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# Screening and Detection SARS-CoV-2 RNA from Buffy Coats

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Coronavirus Method Development Community

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ABSTRACT

The CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the 2019-nCoV in upper and lower respiratory specimens (such as nasopharyngeal or oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate) collected from individuals who meet 2019-nCoV clinical and/or epidemiological criteria. **This protocol is adapted to screen 2019-nCoV in RNA isolated from whole blood.** Results are for the identification of 2019-nCoV RNA. The 2019-nCoV RNA is generally detectable in upper and lower respiratory specimens during infection and very rarely detected in whole blood. Positive results are indicative of active infection with 2019-nCoV but do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Negative results do not preclude 2019-nCoV infection and should not be used as the sole basis of management decisions. The CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel is a molecular *in vitro* diagnostic test that aids in the detection and diagnosis 2019-nCoV and is based on widely used nucleic acid amplification technology. The product contains oligonucleotide primers and dual-labeled hydrolysis probes (TaqMan®) and control material used in rRT-PCR for the *in vitro* qualitative detection of 2019-nCoV RNA in respiratory specimens. The oligonucleotide primers and probes for detection of 2019-nCoV were selected from regions of the virus nucleocapsid (N) gene. The panel is designed for specific detection of the 2019-nCoV (two primer/probe sets). An additional primer/probe set to detect the human RNase P gene (RP) in control samples and clinical specimens is also included in the panel. RNA isolated and purified from whole blood is reverse transcribed to cDNA and subsequently amplified in the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument with SDS version 1.4 software. Detection of viral RNA in whole blood aids in the safe operation of labs working with human blood samples.

ATTACHMENTS

EUA-CDC-nCoV-IFU.pdf purelink_blood_totalrna_man.pdf

GUIDELINES

## Variables during Whole Blood RNA purification

Whole blood, plasma, and sera represent, by far, the most commonly used sample types in the diagnostic field. Because PCR inhibitors in blood samples ~ have been described, generally, it is accepted that a careful purification of nucleic acids is required from such samples before PCR analysis can be performed. Cellular or viral RNA, present in vivo in a protected form in cells or virus, is not readily accessible to all PCR reagents. This barrier is overcome by lysing the cells or virus.

**Erythrocytes lysis:** Since whole blood contains a high number of non-nucleated erythrocytes, purifying total RNA from whole blood without the removal of erythrocytes results in low RNA yields and clogging of purification columns. The depletion of abundant erythrocytes is therefore a key step in the purification of whole blood total RNA and is performed by selective lysis of erythrocytes using hypotonic shock. The erythrocytes are resuspended in a hypotonic buffer that causes an influx of water into erythrocytes and ruptures the erythrocyte cell membranes. Leukocytes are not affected by the hypotonic shock and are easily separated from lysed erythrocytes by centrifugation.

## Warnings and Precautions



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- Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html.
- Specimen processing should be performed in accordance with national biological safety regulations.
- If infection with 2019-nCoV is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.
- Performance characteristics have been determined with human upper respiratory specimens and lower respiratory tract specimens from human patients with signs and symptoms of respiratory infection.
- Perform all manipulations of live virus samples within a Class II (or higher) biological safety cabinet (BSC).
- Use personal protective equipment such as (but not limited to) gloves, eye protection, and lab coats when handling kit reagents while performing this assay and handling materials including samples, reagents, pipettes, and other equipment and reagents.
- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplifications reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon). Workflow in the laboratory should proceed in a unidirectional manner.
- Maintain separate areas for assay setup and handling of nucleic acids.
- Always check the expiration date prior to use. Do not use expired reagent. Do not substitute or mix reagent from different kit lots or from other manufacturers.
- Change aerosol barrier pipette tips between all manual liquid transfers.
- During preparation of samples, compliance with good laboratory techniques is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with nucleic acids.
- Maintain separate, dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g., microcentrifuge tubes, pipette tips) for assay setup and handling of extracted nucleic acids.
- Wear a clean lab coat and powder-free disposable gloves (not previously worn) when setting up assays.
- Change gloves between samples and whenever contamination is suspected.
- Keep reagent and reaction tubes capped or covered as much as possible.
- Primers, probes (including aliquots), and enzyme master mix must be thawed and maintained on cold block at all times during preparation and use.
- Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products such as 10% bleach, "DNA*Zap*™" or "RNase AWAY®" to minimize risk of nucleic acid contamination. Residual bleach should be removed using 70% ethanol.
- RNA should be maintained on cold block or on ice during preparation and use to ensure stability.
- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.

### Reagent Storage, Handling, and Stability

- Store all dried primers and probes and the positive control, nCoVPC, at 2-8°C until re-hydrated for use.
- Store liquid HSC control materials at ≤ -20°C.
- Always check the expiration date prior to use. Do not use expired reagents.
- Protect fluorogenic probes from light.
- Primers, probes (including aliquots), and enzyme master mix must be thawed and kept on a cold block at all times during preparation and use.
- Do not refreeze probes.
- Controls and aliquots of controls must be thawed and kept on ice at all times during preparation and use.

### Primer and Probe Preparation:

1. Upon receipt, store dried primers and probes at 2-8°C.
2. Precautions: These reagents should only be handled in a clean area and stored at appropriate temperatures (see below) in the dark. Freeze-thaw cycles should be avoided. Maintain cold when thawed.



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3. Using aseptic technique, suspend dried reagents in 1.5 mL of nuclease-free water (50X working concentration) and allow to rehydrate for 15 min at room temperature in the dark.
4. Mix gently and aliquot primers/probe in 300 µL volumes into 5 pre-labeled tubes. Store a single aliquot of primers/probe at 2-8oC in the dark. Do not refreeze (stable for up to 4 months). Store remaining aliquots at ≤ -20oC in a non-frost-free freezer.

**2019-nCoV Positive Control (nCoVPC) Preparation:**

1. Precautions: This reagent should be handled with caution in a dedicated nucleic acid handling area to prevent possible contamination. Freeze-thaw cycles should be avoided. Maintain on ice when thawed.
2. Resuspend dried reagent in each tube in 1 mL of nuclease-free water to achieve the proper concentration. Make single use aliquots (approximately 30 µL) and store at ≤ -70oC.
3. Thaw a single aliquot of diluted positive control for each experiment and hold on ice until adding to plate. Discard any unused portion of the aliquot.

**No Template Control (NTC) (not provided)**

1. Sterile, nuclease-free water
2. Aliquot in small volumes
3. Used to check for contamination during specimen extraction and/or plate set-up

## Equipment Preparation

Clean and decontaminate all work surfaces, pipettes, centrifuges, and other equipment prior to use. Decontamination agents should be used including 10% bleach, 70% ethanol, and *DNAzap*™ or *RNase AWAY*® to minimize the risk of nucleic acid contamination.

MATERIALS

| NAME | CATALOG # | VENDOR |
|---|---|---|
| Vortex Mixer | | |
| PureLink™ Total RNA Blood Kit | K156001 | Thermo Fisher |
| Buffycoats / LeukoPaks – Buffy coats made from whole blood | | |
| 2019-nCoV CDC RUO Primers and Probes | 10006713 | IDT |
| 2019-nCoV CDC RUO Plasmid Controls | 10006625 | IDT |
| 70% ethanol | | |
| Promega GoTaq® Probe 1- Step RT-qPCR System | A6121 | Promega |
| Micropipettes (2 or 10 µL 200 µL and 1000 µL) | | |
| Multichannel micropipettes (5-50 µl) | | |
| Racks for 1.5 mL microcentrifuge tubes | | |
| 2 x 96-well -20°C cold blocks | | |
| 7500 Fast Dx Real-Time PCR Systems with SDS 1.4 software (Applied Biosystems; catalog #4406985 or #4 | | |
| Molecular grade water nuclease-free | | |
| 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach) | | |
| Aerosol barrier pipette tips | | |
| 0.2 mL PCR reaction plates (Applied Biosystems; catalog #4346906 or #4366932) | | |
| 1.5 mL microcentrifuge tubes (DNase/RNase free) | | |
| 50-500 µl whole blood (up to 3.5 x 106 leukocytes) | | |
| 96-100% ethanol | | |
| Microcentrifuge capable of centrifuging >8000 x g | | |
| Ice bucket | | |



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| NAME | CATALOG # | VENDOR |
|---|---|---|
| Sterile RNase-free tube (~15 ml) for performing erythrocyte lysis (next page) | | |
| Lysis Buffer (L5) for lysis of erythrocytes (supplied with the kit) | | |
| Lysis Buffer (L3) for lysis of leukocytes (supplied with the kit) | | |
| Wash Buffer (W4) (supplied with the kit) | | |
| Wash Buffer (W5) with ethanol (see below) | | |
| Spin Cartridges with collection tubes Wash Tubes and Elution Tubes (supplied with the kit) | | |

1 Transfer the buffy bag from the sealed NYBC container to a BSL2 hood. Decontaminate the surface of the bag by spraying generous amount of 70% Ethanol. Using a 21 or 22 gauge needle fitted on a 1-5 ml syringe carefully collect ~100 - 200 µl of blood by carefully puncturing the top of the tubing of buffy bag.

2 Carefully transfer the blood from syringe into a 1.5 ml sterile microfuge, RNase-free tube, add 5 volumes of Lysis Buffer (L5) to 1 volume of fresh whole blood sample. For example, add 500 µl of Lysis Buffer (L5) to 100 µl fresh whole blood sample.

2.1 *NEEDLES SHOULD NOT BE RECAPPED, BENT, REMOVED OR OTHERWISE MANIPULATED BY HAND.*

- *However, if it is essential that a needle be recapped due to the nature of the work, the use of a mechanical device or the one-handed scoop method must be used.*

***One-Handed Scoop Method***

1. *Place the cap on the desk or other flat surface with something firm to "push" the needle cap against.*
2. *Holding the syringe with needle attached in one hand, slip the needle into the cap without using the other hand.*
3. *Push the capped needle against a firm object to "seat" the cap onto the needle firmly using only one hand.*

3 Incubate for 10 minutes on ice. Within the BSL2 hood, vortex the tube briefly 2-3 times during the incubation step to allow complete lysis of erythrocytes. The solution turns translucent.
*Note: Guanidine isothiocyanate present in lysis buffer is a chaotropic agent that helps in lysis of the cells and virus particles and is capable of protecting the RNA from endogenous RNases.*

4 Centrifuge the tube at 4°C at 400 x g for 10 minutes. Remove the supernatant completely and discard the supernatant. Do not discard the pellet as the pellet contains leukocytes.

5 Resuspend the leukocyte pellet in volumes of Lysis Buffer (L5). Mix well by vortexing briefly. For example, use 200 µl Lysis Buffer (L5)/100 µl of whole blood from Step 1.

6 Centrifuge the tube at 4°C at 400 x g for 5 minutes. Remove the supernatant completely and discard the supernatant. Do not discard the pellet as the pellet contains leukocytes. The leukocyte pellet should be white with no traces of red. If the pellet is significantly red, wash the pellet with Lysis Buffer (L5).

7 Resuspend the leukocyte pellet in 350 µl Lysis Buffer (L3). Mix well by vortexing briefly to completely resuspend the pellet ensuring the absence of any cell clumps.



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8 Add 350 µl 70% ethanol to the tube and mix well by vortexing briefly.

9 Remove a Spin Cartridge in a Collection Tube from the package. Transfer the leukocyte lysate from Step 7, above, to the Spin Cartridge.

10 Centrifuge the Spin Cartridge at 8,000 × _g for 1 minute at room temperature.

11 Discard the flow through and place the Spin Cartridge into the collection tube.

12 Add 700 µl of Wash Buffer (W4) supplied in the kit to the Spin Cartridge.

13 Centrifuge Spin Cartridge at 8,000 × g for 30 seconds at room temperature.

14 Proceed to DNase I digestion if you need to remove genomic DNA or proceed directly to Step 16.

15 Optional (DNase Digestion): To remove genomic DNA from the samples, add 80 µl of DNase I solution (page 12 for a recipe) to the Spin Cartridge. Incubate at room temperature for 15 minutes.

16 Add 500 µl of Wash Buffer (W4) to the Spin Cartridge. If DNase I digestion is performed, incubate for 5 minutes at room temperature.

17 Centrifuge the Spin Cartridge at 8,000 × _g for 30 seconds at room temperature. Discard the flow through.

18 Add 500 µl of Wash Buffer (W5) with ethanol.

19 Centrifuge the Spin Cartridge at 8,000 × g for 30 seconds at room temperature. Repeat Step 18 once.

20 Discard the flow through and place the Spin Cartridge into the Wash Tube supplied with the kit and centrifuge the Spin Cartridge at 8,000 x g for 1 minute at room temperature to remove any residual Wash Buffer (W5).



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21 Place the Spin Cartridge in a clean 1.7-ml Elution Tube supplied with the kit.

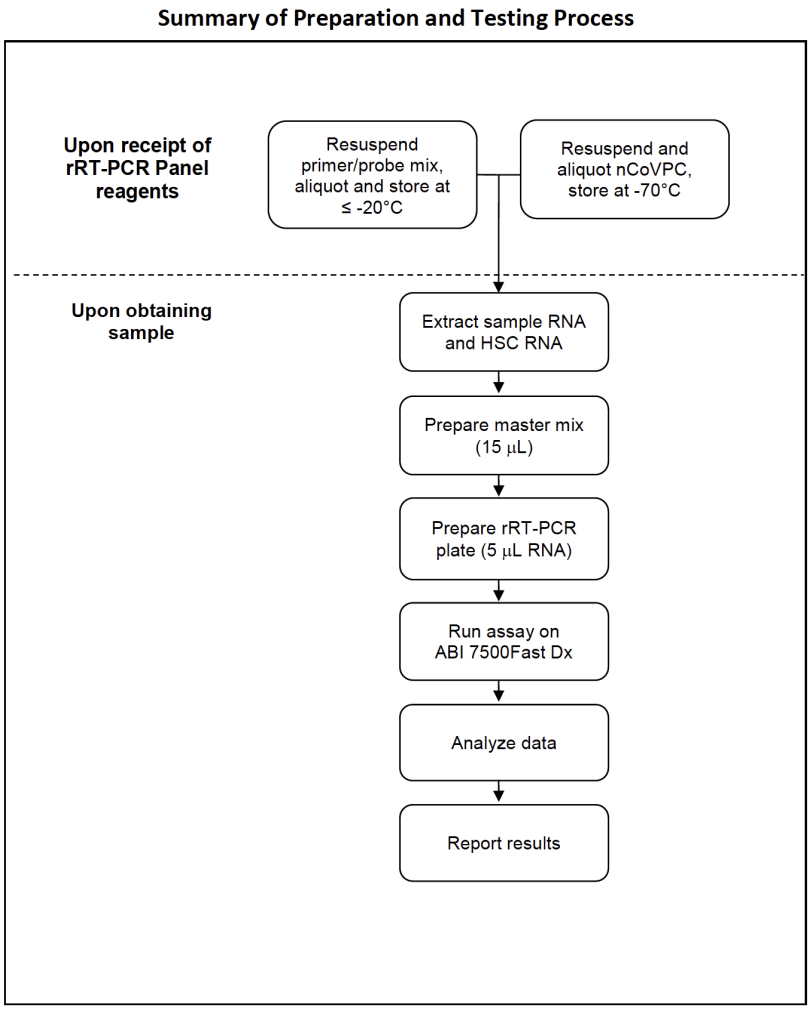
22 Add 20-50 µl of sterile, RNase-free water (supplied with the kit) to the center of the cartridge.

23 Incubate at room temperature for 1 minute. Centrifuge the Spin Cartridge at 8,000 × g for 1 minute at room temperature.

24 The elution tube contains your purified total RNA. Remove and discard the cartridge. Store the total RNA at -80°C or use total RNA for the desired downstream application.

25 The following is the schematic of CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR:

26



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27

**Reaction Master Mix and Plate Set Up**

Note: Plate set-up configuration can vary with the number of specimens and workday organization.
NTCs and nCoVPCs must be included in each run.

- In the reagent set-up room clean hood, place rRT-PCR buffer, enzyme, and primer/probes on ice or cold-block. Keep cold during preparation and use.
- Mix buffer, enzyme, and primer/probes by inversion 5 times.
- Centrifuge reagents and primers/probes for 5 seconds to collect contents at the bottom of the tube, and then place the tube in a cold rack.
- Label one 1.5 mL microcentrifuge tube for each primer/probe set.
- Determine the number of reactions (N) to set up per assay. It is necessary to make excess reaction mix for the NTC, nCoVPC, HSC (if included in the RT-PCR run), and RP reactions and for pipetting error. Use the following guide to determine N: • If number of samples (n) including controls equals 1 through 14, then N = n + 1.
- If number of samples (n) including controls is 15 or greater, then N = n + 2
- For each primer/probe set, calculate the amount of each reagent to be added for each reaction mixture (N = # of reactions).

28

**Promega GoTaq® Probe 1- Step RT-qPCR System**

| Step # | Reagent | Vol. of Reagent Added per Reaction |
|---|---|---|
| 1 | Nuclease-free Water | N x 3.1 µL |
| 2 | Combined Primer/Probe Mix | N x 1.5 µL |
| 3 | GoTaq Probe qPCR Master Mix with dUTP | N x 10.0 µL |
| 4 | Go Script RT Mix for 1-Step RT-qPCR | N x 0.4 µL |
| | **Total Volume** | **N x 15.0 µL** |

29

Set up reaction strip tubes or plates in a 96-well cooler rack.

30

Dispense 15 µL of each master mix into the appropriate wells going across the row as shown below

31

**Figure 1: Example of Reaction Master Mix Plate Set-Up**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | N1 | N1 | N1 | N1 | N1 | N1 | N1 | N1 | N1 | N1 | N1 | N1 |
| B | N2 | N2 | N2 | N2 | N2 | N2 | N2 | N2 | N2 | N2 | N2 | N2 |
| C | RP | RP | RP | RP | RP | RP | RP | RP | RP | RP | RP | RP |
| D | | | | | | | | | | | | |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |



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32 Prior to moving to the nucleic acid handling area, prepare the No Template Control (NTC) reactions for column #1 in the assay preparation area.

33 Pipette 5 µL of nuclease-free water into the NTC sample wells (**Figure 2**, column 1). Securely cap NTC wells before proceeding.

34 Gently vortex nucleic acid sample tubes for approximately 5 seconds. Centrifuge for 5 seconds to collect contents at the bottom of the tube. After centrifugation, place extracted nucleic acid sample tubes in the cold rack.

35 Samples should be added to columns 2-11 (column 1 and 12 are for controls) to the specific assay that is being tested as illustrated in **Figure 2**. Carefully pipette 5.0 µL of the first sample into all the wells labeled for that sample (i.e. Sample "S1" down column #2). *Keep other sample wells covered during addition. Change tips after each addition.* Securely cap the column to which the sample has been added to prevent cross contamination and to ensure sample tracking. Repeat the same steps for the remaining samples.

36 **Figure 2. 2019-nCoV rRT-PCR Diagnostic Panel: Example of Sample and Control Set-up**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11[a] | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | NTC | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | nCoV PC |
| B | NTC | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | nCoV PC |
| C | NTC | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | nCoV PC |
| D | | | | | | | | | | | | |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |

[a]Replace the sample in this column with extracted HSC if necessary

37 If necessary, add 5 µL of Human Specimen Control (HSC) extracted sample to the HSC wells (**Figure 2**, column 11). Securely cap wells after addition. NOTE: Per CLIA regulations, HSC must be tested at least once per day. Cover the entire reaction plate and move the reaction plate to the positive template control handling area.

38
- Pipette 5 µL of nCoVPC RNA to the sample wells of column 12 (**Figure 2**). Securely cap wells after addition of the control RNA.***NOTE:*** *If using 8-tube strips, label the TAB of each strip to indicate sample position.* ***DO NOT LABEL THE TOPS OF THE REACTION TUBES!***
- Briefly centrifuge reaction tube strips for 10-15 seconds. After centrifugation return to cold rack. ***NOTE****: If using 96-well plates, centrifuge plates for 30 seconds at 500 x g, 4° C.*

39 Create a Run Template depending on the available real-time PCR instrument available in the lab and run the machine. Refer to the CDC- document for the detailed set up of the real-time PCR machine.



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40 **Promega GoTaq® Probe 1-Step RT-qPCR System**

a. In Stage 1, Set to 15 min at **45°C**; **1 Rep**.
b. In Stage 2, Set to 2 min at **95°C, 1 Rep.**
c. In Stage 3, Step 1 set to **3 sec** at **95°C**.
d. In Stage 3, Step 2 set to **30 sec** at **55.0°C.**
e. In Stage 3, Reps should be set to **45.**
f. Under **Settings** (**Figure 12**), bottom left-hand box, change volume to 20 µL.
g. Under **Settings**, **Run Mode** selection should be **Standard 7500**.
h. Step 2 of Stage 4 should be highlighted in yellow to indicate data collection (see **Figure 12**).

41 **Primers and Probes:**

**Catalog #2019-nCoVEUA-01 Diagnostic Panel Box #1:**

| *Reagent Label* | *Part #* | *Description* | *Quantity / Tube* | *Reactions / Tube* |
|---|---|---|---|---|
| 2019-nCoV_N1 | RV202001<br>RV202015 | 2019-nCoV_N1 Combined Primer/Probe Mix | 22.5 nmol | 1000 |
| 2019-nCoV_N2 | RV202002<br>RV202016 | 2019-nCoV_N2 Combined Primer/Probe Mix | 22.5 nmol | 1000 |
| RP | RV202004<br>RV202018 | Human RNase P Forward Primer/Probe Mix | 22.5 nmol | 1000 |

**Positive Control (either of the following products are acceptable)**
**Catalog #2019-nCoVEUA-01 Diagnostic Panel Box #2:**

| *Reagent Label* | *Part #* | *Description* | *Quantity* | *Notes* |
|---|---|---|---|---|
| nCoVPC | RV202005 | 2019-nCoV Positive Control (nCoVPC)<br>For use as a positive control with the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel procedure. The nCoVPC contains noninfectious positive control material supplied in a dried state and must be resuspended before use. nCoVPC consists of *in vitro* transcribed RNA. nCoVPC will yield a positive result with each assay in the 2019-nCoV Real-Time RT-PCR Diagnostic Panel including RP. | 4 tubes | Provides (800) 5 µL test reactions |

CDC-Primer and probe infomation



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Interpretation of Results and Reporting

**Extraction and Positive Control Results and Interpretation**
**No Template Control (NTC)**
The NTC consists of using nuclease-free water in the rRT-PCR reactions instead of RNA. The NTC reactions for all primer and probe sets should not exhibit fluorescence growth curves that cross the threshold line. If any of the NTC reactions exhibit a growth curve that crosses the cycle threshold, sample contamination may have occurred. Invalidate the run and repeat the assay with strict adherence to the guidelines.

**2019-nCoV Positive Control (nCoVPC)**
The nCoVPC consists of in vitro transcribed RNA. The nCoVPC will yield a positive result with the following primer and probe sets: N1, N2 and RP.

**Human Specimen Control (HSC) (Extraction Control)**
When HSC is run with the CDC 2019-nCoV rRT-PCR Diagnostic Panel (see previous section on Assay Set Up), the HSC is used as an RNA extraction procedural control to demonstrate successful recovery of RNA as well as extraction reagent integrity. The HSC control consists of noninfectious cultured human cell (A549) material. Purified nucleic acid from the HSC should yield a positive result with the RP primer and probe set and negative results with all 2019-nCoV markers.

**Expected Performance of Controls Included in the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel**

| Control Type | External Control Name | Used to Monitor | 2019 nCoV_N1 | 2019 nCoV_N2 | RP | Expected Ct Values |
|---|---|---|---|---|---|---|
| Positive | nCoVPC | Substantial reagent failure including primer and probe integrity | + | + | + | < 40.00 Ct |
| Negative | NTC | Reagent and/or environmental contamination | - | - | - | None detected |
| Extraction | HSC | Failure in lysis and extraction procedure, potential contamination during extraction | - | - | + | < 40.00 Ct |

**If any of the above controls do not exhibit the expected performance as described, the assay may have been set up and/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run and re-test.**





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## 2019-nCoV rRT-PCR Diagnostic Panel Results Interpretation Guide

The table below lists the expected results for the 2019-nCoV rRT-PCR Diagnostic Panel. If a laboratory obtains unexpected results for assay controls or if inconclusive or invalid results are obtained and cannot be resolved through the recommended re-testing, please contact CDC for consultation and possible specimen referral. See pages 10 and 40 for referral and contact information.

| 2019 nCoV_N1 | 2019 nCoV_N2 | RP | Result Interpretation[a] | Report | Actions |
|---|---|---|---|---|---|
| + | + | ± | 2019-nCoV detected | Positive 2019-nCoV | Report results to CDC and sender. |
| If only one of the two targets is positive | | ± | Inconclusive Result | Inconclusive | Repeat testing of nucleic acid and/or re-extract and repeat rRT-PCR. If the repeated result remains inconclusive, contact your State Public Health Laboratory or CDC for instructions for transfer of the specimen or further guidance. |
| - | - | + | 2019-nCoV not detected | Not Detected | Report results to sender. Consider testing for other respiratory viruses.[b] |
| - | - | - | Invalid Result | Invalid | Repeat extraction and rRT-PCR. If the repeated result remains invalid, consider collecting a new specimen from the patient. |

**[a]Laboratories should report their diagnostic result as appropriate and in compliance with their specific reporting system.**
**[b]Optimum specimen types and timing for peak viral levels during infections caused by 2019-nCoV have not been determined. Collection of multiple specimens from the same patient may be necessary to detect the virus. The possibility of a false negative result should especially be considered if the patient's recent exposures or clinical presentation suggest that 2019-nCoV infection is possible, and diagnostic tests for other causes of illness (e.g., other respiratory illness) are negative. If 2019-nCoV infection is still suspected, re-testing should be considered in consultation with public health authorities.**



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