



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



## SARS-CoV-2 detection by extraction-free qRT-PCR for massive and rapid COVID-19 diagnosis during a pandemic in Armenia

Diana Avetyan<sup>a,b,\*</sup>, Andranik Chavushyan<sup>a</sup>, Hovsep Ghazaryan<sup>a</sup>, Ani Melkonyan<sup>a</sup>, Ani Stepanyan<sup>a</sup>, Roksana Zakharyan<sup>a,b</sup>, Varduhi Hayrapetyan<sup>a</sup>, Sofi Atshemyan<sup>a</sup>, Gisane Khachatryan<sup>a,b</sup>, Tamara Sirunyan<sup>a,b</sup>, Suren Davitavyan<sup>a,b</sup>, Gevorg Martirosyan<sup>a,c</sup>, Gayane Melik-Andreasyan<sup>d</sup>, Shushan Sargsyan<sup>d</sup>, Armine Ghazazyan<sup>d</sup>, Naira Aleksanyan<sup>d</sup>, Xiushan Yin<sup>e,f</sup>, Arsen Arakelyan<sup>a</sup>

<sup>a</sup> Laboratory of Human Genomics and Immunomics, Institute of Molecular Biology, National Academy of Sciences RA, Yerevan, 0014, Armenia

<sup>b</sup> Institute of Biomedicine and Pharmacy, Russian-Armenian University, Yerevan, 0051, Armenia

<sup>c</sup> Davidyants Laboratories, Yerevan, 0054, Armenia

<sup>d</sup> National Center of Disease Control and Prevention, Ministry of Health RA, Yerevan, 0025, Armenia

<sup>e</sup> Applied Biology Laboratory, Shenyang University of Chemical Technology, Shenyang, 110142, China

<sup>f</sup> Biotech & Biomedicine Science (Shenyang) Co. Ltd, Shenyang, 110000, China

### ARTICLE INFO

#### Keywords:

COVID-19  
SARS-CoV-2  
Virus detection  
Direct sample qRT-PCR  
Heat-inactivation  
Pelleting

### ABSTRACT

COVID-19 pandemic severely impacted the healthcare and economy on a global scale. It is widely recognized that mass testing is an efficient way to contain the spread of SARS-CoV-2 infection as well as aid in the development of informed policies for disease management. However, the current COVID-19 worldwide infection rates increased the demand for rapid and reliable screening of infection.

We compared the performance of qRT-PCR in direct heat-inactivated (H), heat-inactivated and pelleted (HC) samples against RNA in a group of 74 subjects (44 positive and 30 negative). Then we compared the sensitivity of HC in a larger group of 196 COVID-19 positive samples.

Our study suggests that HC samples show higher accuracy for SARS-CoV-2 detection PCR assay compared to direct H (89 % vs 83 % of the detection in RNA). The sensitivity of detection using direct samples varied depending on the sample transport and storage media as well as the viral loads (as measured by qRT-PCR Ct levels).

Altogether, all the data suggest that purified RNA provides more accurate results, however, direct sample testing with qRT-PCR may help to significantly increase testing capacity. Switching to the direct sample testing is justified if the number of tests is doubled at least.

### 1. Introduction

Coronavirus disease (COVID-19) is a respiratory tract infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Wang et al., 2020). It was first identified in Wuhan, Hubei Province, China, in December 2019 (Wu et al., 2020; Zhou et al., 2020). The current COVID-19 worldwide infection rates and a high proportion of asymptomatic cases forced an unexpected burden on the health care systems worldwide and led to the need for a rapid, affordable and efficient diagnostic test for massive routine screening.

The current standard method for the detection of RNA viruses in clinical diagnostics is based on the extraction of RNA from a nasopharyngeal swab in viral transport media (VTM) followed by one- or two-step reverse transcription quantitative polymerase chain reaction (qRT-PCR) (Alcoba-Florez et al., 2020; Centers for Disease Control and Prevention, 2020). Currently, available diagnostic options also include several sample-to-answer platforms, however, conventional qPCR workflow dominates over all other diagnostic platforms due to its affordability (Zhen et al., 2020). One of the key factors for successful qRT-PCR is the quality of nucleic acid extraction. However, in many

\* Corresponding author at: Laboratory of Human Genomics and Immunomics, Institute of Molecular Biology, National Academy of Sciences RA, Yerevan, 0014, Armenia.

E-mail address: [d.avetyan@mb.sci.am](mailto:d.avetyan@mb.sci.am) (D. Avetyan).

<https://doi.org/10.1016/j.jviromet.2021.114199>

Received 13 January 2021; Received in revised form 25 May 2021; Accepted 25 May 2021

Available online 4 June 2021

0166-0934/© 2021 Published by Elsevier B.V.

sites, automatic isolation is not available, while manual isolation takes 2–4 hours and requires significant work that can result in additional experimental errors as well as limiting the possibilities to extend testing capacity. Therefore, the ability to omit the RNA purification from diagnostic protocols would be not only affordable, operative, and efficient for COVID-19 screening, but also keeps the use of commercial kits to the minimum. The options of detection of SARS-CoV-2 by qRT-PCR directly from VTM, UTM (Universal Transport Medium) media have already been explored with certain promising data (Alcoba-Florez et al., 2020; Centers for Disease Control and Prevention, 2020; Li et al., 2020; Mancini et al., 2020; Merindol et al., 2020). However, it has been shown that detecting sensitivity in those samples is decreased compared to purified RNA samples, leading to the increase of “false-negative” results. Here we optimized two different protocols for qRT-PCR with direct samples and systematically compared them with the current detection assay.

## 2. Materials and methods

### 2.1. Samples

We performed the study enrolling subjects during the period June–July 2020. We collected paired nasopharyngeal swabs aliquots and RNA samples extracted from the same nasopharyngeal swabs from 270 subjects collected in two phases. The first group consisted of 74 positive ( $n = 44$ ) and negative ( $n = 30$ ) samples. The second group of 196 samples was collected from SARS-CoV-2 positive subjects. The extracted RNA and nasopharyngeal swab sample aliquots were obtained from the National Center for Disease Control and Prevention of MH RA, National Centre for Aids Prevention of MH RA, and Davidyants Laboratories. Subject status was diagnosed at testing sites using commercially available kits (Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit, Sansure Biotech, China and SARS-CoV-2/SARS-CoV, DNA technology, Russia). Both kits contain internal control (human RNase P gene) and SARS-CoV-2/SARS-CoV additionally contains extraction control. Swab samples were stored in three different transport media types: Sample Storage Reagent (Sansure Biotech, China) ( $n = 45$ ) media, PBS ( $n = 91$ ) and STORE-F UTM (DNA technologies, Russia) ( $n = 134$ ). No additional clinical or demographic information was collected. The study was approved by the Institutional Review Board of the Institute of Molecular Biology NAS RA (IRB#: 00004079).

### 2.2. RNA extraction

Extracted RNA samples along with corresponding swab samples were provided by three testing facilities in Armenia (See above). RNA extraction was performed using both automated and manual extraction protocols. Automated extraction (45 samples) was performed using Maxwell RSC Viral Total Nucleic Acid Purification Kit (Promega Corporation Inc, US). Manual extraction was performed using a triazole based PREP-NA kit (114 samples) (DNA Technologies Ltd, Russian Federation) and magnetic bead-based ZipPrime nucleic acid isolation kit (111 samples) (ZipPrime Ltd, Turkey) according to the manufacturer's manuals.

### 2.3. Direct sample with heat-inactivation

To skip RNA extraction, 20  $\mu$ l of swab samples in transport media were heat-inactivated at 95 °C for 5 min before loading into the PCR.

### 2.4. Direct sample with heat-inactivation and pelleting

To skip RNA extraction, 100  $\mu$ l of swab samples in transport media were heat-inactivated at 95 °C for 5 min and centrifuged for 10 min at 12,000 g. The supernatant was aspirated to leave 20–30  $\mu$ l liquid in a tube. Pellet was mixed by pipetting or vortexing and used in PCR.

### 2.5. qRT-PCR

We performed qRT-PCR (both using extracted RNA and direct samples) targeting the N gene and ORF1ab gene in the conserved region of the SARS-CoV-2 genome (Wu et al., 2020). The final reaction volume consisted of 10  $\mu$ l PCR reaction mix (Vazyme Ltd, China), 1.5  $\mu$ l primer/probe mix (ORF-F: 5'-CCCTGTGGGTTTACACTTAA-3' (2  $\mu$ M final concentration); ORF-R: 5'-ACGATTGTGCATCAGCTGA-3' (2  $\mu$ M final concentration); ORF-P: 5'-FAM-CCGCTGCGGTATGTGGAAAGGTTATGG-BHQ1-3' (0.5  $\mu$ M final concentration); N-F: 5'-GGGAGCCTTGAATACACCAAAA-3' (2  $\mu$ M final concentration); N-R: 5'-TGTAGCAGATTGCAGCATTG-3' (2  $\mu$ M final concentration); N-P: 5'-HEX-AT CACATTGGCACCCGCAATCTG-BHQ2-3' (0.5  $\mu$ M final concentration)), 5  $\mu$ l template (sample, negative or positive control) was added to the final reaction volume of 20  $\mu$ l. Thermal cycling was performed as follows: 55 °C for 5 min (reverse transcription), followed by 94 °C for 2 min and 45 cycles of 94 °C for 5 s, 55 °C for 10 s. A final cooling step at 25 °C for 20 s was also included. The reaction took 1 h and 10 min in total and was performed on Rotor-Gene Q thermal cycler (Qiagen N.V., Germany). The protocol was described in detail elsewhere (Li et al., 2020). Samples were considered positive when a signal was detected at Ct < 40 for any gene.

### 2.6. Data analysis

Accuracy, sensitivity, and specificity for PCR assays were calculated using *caret* and *epiR* packages available in the R software environment for statistical computing. The Wilcoxon matched rank test was used to compare differences in Ct values in matching samples.

## 3. Results

For this part of the study, we performed qRT-PCR assay for SARS-CoV-2 using matched extracted RNA, heat-inactivated (H), and heat-inactivated and pelleted (HC) samples from 74 subjects. Nasopharyngeal swabs were stored in PBS ( $n = 47$ ) and STORE-F UTM ( $n = 27$ ) for 12–72 hours at +4 °C before PCR. There were no significant differences in transportation time for samples from different testing facilities. In-laboratory results of qRT-PCR were in complete concordance with results obtained at testing facilities (data not shown). Then, the qRT-PCR was performed with the direct addition of heat-inactivated samples with and without centrifugation. The results suggest that the centrifugation step considerably improved accuracy and sensitivity compared with the direct heat-inactivation (Table 1).

The low sensitivity values in both HC and H could be partially attributed to the issues of detection in samples with low viral loads (as measured by qRT-PCR cycle threshold (Ct) levels) close to the detection limit. Interestingly, in these samples, Ct values were mostly detected only by one channel. In fact, for samples with Ct values < 34 (purified RNA), the sensitivity for H was 93 % (28/30) and for HC was 97 % (29/30). Only one sample with Ct value < 20 was missed using HC samples compared to purified RNA.

On the other side, we report three positive HC samples that hadn't been detected in the extracted RNA. These samples were positive with Ct values around 22 (not detected in extracted RNA), 32 (Ct > 40 in

**Table 1**  
COVID-19 detection qRT-PCR results using direct sample and purified RNA.

		Extracted RNA		Performance evaluation
		Positive	Negative	
HC	Positive	39	3	Accuracy: 89 % (95 % CI: 80–95 %) Sensitivity: 89 % (95 % CI: 75–96 %) Specificity: 90 % (95 % CI: 73–98 %)
	Negative	5	27	
H	Positive	35	3	Accuracy: 83 % (95 % CI: 74–91 %) Sensitivity: 80 % (95 % CI: 65–90 %) Specificity: 90 % (95 % CI: 73–98 %)
	Negative	9	27	

extracted RNA), and 35 (Ct > 40 in extracted RNA). These samples were also positive in H, however, with higher Ct values compared to HC. Samples were extracted using manual extraction kits PROBE-NA (two samples) and a ZipPrime nucleic acid isolation kit (one sample). We suggest that this discrepancy could be a result of a failure during RNA isolation steps using manual extraction protocol.

We also compared the differences between Ct values ( $\Delta$ Ct) obtained from purified RNA and those obtained from heat-inactivation or heat-inactivation and pelleting samples (Fig. 1, Supp. Table 1).

In agreement with the previous studies, we also observed a considerable shift of Ct values towards higher values in direct samples. Meanwhile, the median  $\Delta$ Ct was lower by 1.55 and 2.29 cycles (Wilcoxon signed-rank test  $p = 0.0018$  and  $< 0.0001$  for ORF1ab and N genes, accordingly) in HC samples compared to H samples (Fig. 2A). We also observed 5 out of 44 positive cases (11.4 %) where Ct values for HC and H samples were smaller compared to the detection in corresponding extracted RNA samples (Fig. 2B), which indicates that the omitting of RNA processing steps may reduce degradation. Thus, our results show that additional centrifugation and pelleting steps can improve the detection of viral nucleic acids compared to heat treatment alone without much affecting the workflow of the direct PCR.

Considering the results obtained in the next series of experiments we proceeded only with evaluation of the performance of HC compared to extracted RNA samples. We compared the performance of SARS-CoV-2 PCR detection in HC samples with the detection in extracted RNA in the larger group of COVID-19 positive samples (Table 2). From 196 samples the HC was able to detect 171 positive samples with the overall sensitivity of 88 % (CI95 % 83 %–92 %). We also observed positive correlation between Ct values of extracted RNA and HC samples (Spearman's rank correlation coefficient analysis  $R_s = 0.79$  and  $0.78$ ,  $p < 0.0001$  for ORF1ab and N genes, accordingly) (Supp. Fig. 2).

Further analyses showed that detection sensitivity varies depending on both transportation media and viral loads (as measured by qRT-PCR cycle threshold (Ct) levels). In general, the sensitivity of detection in HC samples increases along with the increase of viral loads (Table 3). Moreover, the sensitivity of detection in HC samples differs depending on the transport media in which a sample was stored. The best performance was obtained with Sample Storage Reagent (Sansure Biotech, China) media, followed by PBS and STORE-F UTM (DNA technologies, Russia). However, it should be also mentioned that sample storage time and conditions were not uniform before PCR assay, and we were not able

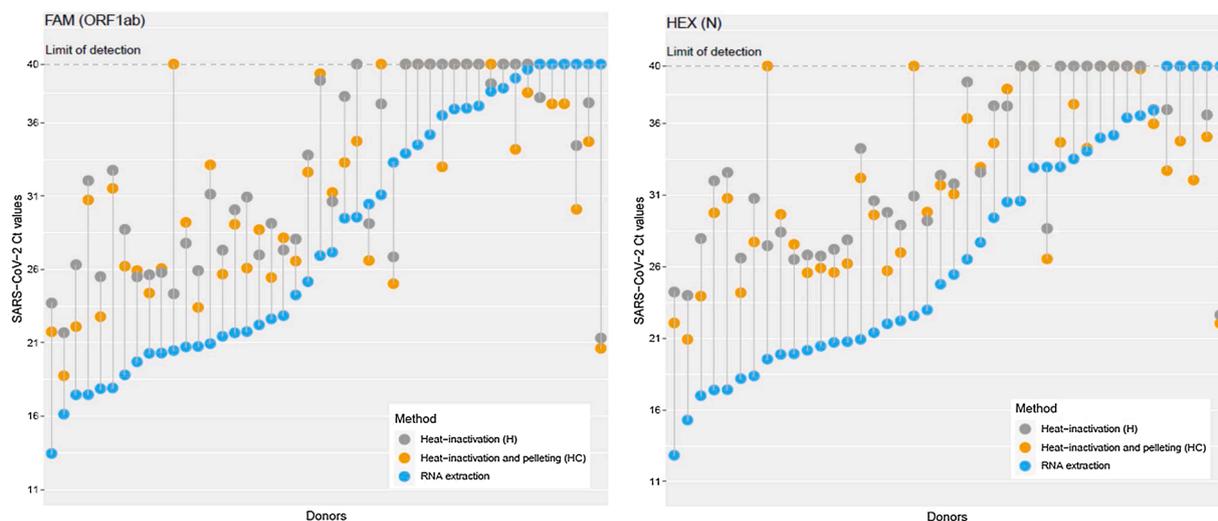
to obtain exact information for each sample. These factors could also affect the performance of the direct assay.

#### 4. Discussion

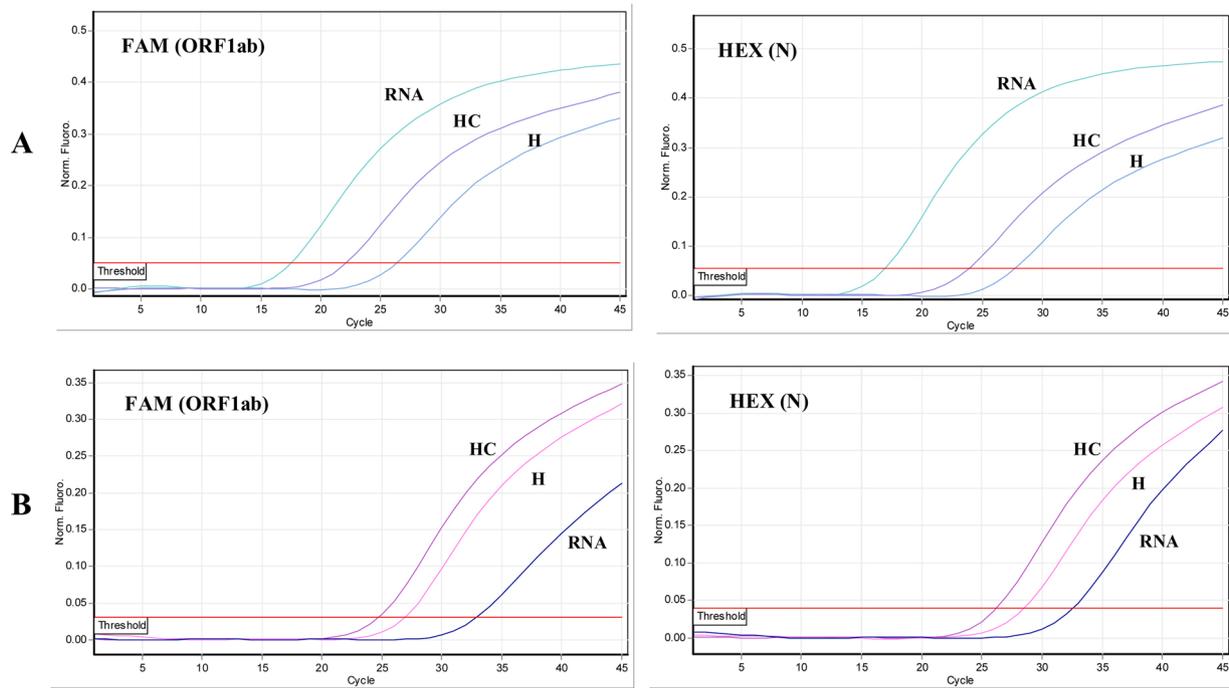
The standard molecular diagnostic test for SARS-CoV-2 is a multistep process that requires viral RNA extraction and qRT-PCR implementation. Due to increased demand in the rapid and reliable screening of this virus, alternative protocols with similar sensitivity are needed. Efforts to simplify the current methods are critical to assess the viral spread and limit the pandemic, as well as could benefit patients' care. Recent attempts have been made to omit RNA extraction to simplify the direct qRT-PCR protocol of COVID-19 detection and also assess the impact of different swab storage media composition on PCR efficiency (Brown et al., 2020; Bruce et al., 2020; Mancini et al., 2020; Merindol et al., 2020). Additionally, methods relying on lysis buffers (Jørgensen et al., 2021) and proteinase K treatment have been proposed to speed up PCR testing (Chu et al., 2020) and increase the sensitivity. However, most of the studies are done with small sample size and needs to be expanded for better understanding performance and caveats associated with direct sample testing.

In this study, we compared different methods of specimen processing for qRT-PCR (without RNA extraction). We demonstrated that heat-inactivation and pelleting approach performs better compared to heat-inactivation only. We speculate that proposed strategy of pelleting, aspiration of supernatant and resuspension/lysis can facilitate the release of viral particles from cells and thus increase the number of viral particles in a sample and sensitivity of detection. These results are further supported when we compared the results of detection in resuspended pellet compared with detection in supernatant (see Supp. Table 2 and Supp. Fig. 1). In addition, there are results pointing at association of viral RNA replication with cell's double membrane organelles, which can form a pellet during low-speed centrifugation (Snijder et al., 2020). Furthermore, low speed centrifugation and pellet lysis have been previously used for increase of cell-associated viral particle concentrations in several studies (Darling et al., 2000; Kim and Park, 2020; Payne, 2017; Prachar et al., 1988).

Overall, we observed consistent results for SARS-CoV-2 detection, however, the average Ct values for both direct methods were higher than for extracted RNA samples. Our study suggests that HC have more sensitivity for SARS-CoV-2 detection PCR assay compared to direct H.



**Fig. 1.** Distribution of Ct values from COVID-19 patients nasopharyngeal swabs following qRT-PCR with standard RNA extraction, heat-inactivation (H), and heat-inactivation and pelleting (HC) methods. The limit of detection (40 Ct) is denoted with a dashed line. Samples with Ct values above this cutoff were considered negative for SARS-CoV-2 RNA. Samples are ordered by the purified RNA qRT-PCR Ct values. Only samples positive at least in one treatment method (RNA extraction, heat-inactivation, or heat-inactivation and pelleting) are presented. The summary of changes in Ct values is provided in Supplementary Table 1.



**Fig. 2.** Representative qRT-PCR amplification plots for the SARS-CoV-2 ORF1ab (FAM) and N (HEX) genes for a nasopharyngeal swab sample subjected to the standard RNA extraction, heat-inactivation (H), and heat-inactivation and pelleting (HC). Amplification plots show normalized value ( $\Delta R_n$ , linear scale) as a function of the qPCR cycle. The horizontal red line denotes the cycle threshold. **(A)** Plot shows the shift of Ct values towards higher values in direct samples. **(B)** Plot shows cases where Ct values for HC and H samples were smaller compared to the detection in extracted RNA samples (in 11.4 % of 44 positive samples) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

**Table 2**  
Detection counts in extracted RNA and HC samples.

	ORF1ab and N	ORF1ab only	N only	Total
RNA	174	9	13	196
HC	145	14	12	171

For the HC method +4.65 (for ORF1ab gene) and +5.53 (for N gene) median Ct differences were detected compared to purified RNA samples. This could be for several reasons: i) for the same input volume of samples nucleic acid extraction yields higher quantity/quality of RNA compared with the direct sample; ii) RNA extraction was performed from fresh swab samples, while the aliquots used for the direct method had been stored at +4 °C for at least one day before heat-inactivation and PCR, which is known to cause the shift of results by 2–3 Ct compared to Ct values for eluates of matched fresh aliquots of the same nasopharyngeal

specimens (Smyrlaki et al., 2020); iii) heat-inactivation might be a reason of viral nucleic acids degradation, that could decrease the qualitative detection of clinical samples, especially weakly positive samples with higher Ct values (Zou et al., 2020); iv) the PCR inhibition is a well acknowledged risk when performing direct PCR from swab samples (Matelski et al., 2020).

We have also noted, that despite the general Ct loss in heat-inactivated and pelleted samples, it was not constant across the samples. We can speculate the variability of Ct loss can be also attributed to the pelleting step, which will be efficient if there is a considerable amount of infected intact cells in a swab sample that can release viral particles during pellet resuspension. Our results suggest that the lower Ct in RNA sample the higher is Ct loss during direct assays. However, the same variability in Ct loss have been also observed previously see Fig. (2 i) in Smyrlaki et al., and this observation worth additional investigations (Smyrlaki et al., 2020). The mean Ct loss in our study was comparable with the ones reported when using direct lysis buffer or treatment with

**Table 3**  
The sensitivity of direct qRT-PCR with HC according to the transportation media used and viral loads (as measured by qRT-PCR cycle threshold (Ct) levels).

Ct	ORF1ab gene (n <sub>RNA</sub> = 183, n <sub>HC</sub> = 155) **					N gene (n <sub>RNA</sub> = 189, n <sub>HC</sub> = 157) **				
	RNA	HC	Sensitivity	Mean $\Delta$ Ct	SD $\Delta$ Ct	RNA	HC	Sensitivity	Mean $\Delta$ Ct	SD $\Delta$ Ct
< 25	46	41	89 %	6.31	4.1	46	38	83 %	7.35	4.49
STOR-F										
25–34	20	16	80 %	-0.23	8.08	24	18	75 %	-1.24	8.86
>34	12	9	75 %	-3.89	4.78	15	8	53 %	-4.33	4.86
<25	21	19	90 %	4.73	3.78	16	14	88 %	6.02	4.28
PBS										
25–34	32	30	94 %	2.61	3.99	32	31	97 %	2.01	5.08
>34	9	7	78 %	-5.68	3.36	9	9	100%	-7.43	4.23
<25	14	14	100 %	3.76	4.49	7	6	86 %	3.38	3.64
SSR*										
25–34	17	17	100 %	1.18	3.38	22	20	91 %	2.17	3.9
>34	12	6	50 %	-0.31	4.52	16	13	81 %	1.6	3.28

\* Sansure sample storage reagent.

\*\* For details see Table 2.

proteinase K. Jørgensen et al. used the in-well direct lysis method, which showed, that an average loss of 3.3 cycles in Ct compared to automated nucleic acids purification happens when using lysis detergent directly to qRT-PCR master mix (Jørgensen et al., 2021). According to Chu et al. paper, the combination of proteinase K and heat (PKH) had significantly higher positive rate than the heat only group and the direct group (no pre-processing steps) (Chu et al., 2020). The Ct values were also significantly earlier for the PKH group than either heat group or direct group, including both nasopharyngeal swab and saliva specimens, however, Ct loss was around 7–10 cycles in PKH compared with RNA samples (see Fig. 1 of (Chu et al., 2020)). Thus, heating and pelleting is a valid approach, which does not require additional reagents for sample treatment and is easy to implement, but markedly improves sensitivity compared to heating only. Moreover, compared with manual RNA extraction protocols, it requires fewer steps which in turn decreases the risks of sample-to-sample contamination.

The overall sensitivity of 88 % in our study was comparable to 87.8 % (n = 41) reported by Alcoba-Florez et al. and was a bit lower than 93 % (n = 77) reported by Brown et al., however the sample size in both studies were much smaller (Alcoba-Florez et al., 2020; Brown et al., 2020). We also demonstrated that detection accuracy with direct sample testing highly depends on the viral load in the sample, and the reliability of direct testing dramatically decreases in the samples with viral load close to the limits of detection.

There are few limitations in our study worth to mention here. Firstly, we were not able to collect all samples in all three types of transport media as well as extract by all three nucleic extraction methods. The reason for this was that we did not perform extraction in the laboratory but obtained samples from three different test sites. Moreover, VTM and extraction kit switching were dictated by overall shortage of reagents and kits. The shortage of reagents and supplies for COVID-19 testing brought up the problem of diversity of transport media and kits. Currently, there are many options for transportation media including commercial solutions from many companies as well as home-made solutions such as VTM protocols by CDC and WHO, PBS, saline and even water. Same situation is with PCR-kits. For countries, especially developing ones, current priority is availability of reagents and supplies (through purchases or donations) rather than “unification” of testing process. Thus, we performed this study in settings very close to the “real-life” situation and the results showed that direct sample testing can still significantly accelerate testing capacity and speed. Secondly, our in-lab developed qRT-PCR assay did not contain internal control, so we were not able to distinguish between PCR reaction inhibition from other reasons causing “false-negatives” during direct sample testing. Addition of internal control will be helpful for direct sample assay, since it will make it more informative. On the other side, our assay result showed full concordance with commercially available kits when used with extracted RNA samples.

In conclusion, omitting the isolation step significantly helps to expand the testing capacity with the minor increase of false-negative ratio. We believe the data presented here is informative for massive routine population-based test, even during the period when vaccine is available. And for certain regions with shortage of standard RNA extraction reagents and automated extraction equipment, direct sample qRT-PCR might be a useful alternative for COVID-19 pandemic control.

## Funding

This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## CRedit authorship contribution statement

**Diana Avetyan:** Methodology, Writing - original draft, Writing - review & editing, Formal analysis, Investigation, Visualization. **Andranik Chavushyan:** Investigation. **Hovsep Ghazaryan:** Investigation.

**Ani Melkonyan:** Investigation. **Ani Stepanyan:** Investigation. **Roksana Zakharyan:** Investigation, Writing - review & editing. **Varduhi Hayrapetyan:** Investigation. **Sofi Atshemyan:** Investigation. **Gisane Khachatryan:** Investigation. **Tamara Sirunyan:** Investigation. **Suren Davitavyan:** Investigation. **Gevorg Martirosyan:** Investigation. **Gayane Melik-Andreasyan:** Investigation. **Shushan Sargsyan:** Investigation, Methodology. **Armine Ghazaryan:** Investigation. **Naira Aleksanyan:** Investigation. **Xiushan Yin:** Writing - original draft, Conceptualization, Methodology. **Arsen Arakelyan:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Supervision.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

The authors are grateful to the National Center for Disease Control of MH RA, National Center for AIDS Prevention of MH RA, and Davidyants Laboratories involved in COVID-19 diagnostic testing for providing nasopharyngeal swabs and RNA samples.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jviromet.2021.114199>.

## References

- Alcoba-Florez, J., González-Montelongo, R., Ínigo-Campos, A., de Artola, D.G.M., Gil-Campesino, H., The Microbiology Technical Support Team, Ciuffreda, L., Valenzuela-Fernández, A., Flores, C., 2020. Fast SARS-CoV-2 detection by RT-qPCR in preheated nasopharyngeal swab samples. *Int. J. Infect. Dis.* <https://doi.org/10.1016/j.ijid.2020.05.099>.
- Brown, J., Atkinson, L., Shah, D., Harris, K., 2020. Validation of an extraction-free RT-PCR protocol for detection of SARS-CoV2 RNA. medRxiv 2020. <https://doi.org/10.1101/2020.04.29.20085910>, 04.29.20085910.
- Bruce, E.A., Huang, M.L., Perchetti, G.A., Tighe, S., Laaguiby, P., Hoffman, J.J., Gerrard, D.L., Nalla, A.K., Wei, Y., Greninger, A.L., Diehl, S.A., Shirley, D.J., Leonard, D.G.B., Huston, C.D., Kirkpatrick, B.D., Dragon, J.A., Crothers, J.W., Jerome, K.R., Botten, J.W., 2020. Direct RT-qPCR detection of SARS-CoV-2 RNA from patient nasopharyngeal swabs without an RNA extraction step. *PLoS Biol.* 18 <https://doi.org/10.1371/journal.pbio.3000896>.
- Centers for Disease Control and Prevention, 2020. CDC 2019–Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. CDC-006-00005.
- Chu, A.W.H., Chan, W.M., Ip, J.D., Yip, C.C.Y., Chan, J.F.W., Yuen, K.Y., To, K.K.W., 2020. Evaluation of simple nucleic acid extraction methods for the detection of SARS-CoV-2 in nasopharyngeal and saliva specimens during global shortage of extraction kits. *J. Clin. Virol.* 129, 104519 <https://doi.org/10.1016/j.jcv.2020.104519>.
- Darling, D., Hughes, C., Galea-Lauri, J., Gäken, J., Trayner, I.D., Kuiper, M., Farzaneh, F., 2000. Low-speed centrifugation of retroviral vectors adsorbed to a particulate substrate: a highly effective means of enhancing retroviral titre. *Gene Ther.* 7, 914–923. <https://doi.org/10.1038/sj.gt.3301201>.
- Jørgensen, R.L., Pedersen, M.S., Chauhan, A.S., Andreasson, L.M., Kristiansen, G.Q., Lisby, J.G., Rosenstjerne, M.W., Schønning, K., 2021. An in-well direct lysis method for rapid detection of SARS-CoV-2 by real time RT-PCR in eSwab specimens. *J. Virol. Methods* 289. <https://doi.org/10.1016/j.jviromet.2021.114062>.
- Kim, J., Park, J.-E., 2020. Porcine epidemic diarrhoea virus purification using low-speed centrifugation. *J. Prev. Vet. Med.* 44, 28–32. <https://doi.org/10.13041/jpvm.2020.44.1.28>.
- Li, X., Liu, J., Liu, Q., Yu, L., Wu, S., Yin, X., 2020. Optimization of a fluorescent qPCR detection for RNA of SARS-CoV-2. *Shengwu Gongcheng Xuebao/Chinese J. Biotechnol.* 36, 732–739. <https://doi.org/10.13345/j.cjb.200088>.
- Mancini, F., Barbanti, F., Scaturro, M., Errico, G., Iacobino, A., Bella, A., Riccardo, F., Marsili, G., Stefanelli, P., Pezzotti, P., Rezza, G., Ciervo, A., 2020. Laboratory management for SARS-CoV-2 detection: a user-friendly combination of the heat treatment approach and rt-Real-time PCR testing. *Emerg. Microbes Infect.* 9, 1393–1396. <https://doi.org/10.1080/22221751.2020.1775500>.
- Matelski, A., Siddique, N., Mead, P., Raimondi, T., Jorgenson, L., Calderon, O., Harman, K., Farrell, J., 2020. Microscopic review of nasopharyngeal swabs as a means of benchside quality assurance. *Ann. Clin. Lab. Sci.* 50, 665–669.

- Merindol, N., Pépin, G., Marchand, C., Rheault, M., Peterson, C., Poirier, A., Houle, C., Germain, H., Danylo, A., 2020. SARS-CoV-2 detection by direct rRT-PCR without RNA extraction. *J. Clin. Virol.* 128, 104423 <https://doi.org/10.1016/j.jcv.2020.104423>.
- Payne, S., 2017. Methods to study viruses. *Viruses*. Elsevier, pp. 37–52. <https://doi.org/10.1016/b978-0-12-803109-4.00004-0>.
- Prachar, J., Hlubinova, K., Kovarik, A., Feldsamova, A., Simkovic, D., 1988. Concentration of retroviruses by low-speed centrifugation. *Neoplasma* 35, 651–655.
- Smyrliaki, I., Ekman, M., Lentini, A., Rufino de Sousa, N., Papanicolaou, N., Vondracek, M., Aarum, J., Safari, H., Muradrasoli, S., Rothfuchs, A.G., Albert, J., Högberg, B., Reinius, B., 2020. Massive and rapid COVID-19 testing is feasible by extraction-free SARS-CoV-2 RT-PCR. *Nat. Commun.* 11 <https://doi.org/10.1038/s41467-020-18611-5>.
- Snijder, E.J., Limpens, R.W.A.L., de Wilde, A.H., de Jong, A.W.M., Zevenhoven-Dobbe, J. C., Maier, H.J., Faas, F.F.G.A., Koster, A.J., Bárcena, M., 2020. A unifying structural and functional model of the coronavirus replication organelle: tracking down RNA synthesis. *PLoS Biol.* 18, e3000715 <https://doi.org/10.1371/journal.pbio.3000715>.
- Wang, C., Horby, P.W., Hayden, F.G., Gao, G.F., 2020. A novel coronavirus outbreak of global health concern. *Lancet*. [https://doi.org/10.1016/S0140-6736\(20\)30185-9](https://doi.org/10.1016/S0140-6736(20)30185-9).
- Wu, F., Zhao, S., Yu, B., Chen, Y.M., Wang, W., Song, Z.G., Hu, Y., Tao, Z.W., Tian, J.H., Pei, Y.Y., Yuan, M.L., Zhang, Y.L., Dai, F.H., Liu, Y., Wang, Q.M., Zheng, J.J., Xu, L., Holmes, E.C., Zhang, Y.Z., 2020. A new coronavirus associated with human respiratory disease in China. *Nature* 579, 265–269. <https://doi.org/10.1038/s41586-020-2008-3>.
- Zhen, W., Smith, E., Manji, R., Schron, D., Berry, G.J., 2020. Clinical evaluation of three sample-to-answer platforms for detection of sars-cov-2. *J. Clin. Microbiol.* 58 <https://doi.org/10.1128/JCM.00783-20>.
- Zhou, P., Yang, X.Lou, Wang, X.G., Hu, B., Zhang, L., Zhang, W., Si, H.R., Zhu, Y., Li, B., Huang, C.L., Chen, H.D., Chen, J., Luo, Y., Guo, H., Jiang, R.Di, Liu, M.Q., Chen, Y., Shen, X.R., Wang, X., Zheng, X.S., Zhao, K., Chen, Q.J., Deng, F., Liu, L.L., Yan, B., Zhan, F.X., Wang, Y.Y., Xiao, G.F., Shi, Z.L., 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579, 270–273. <https://doi.org/10.1038/s41586-020-2012-7>.
- Zou, J., Zhi, S., Chen, M., Su, Xingyu, Kang, L., Li, C., Su, Xiaosong, Zhang, S., Ge, S., Li, W., 2020. 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.* 98, 115109 <https://doi.org/10.1016/j.diagmicrobio.2020.115109>.