Title

The application of Huo-Yan Laboratory mode in high-throughput nucleic acid detection of SARS-CoV-2

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Abstract

Background. The coronavirus disease (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This coronavirus disease has spread globally as a pandemic. Quantitative reverse transcription PCR (qRT-PCR) is established as a diagnostic assay to detect SARS-CoV-2 in patient specimens. The increasing numbers of patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has posed great challenges for the test laboratories.

Methods. The test specimens were collected by the hospitals or CDC in Hubei province. Viral RNA is extracted by manual column extraction and automated magnetic bead extraction method by MGISP-960 automated sample preparation system. qRT-PCR for the novel coronavirus is used in this process and several approaches were applied to prevent contamination and monitor process quality.

Results. We introduced a high-throughput nucleic acid detection mode, Huo-Yan Laboratory, which has an automated process and rigorous quality control measures to ensure the stability and reliability of detection in large-scale specimens. The automated extraction method based on MGISP-960 sample preparation system has comparable results of manual extraction, and requires less time and personnel. The quality control monitoring results suggests that the quality control approaches in testing large-scale specimens are effective, and the test results are stable and reliable. Huo-Yan Laboratory mode can be flexibly adapted to the requirement of different throughput which is contributed to epidemic control in various places.

Introduction

The coronavirus disease (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and has spread globally as a pandemic. As of March 15 2020, infected people have been found in more than 140 countries, territories or areas worldwide, and the number of infected people has exceeded 153,000 (1). The common symptoms of this pneumonia are fever, dry cough, weakness or fatigue and the abnormalities in chest CT images (2, 3). The clinical samples were sequenced by DNBSEQ, Illumina and Oxford Nanopore sequencing, assembled into contigs, and the genome gaps were filled by Sanger sequencing to obtain the viral genome sequence (4). The SARS-CoV-2 genome contains about 30,000 nucleotides. Phylogenetic analysis shows that the genome of SARS-CoV-2 is less similar to severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) with the sequence identity of about 79% and 50% respectively, while it is closely related to bat SARS-like coronavirus bat-SL-CoVZC45 and bat-SL-CoVZXC21 with the sequence identity of 87%. The genome of SARS-CoV-2 can be divided into at least 12 gene segments, which are 1ab, S, 3, E, M, 7, 8, 9, 10b, N, 13, and 14 (5). Another sequencing result indicates that SARS-CoV-2 has much higher similarity to BatCoV RaTG13 with identity of 96.2%. Moreover, protein structure analysis suggests that SARS-CoV-2 and SARS-CoV have similar receptor binding domain and may both bind to human angiotensin-converting enzyme 2 receptor (6, 7).

Quantitative reverse transcription PCR (qRT-PCR) is regarded as the gold standard for identifying coronaviruses. In 2003, Quantitative reverse transcription PCR (qRT-PCR) is established as a diagnostic assay to target SARS-CoV fragment and quantify viral RNA concentration in patient specimens (8, 9). Furthermore, the optimized extraction method and one step quantitative real time PCR can simultaneously detect *ORF1b* gene and 18S ribosomal RNA as an internal positive control, which significantly improves the sensitivity and specificity of virus detection(10). In the detection of MERS-CoV in 2012, the primers for real time PCR were developed based on the sequence of three genes, which are upstream of the envelope gene (*upE*) and open reading frames (*ORF*) *1a*, *ORF1b* or nucleocapsid (*N*) gene(11, 12). Meanwhile, real time PCR kits were also available for testers with a ready-to-use option, which help guarantee much robust results (13, 14). To distinguish SARS-CoV-2 from other pathogens, several countries or regions, such as China, Hong Kong, Thailand, Japan, Germany and the United States, have provided real time PCR detection protocols based on Taqman method (15). qRT-PCR can detect SARS-CoV-2 nucleic acid within 7 days after the symptom onset, then the viral load gradually decrease(5, 16). It is found in serological tests that the median time of detecting the antibody IgG and IgM in patients' plasma specimens is 12 and 14 days after the symptom onset, and the antibodies increase thereafter(17). Compared with serological detection, one-step reverse quantitative PCR can detect pathogens earlier, and has the advantages of high sensitivity and specificity. One-step reverse quantitative PCR include 4 steps: sample nucleic acid extraction, reverse transcription, real time PCR and results interpretation. Due to the infectivity of the virus,

it is necessary to perform pathogen-related experiments in biosafety level 2 (BSL-2) laboratory. Moreover, the detection process requires professional instruments, such as fluorescent quantitative PCR. At present, a sharp rise in the number of patients has brought great challenges to the current test laboratories. The effective detection methods for large-scale specimens are urgently required, to achieve timely isolation and treatment of patients which help minimize the risk of large-scale outbreaks.

To increase the efficacy of detection, we introduced a high-throughput detection mode, Huo-Yan Laboratory, which has been established in Wuhan, China and successfully played an important role in the control of COVID-19 epidemic. This laboratory has a process of automated nucleic acid extraction method and qRT-PCR to test large-scale specimens. Quality control and monitoring measures guarantee the reliability in large-scale testing. This testing mode can be easily applied to different regions and help the local epidemic control.

Materials and Methods

Samples and Controls

The test specimens were collected from hospitals or the Centers for Disease Control and Prevention (CDC) in Hubei province and shipped to laboratory with dry ice. Relevant clinical and epidemiological data for patients are not accessible to this study.

The process positive control pool is equally mixed from chosen specimens which have the C_t value of the Orflab gene of about 25, and the internal control gene from 20 to 25. The positive

control is a mixture of the Orflab gene pseudovirus and the internal control pseudovirus, and provided by qRT-PCR kit. The negative control is nuclease free H₂O. The blank control has only qRT-PCR mix.

Nuclei Acid Extraction Procedure

The samples are inactivated at 56°C for 30 minutes. Viral RNA is extracted by manual column extraction and automated magnetic bead extraction method. Manual extraction method uses QIAamp Viral RNA Mini Kit (Qiagen, 52904) according to the instructions. 140 µL sample is used for RNA extraction and eluted in 60 µL elution buffer with carrier RNA. The automated extraction method uses MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit (MGI, 1000020471). The operation flow is developed based on the MGISP-960 automated sample preparation system, and the script is written in Python (version 3.0). The extraction steps are as follows: add 165µL sample into 96-deep well plate. Prepare Buffer Mixture, each sample needs 160µL Buffer MLB, 200µL ethanol, 15µL Proteinase K, 15µL Magnetic Beads M and 1µL Enhancer Buffer. Buffer mixture should prepare freshly and use in 30 minutes. Place the sample, reagents and pipette tips in the designated positions in the automation system. Initialize and run the MGISP-960 software script. The RNA is eluted in 60 µL nuclease free H₂O.

qRT-PCR

The nucleic acid was tested by Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2 (BGI PathoGenesis). This kit is designed for the detection of *Orflab* gene in patient

specimens and has been approved by NMPA, CE-IVD and FDA. Each qRT-PCR reaction contains 18.5 μ L reaction mixture (including amplification reaction reagents, primers and probes) and 1.5 μ L enzyme. The reaction is performed by a real-time PCR instrument (SLAN-96S Real-Time PCR System, Hongshi, Shanghai). The reaction workflow is reverse transcription at 50°C for 20 minutes, enzyme inactivation at 95°C for 10 minutes, 40 cycles of PCR amplification (95°C for 15 seconds, 60°C for 30 seconds). Fluorescent signals are collected during the 60 °C phase from FAM and HEX channels for detecting SARS-CoV-2 and internal control respectively. Each RT-PCR run takes approximately 1 hour and 40 minutes.

Statistical Analysis

Student's t-test were performed with the data of manual and automated extraction methods. When $P < 0.05$, it was considered statistically significant. The statistical analysis and figure in this study were performed with R software (version 3.5.0 x64).

Results

The Workflow of Huo-Yan Laboratory

In order to accelerate the testing abilities of novel coronavirus, we set up Huo-Yan Laboratory in Wuhan. The testing laboratory is strictly designed at BSL2, with clearly divided areas of reagents preparation, sample preparation and qPCR. The laboratory is equipped with centrifuges, quantitative PCR instruments, MGISP-960 automated sample preparation systems

and biosafety cabinets etc. The workflow includes sample reception, virus inactivation, sample information entry, nucleic acid extraction, qRT-PCR and results report (Figure 1). When the specimens are sent to the laboratory, they are recorded and assigned a serial number according to the sample information form. Then the specimens are inactivated by heat treatment. The serial numbers of specimens need to be checked and arranged to nucleic acid extraction. After extraction, nucleic acid is sent for qPCR amplification. Finally, the qPCR data is analyzed and reports are sent. As of March 15, 2020, over 200 thousand specimens have been tested here and the maximum testing ability has reached 20 thousand per day (<https://huoyan.bgi.com>).

Comparison of Manual and Automated Extraction Methods

Sample extraction is the bottleneck of the existing detection process. We compared the workflow of manual with automated extraction method (Figure 1). The manual extraction can process 24 samples in each run and take 1 hour 50 minutes. The automated extraction can process 192 samples in each run and take 1 hour 8 minutes (Table 1).

We randomly chose 25 samples to compare the results of the manual with automated extraction methods. The cycle threshold (C_t) values of the internal control in all the specimens are less than 32, and there was no significant difference between the C_t values of the internal control in the two methods (Student t -test, $P = 0.5057$). 14 samples with the No C_t value of *Orflab* gene are negative, and 10 samples with the C_t value less than 38 are positive. One sample with the C_t value greater than 38 is suspicious and required re-testing or specimen re-collecting. It is suggested that the detection rate of the manual and automated extraction methods

is consistent (Table 2).

In addition, the analytical sensitivity of the entire detection process is tested by using a serial dilution of positive control (ranging from 5×10^4 to 5×10^0 copies) provided by qRT-PCR kit. The results show that the detection limit of these assays is 10 copies per reaction (Appendix Figure S1A and S1B). The linearity of the detection process is tested by using a serial dilution (ranging from 10 to 2×10^4 -fold) of process positive control pool. A strong linear relationship was observed between the amount of input cDNA and the C_t values (Appendix Figure S1C and S1D).

Quality Control of Detection Process

Several approaches were applied to prevent contamination and monitor process quality. First, contamination prevention measures are carried out to avoid aerosol generation. Second, positive and negative controls are monitored in each run. Each process contains a process positive control pool well A10, and a positive control well H12. Each process contains two negative controls well F10 and G12, a random negative control well and a blank control well F12. To monitor the process, we analyzed the distribution of controls daily. From February 27 to March 7 in 2020, there are over 70,000 detection results and the NoC_t value is set to 40 for statistical analysis. In well H12, the C_t value of *Orf1ab* and the internal control is from 20 to 30 which indicates the detection process is within normal limits (Figure 2A). In well F10, F12 and G12, the NoC_t value of virus *Orf1ab* gene is detected which means no contamination in the test process (Figure 2B). Furthermore, the C_t values of the internal control in most specimens is in the normal range from 15 to 30 (Figure 2C). For the outliers in the test, the possible causes are

analyzed, and the specimens are arranged to re-test. It suggests that the quality control approaches in testing large-scale specimens are effective, and the test results are stable and reliable. Contamination prevention and quality monitoring measures are represented in Appendix materials.

Discussion

In the COVID-19 epidemic, cross-infection in hospitals or in the local community are often caused by SARS-CoV-2 patients who cannot be identified and isolated in time. WHO and Chinese CDC have proposed probes and primers based on the Taqman method for nuclei acid detection for COVID-19 (15). At present, the infected people increase, which result in a sharp rise in the test samples number, and bring great challenge to the current test laboratories. The testing laboratories should meet the corresponding security protection level, have high-throughput detection ability, and stable detection quality. In this study, we introduced an emergency test laboratory mode, Huo-Yan Laboratory, which aims to speed up distinguishing the infected and healthy people, thereby protecting public health(18). The laboratory has scientifically divided areas according to the relevant regulations to ensure the safety of environment, samples and personnel. The automation of detection SARS-CoV-2 can greatly increase the testing ability of laboratory(19). We developed a nucleic acid extraction method using MGISP-960 sample preparation system. The automated extraction method has comparable results of manual extraction, and has the advantages of low cost, short time and high reproducibility. Moreover, MGISP-960 can be easily optimized to

accommodate different throughput and require less personnel. For test laboratory, quality control measures are crucial to prevent contamination(20). We design positive and negative controls in each run, and monitor the distribution of controls daily, which effectively ensure the test of high-throughput specimens. Recently, Huo-Yan Laboratory mode has been established in 13 regions, and a total of 512,000 samples have been tested (<https://huoyan.bgi.com>). This mode is flexibly adapted to the requirement of low, medium and high-throughput and supplied with rigorous quality control measures to performs accurate tests, which is beneficial the control of pandemic.

List of abbreviations

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2;

COVID-19: coronavirus disease;

qRT-PCR: quantitative reverse transcription polymerase chain reaction;

SARS-CoV: severe acute respiratory syndrome coronavirus;

MERS-CoV: Middle East respiratory syndrome coronavirus;

CDC: Centers for Disease Control and Prevention;

BSL2: biosafety-level 2;

NMPA: National Medical Products Administration, China;

CE-IVD: CE-marked in vitro diagnostic;

FDA: Food and Drug Administration, U. S.;

C_t: cycle threshold.

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Table 1. Time consumption of manual and automated extraction method.

Steps	Manual extraction method		Automated extraction method	
	24 samples (two persons)		192 samples (one person, 2 MGISP-960)	
	Hands on*	Total†	Hands on*	Total†
Lysis	26 mins	36 mins	8 mins	68 mins
Binding	36 mins	39 mins		
Wash	15 mins	20 mins		
Elute	8 mins	15 mins		
Total	85 mins	110 mins	8 mins	68 mins

*Hands on time includes the operations of preparing reagents and setting up the instrument.

†Total time includes the reaction time and hands on time.

Table 2. Results of qRT-PCR of manual and automated extraction method in detection of SARS-CoV-2.

Numbers of samples	Manual extraction method with QIAGEN kit	Automated extraction method with MGI kit
Negative	14	14
Positive	10	10
Suspicious	1	1
Detection consistency	100%	

Figure 1. Legend.

The workflow of Huo-Yan Laboratory. The flowchart on the right compares the steps of the manual with automated extraction method.

Figure 2. Legend.

270 **Quality control monitoring for Huo-Yan Laboratory.** (A) The Ct value of *Orflab* and the
271 internal control of positive control H12. (B) The Ct value of the internal control of negative
272 control F10, F12 and G12. (C) The Ct values of the internal control in test specimens.

273 **Appendix Figure. Linear ranges of the detection process.** (A) calibration curve for
274 quantitative analysis of *Orflab* gene of positive control. qRT-PCR results of *Orflab* gene of
275 positive control. (B) calibration curve for quantitative analysis of *Orflab* gene of process
276 positive control pool. RT-PCR results of *Orflab* gene of process positive control pool.

277 Correlation coefficients are indicated.

Sample reception

Virus inactivation

Information entry

Nucleic acid extraction

Real time RT-PCR

Results report

Manual

Sample lysis

Column binding

Wash (Buffer AW1)

Wash (Buffer AW2)

Elute

RNA

Automation

Initialize and load
MGISP-960

Prepare reagents
and consumables

Perform program

RNA

A **C** **B**