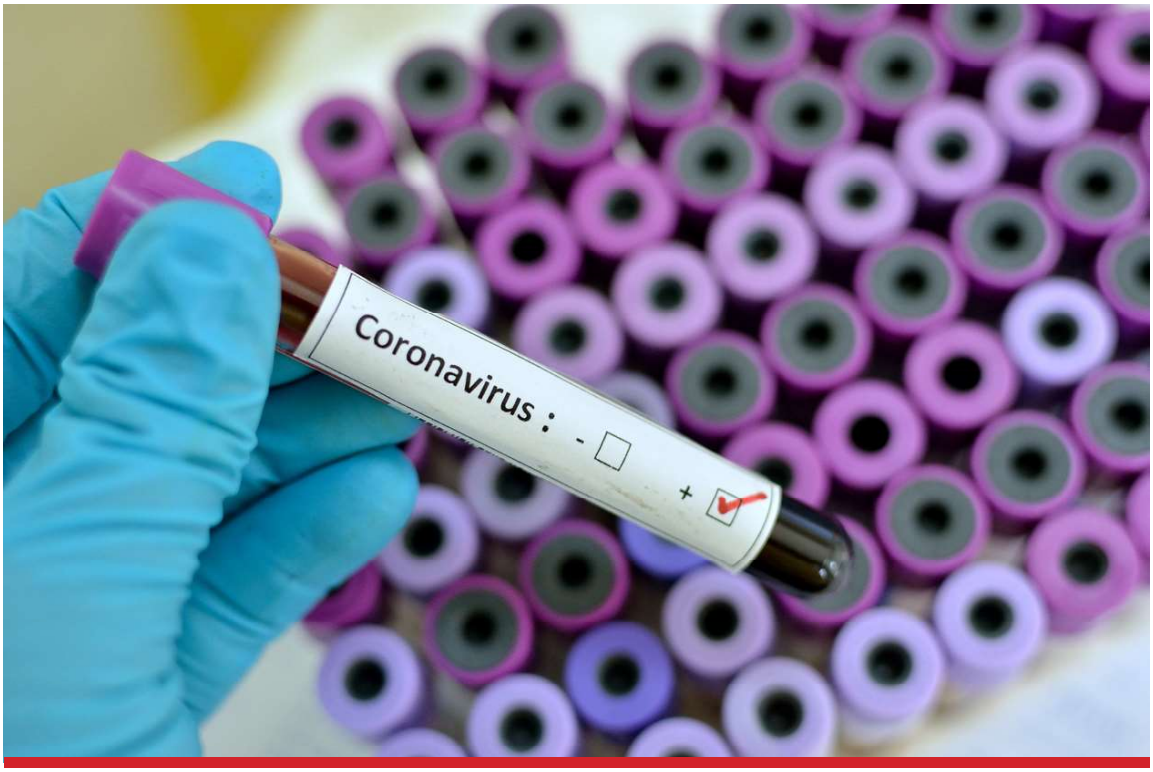
Notes & Details on the 2019 Novel Coronavirus Testing (COVID-19)



The 2019 Novel Coronavirus (2019-nCoV or SARS-CoV-2) is a positive-sense single-stranded RNA (+ssRNA) virus in the coronaviridae family, similar in sequence to the first SARS-CoV virus and bat coronaviruses.[1] The 2019-nCoV virus is a respiratory virus transmitted by respiratory droplets and is the cause of the ongoing 2019-2020 Wuhan coronavirus outbreak of disease now referred to as COVID-19.

The Centers for Disease Control and Prevention developed a laboratory coronavirus test kit based on the full viral sequence published in January 2020.[2] This test was initially available only for use in CDC laboratories. However, beginning in February, CDC-qualified laboratories throughout the United States are able to test for the virus using the coronavirus test kit.[2] Starting with nucleic acid purification, The test follows a standard viral testing protocol. The provided primer and probe are combined with the sample and other standard components for amplification through Real Time reverse transcriptase polymerase chain reaction (Real Time RT-PCR). Kits will be available through the International Reagent Resource (IRR), and are intended for use with the Applied Biosystems 7500 Fast DX Real-Time PCR Instrument with SDS 1.4 software.[3]

Viral Testing Components for the CDC nCoV Protocol

To run the coronavirus test, your lab will need supplies beyond the elements of the virus test kit provided by the CDC. Outlined here are all the components necessary for compliance with protocol, some of which are standard laboratory supplies (like disposable powder-free gloves). If any component is missing in your lab, you should secure it before requesting virus test kits to ensure total compliance.

References

1. 2019-nCoV. Virus Pathogen Resource. Updated Jan 31, 2020. Accessed February 10, 2020. https://www.viprbrc.org/brc/home.spg?decorator=corona_ncov.

2. Patel A, Jernigan DB. Initial Public Health Response and Interim Clinical Guidance for the 2019 Novel Coronavirus Outbreak — United States, December 31, 2019–February 4, 2020. MMWR Morb Mortal Wkly Rep 2020;69:140–146. http://dx.doi.org/10.15585/mwr.mm6905e1

3. CDC Tests for 2019-nCoV. Centers for Disease Control and Prevention. Updated February 5. 2020. Accessed February 10, 2020. https://www.cdc.gov/coronavirus/2019-ncov/about/testing.html

Reagents and Supplies (Consumables)

rRT-PCR primer/probe sets
Primer and probe sets are used during RT-PCR to make copies of nucleic acid. These sets are specific to the target virus and are available from various companies.

Positive template control
A positive template control is specific for the viral test. This control is run during the rRT-PCR assay and should always give a positive result. These are often supplied along with the specific primer/probe sets.

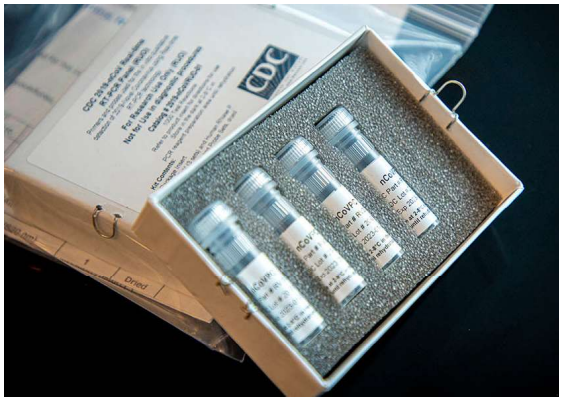
TaqPath™ 1-Step RT-qPCR Master Mix, CG
(ThermoFisher; cat # A15299 or A15300)
This master mix is combined with the primer/probes and samples and run through the RT-PCR machine. Designed to be used with both RNA and DNA amplification, it contains thermostable MMLV reverse transcriptase, dNTPs, UNG, ROXTM dye, and thermostable Fast DNA polymerase.

Molecular grade water, nuclease-free
This sterile distilled water is used to dilute reagents and samples used in the RT-PCR process.

Disposable powder-free gloves
Wearing gloves during molecular processes decreases the likelihood of sample contamination.

P2/P10, P200, and P1000 aerosol barrier tips
Various sterile pipette tip sizes are needed when dispensing reagents, transferring samples, and mixing small volumes of liquids. Tips with filters prevent aerosol contaminants from entering the tip.

Sterile, nuclease-free 1.5 mL microcentrifuge tubes
Reagents are placed in microcentrifuge tubes and gently mixed by micropipette. These tubes are spun in a centrifuge to concentrate the product at the bottom.



0.2 mL PCR reaction tube strips or 96-well real-time PCR reaction plates and optical 8-cap strips
PCR reaction tube strips are strips of eight tubes designed for multiple-sample testing. Well plates are used to efficiently test multiple specimens in one container along with a positive and negative control. Specific caps are needed to work with the optical PCR detection technology.

Laboratory marking pen
A good marking pen allows for easy tube identification and clear results. Do not mark on the tops of microcentrifuge tubes or PCR plate, as this can interfere with the optical detection operation.

Cooler racks for 1.5 microcentrifuge tubes and 96-well 0.2 mL PCR reaction tubes
Cooler racks secure microcentrifuge tubes while reagents and samples area added.

Racks for 1.5 ml microcentrifuge tubes
Convenient location for microcentrifuge tubes while combining reagents for testing.

Acceptable surface decontaminants
DNAZap™ (Life Technologies, cat. #AM9890)

DNA Away™ (Fisher Scientific; cat. #21-236-28)

RNAse Away™ (Fisher Scientific; cat. #21-236-21)

10% bleach (1:10 dilution of commercial 5.25-6.0% sodium hypochlorite)

References

4. CDC Tests for 2019-nCoV. Centers for Disease Control and Prevention. https://www.cdc.gov/coronavirus/2019-ncov/about/testing.html. Updated February 5, 2020. Accessed February 6, 2020.

Equipment

In addition to consumables, your lab will need to create an environment conducive to controlling and completing the viral test. If your lab routinely conducts polymerase chain reactions, you are likely already positioned to comply with these environmental conditions. Otherwise, the following equipment is vital for successful participation in the coronavirus test.

PCR Work Station [UV lamp; Laminar flow (Class 100 HEPA filtered)]
An appropriate laboratory setup should be created when following viral detection protocols. A PCR work station with germicidal UV lamp and laminar flow minimizes contamination of nucleic acid samples. Multiple stations and areas can be set up to prevent cross-contamination.

Vortex mixer
A vortex mixer efficiently mixes small volumes of liquids. Variable speed models allow adjustment from a gentle shaking motion to high speed mixing.

Microcentrifuge
A microcentrifuge uses a spinning mechanism to separate components in microcentrifuge tubes or PCR plates by mass. This results in a nucleic acid pellet at the bottom of the microcentrifuge tube.

Micropipettes (2 or 10 µl, 200 µl and 1000 µl)
Micropipettes are adjustable calibrated instruments designed to be used with disposable sterile tips to transfer small quantities of liquid.

Multichannel micropipettes (5-50 µl)
Multichannel micropipettes are adjustable calibrated instruments designed to be used with disposable sterile tips to transfer multiple samples of small quantities of liquid at a time. These can be hand-held or part of an automated system.

Cold blocks: 2 x 96-well cold blocks
Cold blocks secure sample tubes and maintain a cool temperature while reagents and samples are added.

-20°C (nonfrost-free) and -70°C freezers; 4°C refrigerator
Various temperatures are needed for sample and reagent storage. In the nCoV protocol, three temperatures are specified. Specimens can be stored at 4°C for up to 72 hours after collection, but should be stored at -70°C or lower if a delay in extraction is expected. Extracted nucleic acids should be stored at -70°C or lower.

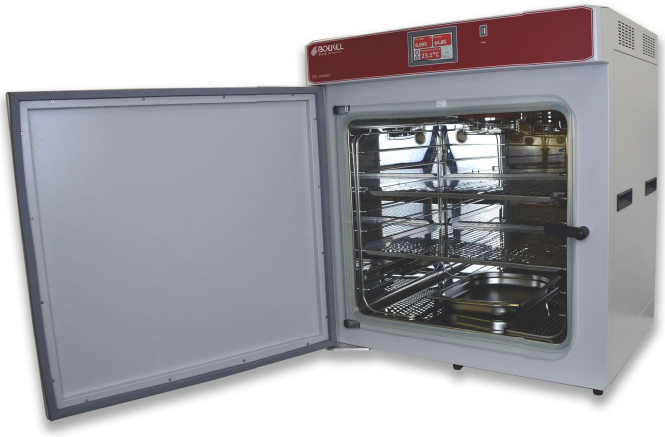
Real-time PCR detection system
A real-time PCR detection system combines adjustable automated PCR cycling with optical detection technology. Associated software can make statistical analysis easier.

Nucleic acid extraction system
Nucleic acid extraction systems simplify the extraction process by providing a kit of reagents to quickly purify nucleic acid from various sources. Appropriate protocols are used to extract specific nucleic acid from tissue, whole blood, or plasma. This can be a manual or automated process.

Notes on Viral Testing - Additional equipment or supplies that may be required for viral testing in research laboratories

Additional processes may be involved in viral testing, including propagating the virus in cell culture or embryonated chicken eggs. Equipment needed for cell culture includes a cell culture hood, incubator (CO2 incubator), water bath, centrifuge, microcentrifuge, refrigerator, freezer (-20°C), cell counter, cryostorage or liquid nitrogen freezer, and a sterilizer such as an autoclave.[5,6] Disposables will include culture flasks, cell culture plates, Petri dishes, pipettes, pipettors, appropriate media, sera, and reagents, and cell cultures. Embryonated chicken eggs will require an egg incubator and egg candler.[7]

No laboratory would be complete without appropriate safety equipment and personal protective equipment appropriate for the biosafety level of the target virus. Universal precautions among all laboratory personnel are key to limiting the risk of infection and contamination. Designated separate areas for clean cell culture and viral cell culture growth should be maintained, and appropriate disposal methods made available.



References

5. ATCC Virology Guide. 2016. Updated 2016. Accessed February 10, 2020. https://www.atcc.org/~/media/PDFs/Culture%20Guides/Virology_Guide.ashx.

6. Gibco Cell Culture Basics Handbook. Invitrogen. Accessed February 10, 2020. https://www.vanderbilt.edu/viibre/CellCultureBasicsEU.pdf

7. Brauer R, Chen P. Influenza virus propagation in embryonated chicken eggs. J Vis Exp. 2015;(97):52421. Published 2015 Mar 19. doi:10.3791/52421 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4401370/

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