Fast and sensitive automated viral RNA extraction and SARS-CoV-2 detection

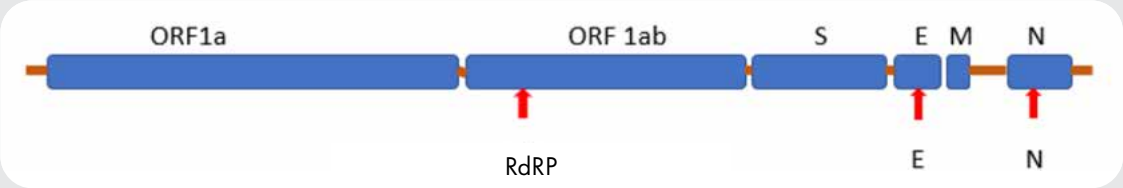
SARS-CoV-2

CASE STUDY

Introduction

SARS-CoV-2 is a single-stranded RNA virus. For accurate and sensitive coronavirus detection, viral RNA must be reliably isolated from the sample. MP Biomedicals offers the MPure-12 Automated Nucleic Acid Extraction Platform with two dedicated kits for medium throughput extraction of viral RNA from swabs in less than one hour.

A multiplex RT-qPCR mastermix, testing for multiple target sequences simultaneously, is then used for the detection of three genes of the SARS-CoV-2 virus: N and E genes encoding structural proteins and the RdRP gene encoding the RNA-dependent RNA polymerase. An internal control system is utilized to monitor the entire PCR process.



Schematic representation of the single-stranded RNA genome of SARS-CoV-2.

The structural genes encode the structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N).
The RNA-dependent RNA polymerase (RdRP) is located in the Open Reading Frame 1ab (ORF1ab).

Workflow

Sample collection & preparation → Viral RNA extraction → RT-qPCR

Materials

Specimen type: Nasopharyngeal swabs collected in universal or viral transport medium and saline solution

RNA isolation system: MPure-12 Automated Nucleic Acid Purification instrument (Cat. No. 117002200) in combination with two kits performing equally well for this application:

- MPure Viral/Pathogen Nucleic Acid Extraction Kit B (Cat. No. 117022130)
- MPure Viral Nucleic Acid Extraction Kit (Cat. No. 117022300)

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Methods

Sample volume: 180 µL to 200 µL

Sample preparation: Briefly vortex samples before adding to sample tubes. Dilute viscous specimens in a saline buffer and vortex prior to use. Add 10 µL of RNA carrier to the sample tubes along with the swab specimen for the enhancement and stabilization of viral RNA recovery.

Internal control: Place internal controls directly in the round well of the reaction chamber.

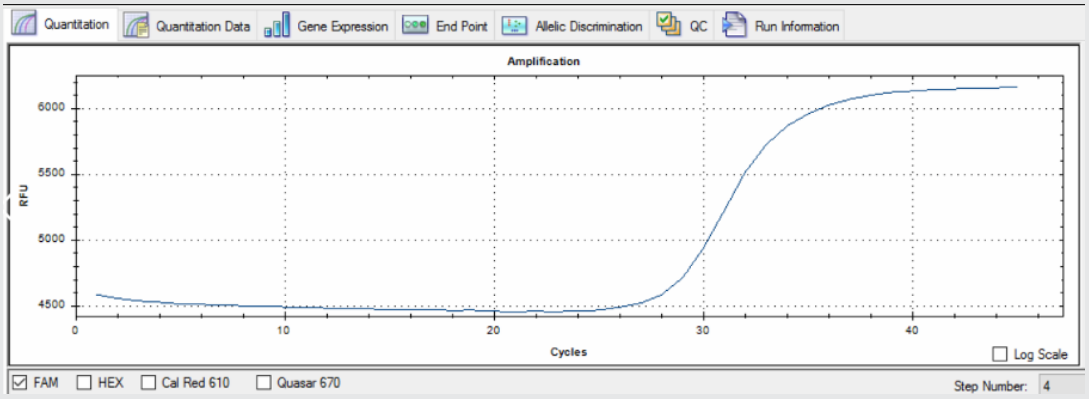
Elution volume: 50 µL

qRT-PCR test: Three genes of SARS-CoV-2, including N, E and RdRP are targeted in the quantitative real-time PCR assay. Primers and TaqMan probes are designed in the conserved region of the SARS-CoV-2 virus specific genome region to allow sensitive and specific amplification and detection of the virus (Allplex™ 2019-nCoV Assay, Seegene).

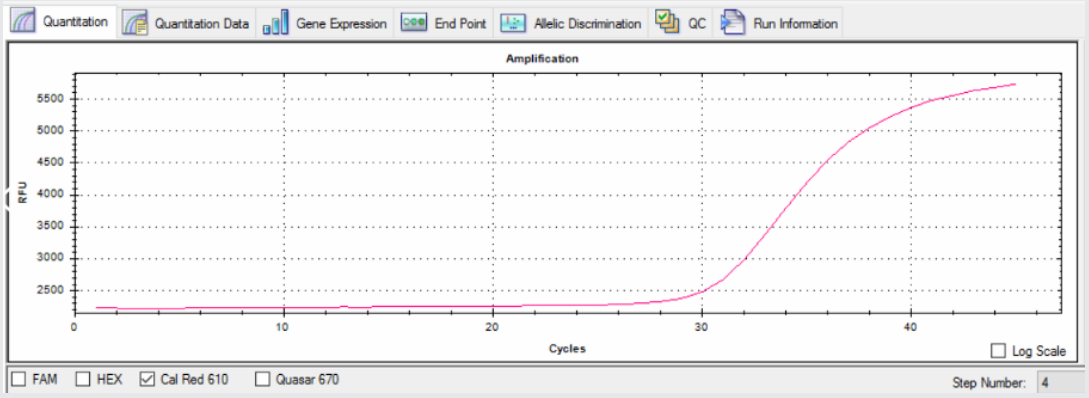
Results

Positive subjects could be accurately detected by this method as shown by the qRT-PCR results displayed below:

E gene (FAM)



RdRP gene (Cal Red 610)



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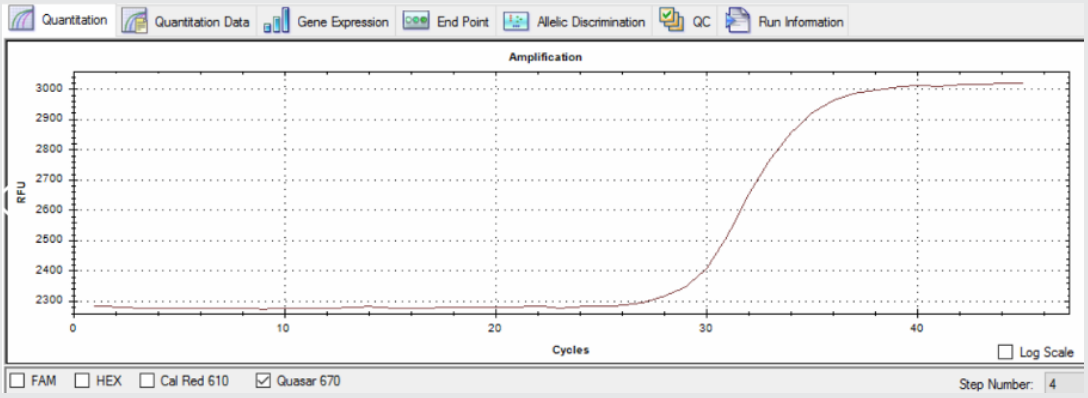
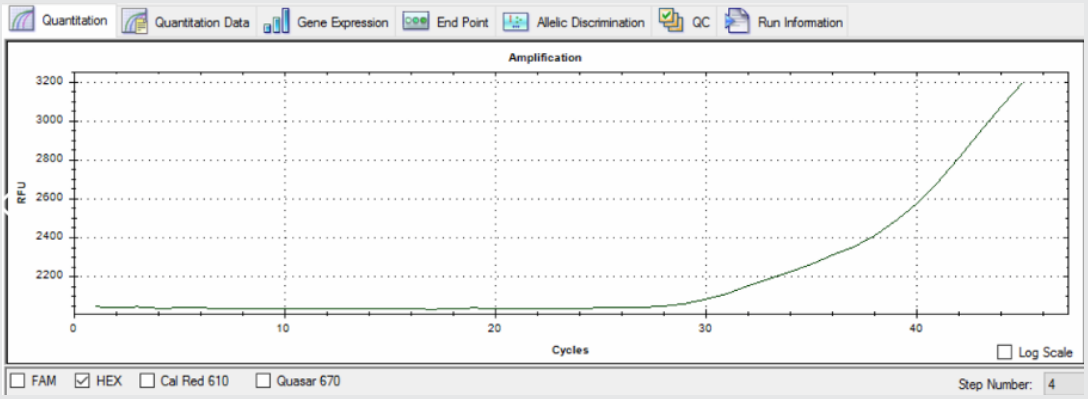
Results

N gene (Quasar 670)



Internal control (HEX)



Conclusion

The MPure-12 Automated Nucleic Acid Purification instrument can be used in combination with an MPure Viral Nucleic Acid Extraction Kit to quickly isolate viral RNA for sensitive SARS-CoV-2 detection via qRT-PCR.

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