SARS-CoV-2 RNA detection in nasal mid-turbinate swabs

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Method Article

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Abstract

In this protocol, we describe a method to investigate the presence of SARS-CoV-2 RNA in nasal swabs, using a commercially available high-throughput Real-Time Polymerase Chain Reaction (RT-PCR) assay (TaqPath™ Covid-19 kit, ThermoFisher Scientific) in a 384-Well Plate. After Viral RNA extraction with QIAamp Viral RNA Mini kit, reverse transcription and cDNA amplification, all samples are assessed for the presence of three specific SARS-CoV-2 viral genomic regions and an internal positive control (IPC), in one Multiplex RT-PCR reaction.

Introduction

Reagents

Nasal Mid-Turbinate Swab (D.I.D. Diagnostic International)	Catalog n° C-503CS01
Universal Transport Medium System (UTM®) (D.I.D. Diagnostic International)	Catalog n° 330C
QIAamp Viral RNA Mini kit (Qiagen)	Catalog n° 52906
TaqPath™ Covid-19 CE-IVD RT-PCR kit (ThermoFisher Scientific)	Catalog n° 48067
MicroAmp™ Optical 96-Well Reaction Plate (ThermoFisher Scientific)	Catalog n° 4306737
MicroAmp™ Optical 384-Well Reaction Plate (ThermoFisher Scientific)	Catalog n° 4309849
MicroAmp™ Optical Adhesive Film (ThermoFisher Scientific)	Catalog n° 4311971

Equipment

MixMate® Vortex (Eppendorf, Hamburg, Germany)
Quant Studio™ 12K Flex Real-Time PCR Instruments (Applied Biosystems, CA, USA)
Microlab Starlet Robot (Hamilton Robotics, Bonaduz, Switzerland)
Centrifuge 5804R (Eppendorf, Hamburg, Germany)
Design and Analysis Software, Version 2.4.3 (Applied Biosystems, CA, USA)

Procedure

Specimens Sampling

A nasal mid-turbinate swab is collected by a supervised onsite self-collection or directly by a researcher, according to the Centers for Diseases Control and Prevention Guideline for “Collecting, Handling, and

Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19)". The nylon swab is immediately placed in a tube of 3.0 ml Universal Transport Medium System (UTM®) and vortexed in MixMate® at 400 rpm, within 3 hours, at room temperature. Then the swab is thrown out and the Viral Transport Medium is frozen at -80 °C until RNA extraction.

Viral RNA isolation

The QIAamp Viral RNA Mini kit is used for RNA isolation, according to the manufacturer's instructions. Prior RNA extraction, an aliquot of 140.0 µL of the Viral Transport Medium is inactivated in lysis buffer (AVL, 560.0 µl) and in each sample, 5.0 µL of MS2 Phage RNA (Internal Positive Control IPC, to monitor extraction, reverse transcription and PCR amplification - included in TaqPath™ Covid-19 kit) and 5.6 µL carrier RNA-AVE (to increase the yield of extracted RNA - included in QIAamp Viral RNA Mini kit) are added. The purified RNA is eluted in 50.0 µL and immediately stored at -80 °C.

SARS-CoV-2 RT-PCR test

To detect SARS-CoV-2 RNA a Multiplex Real Time RT-PCR reaction (TaqPath™ Covid-19 RT-PCR kit) is used. Five µL of Viral RNA are reverse transcribed into cDNA and amplified using the Quant Studio™ 12K Flex Real-Time PCR Instruments (1).

The RT PCR reaction, includes three primer/probe sets specific to different SARS-CoV-2 genomic regions, reducing the risk of detecting other coronaviruses with a certain degree of sequence similarity: 1) ORF-1ab (Open Reading Frame 1ab) with 6-FAM reporter dye, 2); N Protein (Nucleocapsid) with VIC reporter dye and 3) S Protein (Spike) with ABY reporter dye. In addition, a bacteriophage MS2 IPC (with reporter dye JUN) is also amplified to verify the efficacy of the sample preparation and the absence of inhibitors.

The reaction mix added to each Viral RNA sample (5.0 µL) is the following: 5.0 µL of TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (4X), 1.0 µL of TaqPath™ COVID-19 Assay Multiplex and 9.0 µL molecular-grade, nuclease-free water (total volume reaction = 20.0 µL). In each run, a standard curve is added to check the efficiency of the amplification; the 2019-nCoV DNA Control (1×10^4 copies/µL) is diluted serially with Control Dilution Buffer (both included in TaqPath™ COVID-19 kit) 1/4 fold per dilution; five microliters of each standard point are then distributed in the wells with 15.0 µL of reaction mix, to produce five concentrations of copies/reaction (c/r): 6250.00 c/r (ST1), 1562.50 c/r (ST2), 390.62 c/r (ST3), 97.66 c/r (ST4) and 24.41 c/r (ST5). Also 5.0 µL of molecular-grade, nuclease-free water (NTC, No Template Control) is run, to monitor non-specific amplification, cross-contamination and nucleic acid contamination of reagents.

The high-throughput reaction is run in a MicroAmp™ Optical 384-Well Plate prepared by a Microlab Starlet Robot, distributing the reaction mix from a tube and then each Viral RNA, ST and the NTC from a MicroAmp™ Optical 96-Well Plate. The 384-Well Plate is sealed with MicroAmp™ Optical Adhesive Film, vortexed at 800 rcf in MixMate® for 2 minutes, briefly centrifugated at 1200 rcf in 5804R Centrifuge (to collect liquid at the bottom of the plate) and located in Quant Studio™ 12K Flex Real-Time PCR

Instruments for the RT-PCR. The passive reference is set to "None" and "Absolute Quantification" as run type.

The following thermal cycle are applied: 2 min at 25°C for UNG activation, 10 min at 53°C for the Reverse Transcription reaction, 2 min at 95°C for activation, 40 cycles of denaturation at 95°C for 3 seconds and anneal/extension at 60°C for 30 seconds.

The data analysis is performed using the "Design and Analysis Software" 2.4.3 and setting: "baseline start point" = 5, "baseline stop point" = automatic, and "Thresholds" at values fixed, according to the efficiency of amplification. The reaction is considered only if MS2 Ct $\leq$ 38. If any two of the three Sars-CoV-2 genes are positive (Ct $\leq$ 38) the sample are classified as positive; if only one of the assays is positive, the test is repeated. Once the test is repeated, and the result is again positive, the sample is classified positive for Sars-CoV-2. If all three of the assays are negative (Ct = undetermined), the subject is classified as negative.

Troubleshooting

Time Taken

n°360 Specimens Sampling	<i>n° 2 <u>working day</u> for 2 researchers</i>
n°360 Viral RNA isolation/plate preparation	<i>n° 3 <u>working day</u> for 2 researchers</i>
n°360 SARS-CoV-2 RT-PCR test	<i>n° 1 <u>working day</u> for 1 researcher</i>

Anticipated Results

References

. 1. Gregorio Paolo Milani , Emanuele Montomoli , UNICORN Consortium investigators; Valentina Bollati , Benedetta Albetti , Claudio Bandi, Tommaso Bellini , Marco Buscaglia , Carlo Cantarella, Laura Cantone , Michele Carugno, Sergio Casartelli , Guido Cavaletti , Sarah D'Alessandro , Francesca De Chiara , Serena Delbue , Laura Dioni , Ivano Eberini , Chiara Favero , Luca Ferrari , Monica Ferraroni , Laura Galastri , Cristina Galli, Mirjam Hoxha , Simona Iodice, Carlo La Vecchia , Chiara Macchi , Ilaria Manini , Serena Marchi , Jacopo Mariani, Elena Pariani , Angela Cecilia Pesatori, Federica Rota, Massimiliano Ruscica, Tommaso Schioppo , Letizia Tarantini, Claudia Maria Trombetta , Maria Grazia Valsecchi , Marco Vicenzi , Giuliano Zanchetta. SARS-CoV-2 infection among asymptomatic homebound subjects in Milan, Italy.

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