

## LETTER TO THE EDITOR

# Unmatched Virus Preservation Solution may Impede One-Step Reagent for Detection of 2019-nCoV RNA

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### SUMMARY

**Background and Methods:** 2019 Corona Virus Disease (COVID-19) caused by SARS-CoV-2 is still pandemic now. RT-qPCR detection was the most common method for the diagnosis of SARS-CoV-2 infection, facilitated by amounts of nucleic acid testing kits. However, the accuracy of nucleic acid detection is affected by various factors such as specimen collection, specimen preparation, reagents deficiency, and personnel quality.

**Results:** In this study, we found that unmatched virus preservation solution will inhibit N gene and ORF-1ab gene (two independent genes of SARS-CoV-2) amplification in one-step detection reagent.

**Conclusions:** Despite just being a particular phenomenon we found in our work to fight 2019-nCoV, we concluded that unmatched virus preservation solution may have an inhibitory effect on SARS-CoV-2 nucleic acid detection which may lead to incorrect clinical diagnosis.

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#### KEY WORDS

2019-nCoV, virus preservation solution, ribonucleic acid detection, RT-PCR

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The new pneumonia called 2019 novel coronavirus disease (COVID-19) caused by the 2019 novel coronavirus (2019-nCoV) is still epidemic [1,2]. 2019-nCoV nucleic acid testing was designated as the diagnostic method by the National Health Commission of China. However, the nucleic acid detection is affected by various factors such as specimen collection, specimen type, detection reagents, and personnel quality [3]. Recently, many 2019-nCoV nucleic acid detection kits have been applied to clinical practice. However, the positive rate of 2019-nCoV nucleic acid detection is only 30 - 50%, and the false negative rate is extremely high [4].

One-Step 2019-nCoV RNA detection reagent (Shengxiang Biotechnology) is widely used for detection of 2019-nCoV in China. The detection instrument used for

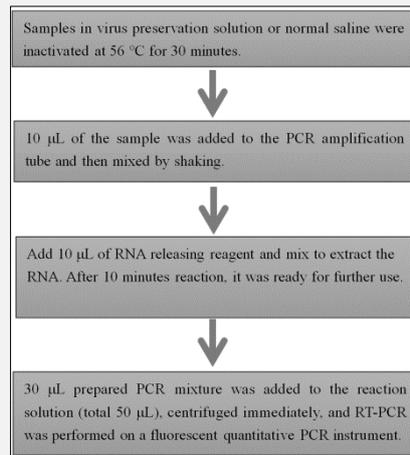


Figure 1. The flow diagram of RT-PCR One-Step Detection Method for 2019-nCoV RNA.

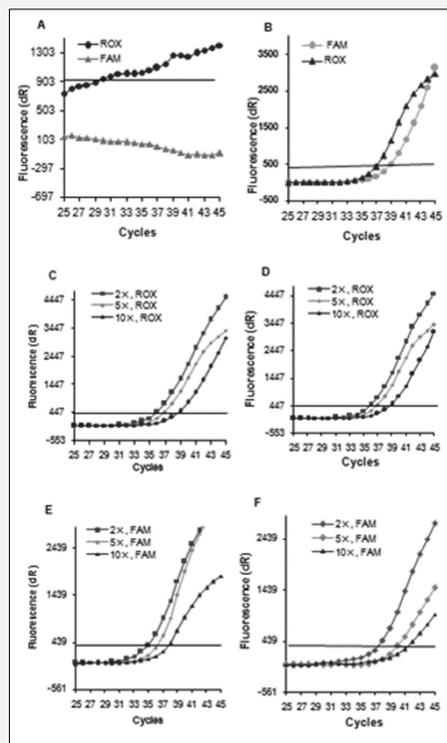


Figure 2. Virus preservation solutions has an inhibitory effect on 2019-nCoV RNA amplification.

(A) The atypical amplification curves by using the One-Step detection method. (B) Typical amplification curves of the sample with saline. (C) Gene N amplification curves using positive control substance with saline. Ct value was 36.84, 37.95, and 39.69 for 2-fold, 5-fold, and 10-fold dilutions, respectively. (D) Gene N Amplification curves using positive control substance with virus preservation solution. Ct value was 37.73, 38.75, and 40.31 for 2-fold, 5-fold, and 10-fold dilutions, respectively. (E) Gene ORF 1ab amplification curves using positive control substance with saline matrix. Ct value was 35.45, 36.56, and 38.15 for 2-fold, 5-fold, and 10-fold dilutions, respectively. (F) Gene ORF 1ab amplification curves using positive control substance with virus preservation solution. Ct value was 37.97, 40.55, and 42.11 for 2-fold, 5-fold, and 10-fold dilutions, respectively.

testing is the Agilent StrataGene MX3005P. The RT-PCR operation process is shown in Figure 1. In this study, we investigated the influence of virus preservation solution (Youkang Hengye Biotechnology, Beijing) on the One-Step detection reagent above. Nasal swab samples, negative control and positive control samples were used for detection. Two independent genes of 2019-nCoV (N gene and ORF 1ab gene) were amplified by the One-Step detection reagent.

We found an atypical amplification curve from a patient's nasal swab sample detected by the detection system (Figure 2A). But typical amplification curves were obtained with the same sample with another detection reagent (Shanghai Zhijiang Biological Company) in Zigong Center for Disease Control and Prevention. Further analysis found that the virus preservation solution used in our system was not produced by the One-Step detection reagent company. The virus preservation solution mainly consists of biological buffering, freezing protection solution, BSA, HEPES, gentamicin, and fungal antibiotics. However, the feasibility has not been fully verified before use. We speculated that the virus preservation solution may have an inhibitory effect on the 2019-nCoV RNA One-Step detection reagent.

In order to further verify the inhibitory effect on the One-Step detection reagent, we collected the patient's nasal swab sample using normal saline instead of virus preservation solution for detecting the 2019-nCoV RNA. A positive result was observed as seen in Figure 2B. For further study, the positive control substance was used because there was no other new positive patient sample in our city. The positive control substance was diluted 2-fold, 5-fold, and 10-fold with virus preservation solution or normal saline, in the meantime. Then, RT-PCR was performed on a fluorescence quantitative PCR instrument as previously described. The amplification curves of N gene and ORF 1ab gene were shown in Figure 2C, Figure 2D, Figure 2E, and Figure 2F. According to the results, all the Ct values of the two genes in the normal saline group were less than virus preservation solution group among 2-fold, 5-fold, and 10-fold dilution.

So, despite just being a particular phenomenon we found in our work to fight with 2019-nCoV, it might be concluded that the virus preservation solution used in the system has an inhibitory effect on the 2019-nCoV RNA One-Step reagent. The virus preservation solution should be verified before use whether it matches the 2019-nCoV RNA detection kit or not. Unmatched virus preservation solutions may have an inhibitory effect on 2019-nCoV RNA detection which might lead to incorrect clinical diagnosis.

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#### Consent for Publication:

Informed consent for publication was obtained from all participants.

#### Availability of Data and Material:

The data and material used to support the findings of this study are available from the corresponding author upon request.

#### Declaration of Interest:

There is no conflict of interest.

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