TODOS 2019-nCoV RT-qPCR Detection Kit

Instructions for Use

Ver 1.0

September 2020

For In vitro Diagnostic Use.
Validation of this test has not been reviewed by FDA. Review under the EUA program is pending.

Catalog # TD-PR02001 (100tests)

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Contents

Intended Use

The TODOS 2019-nCoV RT-qPCR detection kit is a real-time reverse transcription polymerase chain reaction test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swab specimens collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to high complexity laboratory certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests, or by similarly qualified non-U.S. laboratories. The test is d*istributed in accordance with the guidance on Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency, Section IV.C.2*

Results are for the identification of SARS-CoV-2 RNA. This test is for *in vitro* diagnostic use only. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens (e.g. nasopharyngeal swabs) during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The TODOS 2019-nCoV RT-qPCR detection kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. *Validation of this test has not been reviewed by FDA. Review under the EUA program is pending.*

Summary and Explanation

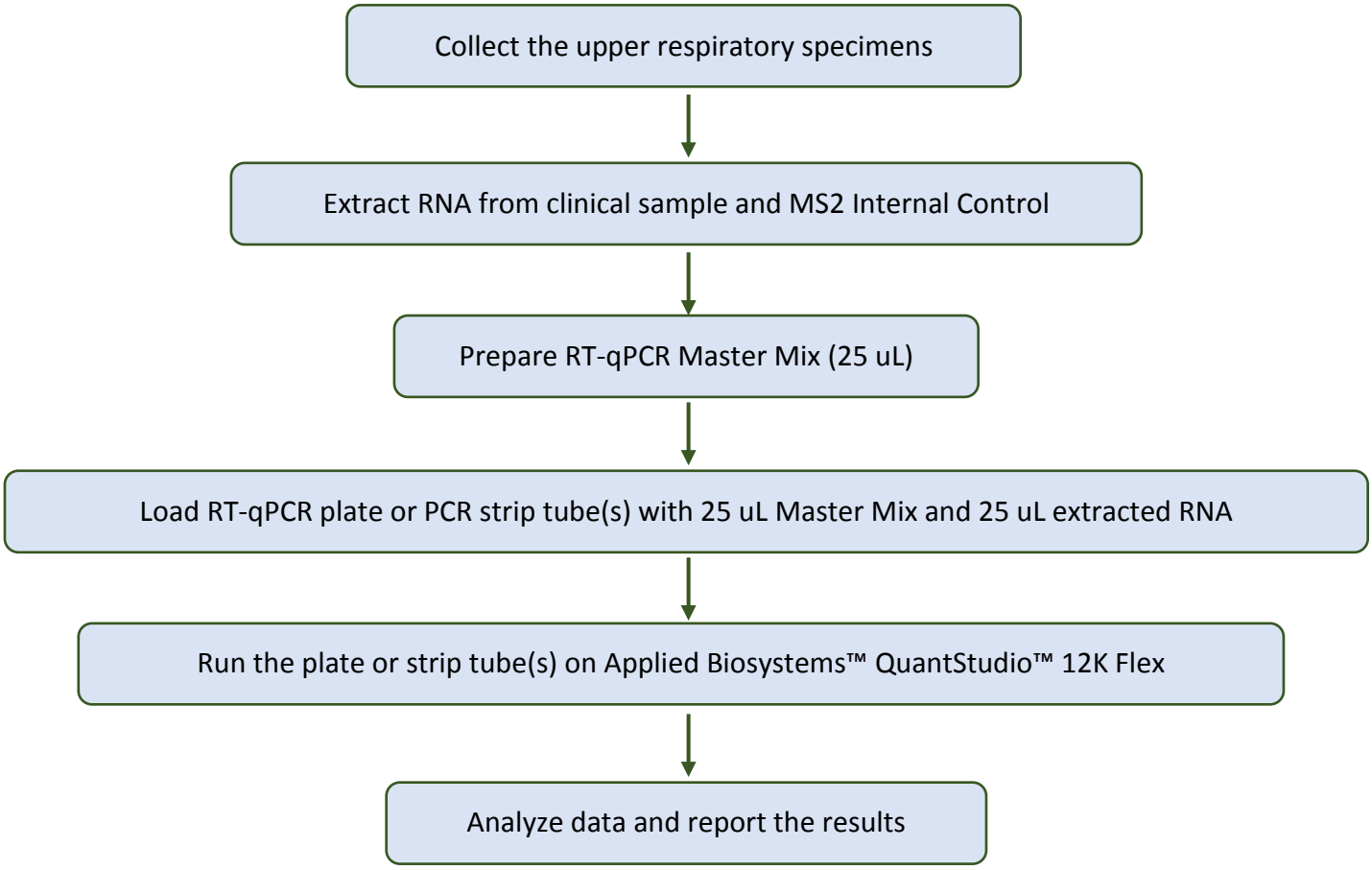
The TODOS 2019-nCoV RT-qPCR Detection Kit is a molecular test that aids in diagnosis of COVID-2019 and is based on widely used nucleic acid amplification technology. The product contains oligonucleotide primers and fluorescent dye labeled probes and control material used in RT-qPCR for the in vitro qualitative detection of SARS-CoV-2 RNA in upper respiratory specimens.

Principles of the Procedure

Three sets of primers and probes are designed to detect three regional sequences in SARS-CoV-2 RNA: ORF1ab, N and E genes. These three regions are selected because they have the least homology with similar viruses, which ensures high sensitivity and specificity of the assay. Additionally, one set of primers and probes are designed to detect the RNA from virus-like particles (VLPs) of bacteriophage MS2. The MS2 RNA serves as an Internal Control for RNA extraction, reverse transcription and PCR amplification.

The RT-PCR assay with the TODOS 2019-nCoV RT-qPCR Detection Kit is a single tube assay that first reverse transcribes specific RNA templates into cDNA copies with gene specific primers, which is then subsequently amplified by Applied Biosystems™ QuantStudio™ 12K Flex Real Time PCR System. In the process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of *Taq* polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by the real time PCR system.

Figure 1. Summary of TODOS 2019 n-CoV RT-qPCR Detection Kit Workflow



Product Description

Materials Provided

One box of TODOS 2019-nCoV RT-qPCR Detection Kit (Catalog # TD-PR02001) contains the reagents and controls summarized in the **Table 1**. This kit is sufficient for 100 reactions.

Table 1. Kit Components

Reagent Label	Description	Volume per tube (uL)	Quantity	Storage
RT-PCR Reaction Mix Reagent	$MgCl_2$, dNTPs	1800	1 tube	-20°C
Enzyme Mix Reagent	*Taq* polymerase, *Taq* Antibody, Thermostable MMLV, UDG, RNasin, 10% BSA	300	1 tube	
2019-nCoV Assay	4 sets of primer/probe – ORF1ab (ROX), E and N (FAM), MS2 (VIC)	200	1 tube	
Negative Control	See below	200	1 tube	
Positive Control		200	1 tube	
Internal Control		100	1 tube	

- ✓ *Protect 2019-nCoV Assay from light.*
- ✓ *Enzyme Mix Reagent must be thawed and kept on a cold block at all times during preparation and use.*
- ✓ *All positive controls should be considered potentially infectious and handled accordingly.*

2019-nCoV assay contains four (4) sets of primers and probes. Two sets of primers and probe target specific regions on N gene and E gene in SARS-CoV-2 genome and the probes are labeled with fluorophore FAM, one set of primers and probe targets specific region on ORF 1ab in SARS-CoV-2 genome and the probe is labeled with fluorophore ROX, and one set of primers and probe targets specific nucleic acid sequence in virus like particles bacteriophage MS2 and the probe is labeled with the fluorophore VIC. Using probes linked to distinguishable dyes enables the parallel detection of SARS-CoV-2 specific RNA and the RNA of Internal Control particle in the corresponding detector channels of the real-time PCR System.

Controls validated and provided with the kit:

- Negative Control

A Negative Control in TODOS 2019-nCoV RT-qPCR Detection Kit contains a buffer that is free of the target virus nucleic acid sequence. This should be included in each RT-PCR run with clinical specimens to verify if reagents are contaminated.

- Positive Control

A positive Control in TODOS 2019-nCoV RT-qPCR Detection Kit consists of virus-like particles of SARS-CoV-2 ORF 1ab, N and E genes. This should be extracted concurrently with test samples and tested during rRT-PCR step to monitor failure in lysis and extraction procedure and verify reagent integrity.

- Internal Control

The internal control in TODOS 2019-nCoV RT-qPCR Detection Kit contains virus like particles bacteriophage MS2 with no homologies to any other known sequences of SARS-CoV-2. It is spiked into clinical specimen for the RNA extraction procedure and is then reverse transcribed, amplified and detected in parallel to the SARS-CoV-2 specific RNA. This control is used to ensure that RNA extraction process and PCR amplification process was conducted correctly.

Materials Required but Not Provided

Table 2. RNA Extraction Equipment

Instrument	Manufacturer	Catalog No.
TODOS Automated Nucleic Acids Extraction System	TODOS Diagnostics, Inc	TD-EQ03001

Table 3. RNA Extraction Kit

Item	Manufacturer	Catalog No.
TODOS Viral RNA Auto Extraction & Purification Kit	TODOS Diagnostics, Inc	16 tests (Cat #. TD-ER01001)
		64 tests (Cat #. TD-ER01002)
		128 tests (Cat #. TD-ER01003)

Table 4. Real Time PCR Instrument

Item	Manufacturer	Catalog No.
QuantStudio™ 12K Flex with QuantStudio™ 12K Flex software (v1.4)	Thermo Fisher Scientific	4471087

Equipment and Consumables Required but Not Provided

- Disposal virus sampling tube
- Biological Safety Cabinet Class II
- PCR workstation (UV lamp, laminar flow)
- Microcentrifuge
- Plate centrifuge (Beckman Coulter, Allegra X-30 microplate rotor package, or equivalent)
- Vortex mixer
- Single- and multi-channel pipettes
- Aerosol barrier pipette tips
- Nuclease-Free Water, Molecular grade
- 1.7 mL microcentrifuge tubes (DNase/RNase free)
- Tube racks for 1.7mL microcentrifuge tubes
- Cool racks or ice block for 1.7 microcentrifuge tube and/ 96 well PCR plates/tubes
- MicroAmp Optical 96-well Reaction Plates, 0.2mL (ThermoFisher, Cat #. N8010560), or equivalent
- MicroAmp Optical adhesive Film (ThermoFisher, Cat #. 4311971), or equivalent
- MicroAmp Optical adhesive Film (ThermoFisher, Cat #. 4311971), or equivalent
- MicroAmp Optical 8-tube Strips, 0.2mL (ThermoFisher, Cat #. A30588), or equivalent
- Surface decontaminants
    - 10% Bleach
    - 70% Ethanol
    - RNase Away, or equivalent
- Biohazard bags

Warnings and Precautions

- For *in vitro* diagnostic use only (IVD)
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- For Prescription Use Only.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- 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/lab-biosafety-guidelines.html

- Specimen processing should be performed in accordance with national biological safety regulation.
- Perform all manipulations of live virus samples in a certified Class II (or higher) biological safety cabinet (BSC).
- If infection with SARS-CoV-2 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 upper respiratory specimens collected from individuals who meet the US Center for Disease Control and Prevention (CDC) clinical and epidemiologic criteria.
- Wear clean, appropriate personal protective equipment (including but not limited to lab coat, gloves, eye and face protection) when handling kit reagents while performing this assay and handling materials including samples reagents, pipettes, and other equipment and reagents
- Change gloves whenever contamination is suspected and before entering another area.
- Use of this product is limited to personnel specifically instructed and trained in the techniques of molecular *in vitro* diagnostic procedures.
- Workflow in the laboratory should proceed in a unidirectional manner.

  - Maintain separate areas for sample receipt, extraction, assay setup, and handling of nucleic acids.
  - Maintain separate, dedicated equipment (e.g. pipettes, centrifuges) and supplies (e.g. microcentrifuge tubes, pipette tips) for assay setup and handling of all extracted nucleic acids. Do not move them around.
- Change aerosol barrier pipette tips between all manual liquid transfers.
- Keep reagent and reaction tubes capped or covered as much as possible.
- 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.
- Purified RNA should be maintained on cold block or on ice during preparation and use to ensure stability.
- Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning product such as 10% Bleach followed by 70% Ethanol and RNase Away to minimize risk of nucleic acid contamination.
- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.

Reagent Storage, Handling, and Stability

- Store the TODOS 2019-nCoV RT-qPCR Detection Kit reagents at -15°C to -25°C.
- Protect 2019-nCoV Assay which contains fluorogenic probes from light.
- Primers, probes (including aliquots), enzyme master mix, and controls must be thawed and kept on a cold block at all times during preparation and use.
- Do not use expired reagents.

Specimen Collection, Handling, and Storage

Inadequate or inappropriate specimen collection, storage, and transport are likely to yield false test results. Training in specimen collection is highly recommended to assure good quality specimens and results.

Collecting the Specimen

- Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (2019-nCoV)

  https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html
- Nasopharyngeal Swab specimens should be collected using only swabs with a synthetic tip, such as nylon or Dacron, and an aluminum or plastic shaft. Place swabs immediately into sterile tubes containing Viral Transport Medium (VTM) or Universal Transport Medium (UTM).

Transporting Specimens

- Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential SARS-CoV-2 specimens.
- Store specimens at 2-8°C and ship overnight to testing facility on ice pack. If a specimen is frozen at -70°C or lower, ship overnight to testing facility on dry ice.

Storing Specimens

- Specimens can be stored at 2-8°C for up to 48 hours after collection.
- If there is a delay in testing or shipping, store specimens at -70°C.
- Extracted nucleic acid should be stored at -70°C or lower.

Assay Procedure

RNA Extraction

RNA extraction is performed with TODOS Viral RNA Auto Extraction and Purification Kit (Cat #. TD-ER01001, TD-ER01002, TD-ER01003) on a TODOS Automated Nucleic Acids Extraction System (Cat. TD-EQ03001).

Table 5. TODOS Viral RNA Auto Extraction and Purification Kit Components

Label	Quantity			Storage
	16 Tests (Cat #.TD-ER01001)	64 Tests (Cat #. TD-ER01002)	128 Tests (Cat #. TD-ER01003)	
Reagent pre-filled 96 deep-well plates (Lysis buffer, Magnetic beads, Wash buffer, Elution buffer)	1 plate	4 plates	8 plates	15-25°C
8-strip rod combs	2 strips	8 strips	16 strips	
Proteinase K	400 µL	1.4 mL	2.8 mL	2-8°C

1. Equilibrate the clinical specimen tube(s) and Positive Control (PC) to room temperature.

***Note**: The PC is included in TODOS 2019-nCoV RT-qPCR Detection Kit, not included in extraction kit.*

2. Vortex samples and spin briefly to collect all specimen on the lid or side to the bottom of the tube.
3. Equilibrate Internal Control (IC) and Proteinase K to room temperature.

***Note**: The IC is included in TODOS 2019-nCoV RT-qPCR Detection Kit, not included in the extraction kit.*

4. Vortex IC and Proteinase K and spin briefly to collect all contents to the bottom of the tube.

5. Prepare and label a 1.7 mL DNase/RNase free tube as “IC Mix”.
6. Mix internal control and Proteinase K by following **Table 6**.

Table 6. Formula of "IC Mix"

Reagent Name	Volume per Test(µL)	Volume per N tests (µL)
Internal Control	1	1 x (N+1)
Proteinase K	20	20 x (N+1)
Total Volume	**21**	**21 x (N+1)**

7. Mix thoroughly by vortex and spin briefly to collect all contents to the bottom of the tube.
8. Invert the 96 deep-well plate 5 times to mix reagents and centrifuge plate briefly to collect all contents to the bottom of each well.
9. Unseal the 96-well pre-filled plate carefully. Ensure the film and foil is completely removed.
10. Add 21 µL of IC Mix and 200 µL of each clinical sample and PC into wells in A1 to H1 and A7 to H7 (The columns containing Lysis buffer).
11. Turn on TODOS Automated Nucleic Acids Extraction System.
12. Ensure the instrument is in idle mode and open the instrument door.
13. Load the 96 deep-well plate onto the heating stand with A1 position in upper left corner.
14. Fit 8-strip rod combs to the magnetic rod cover holder firmly.
15. Close the instrument door.
16. On the touch screen, choose Viral RNA program and ensure the program parameters match with **Table 7**.

Table 7. RNA extraction parameters for TODOS Automated Extraction Instrument

Step	Well Position	Action	Wait (min)	Mixing (min)	Attract (sec)	Volume (uL)	Mixing Speed (1 to 3)	Temperature
1	3	Transfer beads	0	1	20	900	3	OFF (Ambient)
2	1	Lysis	0	20	20	900	3	
3	2	Wash 1	0	2	20	900	3	

4	3	Wash 2	0	2	20	900	3	
5	6	Elution	2	6	20	100	1	60°C
6	3	Discard Beads	0	1	0	900	3	OFF (Ambient)

Note: *If the extraction protocol is not already programmed, go to "Program Setting" – "New" and enter the program parameters according to the table 7. For more information, refer to TODOS Automated Extraction System Instructions for Use.*

17. Start the instrument.
18. After the program is completed, transfer approximately 100µL extracted RNA eluted in A6 to H6 and A12 to H12 wells to clean 1.7mL DNase/RNase free tubes or 96-well plates labeled with sample ID.
19. Store the extracted RNA at -70°C or lower.
20. Discard the used 96 deep-well plates appropriately and decontaminate working surfaces and used equipment with 10% bleach followed by 70% ethanol.

Note: *It is recommended that an extraction control also be extracted with every run. An extraction control is a negative process control. 200 µL of sterile VTM medium (or sample collection medium) is added to the designated well at the time of extraction.*

RT-qPCR preparation

1. Equilibrate all the reagents and controls, except Enzyme Mix Reagent, to room temperature.
2. Thaw and keep the Enzyme Mix Reagent in cool rack or on ice during procedure.
3. Mix all the reagents and controls, except Enzyme Mix, by vortex and spin briefly to collect all contents to the bottom of the tube.
4. Mix the Enzyme Mix Reagent by flicking 5 times and spin briefly to collect all contents to the bottom of tube.
5. Prepare and label two 1.7mL Microcentrifuge tubes as "Master Mix" and "Master Mix + IC".
6. For the extracted RNA containing Internal Control after RNA extraction, prepare RT-qPCR Master Mix according to **Table 8**.

Table 8. Preparation of Master Mix for RNA with Internal Control ("Master Mix")

Reagent Name	Volume in µL per Test	Volume in µL per N Tests
RT-PCR Reaction Mix Reagent	18	18 x (N+1)
Enzyme Mix Reagent	3	3 x (N+1)
2019-nCoV assay	2	2 x (N+1)
Nuclease-free water	2	2 x (N+1)
Total Volume	**25**	**25 x (N+1)**

7. For Negative control and extracted RNA without Internal Control, prepare RT-qPCR Master mix according to **Table 9**.

Table 9. Preparation of Master Mix for RNA without Internal Control ("Master Mix + IC")

Reagent Name	Volume in µL per Test	Volume in µL per N Tests
RT-PCR Reaction Mix Reagent	18	18 x (N+1)
Enzyme Mix Reagent	3	3 x (N+1)
2019-nCoV assay	2	2 x (N+1)
Internal Control	0.5	0.5 x (N+1)
Nuclease-free water	1.5	2 x (N+1)
Total Volume	**25**	**25 x (N+1)**

8. Mix Master mix by inverting 5 times and spin briefly to collect all contents to the bottom of tube.
9. Set up reaction strip tubes/plate in cool rack or on ice.
10. Add 25 µL of RT-PCR Master Mix into the appropriate tubes/wells.
11. Add 25µL of extracted RNA sample or 25µL of the Control (Positive and Negative Control) into each tube/well containing 25µL of RT-PCR Master Mix.

Note: Vortex all samples and controls before adding them to tube/well.

12. Cap the 8-tube strip with optical cap or seal the 96-well reaction plate with optical adhesive film.

Note: When label the tubes/plate, **DO NOT LABEL THE TOPS OF THE REACTION TUBES/PLATES!** It will interfere with detecting the fluorescent signals.

13. Mix the reaction tubes/plate gently and spin briefly to collect all contents to the bottom of tube/well.
14. Ensure one Positive Control and one Negative Control are used in each run.

Real Time (RT) PCR Instrument Setup

Applied Biosystems™ QuantStudio™ 12K Flex (QuantStudio™ 12K Flex software, v1.4)

1. Define the general settings according to **Table 10**.

Table 10. General Setting

Settings	
Block Type	384-well or 96-Well (0.2mL)
Assay Type	Standard curve
Reagent	TaqMan
Ramp Speed	Standard/default

2. Define the Fluorescent Detectors (Dye) according to **Table 11**.
   ***Note**: "None" should be selected for "Passive reference" since the default is "ROX".*

Table 11. Definition of Target with fluorescent dye

Detection	Reporter Dye	Quencher
E gene	FAM	None
N gene		
ORF1ab gene	ROX	None
Internal Control	VIC	None
Passive Reference	None	

3. Set up the RT-qPCR Thermal Cycle Profile according to **Table 12**.
   ***Note:** Collect fluorescent signal at 60°C step.*

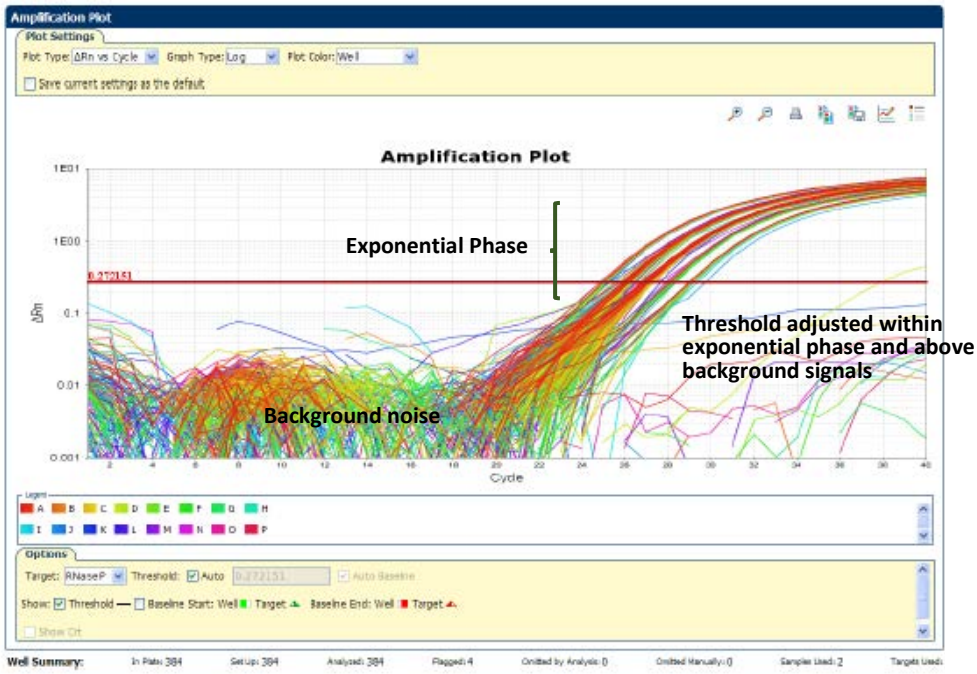
Table 12. TODOS 2019-nCoV RT-qPCR Thermal Cycle Profile

Stage	Temperature	Time	Cycle Number
RT	50°C	10 minutes	1
Hold	95°C	2 minutes	1
PCR	95°C	5 seconds	45
	60°C (Read)	35 seconds	
Reaction volume per well (tube)		50 µL	
Ramp Rate		Default	

Data Analysis

1. After the run has completed, analyze the data using QuantStudio 12K Flex System Software.
2. Threshold adjustment:
   a. In the "Amplification Plot" window, select each target using drop down menu. Then, uncheck the box for "Auto".
   b. Using the mouse, manually drag the threshold line until it lies within the exponential phase of the fluorescence curves and above any background signal. (**Figure 2**).

Figure 2. Threshold adjustment



For more information regarding adjustment of threshold and/or PCR setup, refer to QuantStudio™ 12K Flex.

3. Analyze with adjusted threshold and save the experiment with unique name.
4. Export the run data as an excel file which shows Ct value.

Interpretation of Results and Reporting

Table 13. Expected Results for Test Controls

Control	Used to Monitor	FAM (E and N)	ROX (ORF1ab)	VIC (Internal Control)	Expected Ct
Positive Control (positive process control)	Failure in lysis and extraction procedure; substantial reagent failure including primer and probe integrity	POS	POS	POS	FAM Ct < 40.00 ROX Ct < 40.00 VIC Ct < 40.00

Negative Control	Reagent and/or environmental contamination	NEG	NEG	POS	FAM Ct ≥ 40.00 ROX Ct ≥ 40.00 VIC Ct < 40.00
Extraction Control (Negative process control)	Failure in lysis and extraction procedure; potential contamination during extraction	NEG	NEG	POS	FAM Ct ≥ 40.00 ROX Ct ≥ 40.00 VIC Ct < 40.00

If any of the above controls do not exhibit expected performance as described in **Table 13**, the test is invalid.

Internal Control

- All clinical specimen should exhibit VIC Ct (Threshold Cycle) < 40.00 which indicate the presence of Internal Control. Failure to detect Internal Control in any clinical samples may indicate:
  - Improper extraction of nucleic acid from clinical materials resulting in loss of RNA and/or RNA degradation.
  - Improper test set up and execution.
  - Reagent or equipment malfunction.
- If Internal Control signal is negative for the specimen, the result is invalid for the specimen. If residual specimen is available, repeat the extraction procedure and RT-qPCR test. If Internal Control signal remains negative after re-test, report the results as invalid.

For SARS-CoV-2 gene specific markers

- When all controls exhibit the expected performance, a specimen is considered negative if all the SARS-CoV-2 specific markers (FAM and ROX) have Ct value greater than its respective Ct cutoff value and Internal Control (VIC) have Ct value less than its respective Ct cutoff value.
- When all controls exhibit the expected performance, a specimen is considered positive for SARS-CoV-2 if either or both of the SARS-CoV-2 specific markers (FAM and/or ROX) and Internal Control (VIC) have Ct value less than its respective Ct cutoff value.
- If any one of two SARS-CoV-2 markers and Internal Control are negative for the specimen, the result is invalid for the specimen. If the residual specimen is available, repeat the extraction procedure and repeat the RT-PCR test. If all markers remain negative after re-test, report the results as invalid and a new specimen should be collected if possible.

Table 14. TODOS 2019-nCoV RT-qPCR Result Interpretation

FAM (E and N)	ROX (ORF1ab)	VIC (IC)	Results Interpretation	Report	Action
Positive	Positive	Positive	SARS-CoV-2 detected	SARS-CoV-2 detected	Report results to appropriate public health authority and healthcare provider.
Only 1 of 2 targets is Positive		Positive	SARS-CoV-2 detected	SARS-CoV-2 detected	Report results to appropriate public health authority and healthcare provider.
Negative	Negative	Positive	SARS-CoV-2 not detected	SARS-CoV-2 not detected	Report results to healthcare Provider. Consider testing for other pathogens.
Any Result		Negative	Invalid Result	Invalid Result	Repeat extraction and RT-qPCR. Recollect specimen if needed.

Positive: FAM Ct<40.0 and/or ROX Ct<40.0. Negative: FAM Ct≥40.0 and ROX Ct ≥40.0

Quality Control

- Quality control requirements must be performed in conformance with local, state, and federal regulations or accreditation requirements and the user's laboratory's standard quality control procedures. For further guidance on appropriate quality control practices, refer to 42 CFR 493.1256.
- Quality control procedures are intended to monitor reagent and assay performance.
- Test positive control prior to running diagnostic samples with each new kit lot to ensure all reagents and kit components are working properly.
- Good laboratory practice (cGLP) recommends including a positive extraction control in each nucleic acid isolation batch.
- Always include Negative Control, and Positive control in each amplification and detection run.
- All clinical samples should be tested with a spiked Internal Control to monitor specimen quality and extraction.

Limitations

- .
- The test is distributed in accordance with the guidance on Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency, Section IV.C.2
- The test has been validated but FDA's independent review of this validation is pending.F
- All user, analysts, and any person reporting diagnostic results should be trained to perform this procedure by a competent instructor. They should demonstrate their ability to perform the test and interpret the results prior to performing the test independently.
- Performance of TODOS 2019-nCoV RT-qPCR Detection Kit has only been established in the specimens collected with nasopharyngeal swabs.
- Extraction and amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure. Other extraction approaches and PCR systems have not been evaluated.
- Negative results do not preclude infection of SARS-CoV-2 and should not be used as the sole basis for treatment or other patient management decision. Optimum specimen type and timing for peak viral levels during infections caused by SARS-CoV-2 have not be determined. Collection of multiple specimens (types or time point of infection) from the same patient may be necessary to detect the virus.
- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are in the specimen or if inadequate numbers of viral copies are present in the specimen.
- A false positive result may be observed if cross contamination occurred during the specimen handling or preparation.
- Due to high homology of E and N primer/probes with SARS coronavirus, there is a limited possibility of a false positive result due to infection with SARS coronavirus.
- If the virus mutates in the RT-qPCR target regions, SARS-CoV-2 may not be detected or may be detected less predictively.
- Test performance can be affected because the epidemiology and clinical spectrum of infection caused by SARS-CoV-2 is not fully understood.

- Detection of virus RNA may not indicate the presence of infectious virus or that SARS-CoV-2 is the causative agent for clinical symptoms.
- The performance of this test has not been established for monitoring treatment of SARS-CoV-2 infection.
- The performance of this test has not been established for screening of blood or blood products for the presence of SARS-CoV-2.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

Performance Characteristics

All RNA extraction for the evaluation of TODOS 2019-nCoV RT-qPCR Detection kit were performed by using TODOS Viral RNA Auto Extraction and Purification Kit on the TODOS Automated Nucleic Acids Extraction System. Limit of detection studies was performed for both QuantStudio™ 12k Flex Real-time PCR equipment for the TODOS 2019-nCoV RT-qPCR Detection kit. The real time PCR system with less sensitivity was used for further studies.

Analytical Performance

Limit of Detection (LoD)

The LoD studies determines the lowest detectable concentration of SARS-CoV-2 at which approximately 95% of all replicates (or 19/20 replicates) tested positive using the TODOS 2019-nCoV RT-qPCR Detection.

A. LoD Confirmation Study

A two-phase approach was used to determine LoD. In the first phase, a series of serial dilutions were performed by spiking NR-52287 control material (Gamma-irradiated SARS-Related Coronavirus 2, Isolate USA-WA1/2020; BEI resources, Cat # NR-52287, Lot # 70033322) into pooled negative remnant NP specimens at concentrations-17000 copies/µL, 1700 copies/µL, 170 copies/µL, 17 copies/µl, 5 copies/µL, 1 copies/µL, 0.5 copies/µL, 0.2 copies/µL, 0.15 copies/µL, 0.1 copies/µL, 0.05 copies/µL, 0.025 copies/µL, and 0.0125 copies/µL. Each dilution level was prepared with 5 replicates and extracted. The extracted RNA was further tested

using TODOS 2019-nCoV RT-qPCR Detection kit on Applied Biosystems Quant Studio 12k Flex. This test provided the preliminary LoD (**Table 15**). In the second phase, the preliminary LoD was confirmed by spiking 20 replicates of pooled negative NP swab remnant specimens with control NR-52287 at 1xLoD (**Table 16**).

Table 15. Preliminary LoD Study on QuantStudio 12K Flex by serially diluting NR-52287 in pooled negative NP specimens.

Concentration (viral genomic copies/µL)	E and N assay Detection Rate	ORF1ab assay Detection Rate
17000	5/5(100%)	5/5(100%)
1700	5/5(100%)	5/5(100%)
170	5/5(100%)	5/5(100%)
17	5/5(100%)	5/5(100%)
5	5/5(100%)	5/5(100%)
1	5/5(100%)	5/5(100%)
0.5	5/5(100%)	5/5(100%)
0.2	5/5(100%)	5/5(100%)
0.15	5/5(100%)	5/5(100%)
0.1	5/5(100%)	5/5(100%)
0.05	5/5(100%)	3/5(60%)
0.025	4/5(80%)	0/5(0%)
0.0125	1/5(20%)	0/5(0%)

Table 16. LoD Confirmation Study

Conc. (Copies/µL)	Detection Rate
0.4 (2xLoD)	20/20 (100%)
0.2 (1xLoD)	20/20 (100%)
0.1 (0.5xLoD)	18/20 (90%)

The final LoD was determined to be at 0.2 copies/µL (**Table 15, Table 16**) or **10 copies/reaction. This was referred to as 1xLoD.**

Inclusivity (Analytical Sensitivity)

Sequence alignment was performed with the oligonucleotide primer and probe sequences of the TODOS 2019-nCoV RT-qPCR Detection Kit with all publicly available nucleic acid sequences for SARS-CoV-2 from NCBI GenBank and GISAID as of 24th July, 2020 to demonstrate the predicted inclusivity of the TODOS

2019-nCoV RT-qPCR Detection Kit. All the alignments show 100% identity of the 2019-nCoV Assay to the available 2019-nCoV sequences. The alignment of the 2019-nCoV Assay includes additional sequences for SARS-CoV, MERS-CoV, and other Bat coronaviruses to show that other than SARS coronavirus, the alignment shows low identities and would not predict significant reactivity.

The inclusivity study is conducted *in silico* by mapping the assays to all analyzed SARS-CoV-2 sequences in NCBI (11143 sequences) and GISAID (70615 sequences) database. *In-silico* analysis shows that there are 48, 173 and 11 SARS-COV-2 strains with mismatch at 3' end of primers/probes for 3DMed E, N and ORF1ab genes, respectively. However, all tested SARS-COV-2 strains have perfect alignment for at least 2 out of 3 designed genes for 3DMed design. Therefore, 100% detectability is expected with the published SAR-CoV-2 sequences. The mapping results and the data is available per request.

Cross-reactivity (Analytical Specificity)

The potential cross-reactivity of the assay primers and probes were evaluated using both wet testing and in silico approaches with other respiratory pathogens.

A. In Silico analysis for primers and probes

BLAST analysis queries of the SARS-CoV-2 RT-qPCR assay primers and probes were performed against publicly available nucleotide sequences.

1) ORF1ab Assay
   The probe sequence of 2019-nCoV_ORF1ab assay showed high sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. However, both forward and reverse primers showed no sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. Combining primers and probe, there is no significant homologies with human genome, other coronaviruses or human microflora that would predict potential false positive RT-qPCR results.

2) E Assay

The forward primer, reverse primer and probe sequences of 2019-nCoV_E assay showed high sequence homology to Bat SARS- like coronaviruses. However, these primer and probe sequences showed no significant homology with human genome, other coronaviruses or human microflora. Combining primers and probe, there is a prediction of potential false positive RT-qPCR results in the

presence of human SARS coronavirus and bat SARS coronavirus in samples.

3) N Assay

Analysis of the forward and reverse primer and probe sequences of 2019-nCoV_N assay showed significant homology only to human SARS coronavirus and bat SARS-like coronavirus. No significant homology with human genome, other coronaviruses or human microflora was observed. We predict potential false positive RT-qPCR results in the presence of human SARS coronavirus and bat SARS coronavirus in samples.

In summary, the 2019-nCoV_ORF1ab assay, designed for the specific detection of SARS-CoV-2, showed no significant combined homologies with human genome, other coronaviruses, or human microflora that would predict potential false positive RT-qPCR results. The 2019-nCoV_E and N assays were designed for universal detection of SARS-CoV-2, human SARS coronavirus and bat SARS coronavirus.

B. In Silico analysis for microorganisms

1) An *in-silico* analysis for all the available strains of organisms recommended in FDA EUA template had been conducted.
2) Among the tested organisms listed in **Table 17**, no organisms showed significant sequence homology with the test primer and probes. SARS coronavirus, *Candida glabrata, and Cryptococcus neoformans* showed significant homology with forward primer of ORF 1ab, but not with ORF 1ab reverse primer and probes. Therefore, the risk of non-specific amplification is low. SARS coronavirus showed 100% homology with forward primer and reverse primer and probe for E gene, therefore, a potential false positive result may be reported for a clinical specimen containing SARS coronavirus. As per information provided by CDC, there is no known clinical prevalence of SARS coronavirus since 2004 (https://www.cdc.gov/sars/index.html). Therefore, there is a significantly low possibility of a false-positive result due to SARS coronavirus.

Table 17. Organisms used for in silico analysis

Pathogen - Strain	Pathogen – Strain
Aspergillus fumigatus - Af293	Human Enterovirus D - Enterovirus 70
Bacillus anthracosis (Anthrax) - Ames Ancestor; A2084	Human gammaherpesvirus 4 (EB virus) - B95-8
Bordetella pertussis - BP 165	Human metapneumovirus (hMPV) - 00-1
Candida albicans - SC5314	Human poliovirus 1 – Mahoney
Candida glabrata – CBS138	Influenza A - H5N1
Chlamydia pneumoniae - CWL029	Influenza A - H7N9
Chlamydia psittaci - 6BC	*Klebsiella pneumonia* - HS11286
Corynebacterium diphtheriae - NCTC11397	*Legionella pneumophila* - NCTC12273
Coxiella burneti (Q-Fever) - RSA 493	*Leptospira interrogans* - FMAS_AW1
Cryptococcus neoformans – JEC21	Measles virus - Ichinose-B95a
Haemophilus influenza - Rd KW20	MERS-coronavirus - HCoV-EMC
Human Adenovirus 1 - N/A	*Moraxella cararrhalis* - BBH18
Human Adenovirus 2 - N/A	*Mumps virus* – Miyahara
Human Adenovirus 5 - N/A	*Mycobacterium tuberculosis* - H37Rv
Human Adenovirus 54 - Kobe-H	*Mycoplasma californicum* - ST-6
Human Adenovirus 7 - N/A	*Mycoplasma floccular* - Ms42
Human Adenovirus A - Huie	*Mycoplasma pneumoniae* – FH
Human Adenovirus B1 - GB	*Neisseria elongate* - ATCC 29315
Human Adenovirus B2 - Slobitski	*Neisseria meningitidis* - NCTC10025
Human Adenovirus C - N/A	Parainfluenza virus 1 - Washington 1964
Human Adenovirus D - N/A	Parainfluenza virus 2 - N/A
Human Adenovirus D - Hicks; NIAID V-209-003-014	Parainfluenza virus 3 - N/A
Human Adenovirus F - Dugan	Parainfluenza virus 4 - M-25
Human Adenovirus type 35 - N/A	Parechovirus A – Gregory
Human alphaherpesvirus 3 (Varicella-zoster virus) – Dumas	Parechovirus B - 87-012
Human betaherpesvirus 5 (Human cytomegalovirus) – Merlin	*Pneumocystis jirovecii* - RU7
Human coronavirus - 229E	*Primate Norovirus* - SimianNoV-nj
Human coronavirus - NL63	*Pseudomonas aeruginosa* - PAO1
Human coronavirus - OC43	Respiratory syncytial virus - S2 ts1C
Human coronavirus - HKU1	Rhinovirus A - ATCC VR-1559
Human Enterovirus 68 - Fermon	Rhinovirus B - N/A
Human Enterovirus A - N/A	Rhinovirus C - NAT001
Human Enterovirus B - N/A	Rotavirus A - RVA/Simian-tc/ZAF/SA11-H96/1958/G3P5B [2]
Staphylococcus epidermidis – O47	*Streptococcus pyogenes – NCTC8198*
Streptococcus pneumoniae – NCTC12977	*Streptococcus salivarius – NCT8318*
SARS Cornovirus-Tor2	

C. Wet Lab Analysis

Organisms listed in **Table 18** were spiked into pooled negative NP specimens in triplicates, extracted and tested using TODOS 2019-nCoV RT-qPCR Detection Kit. All high priority organisms likely present in respiratory specimens were not detected by TODOS 2019-nCoV RT-qPCR Detection Kit **(Table 18)**, except for SARS-Coronavirus. There is a limited possibility of a potential false positive or inconclusive result in the presence of SARS Coronavirus.

Table 18. Results for Cross-reactivity Study

Pathogen	Materials Used	TODOS 2019-nCoV RT-qPCR Detection Kit result	Interpretation
Influenza A H1N1 (A/NY/02/09)	ZeptoMetirx, Cat #. NATRPP-1, Respiratory Pathogen Panel-1, Pool 1	0/3	Negative
Parainfluenza Type 4A			Negative
Parainfluenza Type 4B			Negative
Rhinovirus (1A)			Negative
Adenovirus Type 3			Negative
Influenza A H1 (A/New Caledonia/20/99)	ZeptoMetirx, Cat #. NATRPP-1, Respiratory Pathogen Panel-1, Pool 2	0/3	Negative
Respiratory Syncytial Virus A			Negative
Parainfluenza Type 1			Negative
Coronavirus NL63			Negative
Mycoplasma Pneumoniae (M129)			Negative
Influenza A H3 (A/Brisbane/10/07)	ZeptoMetirx, Cat #. NATRPP-1, Respiratory Pathogen Panel-1, Pool 3	0/3	Negative
Respiratory Syncytial Virus B (CH93(18)-18)			Negative
Coronavirus OC43			Negative
Coronavirus HKU-1			Negative
Influenza B(B/Florida/02/06)	ZeptoMetirx, Cat #. NATRPP-1, Respiratory Pathogen Panel-1, Pool 4	0/3	Negative
Parainfluenza Type 3			Negative
Human Metapneumovirus (Peru6-2003)			Negative
Legionella pneumophila (Philadelphia)			Negative
Parainfluenza Type 2	ZeptoMetirx, Cat #. NATRPP-1, Respiratory Pathogen Panel-1, Pool 5	0/3	Negative
Coronavirus 229E			Negative
Human Bocavirus			Negative
Chlamydophila pneumoniae (CWL-029)			Negative
Coronavirus OC43	BEI resources, NR-52725	0/3	Negative
Coronavirus 229E	BEI resources, NR-52726	0/3	Negative
Coronavirus NL63*	BEI resources, NR-44105	0/3	Negative
MERS-coronavirus	BEI resources, NR-50549	0/3	Negative

SARS-coronavirus (Urbani strain)	80,000,000 copies/µL	BEI resources, NR-9548	3/3	Positive
	800,000 copies/µL		3/3	Positive
	800 copies/µL		3/3 (E, N), 0/3(ORF1ab)	Positive
	80 copies/µL		3/3 (E, N), 0/3(ORF1ab)	Positive

*NR-44105 control is non-extractable genomic RNA from Human Coronavirus NL63 (BEI resources). The control was diluted in TE buffer and tested on rRT-PCR.

Interfering Substance Studies

Endogenous interference study was conducted using contrived positive specimen with SARS-CoV-2 concentrations at 2x LoD. The interference substances listed in **Table 19** were spiked into contrived positive samples prior to RNA extraction. Three replicates per each substance was tested in the study.

Table 19. Results for Endogenous Substance Interference Studies

Potential Interfering Substance	Concentration (µg/mL)	(2×LoD) Results (positive or negative)
Nasal spray	N/A	Positive (3/3)
Ky Jelly	N/A	Positive (3/3)
Vaseline	N/A	Positive (3/3)

Clinical Evaluation

Sixty-four nasopharyngeal remnant specimens were obtained from iSpecimen Inc. 30 samples were confirmed negative and 34 samples were confirmed positive specimens. RNA was extracted from the 64 specimens using TODOS extraction device and reagents and tested on TODOS 2019-nCoV RT-qPCR Detection kit. All 30 negative specimens were negative for all targets, and 33 out of 34 positive specimens were positive for all targets detected by the kit. (**Table 20**).

Table 20. Clinical Performance Evaluation with Clinically Derived Nasopharyngeal Swab Specimens

#	Negative Samples	Ct-Value			Expected Result	Observed Result	Positive Samples	Ct-Value			Expected Result	Observed Result
		E, N	ORF1ab	IC				E, N	ORF1ab	IC		
1	CDS-1	U/D	U/D	32.559	Negative	Negative	P-1	34.717	33.765	33.118	Positive	Positive
2	CDS-2	U/D	U/D	32.646	Negative	Negative	P-2	29.493	28.107	32.153	Positive	Positive
3	CDS-3	U/D	U/D	32.063	Negative	Negative	P-3	35.256	34.820	32.704	Positive	Positive
4	CDS-4	U/D	U/D	31.973	Negative	Negative	P-4	18.428	17.059	33.808	Positive	Positive
5	CDS-5	U/D	U/D	32.062	Negative	Negative	P-5	21.713	20.355	32.255	Positive	Positive
6	CDS-6	U/D	U/D	32.516	Negative	Negative	P-6	31.975	30.652	32.805	Positive	Positive
7	CDS-7	U/D	U/D	32.295	Negative	Negative	P-7	36.062	35.623	33.115	Positive	Positive
8	CDS-8	U/D	U/D	31.741	Negative	Negative	P-8	22.149	20.470	32.635	Positive	Positive
9	CDS-9	U/D	U/D	32.492	Negative	Negative	P-9	U/D	U/D	32.919	Positive	Negative
10	CDS-10	U/D	U/D	32.128	Negative	Negative	P-10	37.788	35.683	32.566	Positive	Positive
11	CDS-11	U/D	U/D	31.987	Negative	Negative	P-11	36.340	36.312	32.682	Positive	Positive
12	CDS-12	U/D	U/D	32.499	Negative	Negative	P-12	36.042	35.973	32.740	Positive	Positive
13	CDS-13	U/D	U/D	32.474	Negative	Negative	P-13	21.795	20.086	25.381	Positive	Positive
14	CDS-14	U/D	U/D	32.506	Negative	Negative	P-14	31.669	30.576	32.823	Positive	Positive
15	CDS-15	U/D	U/D	32.600	Negative	Negative	P-15	31.609	30.241	21.973	Positive	Positive
16	CDS-16	U/D	U/D	32.758	Negative	Negative	P-16	36.186	36.446	33.231	Positive	Positive
17	CDS-17	U/D	U/D	32.480	Negative	Negative	P-17	22.895	21.477	31.466	Positive	Positive
18	CDS-18	U/D	U/D	32.826	Negative	Negative	P-18	21.524	19.860	33.769	Positive	Positive
19	CDS-19	U/D	U/D	32.538	Negative	Negative	P-19	17.011	15.689	34.164	Positive	Positive
20	CDS-20	U/D	U/D	32.702	Negative	Negative	P-20	19.243	17.510	34.267	Positive	Positive
21	CDS-21	U/D	U/D	32.520	Negative	Negative	P-21	33.133	31.550	33.033	Positive	Positive
22	CDS-22	U/D	U/D	32.648	Negative	Negative	P-22	38.904	39.322	32.785	Positive	Positive
23	CDS-23	U/D	U/D	32.911	Negative	Negative	P-23	18.490	16.881	34.539	Positive	Positive
24	CDS-24	U/D	U/D	32.276	Negative	Negative	P-24	32.794	31.665	32.702	Positive	Positive
25	CDS-25	U/D	U/D	32.301	Negative	Negative	P-25	32.677	31.113	33.799	Positive	Positive
26	CDS-26	U/D	U/D	32.059	Negative	Negative	P-26	21.444	21.524	17.445	Positive	Positive
27	CDS-27	U/D	U/D	32.042	Negative	Negative	P-27	19.463	17.822	34.125	Positive	Positive
28	CDS-28	U/D	U/D	31.918	Negative	Negative	P-28	17.344	15.814	34.299	Positive	Positive
29	CDS-29	U/D	U/D	32.729	Negative	Negative	P-29	28.826	27.544	32.140	Positive	Positive
30	CDS-30	U/D	U/D	32.108	Negative	Negative	P-30	19.394	17.535	33.720	Positive	Positive
31							P-31	16.302	16.169	33.265	Positive	Positive
32							P-32	31.800	31.735	33.259	Positive	Positive
33							P-33	32.095	32.195	32.709	Positive	Positive
34							P-34	37.163	38.472	33.157	Positive	Positive

One specimen (P-9) was not detected for either N/E gene or ORF1ab gene. Additional tests were performed for P-9. The sample was extracted using other extraction methods and tested on two FDA-EUA approved tests. P-9 remained

undetected for all SARS-CoV-2 targets (**Table 21**). As a result, P-9 was excluded from the validation and PPA/NPA calculations.

Table 21. P-9 sample confirmation on different extraction and PCR methods

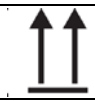
P-9 Sample confirmation	TODOS 2019-nCoV RT-qPCR Detection Kit			Gnomegen COVID-19 RT-qPCR detection kit (FDA-EUA)			CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel (FDA-EUA)		
	E, N	ORF1ab	IC	N1	N2	RNase P	N1	N2	RNase P
Qiagen QIAamp Viral RNA Mini Kit	U/D	U/D	31.624	U/D	U/D	27.233	U/D	U/D	26.482
Omega Bio-tek Mag-Bind Viral DNA/RNA 96 Kit	U/D	U/D	32.578	U/D	U/D	27.491	U/D	U/D	25.816
TODOS Viral RNA Auto Extraction and Purification Kit	U/D	U/D	32.919	U/D	U/D	28.003	U/D	U/D	27.482

In conclusion, the tested SARS-CoV-2 negative, and positive nasopharyngeal specimens tested on TODOS 2019-nCoV RT-qPCR Detection Kit were in 100% agreement to the expected results (**Table 22**).

Table 22. Result Summary of PPA and NPA analysis

N=63 NP Swabs		CDC 2019 Novel Coronavirus(2019-nCoV) Real Time RT-PCR Diagnostic Panel	
		Positive	Negative
TODOS 2019-nCoV RT-qPCR Detection Kit	Positive	33	0
	Negative	0	30
	Total	33	30
Positive Predictive Agreement (PPA)		**(33/33) x100=100%**	
Negative Predictive Agreement (NPA)		**(30/30) x100=100%**	

Symbols

Symbol	Meaning	Symbol	Meaning
	Manufacturer		Number of Tests
	Date of Manufacture		Temperature Limit (Storage Temperature)
	Use-by Date (Expiration Date)		In vitro diagnosis
	Lot number		Keep away from Sunlight
	Catalog Number		Consult Instructions for Use
	This Side Up		Prescription Use Only

Hazards

Corrosion
- Skin Corrosion
- Burns
- Eye Damage
- Corrosive to metals

Health Hazard
- Carcinogen
- Mutagenicity
- Reproductive Toxicity
- Respiratory Sensitizer
- Target Organ Toxicity
- Aspiration toxicity

Exclamation mark
- Irritant (skin & eye)
- Skin Sensitizer
- Acute Toxicity (harmful)
- Narcotic Effects
- Respiratory Tract Irritant
- Hazardous to Ozone Layer (Non-Mandatory)

Flammable
- Flammables
- Pyrophoric
- Self-heating
- Emits Flammable Gas
- Self-Reactive
- Organ Peroxides

Manufacturing/Distribution

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Customer Service

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References

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