Effect of Heat Inactivation for the Detection of Severe Acute Respiratory Syndrome-Corona Virus-2 (SARS-CoV-2) with Reverse Transcription Real Time Polymerase Chain Reaction (rRT-PCR): Evidence from Ethiopian Study

Belete Woldesemayat Hailemariam (✉ beleteweldesemeyat@gmail.com)

Ethiopian Public Health Institute <https://orcid.org/0000-0001-6080-0200>

Gebremedihin Gebremicael

Ethiopian Public Health Institute

Kidist Zealias

Ethiopian Public Health Institute

Amelework Yilma

Ethiopian public health institute

Sisay Adane

Ethiopian Public Health Institute

Mengistu Yimer

Ethiopian Public Health Institute

Gadissa Gutema

Ethiopian Public Health Institute

Altaye Feleke

Ethiopian Public Health Institute

Kassu Desta

Addis Ababa University College of Health Sciences

Research article

Keywords: COVID-19, CT value, Heat inactivation, rRT-PCR, SARS-COV-2

Posted Date: January 28th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-153817/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Coronavirus disease 2019 (COVID-19) specimen handling needs a major concern due to the virus has a potential of easily transmittable to health care workers and laboratory personnel. Heat inactivation before nucleic acid isolation might permit safe testing, even though, the effect of heat inactivation on SARS-CoV-2 RT-PCR detection results needs to be determined.

Methods: An experimental study was conducted in Ethiopian Public Health Institute (EPHI) from September 25 to October 15, 2020. A total of 188 Oro-pharyngeal swabs were collected from COVID-19 suspected cases, referred to EPHI for SARS COV-2 testing during the study period. One group of the sample was inactivated at 56 °C heat for 30 min, and the other group was stored at 4°C for a similar period of time. RNA extraction and detection were done by DAAN Gene extraction and detection kit. Abbott m2000 RT-PCR was used for amplification and detection. Data analysis was done by using SPSS version 23.0; Chi-square and Pearson correlation test for qualitative and semi-quantitative analysis were used. P-value < 0.05 was considered as statistically significant.

Results: Out of 188 total samples, 117 (62.2 %) and 118 (62.8%) were positive for ORF1a/b and N gene respectively before inactivation. Whereas after inactivation, 111 (59 %) was ORF1a/b and 116 (61.7 %) was N gene positive. Rate of positivity between groups was not statistically significant ($p > 0.05$). The mean CT value difference between the two groups of ORF1a/b gene and N gene was 0.042 (95 % CI, -0.247- 0.331; $t = 0.28$; $p = 0.774$) and 0.38 (95% CI, 0.097 - 0.682; $t = 2.638$; $p = 0.010$) respectively.

Conclusion: Heat inactivation at 56 °C for 30 min has not statistically significant effect for the qualitative rRT-PCR detection of SARS-CoV-2. However, the finding also showed that there was statistically significant CT value increment after heat inactivation compared to untreated samples. Therefore, false-negative results with high CT value results ($CT > 35$) were found to be the challenge of this protocol. Hence alternative inactivation methods should be investigated and further studies should be considered.

Background

Coronavirus diseases 2019 (COVID-19) was announced as a global pandemic on March 11, 2020, by World Health Organization (WHO) [1]. As of September 20/2020, severe acute respiratory syndrome-2 (SARS CoV-2) infections have been confirmed in nearly 30.6 million people, and about 950,000 deaths are reported globally [2]. Laboratory testing for COVID-19 is a crucial issue to show a real scenario of the detected case in this pandemic era. The method of COVID-19 testing can be the Nucleic Acid Amplification Test (NAAT) and Serological tests [3]. However, the new recommended point-of-care serological test was failed to be recommended by WHO to use in any setting, including in clinical decision-making rather than in research settings due to window time [4]. Therefore, detection of SARS-CoV-2 RNA with molecular methods (either PCR-based method or deep sequencing) is the gold standard method for the diagnosis of COVID-19. Reverse transcription real-time polymerase chain reaction (rRT-PCR) are using specific sequences of genes that encode the RNA dependent RNA polymerase (RdRP),

nucleocapsid (N), envelope (E), and spike (S) proteins of the virus [5]. However, currently available diagnostic method of COVID-19 either PCR-based or deep sequencing have limitations in terms of reliability, accuracy, and accessibility of testing [6]. Because, these detection methods are depending on the presence of viral genome in sufficient amounts at the site of sample collection that can be amplified [7]. In addition, in the process of the analytical procedure, there are a lot of challenges including sample collection, transportation, treatment, extraction procedure, amplification and detection [6]. Moreover, according to WHO biosafety guideline COVID-19 molecular testing procedures, like sequencing and (NAAT), on clinical specimens should be performed at least in Biosafety Level 2 (BSL-2) laboratory [8]. Therefore, initial sample processing (before inactivation) of all specimens should be performed in a properly maintained and calibrated biosafety cabinet (BSC). In some condition, we are unable to use BSC for extraction procedure, including in point of care testing, specimen handling and testing out of designated testing centers, conducting different field surveys settings and when performing automation extraction. Thus, specimen treatment (inactivation of SARS-CoV-2 virus) prior to sample extraction could be essential to protect laboratory personnel from the nosocomial transmission of COVID-19 [8-10].

Inactivation of SARS-CoV-2 virus can be done by different mechanisms; like chemical inactivation using 0.5 % Povidone-Iodine oral antiseptic, and/or 70 % alcohol can rapidly inactivate the virus within 30 seconds contact time [11]. Lysis buffers which are available in RNA extraction kit are also effective for SARS-CoV-2 inactivation without additional means [8]. However, some detergents using for sample treatment can inhibit PCR reactions. Some of cannot inactivate viruses properly without RNA degradation [6]. Different strain of the virus could be inactivated with related temperature range [12]. The famous method of SARS-CoV-2 inactivation at 56 °C for 30 min prior to extraction procedures was showed that leads to a clear drop of viral infectivity (> 5 Log₁₀ reduction) [10, 13]. The other SARS-CoV-2 inactivation at 60 °C for 60 min, 92 °C for 15 minutes, 80°C for 5 minutes, and 100°C for a minute also could be resulted significantly decrease the viral infectivity in a clinical specimen [13, 14]. However, sample treatment with heat before molecular testing might destroy the viral RNA and lead to false-negative results [15].

Since COVID-19 outbreak occurred in Ethiopia, laboratory testing is conducting with NAAT by RT-PCR technique from Nasopharyngeal or Oro-pharyngeal sample with automated or manual extraction and detection method. In this pandemic time expansion of the testing capacity should be an important issue as the prevention and control strategy of COVID-19 throughout the country. However, resource limitations including level 2 biosafety cabinets are the major challenge. In addition to this, automation extraction and detection are conducted by using a different type of instruments; these instruments have no attached biosafety cabinet. In case of the above conditions to protect laboratory workers from the nosocomial transmission of SARS-CoV-2, viral inactivation is a mandatory process. Currently, in our context, we are using heat at 56 °C for 30 minutes for viral inactivation. But some shreds of evidence showed that viral inactivation for RNA viruses, including SARS-CoV-2 lead to a false-negative result. So, the aim of this study was to evaluate the effect of heat inactivation in the detection of SARS-CoV-2 by using clinical samples in Ethiopian context and also add value as baseline information for further study on the use of inactivation method prior to nucleic acid extraction for COVID-19 testing.

Methods

This experimental study was conducted in EPHI at National HIV Reference laboratory from September 25 to October 15, 2020. A total of 188 Oro-pharyngeal swabs were collected from COVID-19 suspected cases and referred to EPHI for SARS COV-2 testing during the study period. Oro pharyngeal sample was collected by trained sample collectors, with 3-4 ml disposable specimen collection tube. Then the specimen transported to EPHI by triple packaging system. The tertiary containers opened inside the biosafety cabinet level 2 (BSCL-2).

From each, Oro-pharyngeal (throat swab) sample, 500µl sample was aliquoted into two sterilized cryogenic tubes. Then, one group of the sample was inactivated at 56 °C heat for 30 min in a water bath. The other group of the sample was stored at 4°C for a similar period of time. Both groups of the sample were tested with a batch of 20 samples and 2 controls (1 positive, 1 negative) controls were included throughout the procedure (in extraction and detection). During extraction and master mixing procedure, every procedure was performed based on standardized standard operating procedure (SOP), every batch of testing was treated similarly, except one group inactivated with heat and the other is not, to minimize factors affected the result. The RNA extraction was done by DAAN Gene spin column-based manual extraction kit, manufactured by DAAN Gene Co., Ltd of Sun Yat-sen University, Guangzhou China. In this method of nucleic acid isolation/purification 0.2 ml of VTM throat swab sample was used for manual extraction. The final elute for this method was approximately 50µl. The amplification/detection method of this kit is based on one-step rRT-PCR technique. In this method, ORF1a/b and N genes are selected as the conserved region of DAAN technology for amplification and detection of target regions. Specific primers and fluorescent probes are designed (N gene probe is labeled with FAM and ORF1a/b probe with Yellow) for the detection of 2019 Novel Coronavirus RNA in the specimens. The final eluent and master mix preparation were 5µL of the eluate were added to 20 µL of master mix for a final volume of 25 µl. rRT-PCR was performed on the Abbott m2000 RT-PCR (open mode). Based on the manufacturer instruction cycling conditions was as follows: hold for 15 min's at 50°C, hold for 15 min's at 95°C, then 45 cycles of 94°C for 15 seconds, and 55°C for 45 seconds.

The result interpretation was done based on the manufacturer recommendation, the Ct value > 40 and there was no amplification curve in the FAM and VIC channel, but there was amplification curve in Cy5 channel considered as no SARS-CoV-2 RNA in the sample. On the other hand, if the sample has a clear amplification curve in the FAM and VIC channels and CT value ≤ 40 , the sample considered as positive for SARS-CoV-2 RNA. If the CT value of ≤ 40 and amplification curve was detected in a single channel of FAM or VIC, and there was no amplification curve in the other channel, the results were re-tested and the repeated result takes as the final result.

Data processing and analysis

The data entry and analysis was conducted using statistical software version 23.0 of SPSS for descriptive statistics, chi-square and correlation analysis. A paired T-test was used to measure the mean CT difference and to analyze the accuracy of CT and the association between heat inactivated and non-

heat inactivated sample CT values using Pearson correlation coefficient analysis. The consistency analysis value $P < 0.05$ of the CT values is considered statistically significant.

Results

In this study, a total of 188 COVID-19 suspected patients were enrolled and a throat swab sample was collected from September 25 to October 15/2020 referred to EPHI for SARS-CoV-2 testing. Of the total, 108 (57.4%) participants were male and the rest 80 (42.6%) were female. The mean age of the study participants was 32.2 ± 13.1 years. Out of the total samples without heat-inactivated at 56°C for 30 minutes (untreated group), 117 (62.2 %) and 118 (62.8%) were positive for ORF1a/b gene and N gene respectively. A total of 117 ORF1a/b gene-positive samples were also positive for N gene. Only one sample (0.5 %) that treated without heat inactivation was single gene-positive during the original test and also after re-tested. While out of the samples with heat-inactivated at 56°C for 30 minutes, 111 (59%) were ORF1a/b positive and 116 (61.7%) were N gene positive. Out of the total N gene-positive, 111 were ORF1a/b gene also positive. Only one sample (0.5%) that treated with heat inactivation was turned to completely double target gene negative after re-tested; while five samples ($5/188=2.65\%$) was a consistent single gene-positive after re-tested.

Of the total samples, 7 (3.7%) had discordant result between those heat-inactivated and without heat-inactivated matched Oropharyngeal specimen (Table 1). Out of the discordant samples; 5 (71.4 %) Oropharyngeal specimen were double gene negative after the heat-inactivated procedure, but either double or single gene-positive in those treated without heat inactivation procedure. Interestingly, out of the discordant samples; 1 (14.3%) sample was N gene-positive during heat-inactivated, but double gene negative in those treated without the heat-inactivated procedure. Those discordant samples having with single or double gene-positive had more than 35 CT value (Table 1). Based on the DAAN gene assay result interpretation after Chi-square McNemar test indicated that the qualitative detection of SARS-CoV-2 (negativity or positivity) between heat-inactivated at 56 °C for 30 min and non-inactivated group result had no statistically significant difference ($\chi^2 = 1.584$; $p = 0.208$).

Table 1: Discordant result between inactivated and non-inactivated Oropharyngeal specimen in COVID 19 testing, Ethiopia, 2020

Sample number	Heat Inactivated result		Non-heat inactivated result	
	<i>ORF1a/b gene</i> (CT value)	<i>N gene</i> (CT value)	<i>ORF1a/b gene</i> (CT value)	<i>N gene</i> (CT value)
ES 020	Negative	Negative	Positive (36.71)	Positive (36.22)
ES 073	Negative	Negative	Positive (37.84)	Negative
ES 086	Negative	Negative	Negative	Positive (36.62)
ES 150	Negative	Negative	Positive (38.2)	Negative
ES 171	Negative	Positive (36.61)	Positive (39.81)	Positive (36.93)
ES 067	Negative	Positive (37.28)	Negative	Negative
ES 068	Negative	Negative	Negative	Positive (36.38)

The effects of heat inactivation at 56°C for 30 min on the SARS-CoV-2 RT-PCR CT value were assessed. Out of 188 Oropharyngeal swab samples the ORF1a/b gene average CT value of the inactivation group and non-inactivation group were 25.43 (95% CI; 24.369-26.521) and 25.39 (95 % CI; 24.336-26.438) respectively. The range, median and standard deviation of CT value of ORF1a/b gene in the inactivation group were 26.37 (13.83-41.20), 24.73, and 5.96 respectively. Similarly, the range, median and standard deviation of CT value in the non-inactivation group were 27.1 (12.9-40), 25.13 and 5.74 respectively.

Out of the total sample, 32/188 (17.02 %) of samples had low ORF1a/b gene RNA copies, which had the CT values were greater than 30. While 22 (11.7 %) N gene CT values were greater than 30. The mean non-inactivated ORF1a/b and N gene CT values greater than 30 were 33.37 (95 % CI; 32.43 - 34.32) and 32.52 (95 % CI; 31.65 - 33.34) respectively. On the other hand, the inactivated ORF1a/b and N gene CT values greater than 30 were 34.63 (95 % CI; 33.46 - 35.85) and 33.52 (95 % CI; 32.68 - 34.29) respectively (Table: 2).

As shown in table 2, there is no significant difference between the ORF1a/b CT of inactivated and non-inactivated results. The mean difference between the two groups of CT value was 0.042 (95 % CI, - 0.247 to 0.331) and this difference was not statistically significant ($t= 0.28$; $p = 0.774$). On the other hand, the range of N gene CT value in both inactivated and non-treated samples was 29.17 (10.83- 40.0) and 30 (10.0-40.0) respectively. The mean CT value for N gene of the heat-treated group was 24.1585 (95 % CI; 22.925 - 25.301) and the non-inactivated mean CT value was 23.768 (95 % CI; 22.609 - 24.863). The paired t-test analysis showed that the mean difference between both groups of N gene CT was 0.38 (95% CI, 0.097 to 0.682). This difference was indicated that the inactivated and non-inactivated group had a

significant difference in CT value ($t = 2.638$; $p = 0.010$). In addition, as indicated in paired T-test analysis, high CT value (> 30) samples significantly affected with heat inactivation at 56°C for 30 minutes. The mean CT value difference of ORF1a/b and N gene in both inactivated and non-inactivated groups were significantly different ($p < 0.05$) (Table: 2).

Table 2: Analysis of CT value difference between heat inactivated and non-inactivated Oropharyngeal specimen in COVID 19 testing, Ethiopia, 2020

Variables	Mean (95% CI)	Paired T-test differences			Non-parametric Wilcoxon test
		Mean difference (95 % CI)	T-test	P-value	p-value
Inactivated sample ORF1a/b gene CT	25.4397 (24.369,26.521)	0.042 (-0.247 , 0.331)	0.288	0.774	0.871
Non-inactivated sample ORF1a/b gene CT	25.3978 (24.336, 26.438)				
Inactivated sample N gene CT	24.1585 (22.925, 25.301)	0.389 (0.097, 0.682)	2.638	0.010	0.000
Non-inactivated sample N gene CT	23.7689 (22.609, 24.863)				
Inactivated sample ORF1a/b gene CT > 30	34.6269 (33.455, 35.852)	1.262 (0.435, 2.088)	3.114	0.004	0.001
Non-inactivated sample ORF1a/b gene CT > 30	33.3650 (32.427, 34.321)				
Inactivated sample N gene CT > 30	33.5155 (32.6796, 34.293)	1.00 (0.439, 1.561)	3.709	0.001	0.000
Non-inactivated sample N gene CT > 30	32.5155 (31.646, 33.3397)				

In this experimental study the concordance between inactivated and non-inactivated sample results was compared and the, Pearson correlation analysis showed that, the ORF1a/b gene CT values of inactivated sample and non-inactivated sample had excellent correlation ($r = 0.978$; $p < 0.001$); (figure 2). Similarly as figure 3 shows, the N gene CT of inactivated and non-inactivated group had excellent correlation ($r =$

0.969; 95% CI, 0.934 - 0.991; $P < 0.001$). According to figure 3 and 4 revealed that the ORF1a/b gene CT value correlation between inactivated and non-inactivated groups had high positive correlation ($r = 0.749$; 95% CI, 0.434 - 0.935; $p = 0.000$). The N gene correlation between two groups also had strong positive correlation ($r = 0.824$; 95% CI, 0.634 - 0.962; $p = 0.000$).

Discussion

After COVID-19 declared as a global pandemic, the number of suspected cases was increased day today. It needs to maximize laboratory testing by high throughput automated extraction instruments and point of care testing instruments for SARS-CoV-2 detection [6, 9]. So for these types of platforms sample inactivation is an important procedure to protect the exposure of laboratory personnel from SARS-CoV-2 viral infection [9]. However, some studies showed that sample inactivation by using heat leads to false-negative result [15, 16]. Therefore, this study was evaluated the effect of heat inactivation at 56°C for 30 min for SARS-CoV-2 detection. Thus, seven samples (3.7%) were had discordant results between those heat-inactivated and without heat-inactivated matched samples. Of the total discordant samples, 71.4 % samples were double gene negative during the heat-inactivated procedure; but either double or single gene-positive in those without the heat-inactivated procedure. Those discordant samples having with single or double gene-positive were had more than 35 CT value. A similar study performed in Beijing, China, the result showed that the impact of heat inactivation on samples those had high CT values, specifically 33.37 to 36.89 was significant [16]. This indicates heat inactivation may have a negative impact on decreasing viral load of those samples having with greater than 35 CT value. Early identification and detection, early prevention, and control are the currently available method to curbing the rapid spread of the COVID-19 infection. However, this result may have a negative impact on these measures of early identification and detection, early prevention, and control. Although heat inactivation makes safe for laboratory technicians, false-negative individuals in the community may be transmitted to a wide range of people. On the other hand, the positivity and negativity of the original sample and the heat-inactivated sample result in this study was showed that had no statistically significant difference after a chi-square test ($P > 0.05$). Similar results were obtained from Renmin Hospital of Wuhan University, China [17], Zhujiang Hospital, China [18], and a study conducted in USA on sputum sample [9] has no significant difference between the non-inactivated and inactivated sample at 56°C for 30 min for the qualitative detection of SARS-CoV-2. On the contrary, a study conducted in Beijing, China [16] and Yongchuan District Center for Disease Control and Prevention of Chongqing, China [19] were indicated that the qualitative detection of SARS-CoV-2 was significantly impacted with heat inactivation at 56°C for 30 min. This difference might be due to sample size difference between experiments, type of detection/extraction reagent difference, sample type difference and viral strain difference by itself. However, it is difficult to know the impact of this result on the transmission of SARS-COV2, as the virus was new, with lots of unknown characteristics and spreading fast as a forest fire. In this study, the effect of heat inactivation at 56°C for 30 min on the CT value of matched samples was analyzed, and the average CT value difference between non-inactivated and heat-inactivated at 56°C for 30 min were 0.04

and 0.38 for ORF1a/b gene and N gene respectively. It means that after heat inactivation the ORF1a/b gene and N gene CT was increased by 0.04 and 0.38 averagely on each sample respectively. Based on paired T-test analysis, the ORF1a/b gene CT value difference was not statistically significant ($t=0.28$; $p>0.05$), whereas the N gene CT values between inactivated and the non-inactivated group were statistically significant ($t=2.64$; $p=0.01$). In addition to this, the mean CT value difference of heat-treated and untreated group sample greater than 30 CT of ORF1a/b and N gene were 1.26 and 1.00 respectively. These average CT value increment while heat inactivation was statistically significant in both N and ORF1a/b gene ($p<0.05$). Similar studies reported by Pan Y, et al. [16] and Chen H, et al. [15] both were from China indicated that heat inactivation prior to extraction can significantly decrease the number of RNA copies (increased CT values compared to the original sample). This may result in false-negative during heat inactivation procedure which is supportive of transmission. However, the other studies like; the study conducted in Charite University, Berlin, Germany [13], Renmin Hospital of Wuhan University, Huazhong China [17], Republic of South Korea [20] and Zhejiang University School of Medicine, China [21] revealed that CT values of heat-inactivated at 56°C for 30 min group and the non-inactivated group had no statistically significant difference. These difference might be due to sample size difference, type of sample difference or and type of strain difference circulating in the community or additional investigations might be needed to reason out the difference.

In this experimental study, the correlation between inactivated and non-inactivated samples was also examined. As a result, Pearson correlation analysis was performed and the analysis showed that, the ORF1a/b and N gene CT values of inactivated sample and non-inactivated sample had excellent correlation ($r=0.978$; $p<0.001$); and ($r=0.969$; $p<0.001$) respectively (Figure: 1-2). The result reported from the First People's Hospital of Zhaoqing, Zhao Qing City, China, the CT value of ORF1a/b gene and N gene were perfectly correlated between inactivated at 56°C for 30 min and non-inactivated samples [15].

Limitation Of The Study

Our study has some limitations;

1. We have used only Oropharyngeal swabs, so we cannot conclude for another type of samples; like sputum, saliva, blood and stool samples
2. In this study we didn't perform SARS-CoV-2 viral quantification to show the exact viral copy difference between inactivated and non-inactivated sample results and
3. Our study was not performed viral infectivity analysis because of lack of infrastructure

Conclusion

In conclusion, our result showed that heat inactivation at 56°C for 30 min has not statistically significant effect for the qualitative rRT-PCR detection (positivity or negativity rate of detection) of SARS-CoV-2 viral infection. However, this study showed that there was statistically significant CT value increment after heat inactivation at 56 °C for 30 minutes compared to untreated samples. So, a false negative result in

high CT value (especially the result greater than 35) might be the challenge of this protocol. Finally for SARS-CoV-2 viral inactivation prior to sample extraction other inactivation methods rather than heat inactivation and further studies should be considered. By replicating this study the real effect of heat inactivation on the detection of SARS-CoV-2 viral genomic materials could be known which will have an impact on the diagnosis and prognosis of COVID-19 patients specifically in resource-limited settings.

Abbreviations

BSC: Biological Safety Cabinet; BSCL2: Biosafety Cabinet Level 2; CDC: Center for Disease Control; COVID-19: Coronavirus disease 19; CT: Cycle Threshold; EPHI: Ethiopian Public Health Institution; IC: Internal Control; NAAT: Nucleic Acid Amplification Test; ORF1a/b: Open Reading frame 1a/b; POC: Point of Care; rRT-PCR: reverse transcriptase Real Time Polymerase Chain Reaction; SARS CoV-2: Sever Acute Respiratory Syndrome Corona Virus -2; SOP: Standard Operation Procedure; VTM: Viral Transport Media; WHO: World Health Organization

Declarations

Ethics approval and consent to participate

Ethical clearance was received from Ethiopian public health institute scientific ethical review committee with reference number EPHI-IRB-279-2020. Written informed consent was obtained from all participants prior to participation and we did not use any personal identifiers and all information's are kept confidential.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article. The data that support the findings of this study are available from the corresponding author on reasonable request.

Competing interests

All authors declare that they have no conflict or competing interests.

Funding

Ethiopian public health institute was support the reagents and materials used for this study.

Authors' contributions

BW: Conception of the study, writing the original draft, study design, data analysis and interpretation; GG: study design, data analysis, critical review of the manuscript; KZ: writing the original draft, study design and critical review of the manuscript; AY: laboratory investigation and critical review of the manuscript; SA: laboratory investigation, data acquisition and study design; MY: laboratory investigation, data acquisition and study design; GGJ: writing the original draft and study design; AF: study design; KD: Manuscript critical review and final approval of the version to be submitted. All authors read and approved the final manuscript.

Acknowledgements

We would like to acknowledge Ethiopian Public Health Institute (EPHI) for material and reagent support. My appreciation also goes to all staffs of EPHI, HIV/AIDS disease research team for their cooperation on specimen handling and laboratory analysis.

Authors' information

¹HIV/AIDS disease research team, TB and HIV/AIDS Disease Research Directorate, Ethiopian Public Health Institute, Addis Ababa, Ethiopia. ²Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences, Addis Ababa, Ethiopia.

References

1. Arun Zanke A, R Thenge R, S Adhao V. COVID-19: A pandemic declare by world health organization. *IP Int J Compr Adv Pharmacol*. 2020; 5(2):49–57.
2. World Health Organization (WHO). Coronavirus disease (COVID-19), weekly update.2020; available from; <https://www.who.int/publications/m/item/weekly-epidemiological-update—7-september-2020>
3. Esbin MN, Whitney ON, Chong S, Maurer A, Darzacq X, Tjian R. Overcoming the bottleneck to widespread testing: A rapid review of nucleic acid testing approaches for COVID-19 detection. *Rna*. 2020; 26(7):771–83.
4. World Health Organization (WHO). Advice on the use of point-of-care immunodiagnostic tests for COVID-19. WHO. 2020; available from; (<https://www.who.int/news-room/commentaries/detail/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-covid-19>).
5. Kumar R, Nagpal S, Kaushik S, Mendiratta S. COVID-19 diagnostic approaches: different roads to the same destination. *Virus Disease*. 2020; 31(2):97–105. Available from: <https://doi.org/10.1007/s13337-020-00599-7>
6. Feng W, Newbigging AM, Le C, Pang B, Peng H, Cao Y, et al. Molecular Diagnosis of COVID-19: Challenges and Research Needs. *Anal Chem*. 2020; 92(15):10196–209.
7. Udugama B, Kadhiresan P, Kozlowski HN, Malekjahani A, Osborne M, Li VYC, et al. Diagnosing COVID-19: The Disease and Tools for Detection. *ACS Nano*. 2020; <https://dx.doi.org/10.1021/acsnano.0c02624>

8. World Health Organization (WHO). Laboratory biosafety guidance related to the novel coronavirus (2019-nCoV). 2020 ;(World Health Organization; License: CC BY-NC-SA 3.0 IGO). Available from: https://www.who.int/docs/default-source/coronaviruse/laboratory-biosafety-novel-coronavirus-version-1-1.pdf?sfvrsn=912a9847_2
9. Auerswald H, Yann S, Dul S, In S, Dussart P, Martin NJ, et al. Assessment of Inactivation Procedures for SARS-CoV-2. *bioRxiv*. 2020; doi: <https://doi.org/10.1101/2020.05.28.120444>.
10. Wang T, Lien C, Liu S, Selveraj P. Effective Heat Inactivation of SARS-CoV-2. 2020; 2020.04.29.20085498. Available from: <https://www.medrxiv.org/content/10.1101/2020.04.29.20085498v1>
11. Bidra AS, Pelletier JS, Westover JB, Frank S, Brown SM, Tessema B. Rapid In-Vitro Inactivation of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Using Povidone-Iodine Oral Antiseptic Rinse. *J Prosthodont*. 2020; 29(6):529–33.
12. Abraham JP, Plourde BD, Cheng L. Using heat to kill SARS-CoV-2. 2020; *Rev Med Virol*. 2020; 30:e2115. <https://doi.org/10.1002/rmv.2115>
13. Pastorino B, Touret F, Gilles M, Lamballerie X De. Heat Inactivation of Different Types of SARS-CoV-2 Samples: What Protocols for Biosafety, Molecular Detection and Serological Diagnostics? 2020; 12 (735):6–13.
14. Hessling M, Hoenes K, Lingenfelder C. Selection of parameters for thermal coronavirus inactivation - a data-based recommendation. *GMS Hyg Infect Control*. 2020; 15: ISSN 2196-5226.
15. Chen H, Wu R, Xing Y, Du Q, Xue Z, Xi Y, et al. Influence of different inactivation methods on severe acute respiratory syndrome coronavirus 2 RNA copy number. *J Clin Microbiol*. 2020; 58(8).
16. Pan Y, Long L, Zhang D, Yuan T, Cui S, Yang P, et al. Potential False-Negative Nucleic Acid Testing Results for Severe Acute Respiratory Syndrome Coronavirus 2 from Thermal Inactivation of Samples with Low Viral Loads. *Clin Chem*. 2020; 66(6):794–801.
17. Wu Z gang, Zheng H yun, Gu J, Li F, Lv R long, Deng Y yun, et al. Effects of Different Temperature and Time Durations of Virus Inactivation on Results of Real-time Fluorescence PCR Testing of COVID-19 Viruses. *Curr Med Sci*. 2020; 40(4):40–3.
18. liu yanxia, Cao Z, Chen M, Zhong Y, Luo Y, Shi G, et al. Effect of Heat inactivation on Real-Time Reverse Transcription PCR of the SARS-COV-2 Detection. *medRxiv* 2020;(<https://doi.org/10.1101/2020.05.19.20101469>).
19. Zou J, Zhi S, Chen M, Su X, Kang L, Li C, et al. Heat inactivation decreases the qualitative real-time RT-PCR detection rates of clinical samples with high cycle threshold values in COVID-19. *Diagn Microbiol Infect Dis*. 2020; 98(1).
20. Kim Y Il, Casel MAB, Kim SM, Kim SG, Park SJ, Kim EH, et al. Development of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) thermal inactivation method with preservation of diagnostic sensitivity. *J Microbiol*. 2020; 58(10):886–91.
21. Duan Xiuzhi, Wang Xuchu YP. The effect of virus inactivation treatment on the weak positive results of the 2019 new coronavirus nucleic acid test. *Chinese Journal of Inspection Medicine*. 2020; 43(4):

Figures

Figure 1

Correlation of inactivated and non-inactivated ORF1a/b gene CT for Oropharyngeal specimen in COVID 19 testing, Ethiopia, 2020

Figure 2

Correlation of inactivated and non-inactivated N gene CT for Oropharyngeal specimen in COVID 19 testing, Ethiopia, 2020

Figure 3

Correlation of inactivated and non-inactivated ORF1a/b gene CT value greater than 30 for Oropharyngeal specimen in COVID 19 testing, Ethiopia, 2020

Figure 4

Correlation of inactivated and non-inactivated N gene CT value greater than 30 for Oropharyngeal specimen in COVID 19 testing, Ethiopia, 2020