ORIGINAL RESEARCH PAPER
Volume - 11 | Issue - 02 | February - 2022 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

SIMPLIFIED COVID -19 TESTING BY SKIPPING THE RNA EXTRACTION CHECK POINT BY USING HEAT INACTIVATION METHOD.

Medical Microbiology

Sulmaz Reshi* Senior Resident Department of Microbiology, Govind Ballabh Pant Institute of Medical Education and Research, New Delhi, India. *Corresponding Author

Abha Sharma Associate Professor, Department of Microbiology, Govind Ballabh Pant Institute of Medical Education and Research, New Delhi, India.

Poonam Loomba Professor and Head of the department, Department of Microbiology, Govind Ballabh Pant Institute of Medical Education and Research, New Delhi, India.

Bibha Mishra Consultant, Department of Microbiology, Govind Ballabh Pant Institute of Medical Education and Research, New Delhi, India.

Ashna Bhasin Senior Resident, Department of Microbiology, Govind Ballabh Pant Institute of Medical Education and Research, New Delhi, India.

ABSTRACT

Background and Objectives: The ongoing Covid -19 Pandemic is posing a threat of fresh waves, important measure to contain this pandemic is early detection. It is challenging to keep up the supply of reagents and inevitably shortages of the supply chain crop up when demand is so high. RNA extraction, for doing RT PCR for diagnosing SARS CoV 2 , is either done by manual or by automated extractors. [1] . Alternative methods are being explored and one such technique is the use of heat inactivation method . This study was done to validate the heat inactivation method and compare its performance with the Gold standard method of extraction.

Method : Nasopharyngeal swab in VTM, which had previously been processed were preserved in – 70^{0}C freezer were thawed . Heat inactivation was done in water bath at 65ºC for 1 hour. Samples were then placed at room temperature for 15 mints, vortexed for 10 seconds and subjected to centrifugation at 1000g for 1 minute. 5 µl of supernatant was directly put into RT qPCR reaction and loaded in thermocycler . Results obtained by Heat inactivation were compared with the previously reported patients samples result based on Real time RT-PCR.

Results : Total of 200 samples were compared,110 were known positive and 90 were known negative. Out of 110 known positive samples ,106 were positive by both methods. Four known positive samples were negative by heat inactivation method,these four samples had low amounts of RNA corresponding to Ct value of more than 30 . All 90 samples were negative by both the methods . Kappa analysis showed 98 % agreement (k = 0.960) . Correlation coefficient analysis of CT values obtained by RT PCR of both the methods was done , the results showed that CT values between both the methods were correlated for both E gene and RdRP gene.

Conclusion : This study suggests that in the absence of adequate supply of RNA extraction kits, the heat inactivation protocol is a feasible and viable alternative.

KEYWORDS

Pandemic , SARS CoV 2, Heat inactivation , RT PCR .

INTRODUCTION:-

The ongoing Covid -19 Pandemic is posing a threat of fresh waves over the subsequent years, which might have the potential of overwhelming the healthcare system. The second wave of the pandemic laid bare the inadequacies of healthcare facilities both in the developed as well as the developing world. The most important measure to contain this pandemic is early detection of both the asymptomatic and symptomatic cases. However, this is technically challenging in a country like India with a huge population, which imposes considerable pressure on both the public as well as private sectors. It is very challenging to keep up the supply of required reagents and inevitably shortages of the supply chain crop up when demand is so high and ever increasing. Even our institute, though being one of the prime centres for SARS CoV-2 19 testing, has faced the brunt of increased demands of testing in terms of materials and manpower. The situation is even more difficult in peripheral cities and laboratories. Moreover the bio-safety precautions required for testing are considerable, which many peripheral labs are not able to maintain, resulting in many cases going undetected in peripheral areas. While performing a molecular testing for SARS CoV2 , which is the gold standard test, the first step to be performed is RNA extraction, which is either performed by manual method (using silica column based method or magnetic beads method) , or can be done by automatic Nucleic acid extractors. [1] However, due to limited resources many a times, laboratories fall short of the reagents and other consumables. Moreover, Nucleic acid extraction is not only time consuming but demands bio safety precautions, reagents are expensive, requires trained laboratory technicians and liable to error due to manual handling which might cause carry over in samples giving false positive results. So alternative methods to RNA extraction are being explored and one such technique is the use of heat inactivated clinical specimen subjected directly to qRT- PCR. [2] Heat inactivation of samples bypasses the labour intensive and time consuming extraction process by an affordable, quick, efficient , simple and easy to perform method. [3]

We compared the diagnostic sensitivity of the heat inactivation method for detection of SARS-CoV-2 using NP swabs in viral transport medium (VTM) to results obtained using an automated RNA extraction system. This study was done with the aim to validate the heat inactivation method instead of using the Nucleic acid extraction procedure and to compare its performance with the Gold standard method of nucleic acid extraction.

MATERIAL AND METHODS:-

This study was conducted in the department of Microbiology, Govind Ballabh Pant Institute of Medical Education and Research (GIPMER), New Delhi over a period of two months (July 2021 to August 2021) .Ethical clearance was obtained from Ethics committee for performing the study (IEC- 560 / 3.6.2021) .

Sample Collection:-

Nasopharyngeal / oropharyngeal swab in VTM, which had previously been processed , RNA extraction had been performed using MagMax - 96 Viral RNA isolation Kit samples (Thermo Fisher Scientific , USA) in an automated RNA extractor and reported by Real Time RT-PCR and had been preserved at - 70 °C, were used as the samples for further processing by heat inactivation method. The samples were considered positive or negative for SARS- CoV -2 by Q-Line (q RT-PCR) assay performed on extracted RNA and was performed on a CFX 96 Real-Time System (BioRad) .

VTM was taken out of – 70^{0}C freezer and thawed. Subsequently it was vortexed and heat inactivation was done in a water bath at -65 °C for 1 hour. Samples were then placed at room temperature for 15 mints, vortexed for 10 seconds and subjected to centrifugation at 1000g for 1 minute. 5 µl of supernatant was directly put into RT qPCR reaction and loaded in thermocycler. To avoid any bias the same PCR kit was used by which these samples were processed earlier and RT-qPCR was performed on a CFX 96 Real-Time System (BioRad).Results obtained

by Heat inactivation were compared with the previously reported patients samples result based on Real time RT-PCR.

Statistical Analysis

Significant P value by fishers exact test and kappa analysis using 2021 GraphPad software (www.graphad.com /quickcases) was used in this study. Correlation between different specimen CT values were analyzed using Pearsons product moment correlation coefficient analysis .P value of < 0.05 is considered statistically significant.

RESULTS:-

We compared the automated extraction of SARS-CoV-2 versus in-house heat inactivation method (Table 1 and 2) followed by q RT PCR using the same PCR kit for both extraction methods. Total 200 samples were compared, out of which 110 were known positive and 90 were known negative. Out of 110 known positive samples ,106 were positive by both methods. Four known positive samples that were negative by heat inactivation RNA release method had low amounts of RNA corresponding to Ct value of more than 30 in each . All 90 known negative samples were negative by both the methods . Kappa analysis showed 98 % agreement (k = 0.960) between both the extraction methods.

Correlation analysis : RT-PCR results of Automated extraction method and heat inactivation method using Pearson product moment correlation coefficient analysis was done, the results showed that CT values between both the methods were correlated with both E gene and RdRP gene (P < 0.001) (Table 3), E gene(fig 1) , RdRP gene (fig 2).

Table1: Sensitivity and specificity of detection of SARS-CoV2 using heat inactivation method followed by RTPCR versus automated extraction followed by RTPCR

Method	Positive Samples			Negative Samples			Total samples
	Positive by test	Total Positive	Sensitivity	Negative by test	Total Negative	Specificity	
Automated	110	110	100.00%	90	90	100.00%	200
Heat Inactivation	106	110	96.36%	90	90	100.00%	200

Table2: Comparison of detection of SARS-CoV2 using heat inactivation method followed by RTPCR versus automated extraction followed by RTPCR(kappa analysis)

		Automation		Total	Kappa statistics
		Positive	Negative		
Heat Inactivation	Positive	106	0	106	
	Negative	04	90	94	
Total		110	90	200	0.960

Kappa analysis : κ = 0.960

Confidence interval (CI) =95 % (0.921 to 0.999)

Table 3: Analysis of correlation of CT values obtained by heat inactivated method and automated extraction method.

Variable	Heat Inactivation Method	
	E gene	RdRP
R	0.6415	0.5265
P	<0.001	<0.001

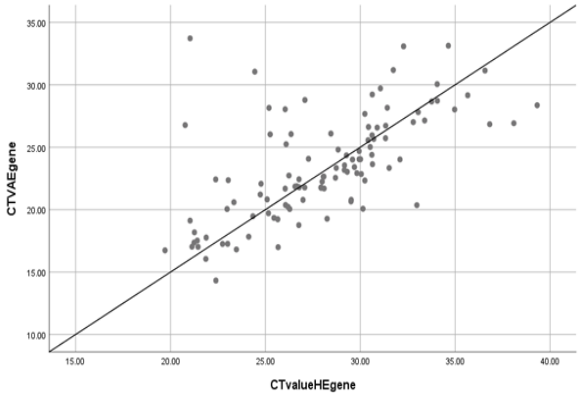

Fig .1: Analysis of correlation between heat inactivation method and automated extraction (E gene)

Figure Legend: Horizontal axis represent Heat inactivation CT values for E gene, and vertical axis represent CT values of E genes for automated extraction

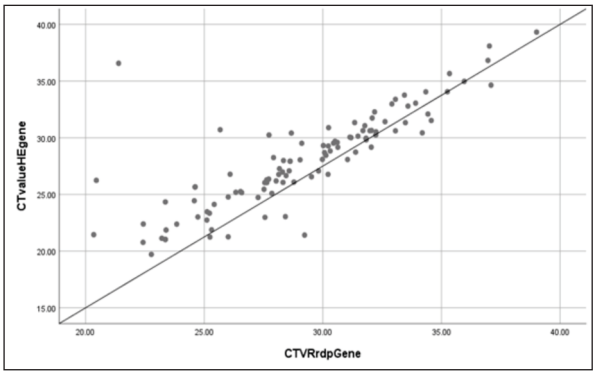

Fig 2 : Analysis of correlation between heat inactivation and automated extraction (RdRP)

Figure Legend: Horizontal axis represent Heat inactivation CT values for RdRP gene, and vertical axis represent CT values of RdRP genes for automated extraction.

Table 4: Comparison of Ct values for heat inactivation method at 65 o for 1 hour and automated extraction

S No	Lab No	Ct values for heat inactivation method at 65 ° for 1 hour			Ct values for automated extraction			S No	Lab No	Ct values for heat inactivation method at 65 ° for 1 hour			Ct values for automated extraction		
		E gene	RdRp	IC (RNAse P)	E gene	RdRp	IC(RNAse P)			E gene	RdRp	IC (RNAse P)	E gene	RdRp	IC(RNAse P)
1	443	22.39	22.43	30.24	14.32	18.53	29.80	51	11241	30.61	33.06	35.94	24.39	26.25	30.65
2	484	30.89	30.24	32.43	26.57	28.48	29.99	52	11263	31.33	33.48	34.09	25.72	27.01	30.66
3	473	26.77	30.22	32.37	22.43	26.22	29.69	53	11251	25.19	26.33	31.49	28.15	29.66	31.79
4	471	30.43	34.19	33.08	26.62	29.11	30.97	54	11195	21.24	25.24	28.01	17.36	20.57	28.92
5	453	32.28	32.18	29.33	33.08	34.43	31.08	55	11214	30.41	28.67	27.82	25.56	26.98	27.57
6	449	28.73	31.38	28.19	23.34	27.11	25.67	56	11238	30.71	25.67	30.17	25.66	23.21	27.37
7	422	31.74	32.08	30.52	31.18	34.06	30.01	57	11244	33.76	33.44	31.05	28.66	29.46	32.33
8	430	30.62	31.98	30.03	25.96	29.02	31.08	58	11264	35.66	35.34	34.19	29.16	30.98	31.23
9	410	24.44	24.58	30.08	31.05	32.22	32.05	59	11266	25.09	27.85	27.58	20.82	24.15	26.13
10	501	30.14	31.48	31.08	20.07	28.03	30.17	60	11208	25.67	24.62	30.09	16.99	19.25	29.45
11	425	27.27	28.17	33.97	24.07	27.21	32.23	61	11220	19.71	22.77	30.25	16.74	19.28	28.87
12	516	26.35	27.72	35.06	26.05	23.46	31.51	62	11223	27.07	29.82	32.11	21.77	24.02	28.07
13	413	30.52	32.24	30.01	25.02	28.04	28.13	63	11228	24.73	27.27	33.34	21.21	23.05	29.95
14	408	26.66	28.45	33.02	21.87	24.04	30.91	64	11230	28.83	30.31	30.03	24.81	27.11	28.73
15	424			37.23	33.93	34.01	31.03	65	11248	26.19	28.03	31.55	20.23	22.53	27.63
16	459	31.42	32.62	30.08	28.16	30.38	27.37	66	11219	21.03	23.36	30.63	19.12	22.07	31.99
17	421	25.45	27.52	27.78	19.34	22.99	24.07	67	11255	21.03	23.36	30.63	33.72	34.91	32.99

18	469	21.45	20.34	24.47	17.02	19.81	25.07	68	11204	36.82	36.96	31.65	26.84	28.66	30.03
19	450	28.69	30.07	29.29	22.55	26.91	31.12	69	11247	33.05	33.91	31.33	27.81	29.02	29.07
20	498	21.26	26.01	26.83	18.18	22.6	22.81	70	11260	28.09	29.98	29.67	21.69	23.85	27.67
21	512	20.77	22.42	29.69	26.77	29.28	31.78	71	11239	36.57	21.39	31.91	31.14	31.07	30.01
22	514	22.38	23.84	29.47	22.42	26.42	31.02	72	11216			34.45	35.39	32.95	32.16
23	447	25.25	26.53	30.44	26.03	27.42	30.44	73	11240	23.01	24.73	25.08	17.26	19.86	25.37
24	464	26.04	27.55	26.07	28.04	29.69	28.44	74	11259	26.24	20.45	30.24	22.73	24.69	28.85
25	515	27.08	28.58	30.27	28.78	31.47	29.81	75	11267			35.12	33.96	32.71	31.46
26	452	26.78	26.09	28.11	21.79	23.76	26.27	76	11261	34.05	34.34	28.41	30.05	31.69	28.41
27	454			35.55	35.28	33.02	32.77	77	11207	33.39	33.06	30.95	27.14	28.28	29.06
28	461	27.99	28.34	28	22.24	24.98	30.24	78	11249	26.09	28.78	34.04	25.24	27.26	32.24
29	416	28.45	30.13	25.27	26.09	26.09	24.31	79	11250	23.34	25.22	33.34	20.59	23.04	32.34
30	409	24.77	26.01	25.76	22.07	24.68	28.85	80	11265	28.08	31.04	29.55	22.65	25.27	28.56
31	427	31.06	31.77	28.26	29.71	31.97	30.07	81	693	24.34	23.36	31.95	19.47	17.14	32.55
32	494	21.41	29.23	25.02	17.52	23.01	27.92	82	644	25.65	24.61	31.33	19.23	19.89	29.95
33	426	29.51	29.11	26.46	20.66	23.39	25.89	83	639	27.93	28.62	26.01	21.76	24.43	26.97
34	412	30.63	31.69	27.38	29.22	28.21	29.28	84	676	29.31	30.02	30.52	23.03	25.07	29.83
35	496	23.04	28.42	28.95	22.36	24.78	24.88	85	656	24.12	25.42	29.42	17.83	20.16	28.15
36	474	30.01	31.19	30.03	24.04	26.64	26.43	86	657	32.79	33.59	31.12	27.01	28.46	30.85
37	70	28.25	27.92	25.23	19.27	22.16	25.48	87	633	21.86	23.39	24.83	16.05	19.28	24.92
38	3	29.17	32.04	25.66	23.55	26.68	26.34	88	714	34.64	37.09	29.31	33.13	32.77	29.14
39	79	29.69	30.51	27.62	23.41	25.59	26.04	89	627	29.82	31.83	33.76	22.92	26.03	33.86
40	518	30.24	27.73	28.01	22.33	25.98	27.36	90	652	39.32	39.01	24.14	28.36	30.94	26.96
41	11157	34.06	35.25	36.97	28.72	29.75	33.53	91	681	26.76	28.14	26.14	18.76	21.15	26.39
42	11158	29.16	30.63	29.47	23.17	25.82	28.05	92	629	30.05	31.14	27.73	22.85	24.08	29.61
43	11167	26.28	27.63	36.98	20.05	22.47	32.44	93	654	29.59	30.61	28.75	24.01	25.73	31.66
44	11188	31.52	34.57	32.42	23.34	26.11	30.96	94	602	25.16	26.58	26.32	19.71	21.89	28.17
45	11176	26.56	29.52	35.02	21.85	22.85	29.78	95	560	32.09	34.43	27.23	24.02	26.74	27.86
46	11184	26.06	28.32	32.68	20.37	23.01	29.27	96	579	21.88	25.31	28.02	17.76	20.36	30.07
47	11189	29.94	31.82	33.22	24.04	26.68	30.02	97	524	30.64	32.05	29.29	23.64	26.42	27.88
48	11190	29.94	31.82	33.22	24.69	27.02	31.86	98	589	23.47	25.12	24.29	16.81	19.17	26.01
49	11172	30.24	32.23	32.33	27.67	29.41	31.35	99	592	34.97	35.96	28.78	28.02	29.41	30.14
50	11199	29.27	30.23	36.33	24.35	26.09	31.11	100	567	32.98	32.91	26.17	20.36	23.14	30.18

S No	Lab No	Ct values for heat inactivation method at 65 ° for 1 hour			Ct values for automated extraction			S No	Lab No	Ct values for heat inactivation method at 65 ° for 1 hour			Ct values for automated extraction		
		E gene	RdRp	IC(RNAse P)	E gene	RdRp	IC (RNAse P)			E gene	RdRp	IC (RNAse P)	E gene	RdRp	IC(RN Ase P)
101	594	22.98	27.56	28.94	20.05	23.37	30.36	151	20306			30.24			29.86
102	590	22.74	25.11	23.79	17.25	19.54	25.23	152	20307			31.25			31.99
103	550	26.04	27.64	29.37	21.68	19.38	30.82	153	20308			30.25			29.73
104	601	31.34	31.34	27.47	26.72	26.69	30.81	154	20309			30.25			29.73
105	525	21.13	23.21	26.68	17.03	19.07	27.91	155	20310			28.45			27.67
106	553	29.52	30.45	29.82	20.78	24.61	30.23	156	20311			32.45			31.58
107	537	26.98	28.3	28.94	20.78	22.79	28.94	157	20312			29.45			28.02
108	528	28.25	25.55	28.66	26.92	20.92	28.19	158	20313			28.25			26.87
109	539	28.05	29.04	27.78	22.64	22.43	29.65	159	20314			26.45			25.87
110	20265	27.93	28.62	26.01	21.76	24.43	26.97	160	20315			30.25			29.16
111	20266			34.98			34.63	161	20316			30.25			28.53
112	20267			34.45			39.55	162	20317			25.25			24.51
113	20268			30.23			31.02	163	20318			30.25			29.32
114	20269			31.23			30.01	164	20319			33.45			32.03
115	20270			32.23			33.78	165	20320			28.25			27.57
116	20271			31.34			32.45	166	20321			30.25			29.47
117	20272			28.78			26.84	167	20322			30.45			29.47
118	20273			31.34			30.45	168	20323			28.45			27.57
119	20274			32.23			34.22	169	20324			31.25			29.47
120	20275			28.25			26.05	170	20325			30.25			27.05
121	20276			30.23			31.41	171	20326			32.45			31.05
122	20277			29.28			30.23	172	20327			32.25			33.82
123	20278			32.28			35.52	173	20328			36.25			35.83
124	20279			32.34			31.84	174	20329			28.45			26.34
125	20280			28.24			26.28	175	20330			33.25			32.24
126	20281			27.28			26.88	176	20331			26.25			27.66
127	20282			27.99			26.89	177	20332			28.55			27.09
128	20283			31.22			30.26	178	20333			29.45			30.01
129	20284			30.23			29.56	179	20334			30.25			31.29
130	20285			33.45			34.44	180	20335			32.25			35.24
131	20286			32.34			31.45	181	20336			32.45			34.63
132	20287			29.85			28.57	182	20337			31.45			32.98
133	20288			33.45			32.94	183	20338			32.45			34.19

134	20289			32.25			30.89	184	20339			28.45			27.09
135	20290			31.45			30.09	185	20340			28.45			26.97
136	20291			30.25			31.78	186	20341			31.55			32.03
137	20292			30.25			29.07	187	20342			31.55			30.04
138	20293			28.25			26.77	188	20343			30.55			28.36
139	20294			30.25			30.96	189	20344			28.45			27.37
140	20295			31.25			32.53	190	20345			26.45			28.24
141	20296			28.55			29.75	191	20346			28.25			29.99
142	20297			30.25			31.03	192	20347			30.45			30.28
143	20298			30.55			31.55	193	20348			30.25			31.41
144	20299			30.55			29.18	194	20349			28.25			27.83
145	20300			25.55			23.51	195	20350			26.55			23.55
146	20301			30.55			29.61	196	20351			24.55			27.82
147	20302			28.45			26.02	197	20352			24.25			23.55
148	20303			31.25			30.04	198	20353			26.25			27.83
149	20304			31.25			30.91	199	20354			25.55			23.55
150	20305			32.25			31.03	200	20355			28.44			29.82

DISCUSSION:

The SARS-CoV-2 pandemic is far from over. Though case loads have been steady, but there has been no explosive spurt in cases as was the case during the second wave in the month of April- May 2021 in India. Globally the third wave of the pandemic is again stretching healthcare resources. Although, such effects haven't been felt in India yet, however, there is no room for complacency and the harsh lessons meted out by the second wave of the pandemic stand as a testimony to that end. The means to control the pandemic remain essentially the same; personal protection, social distancing, masking up and testing. Education and health awareness have contributed to a significant proportion of the population following COVID appropriate behavior, yet an area of significant concern is still testing. Even in a tertiary care centre like ours, resources get stretched with regard to the testing kits and reagents. RNA extraction, the first step of the process, is either performed by manual method (using silica column based method or magnetic beads method), or by automatic Nucleic acid extractors. [1] Moreover, Nucleic acid extraction is not only time consuming but demands bio safety precautions, expensive and is liable to error due to manual handling which might cause carry over in samples giving false positive results.

In order to alleviate the same, a new protocol has emerged, wherein, heat inactivation of VTM is done, which bypasses the labor intensive, expensive and time consuming extraction process. It also reduces the possibility of spread of the contagious virus in laboratory workers. [4]

We tested 200 nasopharyngeal swab samples by using the standard protocol of automated extraction followed by RT-PCR. 110 of these samples tested positive by RT-PCR and 90 were negative. The same samples were later processed by heat inactivation protocol, samples were placed in a water bath at 65 ^{0} for 1 hour. The samples were subsequently centrifuged and the supernatant was subjected to a RT-PCR. The results were compared to the gold standard of automated extraction followed by RT-PCR.

Among the 110 positive samples 106 tested positive by the Heat inactivation method, while 4 samples were Negative by heat inactivation method. The effect of heat inactivation on CT value was observed and compared to automated extraction method.

We tabulated the Ct value of samples for E gene and RdRp gene for both the automated extraction and Heat inactivation method. Ct values of both E gene and RdRp for hit-RT-qPCR samples were higher than for automated RNA extraction elutes of the same samples (mean difference =1- 6 Ct). Heat inactivation method gave 100 percent results for samples with Ct value < 20 by automated extraction, (12/ 12) , 100 % results for Ct values in the range of 20- 30 by automated extraction (81 / 81), and 76 .47 % (13 / 17) for samples with Ct value of 30- 38 with automated extraction. These results are in concordance with Barza, et al. [3]

Our protocol correctly identified all except four clinical samples with Ct value in the range of 30- 38. This is in concordance with findings of Alagarasu et al [1] and Barza et al.[3] They also got false negative results for heat inactivation methods in samples with Ct value greater than 30. [3] The 4 samples that were missed by heat inactivation method had a Ct value of > 30 by automated extraction method corresponding to a lower viral load .It was noted that for all the samples CT value obtained by heat inactivation method was slightly higher compared to the CT values of the same samples done with automated extraction, the Higher Ct values obtained by heat inactivation method and missing out of 4 samples with Ct value more than 30 could be explained by the fact that in heat inactivation method the final volume of elute taken is only 5 µl in comparison to 200 µl taken in automated extraction ,that is automated extraction method uses 40 times(200 µl) the elute compared to heat inactivation where we use only 5 µl this can be a reasonable explanation for such results. [3] This could be due to the inhibitors and could be overcome by dilution by addition of RNAse free water and diluting the sample. Such a method was reported by Alagarasu et al as well. [1]

Using heat-treatment method sensitivity approached to nearly 96.8% and specificity to 100%. This finding is in corcordance with the findings of Khelil et al. [2]

Correlation analysis was done and RT-PCR results of both automated extraction method and heat inactivation method using Pearson product moment correlation coefficient analysis was done, the results showed that CT values between both the methods were correlated with both E gene and RdRP gene ($P < 0.001$). This finding is similar to the findings of Y .Wang et al. [4] These results showed that CT values between the inactivated methods and automated extraction were correlated with both N gene and ORF1 gene. [4]

Comparably, aliquots from 45 known positive nasopharyngeal swab samples were subjected to heat-treatment but with decreasing heating time to 30 min. Only 18 out of 45 were detected positive (18/45 = 40 % sensitivity), while as 27 out of 45 (27 / 45) were invalid by heat inactivation method at decreased time whereas same samples when thermally treated with increased time from 30 to 60 mints yielded 100 percent sensitivity.

The sensitivity of the method was very less when the same samples were treated at lower temperature and when the heating time was increased to (60 min) sensitivity increased. Our findings are consistent with the findings of Khelil et al.They also found sensitivity increases with increase in time of heat inactivation. [2]

The present study suggests that the heat inactivation protocol at 65 $^{\circ}$C for 1 hour can be used in the absence of RNA extraction kits. Widespread adoption of this technique may circumvent resource shortage and testing inadequacies. This may be a turning point in this fight against COVID and may bridge the distance between testing requirements and testing availability. This testing method is also attractive in settings where repeated, cheaper and quicker testing is desirable e.g. testing of healthcare personnel and screening of patients in OPD settings. The results also show that this can be achieved without major sacrifice in accuracy of determining negative and positive cases. The procedure could be especially useful for massively scaling up SARS-CoV-2 testing, as the cost of RNA purification could be unworkable in mass testing.

CONCLUSION :-

This study suggests that in the absence of adequate supply of RNA extraction kits, the heat inactivation protocol is a feasible alternative. Heat inactivation of VTM in a water bath at 65 ^{0} for 1 hour is a simple and viable substitute which bypasses the labor intensive, expensive and time consuming extraction process, without compromising much on sensitivity and specificity of the subsequent RT PCR. We propose

that such a method can be employed for mass testing in resource constrained settings. However, individuals with high disease suspicion who return a negative test should be re-verified using conventional extraction methods.

Conflict of interest: None

REFERENCES:-

(1) Alagarasu K, Potdar VA, Vipat V, Hundekar S, Gunjikar R, Choudhary ML, et al. Utility of a modified heat inactivation method for direct detection of SARS-CoV-2 by RT-qPCR in viral transport medium bypassing RNA extraction: A preliminary study. Indian J Med Res. 2020; 152:108-10.
(2) Mokhtar KM. Improved COVID-19 Testing by Extraction-Free SARS-CoV-2 RT-PCR. EJIFCC. 2020;31(4):362-368.
(3) Barza R, Patel P, Sabatini L, Singh K. Use of a simplified sample processing step without RNA extraction for direct SARS-CoV-2 RT-PCR detection. J Clin Virol. 2020; 132: 104 587.
(4) Wang Y, Song W, Zhao Z, Chen P, Liu J, Li C. The impacts of viral inactivating methods on quantitative RT-PCR for COVID-19. Virus Res. 2020;285:197988.