO	TRUE NEGATIVE TRUE			
288450 NON-REA			NON-REACTIVE	NON-REACTIVE	NO NO	NEGATIVE			
66 28850 NON-REA 67 28851 NON-REA 68 28852 REACT 69 28853 NON-REA 69 28854 NON-REA 69 28855 NON-REA 69 28855 NON-REA 69 28856 NON-REA 69 28856 NON-REA 69 28856 NON-REA 69 28866 NON-REA 69 28866 NON-REA 69 28866 NON-REA 68 28867 NON-REA 68 28869 NON-REA 68 28873 NON-REA				NON-REACTIVE	10	NEGATIVE TRUE NEGATIVE	-		
87 28851 NON. REA 88 28852 NON. REA 89 28853 NON. REA 70 28854 NON. REA 71 28855 NON. REA 72 28855 NON. REA 73 28857 NON. REA 74 28857 NON. REA 75 28850 NON. REA 76 28850 NON. REA 77 28851 NON. REA 78 28852 REACT 79 28853 NON. REA 88 28854 NON. REA 88 28856 NON. REA 88 28856 NON. REA 88 28856 NON. REA 88 28857 NON. REA 88 28857 NON. REA 88 28857 NON. REA 88 28857 NON. REA 89 28857 NON. REA			NON-REACTIVE	NON-REACTIVE	NO	TRUE NEGATIVE	-		
28852 REACT 28853 NON.REA 28855 NON.REA 28855 NON.REA 28855 NON.REA 28855 NON.REA 28855 NON.REA 28855 NON.REA 28856 NON.REA 28856 NON.REA 28856 NON.REA 28866 REACT 28866 NON.REA 28866 NON.REA 28866 REACT 28866 NON.REA 28866 NON.REA 28866 NON.REA 28866 NON.REA 28866 NON.REA 28866 REACT 28867 NON.REA 28868 NON.REA 28868 NON.REA 28869 NON.REA 28869 NON.REA 28869 NON.REA 28869 NON.REA 28869 NON.REA 28869 NON.REA 28870 NON.REA 28871 NON.REA 28871 NON.REA 28872 NON.REA 28872 NON.REA 28873 NON.REA 28873 NON.REA 28874 NON.REA			NON-REACTIVE	NON-REACTIVE	NO	TRUE NEGATIVE			
70 28855 NON-REA 71 28855 NON-REA 72 28856 NON-REA 73 28856 NON-REA 74 28858 NON-REA 75 28857 NON-REA 76 28860 NON-REA 76 28860 NON-REA 77 28861 NON-REA 78 28862 REACT 78 28863 NON-REA 80 28864 NON-REA 81 28865 NON-REA 82 28866 NON-REA 83 28867 NON-REA 84 28867 NON-REA 85 28867 NON-REA 86 28870 NON-REA 87 28871 NON-REA 88 28872 NON-REA 89 28873 NON-REA 89 28873 NON-REA 89 28874 NON-REA 89 28874 NON-REA			REACTIVE	REACTIVE	NO	POSITIVE			
71 28855 NON-REA 72 28856 NON-REA 73 28857 NON-REA 74 28857 NON-REA 75 28859 NON-REA 75 28859 NON-REA 76 28860 NON-REA 77 28860 NON-REA 77 28861 NON-REA 78 28862 REACT 78 28862 NON-REA 88 28866 NON-REA 88 28866 NON-REA 88 28866 NON-REA 88 28866 NON-REA 88 28867 NON-REA 88 28868 NON-REA 88 28869 NON-REA 88 28870 NON-REA 88 28871 NON-REA 88 28872 NON-REA 89 28873 NON-REA 89 28873 NON-REA 89 28873 NON-REA 89 28874 NON-REA				NON-REACTIVE	NO	TRUE NEGATIVE TRUE			
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74 28858 NON-REA 75 28859 REACT 76 28860 NON-REA 77 28861 NON-REA 77 28861 NON-REA 78 28862 REACT 79 28863 NON-REA 80 28864 NON-REA 80 28866 REACT 80 28867 NON-REA 80 28869 NON-REA 80 28870 NON-REA 80 28870 NON-REA 80 28870 NON-REA 80 28871 NON-REA 80 28872 NON-REA 80 28873 NON-REA 80 28874 NON-REA			NON-REACTIVE	NON-REACTIVE	NO	NEGATIVE			
75 28859 REACT 76 28860 NON-REA 777 28861 NON-REA 78 28862 REACT 79 28862 REACT 80 28864 NON-REA 81 28865 NON-REA 82 28865 NON-REA 83 28865 NON-REA 84 28866 NON-REA 85 28869 NON-REA 86 28879 NON-REA 87 28871 NON-REA 88 28873 NON-REA 89 28873 NON-REA 89 28873 NON-REA 89 28873 NON-REA 89 28874 NON-REA	74	28858		NON-REACTIVE	NO	TRUE			
77 28861 NON.REA 28862 REACT 78 28863 NON.REA 80 28864 NON.REA 81 28865 NON.REA 82 28866 REACT 83 28866 NON.REA 84 28866 NON.REA 85 28869 NON.REA 86 28870 NON.REA 86 28871 NON.REA 87 28872 NON.REA 88 28872 NON.REA 89 28873 NON.REA 89 28874 NON.REA 89 28875 NON.REA			REACTIVE	REACTIVE	NO	POSITIVE			
78 28862 REACT 79 28863 NON-REA 80 28864 NON-REA 81 28865 NON-REA 82 28866 REACT 83 28867 NON-REA 84 28868 NON-REA 85 28867 NON-REA 86 28871 NON-REA 87 28871 NON-REA 88 28872 NON-REA 89 28873 NON-REA 89 28874 NON-REA 89 28874 NON-REA 89 28874 NON-REA 89 28875 NON-REA 89 28875 NON-REA			NON-REACTIVE	NON-REACTIVE	NO	TRUE NEGATIVE TRUE			
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28864 NON-REA			REACTIVE	REACTIVE	NO .	POSITIVE	-		
81 28865 NON REA 82 28866 REACT 83 28867 NON-REA 84 28868 NON REA 85 28869 NON-REA 86 28870 NON-REA 87 28871 NON-REA 88 28873 NON-REA 89 28873 NON-REA 90 28874 NON-REA 90 28874 NON-REA 91 28875 NON-REA			NON-REACTIVE	NON-REACTIVE	NO	TRUE NEGATIVE	-		
### 28866 REACT ### 28867 NON-REA ### 28868 NON-REA ### 28868 NON-REA ### 28870 NON-REA ### 28871 NON-REA ### 28873 NON-REA ### 28873 NON-REA ### 28873 NON-REA ### 28874 NON-REA ### 28875 ### 28875 NON-REA ### 28875 ### 28875 NON-REA ### 28875	11	28865	NON-REACTIVE	NON-REACTIVE	NO	TRUE NEGATIVE			
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Analyte		MPARISON S SARS COV				DR.SMITA SHAR		
nce Gene	LIGHT	CYCLER 48		Comparis		REAL TIME PCR	7500(S)	
	Sample ID	Light Cycler 480 (Rdrp gene)	ments Real Time PCR	Discrepant	Final Interpretati on	Sensitivity	Summary 100%	1
1.	30916	gene) NON REACTIVE		NO	TRUE NEGATIVE TRUE	Specificity	100%	
2	30917	REACTIVE	REACTIVE	NO	POSITIVE			
	30918	NON REACTIVE		70	TRUE NEGATIVE TRUE	tal Number of sampl	94	
8	30919	REACTIVE	REACTIVE	NO	POSITIVE TRUE POSITIVE	True Positive True Negative	77	t
6	30921	NON REACTIVE	REACTION	NO	TRUE NEGATIVE	False Positive		İ
7	30921			NO	TRUE NEGATIVE	False Negative	0	
		NON REACTIVE		NO	TRUE NEGATIVE			
9	30923	NON REACTIVE		NO	TRUE	•		
10	30924	NON REACTIVE	NON REACTIVE	NO	TRUE			ī
1.1	30926	NON REACTIVE		70	TRUE NEGATIVE	Note: Any discrepa verified by suitable c before selecting the	nt result should be onfirmatory method	
12	30927	NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE TRUE NEGATIVE TRUE	before selecting the	final interpretation.	-
14	30928	NON REACTIVE	REACTIVE	70	TRUE POSITIVE			'
1.5	30930	NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE			
16	30931	NON REACTIVE		70	TRUE NEGATIVE			
17	30932	NON REACTIVE		70	TRUE NEGATIVE	•		
18	30933	NON REACTIVE		20	TRUE NEGATIVE			
19	30934	NON REACTIVE		20	TRUE NEGATIVE			
20	30935		NON REACTIVE	NO	TRUE NEGATIVE	•		
21				NO	TRUE	•		
22	30936			NO	TRUE	•		
23	30937	NON REACTIVE		NO	TRUE NEGATIVE			
24	30938			NO	TRUE	-		
25	30939	NON REACTIVE	NON REACTIVE	NO.	TRUE	-		
26	30940	NON REACTIVE		NO -	TRUE NEGATIVE			
26	30941	NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE	-		
29	30942	NON REACTIVE	NON REACTIVE	NO.	TRUE POSITIVE			
29	30943	BEACTIVE	REACTIVE	NO	TRUE NEGATIVE	•		
ao	30944	NON REACTIVE		200	TRUE	•		
81				NO	TRUE	•		
32	30946	NON REACTIVE		NO	TRUE NEGATIVE			
88	30947	NON REACTIVE	NON REACTIVE	710	TRUE POSITIVE			
84		NON	DICORI COLO	NO	TRUE NEGATIVE			
35	30949	NON REACTIVE	NON REACTIVE	NO	TRUE	•		
36		NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE			
37	30951	NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE	-		
38	30952	NON REACTIVE	NON REACTIVE	NO	TRUE	•		
39	30953	NON REACTIVE	NON REACTIVE	NO	TRUE			
40	30954	NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE			
41	30955	NON REACTIVE	NON REACTIVE		TRUE NEGATIVE			
42	30956	NON REACTIVE	NON REACTIVE			-		
	30957	NON REACTIVE	NON REACTIVE	NO	NEGATIVE			
4.8	30958	NON REACTIVE	NON REACTIVE	20	NEGATIVE	-		
44	30959	NON REACTIVE	NON REACTIVE	NO	NEGATIVE	-		
46	20960	NON REACTIVE		NO	TRUE NEGATIVE TRUE			
47	30961	REACTIVE	REACTIVE	700	POSITIVE POSITIVE POSITIVE			
48	30963	NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE	·		
49	30964	NON REACTIVE		70	TRUE NEGATIVE			
50	30965	NON REACTIVE		70	TRUE NEGATIVE	•		
5.1	30966	NON REACTIVE		20	TRUE NEGATIVE			
52	30967	NON REACTIVE		20	TRUE NEGATIVE			
88				NO	TRUE NEGATIVE	•		
5.4	30968	NON REACTIVE		NO	TRUE NEGATIVE	•		
55	30969			NO.	TRUE NEGATIVE			
56	30970			NO	TRUE NEGATIVE			
57	30971	NON REACTIVE	NON REACTIVE	NO	TRUE	-		
5.8	30973	NON REACTIVE	NON REACTIVE	70	NEGATIVE			
59	30974	NON REACTIVE		70	TRUE NEGATIVE	•		
60		REACTIVE	REACTIVE	NO	POSITIVE			
61	30976	NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE	-		
62	30977	NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE			
68	30978	NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE			
64	30979	NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE			
65	30980	REACTIVE	REACTIVE	NO	POSITIVE			
66	30981	NON REACTIVE	NON REACTIVE	NO	NEGATIVE	-		
67	30982	NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE			
68	30983	NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE			
69	30984	NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE			
70	30985	NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE			
71	30986	NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE TRUE NEGATIVE			
72	30987	NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE			
78	30988	REACTIVE	REACTIVE	NO	POSITIVE			
74	30989	NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE TRUE POSITIVE			
76	30990	REACTIVE	REACTIVE	NO	POSITIVE			
77	30992	NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE			
78	30993	NON REACTIVE	NON REACTIVE	70	TRUE NEGATIVE			
79	30994	NON REACTIVE	NON REACTIVE	20	TRUE NEGATIVE			
во	30995	REACTIVE	REACTIVE	NO	POSITIVE			
81	30996	NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE			
82	30997	NON REACTIVE		20	TRUE NEGATIVE			
88	30998	NON REACTIVE		NO	TRUE NEGATIVE			
84	30999	REACTIVE	REACTIVE	NO	POSITIVE			
85	31000	NON REACTIVE	NON REACTIVE	NO	NEGATIVE			
86	31001	NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE			
87	31002	NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE			
88	31003	NON REACTIVE		NO	TRUE NEGATIVE			
89	31004	REACTIVE	REACTIVE	NO	POSITIVE			
90	31008	NON REACTIVE	NON REACTIVE	NO	NEGATIVE			
91	31006	NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE			
	31007	NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE			
92		NON REACTION	NON REACTIVE	20	TRUE NEGATIVE			
92	81008							
	31008	NON REACTIVE	NON REACTIVE	NO	TRUE NEGATIVE			
93	31008 31009 NEG.CONTR	NON REACTIVE NON-REACTIVE	NON REACTIVE NON-REACTIVE BLACTIVE	2 0 2 2 2 2 2 2 2 2 2 3	NEGATIVE			

DISCUSSION

Regarding the different types of clinical specimens, the COVID-19-RdRp/Hel assay was significantly more sensitive than the RdRp-P2 assay for the detection of SARS-CoV-2 RNA in nasopharyngeal aspirates/swabs or throat swabs, saliva specimens, and plasma specimens. False-negative results might arise from testing nasopharyngeal aspirate/swabs or throat swabs with low viral loads in COVID-19, SARS, and MERS patients (9-13). RT-PCR assays with higher sensitivity, such as the COVID-19-RdRp/Hel assay, might help to reduce the false-negative rate among these specimens which are frequently the only specimens available for establishing the diagnosis of COVID-19.

The WHO recommended that the E gene assay followed by a confirmatory assay using the RdRp gene can be utilized for first line screening of COVID-19 cases; [14] and in the United States the CDC asked to use two nucleocapsid protein targets [N1 and N2] as a molecular assay [15]. It is advisable to use, at least two molecular targets to avoid the situation of a potential genetic drift of SARS-CoV-2 and the cross-reaction with other endemic coronaviruses as well, However, the ideal design would include at least one conserved region and one specific region to mitigate against the effects of genetic

Wong et al., used RdRp/Helicase gene combination in their study on 64 (51 known positives) patients and found sensitivity at 91% [16]. Yip et al., on the other hand used Non-Structural Protein 2 (nsp2) with as target 100% analytical sensitivity [17]. In both sensitivity could not be measured. He et al. did with ORF 1ab (Open Reading Frame 1ab) 79% sensitivity only 34 patients. The specificity was 100% [18]. Fang et al., studied it in 2 phases. First up they tested with ORF 1ab, envelope gene (e-gene), and Nucleocapsid gene (N) and had a sensitivity of 71%. A study with a solo target (ORF1ab) in a large study had no significant analytical sensitivity/ specificity data [19]. A study by Liu et al., with Nucleocapsid Protein (NP) as a target has a similar sample size and outcome [20]. Ishige et al., in their study developed a multiplex PCR targeting 3 genes Sarbeco-e gene, N-gene, and human abl1 as an internal control. This kit results perfectly matched with simplex PCR results with different targets [21]. Muenchoff et al., in a multicenter comparative study (seven laboratories) found RdRp to be lower sensitivity with the need to improve its sensitivity. However, the same study found CDC N1 primer/probe-based kits highly useful and sensitive [22].

CONCLUSION

Detection of Rdrp gene of SARS CoV 2 is more sensitive and specific than S gene.

Conflicts of Interests: There is no conflicts of interests.

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