LabTurbo Automation for Gene Detection

**User Manual**

# LabTurbo™ AIO COVID-19 RNA Testing Kit

CE IVD

REF **Acov11240 and Acov13000**

**480 tests** **Store at -20 ℃**

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# I. Intended Use

**LabTurbo™ AIO COVID-19 RNA Testing Kit** is a real-time reverse transcription polymerase chain reaction, (RT-PCR) diagnostic product for SARS-CoV-2 RNA (COVID-19) detection.

The positive detection result represents that SARS-CoV-2 RNA has been identified. Laboratories are required to report all positive results to the appropriate public health authorities. The **LabTurbo™ AIO COVID-19 RNA Testing Kit** does not preclude the possibility of false positive results. Please contact your healthcare provider to determine how best to care for you based on the test results, medical history, and your symptoms.

The negative results could not fully exclude the infection of SARS-CoV-2. Additional clinical observations, epidemiological information, and traveling history are recommended for comprehensive evaluation. It is possible for this test to give some people with COVID-19 an incorrect negative result (false negative). This means that you could still possibly have COVID-19 even though the test is negative. If this is the case, your healthcare provider will consider the test result together with your symptoms, possible exposures, and the geographical location of places you have recently traveled, in deciding your treatment options.

# II. Product Description

**LabTurbo™ AIO COVID-19 RNA Testing Kit** includes two products, Acov11240 and Acov13000. They contain oligonucleotide primers, dual-labeled hydrolysis probes, and control material used in RT-qPCR for the *in vitro* qualitative detection of SARS-CoV-2 (COVID-19) RNA in respiratory specimens. They are designed for assay and validation:

## • Table 1. Detection Target in LabTurbo™ AIO COVID-19 RNA Testing Kit

| Assay | N gene | E gene | Endogenous IC | RdRP |
|---|---|---|---|---|
| Validation | COVID-19 | SARS-CoV | Patient swab cell | COVID-19 |
| Dye | FAM | HEX | Cy5 | FAM |
| Kit | Acov11240 | | | Acov13000 |

The product, Kit No. Acov11240, is a multiplex RT-qPCR assay for N gene, E gene, and endogenous IC in the specimens. N gene is specifically used to validate SARS-CoV-2 (COVID-19) in specimens; the E gene is used to validate universal SARS-CoV RNA. RNase P (RP) is used as an endogenous Internal Control (IC) to validate successful specimen collection from individual patients. The Acov13000 kit is a singleplex RT-qPCR assay for the RdRP gene as a confirmatory validation for the presence of SARS-CoV-2 from specimens with negative N gene detection and positive E gene detection. Furthermore, the



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Positive Extraction Control (PEC) can validate the workflow and performance of the assay. The RNase-free water is used as the negative extraction control (NEC) to validate the reagent stability and monitor cross-contamination. Both PEC and NEC have to be validated in every batch of the assay. The positive control (PC) is used to validate the RT-qPCR performance if the PEC fails due to extraction; the negative control (NC) is used to confirm cross-contamination if the NEC fails due to extraction.

The assays could be completed using the fully automated instrument LabTurbo AIO SP-qPCR System (LabTurbo™ AIO), or using the semi-automation LabTurbo 48 compact system (CE IVD), as an extractor, and with a RT-qPCR machine, such as the QuantStudio™ 5 Real-Time PCR Systems, manufactured by ThermoFisher. All systems can use the LabTurbo™ Viral DNA/RNA Mini kit (LVX480-500) for nucleic acid extraction and the LabTurbo™ AIO COVID-19 RNA Testing Kit (Acov11240 and Acov13000) for RT-qPCR assay.

## • Table 2. The Assay Workflow of LabTurbo AIO COVID-19 RNA Testing Kit

| Step | Description |
|---|---|
| Sample Identification | Use barcode reader or manually key in the sample barcodes |
| Viral RNA extraction | Nucleic extraction from samples<br>Extraction kit: LVX480-500<br>Sample Input 500 µl<br>Elution volume 60 µl |
| RT-qPCR setup | RT-qPCR reaction preparation and liquid-handling<br>RNA template: 6 µl<br>Master mix: 19 µl<br>Total volume: 25 µl |
| RT-qPCR reaction | RT-PCR reaction including reverse transcription, thermocycling and fluorescence detection of each gene<br>Kits and detection targets:<br>Acov11240: for N and E genes of SARS-CoV-2 and internal control (human RNase P gene)<br>Acov13000: for RdRp gene of SARS-CoV-2 |
| CT report | Summarizing the final CT values of each gene from each sample |

The kit will be shipped on wet ice and should be stored at -20°C, prior to use. After opening, it could be stored at 4-8°C for up to 5 days and the number of freeze-thaw cycles should not be more than five times. **The in-use mixture is prepared from components that are stable at 4-8°C for 5 days, so it will be convenient for the user to prepare a larger amount of the mixture that is intended for several days use.**

LabTurbo™ Viral DNA/RNA Mini Kit (LVX480-500) utilizes the chaotropic salts silica membrane column and vacuum method, (Boom Method). The kit is manufactured by GMP-certified facility (GMP1339) and suitable for IVD use (TFDA 006288). It is designed for LabTurbo automation systems that has been widely used for SARS-CoV-2 RNA extraction from the clinical samples of OP/NP swab or sputum, and stool[1,2,3]. It inherits numerous



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advantages from the conventional membrane column vacuum extraction method including: 1) effective washing to get rid of PCR inhibitors from challenging clinical samples, 2) robust binding capability by multi-layer membrane structure to capture free nucleic acid in liquid and get the best nucleic acid recovery, 3) highly concentrated elution by using larger sample input 500 µl and less elution volume 60 µl to increase the detection sensitivity. Thus the kit can be widely used for clinical applications to achieve great detection sensitivity.

LabTurbo™ AIO SP-qPCR System was developed based on the Taigen Biosciences Automated Isolation System[4] that had been used for a hundreds of thousand of sample screenings for *babesia microti* in the U.S. Blood Supply, as published in NEJM 2016, and obtained Pre-market Approval (PMA) by FDA in 2019 (BLA/ STN#: 125588). LabTurbo™ AIO was further integrated with traditional Peltier element PCR system and optical detection system to achieve full automation of nucleic acid testing workflow from sample preparation, nucleic acid extraction, reaction setup liquid-handling, qPCR reaction to Ct report. It comes in two models: LabTurbo™ AIO 24 and AIO 48. The AIO 24 runs 1 to 24 samples per batch which is up to 500 samples per day per unit under 24/7 operation. The AIO 48 runs 1 to 48 samples per batch, totaling 1000 samples per day per unit under 24/7 operation. Data analysis of LabTurbo™ AIO has been optimized for the CT report that can be exported. However, it also allows technicians to adjust the threshold for the analysis of weak positive results.

## III. Warnings and Precautions

⚠ For *in vitro* diagnostic use.

⚠ For prescription use only.

- All samples shall be considered potentially infectious and shall be operated and handled in strict accordance with the laboratory's bio-safety requirements. The experimental personnel should receive professional training (including sample processing, reagent preparation, instrument operation, and software setting, etc.). For the laboratory management specifications, please strictly follow the relevant management specifications for gene amplification test laboratories issued by local regulatory agencies.

- The laboratory should be divided into reagent preparation area, sample preparation area, and LabTurbo™ AIO area. All the articles in each area are for special purposes, and they shall not be used for other purposes, as to avoid contamination.

- The suggested PPE (Personal Protective Equipment) for a laboratory worker are gowns or closed lab coats, hairnets, gloves, eye protection (face shield or goggles) and surgical facemasks or fit-tested N95 masks. Laboratory clothes, hats, shoes, gloves, etc. shall be fully equipped during operation to avoid direct contact of reagents or samples with skin. In case



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of liquid leakage, rinse with plenty of water immediately. In case of contact with skin wounds, please notify the local health and epidemic prevention department in time.

- This product is an in vitro diagnostic reagent kit. In all test procedure, well laboratory and LabTurbo™ AIO training for laboratory personnel are essential to guarantee the test accuracy and safety. Please read the manual carefully before the experiment.

- Specimen sampling and processing should be performed according to the recommendation of local regulation. Sample processing shall be carried out in the biosafety cabinet to protect the safety of operators and prevent environmental contamination.

- RNase / DNase free pipette tips and water are recommended in all test procedure.

- Avoid swallowing or contacting skin and eyes with **Proteinase K**/**Lysis/Wash Buffer**. In case of accidental swallowing or contact, please rinse with tap water and seek medical care.

# IV. Materials

## • Table 3. Acov11240 Kit Contents for 480 Assays

| Component | Description | Quantity | Storage |
|---|---|---|---|
| Primer/Probe mixture 124 (PM NEI) | For N gene, E gene, and IC | 4 X 300 µl (10 X) | Store at -20 ºC for up to one year<br><br>Ship at 4 to 8ºC<br><br>Store at 4 to 8ºC for up to 5 days after open<br><br>Avoid freeze-thaw cycle for more than 5 times. |
| Reverse Transcriptase (RT) | For Reverse Transcription | 2 X 300 µl (20 X) | |
| PCR Master Mix (MM) | For polymerase chain reaction | 4 X 1.5 ml(2 X) | |
| Positive Extraction Control (PEC) | RNA mixture of N, E, RdRP, IC | 1 x 200 µl (50 X) | |
| Positive Control (PC) | RNA mixture of N, E, RdRP, IC | 1 x 120 µl (50 X) | |
| RNase-free Water | For component of RT-qPCR | 2 x 1.5 ml | |

## • Table 4. Acov11300Kit Contents for 480 Assays

| Component | Description | Quantity | Storage |
|---|---|---|---|
| Primer/Probe mixture 300 (PM RdRP) | For RdRP gene | 4 X 300 µl (10 X) | Store at -20 ºC for up to one year<br><br>Ship at 4 to 8ºC<br><br>Store at 4 to 8ºC for up to 5 days after open<br><br>Avoid freeze-thaw cycle for more than 5 times. |
| Reverse Transcriptase (RT) | For Reverse Transcription | 2 X 300 µl (20 X) | |
| PCR Master Mix (MM) | For polymerase chain reaction | 4 X 1.5 ml(2 X) | |
| Positive Extraction Control (PEC) | RNA mixture of N, E, RdRP, IC | 1 x 200 µl (50 X) | |
| Positive Control (PC) | RNA mixture of N, E, RdRP, IC | 1 x 120 µl (50 X) | |
| RNase-free Water | For component of RT-qPCR | 2 x 1.5ml | |

## • Table 5. LabTurbo™ Viral DNA/RNA Mini Kit LVX480-500

| Component | Description | Quantity | Storage |
|---|---|---|---|
| Buffer VXL | Lysis buffer | 2 x 150 ml/bottle | Room temperature |
| BE solution | Carrier RNA | 2 x 1.5 ml | Room temperature |
| Proteinase K | For sample lysis | 3 x 4.4 ml/bottle | Room temperature |



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| | | | |
|---|---|---|---|
| Buffer LW1 (concentrated) | Washing buffer | 38 ml/bottle | Room temperature |
| | | 2 x 175 ml/bottle | |
| CCEB | Elution buffer | 1 x 250 ml/bottle | Room temperature |
| Sample 6-Tube Strip | For sample lysis | 4 x 20 strip/PKG | Room temperature |
| Single Elution Tube | For elution | 4 x 120 pics/PKG | Room temperature |
| 6-Adaptor Strip | For extraction | 4 x 20 strip/PKG | Room temperature |
| LVX Column | For extraction | 4 x 120 pics/PKG | Room temperature |

## • Table 6. Required Materials but Not Supplied

| Equipment | Description | P/N |
|---|---|---|
| LabTurbo™ AIO 24 SP-qPCR System | Full automation for COVID-19 testing from sample-in and CT report-out; Throughput 1-24 / batch. | A2410 / A2420 Taigen |
| LabTurbo™ AIO 48 SP-qPCR System | Full automation for COVID-19 testing from sample-in and CT report-out; Throughput 1-48 / batch. | A48S10 / A48S20 Taigen |
| LabTurbo 48 Compact System | Full automation for nucleic acid extraction Throughput 1-48 / batch. | C4810/ C4820 Taigen |
| QuantStudio™ 5 Real-Time PCR System | For RT-qPCR reaction | Thermo Fisher |
| Laboratory Refrigerators 4°C to 10°C | For sample and reagent storage | N/A |
| Laboratory freezers –20°C | For sample and reagent storage | N/A |
| **Reagent** | **Description** | **P/N** |
| LabTurbo™ Viral DNA/RNA Mini Kit | Kit for COVID-19 RNA extraction | LVX480-500 |
| 100% Ethanol | For RNA extraction | N/A |
| **Consumables** | **Description** | **P/N** |
| PCR Tube and Cap | For RT-QPCR reaction | A0130, Taigen |
| Screw Cap Tube (2 ml) | 480/pack | A0220, Taigen |
| LabTurbo™ AIO Filter Tip | For liquid handling (4800) | S0650, Taigen |
| LabTurbo™ 48 Filter Tip | For liquid handling (4800) | S0550, Taigen |
| LabTurbo™ Specimen Collection and Transport Kit | For sampling specimen from nasopharyngeal | LS0610, Taigen |



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# V. Preparations before Running COVID-19 RNA Testing on LabTurbo™ AIO SP-qPCR System

## A. The Preparation of Sample

1. For specimen sampling, storage, shipping, and handling, please follow the guidance issued by the national competent authorities of the Member States of the European Union (EU) or CDC. (https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html).

2. LabTurbo™ AIO can only use sample 6-tube strips (in the kit LVX480-500) or 2 ml screw tubes (A0220) as the tube. Tubes with diameters in 0.75±0.05 mm and a height within 3~6 cm are also compatible.

Step 1: Transfer 0.5 ml buffer/medium of NP swab specimen, 25 µl proteinase K, and 0.5 ml VXL buffer into a tube. Pipet gently 3-5 times. (sample 6-tube strip, VXL buffer, and proteinase K are supplied in the kit LVX480-500)

Step 2: Ready for LabTurbo™ AIO for fully automated COVID-19 RNA assay.

## B. The Preparation of Positive Extraction Control (PEC) and Negative Extraction Control (NEC)

Step 1: Prepare one tube for PEC and another tube for NEC.

Step 2: Transfer 500 µl RNase-free water or negative sample into NEC tubes, and transfer 10 µl of 50x PEC and 490 µl RNase-free water into PEC tubes. Then, add 500 µl VXL buffer and 25 µl proteinase K into both tubes. (Using positive clinical specimen as a PEC in an appropriate concentration is acceptable.)

Step 3a: Ready for LabTurbo™ AIO for fully automated COVID-19 RNA assay.

Step3b: Ready for LabTurbo™ 48 for automated viral RNA extraction

## C. The Preparation of RT-qPCR Reagents

1. Clear and RNase-free dedicated space is needed to prevent contamination. Reagents should avoid direct exposure to light. Each new reagent should be centrifuged before opening.
2. Prepare one screw tube (A0220) for RT-qPCR preparation.
3. The formula is as below:



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## • Table 7. Formula of RT-qPCR Preparation

| Component | Acov11240 | Acov11300 | Total volume of in-use mixture* per test | Total volume for n reaction |
|---|---|---|---|---|
| Reverse transcription mixture **(RT)** | 1.25 µl | 1.25 µl | 19 µl | 19 x (n +3) (+3 are for PEC, NEC, and dead volume) |
| 2X PCR Master Mix **(MM)** | 12.5 µl | 12.5 µl | | |
| Primer/probe mixture 124 **(PM NEI)** | 2.5 µl | - | | |
| Primer/probe mixture 300 **(PM RdRP)** | - | 2.5 µl | | |
| RNase-free water | 2.75 µl | 2.75 µl | | |
| Elute from each extraction | 6 µl | 6 µl | | |
| Total volume for each reaction | 25 µl | 25 µl | | |

***The storage for in-use mixture is at 4 ºC~8 ºC for 5 days.**

## D. The Preparation of Extraction Reagents for Kit LVX480-500

LabTurbo™ Viral DNA/RNA Mini Kit has been approved by TFDA (registration number: TFDA00626) and manufactured under GMP regulation (GMP certificate number: 1339). LabTurbo™ AIO SP-qPCR System and LabTurbo 48 Compact system all equips with active reagent import function to manage the reagent supply in each batch assay and will alert if there is insufficient reagent available. The three reagents, LW1, Ethanol, and CCEB, are stable at room temperature for one month after opening. Please load the reagents with their original bottles to the reagent bottle holders on the system safety door by following the instruction below:

1. Follow the LW1 buffer reconstitute instruction provided on the LW1 reagent label. Add the correct amount of absolute EtOH into the bottle and mix it well. Place the LW1 bottle on the LW1 bottle holder.
2. Add absolute EtOH to the EtOH reagent bottle and place the EtOH bottle on the EtOH bottle holder.
3. Place the CCEB bottle (ready to use) on the CCEB bottle holder.


Note: The first and second bottles are to be left empty.



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# VI. Run COVID-19 RNA Testing on LabTurboTM AIO SP-qPCR System

1. Turn on the machine, and then click the **LabTurbo™ AIO** icon on the desktop to launch the system software.

2. Select the sample numbers and choose the program (For Acov11240 or Acov13000), and then click "**Next**." (Table 8 provides the details of RT-qPCR condition in the program)

3. Enter specimen ID documentation by barcode reader, manual key in, or barcode file import.

4. Follow the worktable instruction on the monitor step by step to place extraction consumables, samples, PEC, NEC, and assay in-use mixtures onto the worktable correctly, and then click "**Next**."

5. Close the safety door. Press "**Start**" to execute fully automated program.

6. When the program finishes, the CT result with sample barcode will display on the monitor and can be exported.

## • Table 8. Conditions for RT-qPCR

| | RT reaction | Heat Activation | Denaturation | Annealing/ Extension |
|---|---|---|---|---|
| **Temperature (ºC)** | 55 | 95 | 95 | 60 |
| **Time (sec)** | 600 | 60 | 10 | 15 |
| **Cycle (s)** | 1 | 1 | 45 | |



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# VII. Results and Interpretation

FAM signal indicates that the N gene of SARS-CoV-2 (COVID-19) has been detected; HEX signal indicates that the E gene of universal coronavirus has been detected; Cy5 signal indicates that the human cell endogenous Internal Control has been detected. The Ct of PEC should be within the range of 25-30 and the Ct of NEC should be higher than 36 for the validation of each batch before result interpretation of each sample. The interpretation of the results of individual samples is shown below:

## • Table 9. Acov11240 Result Interpretation and Actions

| N gene (FAM) | E gene (HEX) | IC (Cy5) | Result | Interpretation and Action |
|---|---|---|---|---|
| **+** (CT < 36) | **+** or **-** | **+** or **-** | Valid | **COVID-19 RNA was detected** |
| **-** (CT > 36) | **+** (CT < 36) | **+** or **-** | Valid | **Universal coronavirus or presumptive COVID-19 RNA was detected*** |
| **-** (CT > 36) | **-** (CT > 36) | **+** | Valid | **COVID-19 RNA was not detected** |
| **-** (CT > 36) | **-** (CT > 36) | **-** (CT > 36) | Invalid | **Repeat extraction and RT-qPCR. If the repeated result remains invalid, consider collecting a new specimen from the patient.** |

*The LabTurbo Acov13000 kit is for the single-plex RT-qPCR assay for the detection of RdRP gene. It is used to confirm and validate the presence of SARS-CoV-2 in specimens with negative N gene detection and positive E gene detection from LabTurbo Acov11240 assay.

## • Table 10. Acov13000 Result Interpretation and Actions

| RdRP gene (FAM) | Result | Interpretation and Action |
|---|---|---|
| **+** (CT < 36) | Valid | **COVID-19 RNA was detected** |
| **-** (CT > 36) | Valid | **COVID-19 RNA was not detected**** |

** For positive E gene detection with negative RdRP detection, please report to appropriate public health authorities.

**Note: Regarding the ambiguous detection results, we recommend reviewing the fluorescent curves following the associated system user manuals and/or repeat testing of that particular sample.**



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# VIII. Performance Evaluation

## 1. Analytical Sensitivity:

To determine the Limit of Detection (LoD) and the analytical sensitivity of the LabTurbo™ AIO COVID-19 RNA Testing Kit, studies were performed following the procedures of the "Run COVID-19 RNA Testing on LabTurbo™ AIO SP-qPCR System" test from sample preparation to detection. The LoD was determined to be the lowest concentration of target genes with 95% detection rate or above. The genomic RNA of heat inactivated SARS-CoV-2 (3.75 x $10^5$ genome copies /µl, ATCC no. VR-1986HK™, lot number 70035039) was spiked and diluted into pooled VTM (Viral transport medium) from NP swab specimens collected from SARS-CoV-2 negative individuals to the several concentrations for LoD determination.

To determine the possible LoD, the concentrations of 200, 1000, 5000, and 25000 copies/ml were tested first. Each concentration was tested in 5 replicates. The minimum concentration with the positive detection rate of 100% was considered as the possible LoD. The results of possible LoDs were 1000, 5000, and 1000 copies/mL for N, E, and RdRP of kit Acov11240 and Acov13000, respectively.

The LoDs were further confirmed by testing 20 replicates at the concentration of 1 x, 2 x, and 4 x of the possible LoD, (1000, 2000, and 4000 copies/mL). The results of the final LoD concentration are summarized in the Tables 11 and 12 below:

### • Table 11. The Final Results of LoD Confirmation Data

| Sample number | N gene 1000 copies/mL | E gene 2000 copies/mL | RdRP 1000 copies/mL | Result | Overall % Positivity |
|---|---|---|---|---|---|
| 1 | 33.2 | 31.3 | 32.4 | + | 100% |
| 2 | 32.1 | 31.1 | 33.0 | + | |
| 3 | 31.6 | 31.4 | 32.1 | + | |
| 4 | 32.5 | 31.0 | 32.8 | + | |
| 5 | 32.3 | 30.9 | 32.8 | + | |
| 6 | 31.3 | 31.8 | 32.4 | + | |
| 7 | 32.7 | 32.0 | 33.1 | + | |
| 8 | 32.2 | 30.6 | 32.6 | + | |
| 9 | 31.8 | 31.2 | 32.4 | + | |
| 10 | 32.1 | 30.6 | 32.8 | + | |
| 11 | 31.1 | 30.9 | 33.4 | + | |
| 12 | 32.6 | 31.8 | 33.6 | + | |
| 13 | 32.2 | 31.4 | 32.1 | + | |
| 14 | 33.0 | 32.3 | 33.7 | + | |
| 15 | 31.4 | 31.2 | 32.5 | + | |
| 16 | 32.5 | 31.4 | 32.4 | + | |
| 17 | 32.1 | 31.3 | 32.5 | + | |
| 18 | 32.7 | 31.2 | 32.8 | + | |



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| | | | | | |
|---|---|---|---|---|---|
| 19 | 32.5 | 32.1 | 32.2 | + | |
| 20 | 32.9 | 32.0 | 32.6 | + | |
| PEC | 26.7 | 29.4 | 30.0 | + | |
| NEC | nd | nd | nd | - | |

**• Table 12. LoD Confirmation Summary**

| | N Gene | E Gene | RdRP | IC |
|---|---|---|---|---|
| **LoD (copies/ mL)** | 1000 | 2000 | 1000 | 100 * |
| **Replicate** | 20 | 20 | 20 | 20 |
| **Detection rate** | 100% | 100% | 100% | 100% |
| **Mean CT** | 32.3 | 31.4 | 32.7 | 30.3 |
| **95% CI** | (31.4; 33.1) | (30.5; 32.3) | (31.8; 33.6) | (29.6.; 31.0) |

The confirmed LoDs for the LabTurbo™ AIO COVID-19 RNA Testing Kits, Acov11240 and Acov13000, are 1000 copies/ml. The confirmed LoDs are 1000, 2000, 1000, and 100 copies/ ml for N, E, RdRP, and IC, respectively.

*The LoD of the IC was determined by synthetic RNA of 100 copies/ml tested in 20 replicates (data not shown).

# 2. Analytical Specificity

## Inclusivity:

### • N gene

There are 8895 entries of SARS-CoV-2 sequences, up until 6/22/2020,from the NCBI (6070 entries) and the GISAID (2825 entries) database have been analyzed for ensuring the specificity of N gene primer/probe set in **LabTurbo™ AIO COVID-19 RNA Testing Kit**. The 8604 entries had a 100% match to the target sequence of the N gene. A single nucleotide mismatch was found in 276 entries, and two nucleotides mismatch were found in 15 entries. Most of the mismatches are close to the 3'-end of the probe or 5'-end of forward/ reverse primer binding region. None of these single or two base mismatches are predicted to impact the performance.

### •E gene

There are 8895 entries of SARS-CoV-2 sequences, up until 6/22/2020,from the NCBI (6070 entries) and the GISAID (2825 entries) database have been analyzed for ensuring the specificity of E gene primer/probe set in **LabTurbo™ AIO COVID-19 RNA Testing Kit**. The 8831 entries had a 100% match to the target sequence of the E gene. A single nucleotide mismatch was found in 57 entries, and two nucleotides mismatch were found in 7 entries. Most of the mismatches are close to the 3'-end of the probe or 5'-end of forward/ reverse primer binding region. None of these single or two base mismatches are predicted to impact the performance.



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## • RDRP gene

There are 8895 entries of SARS-CoV-2 sequences, up until 06/22/2020, from the NCBI (6070 entries) and the GISAID (2825 entries) database have been analyzed for ensuring the specificity of RDRP gene primer/probe set in **LabTurbo™ AIO COVID-19 RNA Testing Kit**. 8846 entries had a 100% match to the target sequence of the RDRP gene. A single nucleotide mismatch was found in 47 entries, and two nucleotides mismatch were found in 2 entries. Most of the mismatches are close to the 3'-end of the probe or 5'-end of forward/ reverse primer binding region. None of these single or two base mismatches are predicted to impact the performance.

Although the in-silico analysis predicts that the specificity for both complete match and single-base mismatch of each gene can be higher than 99.8%, it still cannot completely exclude the false negative results caused by the N gene mutation. This method is designed to multiplex the N gene and the E gene in the same tube for detection. When the detection result of the N gene is negative and the E gene is positive, RdRP (Acov13000) detection can be used as a confirmatory validation of the presence of SARS-CoV-2. The detection method involving detecting N, E and RdRP genes should help avoid false negative results caused by single-gene mutations.

## • Table 13. Specificity Summary

| Target | Total Entries (%) | Complete Match (%) | Single-base Mismatch (%) | Two-base Mismatch (%) |
|---|---|---|---|---|
| N | 8895 (100%) | 8604 (96.7%) | 276 (3.1%) | 15 (0.2%) |
| E | | 8831(99.3%) | 57 (0.6%) | 7 (0.1%) |
| RdRP | | 8846 (99.4%) | 47 (0.5%) | 2 (0.1%) |

# 3. Cross-Reactivity:

## *In-silico* Analysis

The *in-silico* analysis for the N, E, and RdRP primer/probe sets of the **LabTurbo™ AIO COVID-19 RNA Testing Kit** was conducted to assess cross-reactivity. *In-silico* cross-reactivity is defined as positive when the aliment is greater than 80% homology between primer and probe set and any sequence present in the targeted microorganism. The results were as below Table 14, 15, and 16:

## •Table 14. *In silico* cross-reactivity analysis for N gene primer/probe set.

| Strains | N Gene Forward Primer | N Gene Probe | N Gene Reverse Primer |
|---|---|---|---|
| **Human coronavirus 229E** | 50% | 33% | 45% |



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| | | | |
|---|---|---|---|
| **Human coronavirus OC43** | 50% | 41% | 41% |
| **Human coronavirus HKU1** | 50% | 41% | 41% |
| **Human coronavirus NL63** | 45% | 41% | 41% |
| **SARS-coronavirus** | 40% | 91% | 91% |
| **MERS-coronavirus** | 50% | 37% | 41% |
| **Adenovirus type 1** | 45% | 37% | 37% |
| **Adenovirus type 7** | 45% | 37% | 37% |
| **Human Metapneumovirus** | 55% | 37% | 45% |
| **Parainfluenza virus 1** | No alignment* | No alignment* | No alignment* |
| **Parainfluenza virus 2** | No alignment* | No alignment* | No alignment* |
| **Parainfluenza virus 3** | No alignment* | No alignment* | No alignment* |
| **Parainfluenza virus 4** | 40% | 37% | 37% |
| **Influenza A** | 80% | 58% | 58% |
| **Influenza B** | 50% | 50% | 45% |
| **Enterovirus** | 65% | 45% | 50% |
| **Respiratory syncytial virus type B** | 50% | 37% | 33% |
| **Rhinovirus** | 70% | 50% | 45% |
| ***Chlamydia pneumoniae*** | 60% | 50% | 50% |
| ***Haemophilus influenzae*** | 65% | 58% | 50% |
| ***Legionella pneumophila*** | 70% | 54% | 54% |
| ***MycobaCTerium tuberculosis*** | 60% | 50% | 50% |
| ***Streptococcus pneumoniae*** | 65% | 54% | 66% |
| ***Streptococcus pyogenes*** | 70% | 54% | 58% |
| ***Bordetella pertussis*** | 65% | No alignment* | No alignment* |
| ***Mycoplasma pneumoniae*** | 60% | 54% | 45% |
| ***Pneumocystis jirovecii* (PJP)** | 60% | 54% | 54% |
| ***Candida albicans*** | 70% | 58% | 79% |
| ***Pseudomonas aeruginosa*** | 75% | 62% | 54% |
| ***Staphylococcus epidermis*** | 60% | 50% | 50% |
| ***Streptococcus salivarius*** | 45% | 54% | 54% |

## •Table 15. *In silico* Cross-Reactivity Analysis for E Gene



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## Primer/probe Set.

| Strains | E Gene Forward Primer | E Gene Probe | E Gene Reverse Primer |
|---|---|---|---|
| **Human coronavirus 229E** | 46% | 50% | 40% |
| **Human coronavirus OC43** | 42% | 34% | 45% |
| **Human coronavirus HKU1** | 38% | 42% | 40% |
| **Human coronavirus NL63** | 42% | 42% | 45% |
| **SARS-coronavirus** | 100% | 100% | 100% |
| **MERS-coronavirus** | 38% | 38% | 45% |
| **Adenovirus type 1** | 46% | 34% | 45% |
| **Adenovirus type 7** | 34% | 38% | 40% |
| **Human Metapneumovirus** | 42% | 34% | 45% |
| **Parainfluenza virus 1** | No alignment* | No alignment* | No alignment* |
| **Parainfluenza virus 2** | No alignment* | No alignment* | No alignment* |
| **Parainfluenza virus 3** | No alignment* | No alignment* | No alignment* |
| **Parainfluenza virus 4** | 38% | 34% | 59% |
| **Influenza A** | 57% | 46% | 81% |
| **Influenza B** | 42% | 53% | 63% |
| **Enterovirus** | 50% | 53% | 59% |
| **Respiratory syncytial virus type B** | 34% | 38% | 31% |
| **Rhinovirus** | No Alignment* | 50% | 54% |
| ***Chlamydia pneumoniae*** | 42% | 50% | 77% |
| ***Haemophilus influenzae*** | 46% | 53% | 59% |
| ***Legionella pneumophila*** | 53% | 61% | 59% |
| ***MycobaCTerium tuberculosis*** | No Alignment* | 50% | 53% |
| ***Streptococcus pneumoniae*** | 50% | 53% | 77% |
| ***Streptococcus pyogenes*** | 53% | 46% | 59% |
| ***Bordetella pertussis*** | No Alignment* | 53% | 63% |
| ***Mycoplasma pneumoniae*** | 54% | 46% | 54% |
| ***Pneumocystis jirovecii* (PJP)** | 46% | 50% | 50% |
| ***Candida albicans*** | 57% | 50% | 54% |
| ***Pseudomonas aeruginosa*** | 50% | 53% | 68% |
| ***Staphylococcus epidermis*** | 53% | 61% | 59% |



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| | | | |
|---|---|---|---|
| ***Streptococcus salivarius*** | 57% | 50% | 54% |

## •Table 16. *In silico* Cross-Reactivity Analysis for RdRP Gene Primer/probe Set

| Strains | RdRP Gene Forward Primer | RdRP Gene Probe | RdRP Gene Reverse Primer |
|---|---|---|---|
| **Human coronavirus 229E** | 45% | 36% | 38% |
| **Human coronavirus OC43** | 81% | 40% | 81% |
| **Human coronavirus HKU1** | 90% | 52% | 81% |
| **Human coronavirus NL63** | 50% | 32% | 88% |
| **SARS-coronavirus** | 95% | 88% | 97% |
| **MERS-coronavirus** | 50% | 40% | 71% |
| **Adenovirus type 1** | 45% | 36% | 34% |
| **Adenovirus type 7** | 45% | 40% | 42% |
| **Human Metapneumovirus** | 40% | 40% | 42% |
| **Parainfluenza virus 1** | No alignment* | No alignment* | No alignment* |
| **Parainfluenza virus 2** | No alignment* | No alignment* | No alignment* |
| **Parainfluenza virus 3** | No alignment* | No alignment* | No alignment* |
| **Parainfluenza virus 4** | 45% | 40% | 42% |
| **Influenza A** | 54% | 56% | 53% |
| **Influenza B** | 50% | 48% | 42% |
| **Enterovirus** | 63% | 52% | 53% |
| **Respiratory syncytial virus typeB** | 36% | 40% | 38% |
| **Rhinovirus** | 52% | 52% | 50% |
| ***Chlamydia pneumoniae*** | 50% | 52% | 53% |
| ***Haemophilus influenzae*** | 59% | 52% | 53% |
| ***Legionella pneumophila*** | 59% | 56% | 73% |
| ***MycobaCTerium tuberculosis*** | 59% | 48% | No Alignment* |
| ***Streptococcus pneumoniae*** | 54% | 56% | 53% |
| ***Streptococcus pyogenes*** | 59% | 68% | 50% |
| ***Bordetella pertussis*** | 54% | 59% | No Alignment* |
| ***Mycoplasma pneumoniae*** | 54% | 44% | 50% |
| ***Pneumocystis jirovecii* (PJP)** | 59% | 52% | 61% |
| ***Candida albicans*** | 59% | 52% | 65% |



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| | | | |
|---|---|---|---|
| ***Pseudomonas aeruginosa*** | 68% | 60% | 65% |
| ***Staphylococcus epidermis*** | 54% | 48% | 53% |
| ***Streptococcus salivarius*** | 59% | 64% | 65% |

*The target sequences were blasted against NCBI Database and no alignment results were found. No potential unintended cross-reactivity is expected based on this *in silico* analysis.

The *in silico* analysis is performed for analyzing the homology of primer/probe set in 31 organisms. Most of homologies in above organisms are below 80%, indicating no concerns for the occurrence of cross-reactivity. However, the wet testing is still needed to preclude the possibility of cross-reactivity for the organisms that is over 80% homology in *in silico* analysis.

## Wet testing

The organisms with over 80% homology to primer/probe in *in silico* study were further analyzed by wet testing to preclude the occurrence of cross-reactivity or microbial interference with the assay. The wet testing study was performed using COVID-19 RNA Testing kit on LabTurbo™ AIO SP-qPCR System and the potential cross-reactive organisms at concentration in 1 x $10^6$ copies/ml. The organisms listed below were purchased from ATCC. The results were as below Table 17 and 18:

### •Table 17. Wet Testing of Potential Cross-Reactive Respiratory Organisms

| Sample | Source | Sample ID | Assay | Replicates Detected/Total | Result |
|---|---|---|---|---|---|
| Coronavirus OC43 | ATCC | VR-1558D/70033324 | N Gene | 0/3 | Negative |
| Coronavirus HKU1 | ATCC | VR-3262SD/70034816 | | 0/3 | Negative |
| Coronavirus NL63 | ATCC | VR-3263SD/70034817 | | 0/3 | Negative |
| MERS-coronavirus | ATCC | VR-3248SD/70034423 | | 0/3 | Negative |
| Influenza A | ATCC | VR-1738D/59057575 | | 0/3 | Negative |
| Pooled Human Nasal Wash | Nature | | | 0/3 | Negative |

| Sample | Source | Sample ID | Assay | Replicates Detected/Total | Result |
|---|---|---|---|---|---|
| Coronavirus OC43 | ATCC | VR-1558D/70033324 | E Gene | 0/3 | Negative |
| Coronavirus HKU1 | ATCC | VR-3262SD/70034816 | | 0/3 | Negative |
| Coronavirus NL63 | ATCC | VR-3263SD/70034817 | | 0/3 | Negative |
| MERS-coronavirus | ATCC | VR-3248SD/70034423 | | 0/3 | Negative |
| Influenza A | ATCC | VR-1738D/59057575 | | 0/3 | Negative |
| Pooled Human Nasal Wash | Nature | | | 0/3 | Negative |

| Sample | Source | Sample ID | Assay | Replicates Detected/Total | Result |
|---|---|---|---|---|---|
| Coronavirus OC43 | ATCC | VR-1558D/70033324 | RdRP | 0/3 | Negative |



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| | | | | | |
|---|---|---|---|---|---|
| Coronavirus HKU1 | ATCC | VR-3262SD/70034816 | Gene | 0/3 | Negative |
| Coronavirus NL63 | ATCC | VR-3263SD/70034817 | | 0/3 | Negative |
| MERS-coronavirus | ATCC | VR-3248SD/70034423 | | 0/3 | Negative |
| Influenza A | ATCC | VR-1738D/59057575 | | 0/3 | Negative |
| Pooled Human Nasal Wash | Nature | | | 0/3 | Negative |

## •Table 18. Wet Testing of Potential Interference Respiratory Organisms with 3x LOD the Genomic RNA of Heated inactive SARS-CoV-2 virus (3000copies/ml)

| Sample | Source | Sample ID | Assay | Replicates Detected/Total | Result |
|---|---|---|---|---|---|
| Coronavirus OC43 | ATCC | VR-1558D/70033324 | N Gene | 3/3 | Positive |
| Coronavirus HKU1 | ATCC | VR-3262SD/70034816 | | 3/3 | Positive |
| Coronavirus NL63 | ATCC | VR-3263SD/70034817 | | 3/3 | Positive |
| MERS-coronavirus | ATCC | VR-3248SD/70034423 | | 3/3 | Positive |
| Influenza A | ATCC | VR-1738D/59057575 | | 3/3 | Positive |
| Pooled Human Nasal Wash | nature | | | 3/3 | Positive |

| Sample | Source | Sample ID | Assay | Replicates Detected/Total | Result |
|---|---|---|---|---|---|
| Coronavirus OC43 | ATCC | VR-1558D/70033324 | E Gene | 3/3 | Positive |
| Coronavirus HKU1 | ATCC | VR-3262SD/70034816 | | 3/3 | Positive |
| Coronavirus NL63 | ATCC | VR-3263SD/70034817 | | 3/3 | Positive |
| MERS-coronavirus | ATCC | VR-3248SD/70034423 | | 3/3 | Positive |
| Influenza A | ATCC | VR-1738D/59057575 | | 3/3 | Positive |
| Pooled Human Nasal Wash | Nature | | | 3/3 | Positive |

| Sample | Source | Sample ID | Assay | Replicates Detected/Total | Result |
|---|---|---|---|---|---|
| Coronavirus OC43 | ATCC | VR-1558D/70033324 | RdRP Gene | 3/3 | Positive |
| Coronavirus HKU1 | ATCC | VR-3262SD/70034816 | | 3/3 | Positive |
| Coronavirus NL63 | ATCC | VR-3263SD/70034817 | | 3/3 | Positive |
| MERS-coronavirus | ATCC | VR-3248SD/70034423 | | 3/3 | Positive |
| Influenza A | ATCC | VR-1738D/59057575 | | 3/3 | Positive |
| Pooled Human Nasal Wash | Nature | | | 3/3 | Positive |

The wet testing of cross-reactivity and microbial interference studies were performed in triplicate. In the cross-reactivity study, the detections of SARS-CoV-2 are all negative, indicating that the primer/probe sets do not react with potential organisms. In the microbial interference study, the detections of SARS-CoV-2 are all positive, indicating that the co-infection with potential organisms would not interfere with the efficiency of SARS-CoV-2 detection. Thus, the SARS-CoV-2 detection by using this kit would not be interfered by the organisms listed above.

*The results of both cross-reactivity studies were interpreted as negative detections as the CT values were all higher than 36.



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# 4. Interference Substance Study*:*

An Interference Substance study was performed by following the procedures listed on "Run COVID-19 RNA Testing on LabTurbo™ AIO SP-qPCR System" from sample preparation to detection to assess the performance of the assay with common respiratory substances. Contrived specimens were prepared by spiking genomic RNA of heat inactivated SARS-CoV-2 into pooled VTM of NP swab specimens from patients confirmed negative of SARS-CoV-2 to form specimens of 3x LoD. The contrived specimens were tested with interfering substances at relevant concentrations in triplicate. The results are summarized in Table 19 as below:

## • Table 19. Interference Substances Studies

| No. | Interference Substance | Main ingredient | Concentration | Result | |
|---|---|---|---|---|---|
| | | | | Acov 11240 | Acov 13000 |
| 1 | Untreated | | **0** | **+** | **+** |
| 2 | Nasal Spray (Afrin) | Xylometazoline Hydrochloride 1.0 mg/ml | **10% (v/v)** | **+** | **+** |
| 3 | Nasal Spray (CVS Nasal Spray) | Oxymetazoline Hydrochloride 1.0 mg/ml | **10% (v/v)** | **+** | **+** |
| 4 | Nasal Corticosteroid (Rhinocort) | Budesonide Micronized 1.28 mg/ml | **1.0% (w/v)** | **+** | **+** |
| 5 | Nasal Corticosteroid (Flonase) | Fluticasone Furoate 27.5ug/ 50ul | **1.0% (w/v)** | **+** | **+** |
| 6 | Homeopathic Allergy Relief Medicine (Alkolol) | Benzalkonium Chloride 0.0035 % | **10% (w/v)** | **+** | **+** |
| 7 | Throat Lozenge (Cepacol) | Cetylpyridinium Chloride 2 mg/tablet | **10% (w/v)** | **+** | **+** |
| 8 | Oral Anesthetic and Analgesic (Chloraseptic Spray) | Benzydamine HCl 3.0 mg/ml | **10% (w/v)** | **+** | **+** |
| 9 | Antibiotic Nasal Ointment (Mupirocin) | Mupirocin 20 mg/g | **10% (w/v)** | **+** | **+** |
| 10 | AntibaCTerial, Systemic (Tobramycin) | Tobramycin 500 mg/Caps. | **10% (w/v)** | **+** | **+** |
| 11 | AntibaCTerial, Systemic (Amoxicillin) | Amoxicillin 500 mg/Caps. | **10% (w/v)** | **+** | **+** |
| 12 | Antiviral (Oseltamivir) | Oseltamivir phosphate 75 mg/caps. | **10% (w/v)** | **+** | **+** |
| 13 | Sputum | | **10% (v/v)** | **+** | **+** |
| 14 | Whole Blood | | **10% (v/v)** | **+** | **+** |
| 15 | Stool | | **1.0% (w/v)** | **+** | **+** |
| 16 | Concentrated IC (synthetic RNA) | | **1 x $10^9$ copies/ml** | **+** | **+** |



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The interference substance study with interfering substances at relevant concentrations in triplicate demonstrated that the positive detection rate were all 100% at 3X LoD by LabTurbo™ AIO SP-qPCR System from sample preparation (LVX480-500) to detection (kit Acov11240 and Acov13000). The results showed that the nucleic acid can be effectively purified from interfering substances by LabTurbo™ Viral DNA/RNA Mini Kit (LVX480-500).

# 5. Clinical Evaluation

In the clinical evaluation study, the individual leftover clinical NP swab samples were from two independent laboratories (50 positive / 7 negative and 76 positive / 109 negative, respectively). A total 242 specimens, including 126 positive and 116 negative, were tested with the **LabTurbo™ AIO COVID-19 RNA Testing Kit using the LabTurbo™ AIO system**. According to the results, as displayed in Table 20, both the Positive Percent Agreement, (PPA), and Negative Percent Agreement, (NPA), are greater than 99% by using Acov11240 and Acov13000 kits. Hence, the LabTurbo™ COVID-19 RNA Testing Kit proved to be a reliable method for detecting the COVID-19 RNA in clinical evaluations.

## • Table 20. Summary of Clinical Study

| N gene (Acov11240)<br>RdRP Gene (Acov13000) | | Individual leftover clinical specimens tested by EUA RT-PCR assay from two independent laboratories<br>Comparator device:<br>**CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel** | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| **LabTurbo™ AIO COVID-19 RNA Testing Kit** | Positive | 125 | 0 | 125 |
| | Negative | 1 | 116 | 117 |
| | Total | 126 | 116 | 242 |
| Positive Percent Agreement | | 99.2%*(125/126) *Criteria: 97-100% | | |
| Negative Percent Agreement | | 99.1%*(116/117) *Criteria: 97-100% | | |

# IX The Bridge Study

(1) The bridge study was conducted in four independent laboratories, including three sets of LabTurbo™ AIO 48 and three sets of LabTurbo™ AIO 24, a total of six systems. This test is based on the LoD at 1000 copies/ml for this kit. Four concentrations at 4000, 2500, 1000 and 200 copies/ml were tested respectively; each concentration is tested in five replicates. The samples were prepared by spiking the genomic RNA of heat inactivated SARS-CoV-2 ($3.75 \times 10^5$ genome copies /µl, ATCC no. VR-1986HK™, lot number 70035039) into VXL buffer and pooled NP swab specimens from SARS-CoV-2 negative individuals with the aforementioned concentration. According to results shown below in Table 21, the performances of the six LabTurbo™ AIO systems, as tested at different laboratories were considered as "comparable". The lowest



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concentration with five positive replicates in IC, E, N and RdRP genes is 1000 copies/ml, which is consistent with the performance of Acov12400 and Acov13000 and pass the acceptance criteria (3x LoD= 3000 copies/ml) of the bridge study.

## • Table 21. The Results of Bridge Study

| Concentration | IC | E | N | RdRP | Result |
|---|---|---|---|---|---|
| 4000 copies / ml | Overall 100% Positive detection from all six LabTurbo™ AIO systems | | | | Pass |
| 2500 copies / ml | Overall 100% Positive detection from all six LabTurbo™ AIO systems | | | | Pass |
| 1000 copies / ml | Overall 100% Positive detection from all six LabTurbo™ AIO systems | | | | Pass |

(2) The bridge study was performed on semi-automation system by utilizing LabTurbo 48 Compact System, made by Taigen Biosciences, for RNA extraction, in conjunction with the QuantStudio™ 5 Real-Time PCR Systems, made by ThermoFisher, for RT-qPCR reaction to test the efficiency of LabTurbo™ AIO COVID-19 RNA Testing Kit. The samples were prepared by spiking the genomic RNA of heat inactivated SARS-CoV-2 ($3.75 \times 10^5$ genome copies /µl, ATCC no. VR-1986HK™, lot number 70035039) into VXL buffer and pooled NP swab specimens from SARS-CoV-2 negative individuals to be the recommended concentration. The method was detailed below in Table 22.

## • Table 22. The Assay Method for the Bridge Study

| Step | | Description | | |
|---|---|---|---|---|
| Sample Identification | | Manual barcode key in, barcode reader, barcode file import | | |
| Viral RNA Extraction | | Instrument:LabTurbo 48 Compact System<br>Reagent: LVX480-500:<br>Input 500 µl and Elution 60 µl | | |
| RT-qPCR Setup | | Reagent: Acov11240 / Acov13000<br>Template 3.5 µl + in use mixture 11.5 µl = 15 ul | | |
| RT-qPCR Reaction | | Instrument:QuantStudio™ 5 Real-Time PCR Systems (ThermoFisher) | | |
| **Program** | **RT Reaction** | **Heat Activation** | **Denaturation** | **Annealing/Extension** |
| **Temperature (ºC)** | 55 | 95 | 95 | 60 |
| **Time (sec)** | 600 | 60 | 10 | 15 |
| **Cycle (s)** | 1 | 1 | 45 | |

In Table 23, the semi-automated method results were comparable to the fully automated method. The lowest level of 250 copies / ml, at which 4/4 replicates were all positive within 3-fold (3000 copies / ml) of the claimed LoD (1000 copies / ml) for the kit Acov11240 and 13000.

## • Table 23. The Results of the Bridge Study

| Concentration | Tested Kits: Acov11240 and Acov13000 | Result |
|---|---|---|



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| | | |
|---|---|---|
| 5000 copies / ml | Overall 100% Positive detections for all 4 replicates | Pass |
| 2500 copies / ml | Overall 100% Positive detections for all 4 replicates | Pass |
| 1000 copies / ml | Overall 100% Positive detections for all 4 replicates | Pass |
| 250 copies / ml | Overall 100% Positive detections for all 4 replicates | Pass |

# X. Quality Control

- The performance of LabTurbo™ AIO COVID-19 RNA Testing Kit is inspected and tested routinely on a lot-to-lot basis in our QC laboratory. All components of the product are validated by visual, physical, RNase free, and functional tests with appropriate sample types, reagents, physical, and biochemical methods prior to the shipments.
- Test all positive controls prior to running diagnostic samples with each new kit lot to ensure all reagents and kit components are working properly.
- Always include a negative control (NC or NEC) and all positive controls (PC, PEC, IC) in each PCR run for clinical samples to confirm the quality of workflow including specimen collection, RNA extraction, Reaction setup, and RT-qPCR performance.

# XI. Limitations

- The test was limited for use with nasopharyngeal (NP) swab specimen.
- False negative results might be caused by incorrect specimen sampling, handling, and transportation.
- False positive results might be caused by specimen cross-contamination during sampling or handling and/or RT-qPCR reagents contamination.
- Do not use any expired reagent for testing.



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# XII. Reference


1. **Novel rapid identification of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by real-time RT-PCR using BD Max Open System in Taiwan**
   **https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7305773/**
   **PeerJ. 2020; 8: e9318; PMID: 32596046**
   Nucleic acid extraction Total nucleic acid containing viral RNA was extracted from 0.3 mL of the **throat swab supernatant** or **liquefied sputum**, by using LabTurbo Viral nucleic acid extraction kits on a LabTurbo 48 automatic extractor (Taigen Bioscience Corp., Taipei, Taiwan). RNA was eluted with 60 µL of RNase-free water.
2. **Culture-Based Virus Isolation to Evaluate Potential Infectivity of Clinical Specimens Tested for COVID-19**
   **J Clin Microbiol. 2020 Aug; 58(8); PMID: 32518072**
   **https://jcm.asm.org/content/jcm/early/2020/06/08/JCM.01068-20.full.pdf**
   "RNA was extracted from clinical specimens by the automatic LabTurbo system (Taigen, Taiwan)".
3. **Incidence of and Factors Associated with False Positives in Laboratory Diagnosis of Norovirus Infection by Amplification of the RNA-Dependent RNA Polymerase Gene**
   **PLoS One. 2014; 9(9): e109876; PMID: 25264621**
   **https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4181653/**
   Preparation of **stool samples** and viral RNA extraction During the period of study, a LabTurbo Mini Kit (Cat. No. LVN480-300, Taigen Bioscience Corporation, Taipei, Taiwan) was used to extract viral RNA in the clinical virology laboratory. The procedure was accomplished with the automatic nucleic acid extraction system LabTurbo 48 Compact (Taigen Bioscience Corporation, Taipei, Taiwan).
4. **Screening for *Babesia microti* in the U.S. Blood Supply**
   **N Engl J Med 2016; 375:2236-2245; DOI: 10.1056/NEJMoa1600897**
   **https://www.nejm.org/doi/full/10.1056/nejmoa1600897**
   DNA was extracted with the use of an automated membrane-based isolation system (Taigen Bioscience). PCR primers and probes targeted the *B. microti* 18S ribosomal RNA gene; the 95% lower limit of detection was 66 piroplasms per milliliter.



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