



iAMP[®] COVID-19 Detection Kit

REF iAMP-COVID19-100 Instructions For Use

Rx Only

**For use under an
Emergency Use Authorization (EUA) only**

IVD For In Vitro Diagnostic Use



V2.0
July 2020

Table of Contents

SYMBOLS	3
INTENDED USE	4
SUMMARY AND EXPLANATION OF THE TEST	4
TEST WORKFLOW OVERVIEW	5
KIT COMPONENTS (100 Reactions).....	5
EQUIPMENTS & MATERIALS REQUIRED BUT NOT SUPPLIED with the kit.....	6
KIT STORAGE INFORMATION	7
SPECIMENS.....	7
Biosafety Precautions.....	7
Acceptable Specimens	7
Specimen Handling and Storage	7
Specimen Rejection criteria	7
WARNING and PRECAUTIONS	8
QUALITY CONTROL	8
General Considerations.....	8
Assay Controls	9
TEST PROCEDURE	9
Specimen Processing.....	9
Reaction assembly:	9
Run setup:	10
A. Using Biorad CFX96 Manager Software (3.0 or higher) connected to the Biorad CFX96 Real-Time PCR System:	10
B. Using Roche LightCycler 480 Software Release 1.5.0 connected to the Roche LightCycler® 480 Instrument II Real-Time PCR System:	11
C. Using PowerGene9600 Software connected to the Atila PowerGene 9600 Plus Real-Time PCR System:	13
D. Using Applied Biosystems 7500 Software connected to the Applied Biosystems™ 7500 Real-Time PCR Systems:	16
RESULT INTERPRETATION.....	18
1. INTERPRETATION OF TEST CONTROLS.....	18
iAMP COVID-19 Detection Kit Controls – Positive, Negative.....	18
2. EXAMINATION AND INTERPRETATION OF PATIENT SPECIMEN RESULTS:	18
PERFORMANCE CHARACTERISTICS.....	21
Limit of Detection (LoD) – Analytical Sensitivity	21

a. Tentative LoD Study.....	21
b. Confirmatory LoD Study	22
Inclusivity.....	22
Cross-Reactivity (Analytical Specificity)	22
Endogenous Interference Substances Studies:	24
Clinical Evaluation	25

IMPORTANT NOTICE

The instruction for use must be read carefully prior to use and followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from these instructions.

SYMBOLS



Consult instructions for use



In vitro diagnostic medical device



Temperature limitation



Sufficient for <n> tests



Use by



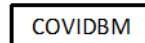
Catalogue number



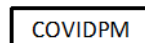
Batch code



Manufacturer



Buffer Mix



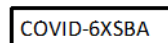
Primer Mix



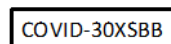
Negative control



Positive control



6X Sample Buffer A



30X Sample Buffer B



Reaction Mix

INTENDED USE

The iAMP COVID-19 Detection Kit is a real-time fluorescent reverse transcription isothermal assay intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasal, nasopharyngeal (NP), and oropharyngeal (OP) swabs from patients suspected of COVID-19 by their health care provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens 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. 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 iAMP COVID-19 Detection Kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time nucleic acid amplification and in vitro diagnostic procedures. The iAMP COVID-19 Detection Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

SUMMARY AND EXPLANATION OF THE TEST

The iAMP COVID-19 Detection Kit is a real-time reverse transcription isothermal amplification test. The test is based on a proprietary isothermal amplification technology termed OMEGA amplification (Patent: WO 2017/205510 A1; publication: The Journal of Molecular Diagnostics, Vol.22, No 3, 419-428, 2020). OMEGA primer sets are designed to specifically detect RNA and later cDNA from the N and ORF-1ab genes of the SARS-CoV-2 virus in nasal, nasopharyngeal and/or oropharyngeal swabs from patients with signs and symptoms of infection who are suspected of COVID-19.

The iAMP COVID-19 assay's key differentiator from current rRT-PCR COVID-19 assays is its ability to detect SARS-CoV-2 RNA directly from samples without prior RNA extraction process. Swab specimens are inserted directly into our 1X iAMP COVID-19 Sample Buffer Mix with a 15 min incubation at room temperature and can be directly used for OMEGA isothermal amplification and signal detection. Sample to result takes less than 1.5 hours.

Primers and probes are designed to detect nucleic acid sequences from the nucleocapsid (N) gene and the ORF-1ab gene (one target sequence for each gene). Both genes are

detected within the same reaction vessel in the FAM channel without being differentiated. Primers and probes targeting the human *Gapdh* gene are also used on each sample as an internal control, and amplification signal is detected in HEX channel.

Both reverse transcription and nucleic acid amplification take place at 61°C. Target sequence in the specimens is amplified with N/ORF-1ab primer sets that are specific to SARS-CoV-2. During the amplification, fluorescence resonance energy transfer (FRET) probes can be incorporated in the amplification products. Upon the incorporation, fluorescence is generated and can be monitored by the fluorescence reader in a real time fashion.

TEST WORKFLOW OVERVIEW

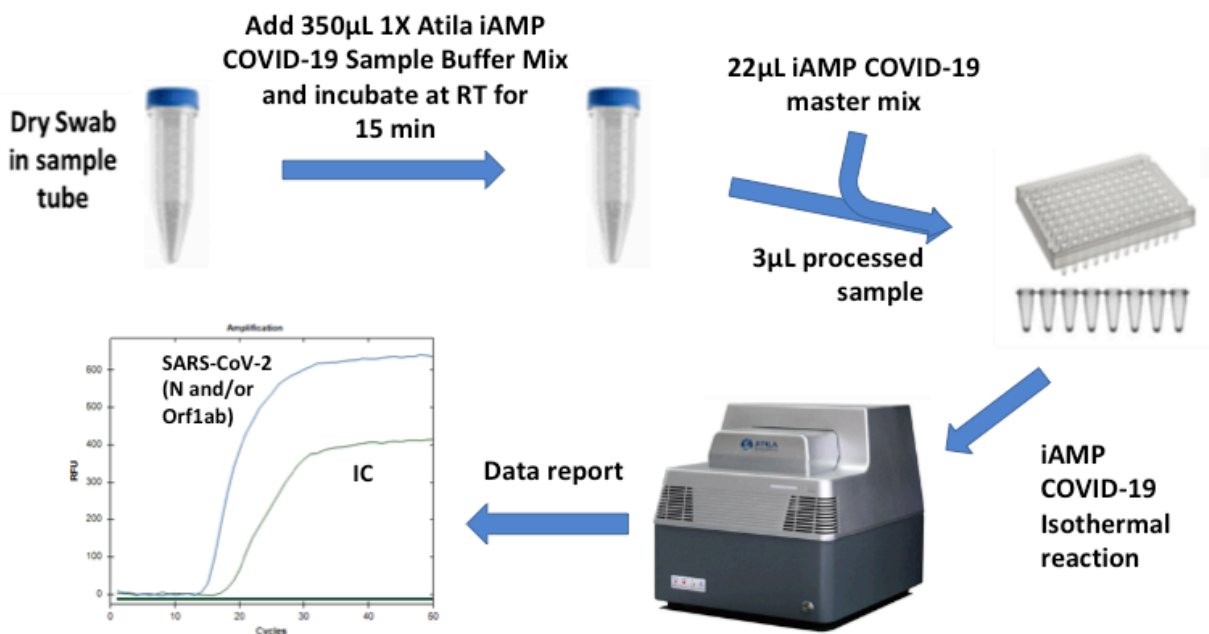


Figure 1. Test workflow.

KIT COMPONENTS (100 Reactions)

- | | |
|---|-----------------|
| 1. Primer Mix (COVIDPM) | 540 µL X 1 tube |
| 2. Buffer Mix (COVIDBM) | 540 µL X 1 tube |
| 3. Reaction Mix (COVIDRM) | 1.3 mL X 1 tube |
| 4. Positive Control Template (COVIDPC) | 300 µL X 1 tube |
| 5. Negative Control Template (COVIDNC) | 300 µL X 1 tube |
| 6. Atila Sample Collection Device (iAMP-COVID19-SCD) | 100 units |
| <ul style="list-style-type: none"> • Synthetic fiber swabs with plastic shafts (100 individually packed swabs for the swab type as indicated) • Collection tubes (100 tubes for each kit) | |

- 6X Atila COVID-19 Sample Buffer A (COVID-6XSBA, 1.25 mL X 5 tubes enclosed in the kit)
 - 30X Atila COVID-19 Sample Buffer B (COVID-30XSBB, 240 µL x 5 tubes enclosed in the kit).
7. Instructions for use 1 booklet

EQUIPMENTS & MATERIALS REQUIRED BUT NOT SUPPLIED with the kit

1. Water: nuclease-free H₂O (ThermoFisher 10977015, or equivalent)
2. One of the following validated Real-time PCR system with **FAM/HEX** fluorescence channels: Biorad CFX96 Real-Time System, Roche LightCycler® 480 Instrument II, Atila PowerGene 9600 Plus Real-Time PCR System, and Applied Biosystems™ 7500 Real-Time PCR Systems.
3. PCR strips, cap strips, plates, and plate sealing film compatible with CFX96 Real-Time System.
 - PCR strips (MicroAmp™ Fast Reaction tubes, ABI, 4358293)
 - PCR cap strips (MicroAmp™ Optical 8-Cap Strips, ABI, 4323032)
 - PCR plates (Multiplate™ 96-Well PCR Plates, high profile, unskirted, clear; Biorad, MLP9601)
 - PCR Plate Sealing Film (Microseal 'B' PCR Plate Sealing Film, adhesive, optical; Biorad, MSB1001)
4. PCR strips, cap strips, plates, and plate sealing film compatible with Roche LightCycler® 480 Instrument II .
 - PCR strips and caps (LightCycler 8-tube strips, white, Roche, 06612601001)
 - PCR plates and sealing film (LightCycler 480 Multiwell Plate with foils, Roche, 05102413001)
5. PCR strips, cap strips, plates, and plate sealing film compatible with Atila PowerGene 9600 Plus Real-Time PCR System.
 - PCR strips and caps (8-tube strips, Atila BioSystems, Powerstrip)
 - PCR plates (96 well PCR plate, Atila BioSystems, Powerplate)
 - PCR plate sealing film (Atila Biosystems, SEAL)
6. PCR strips, cap strips, plates, and plate sealing film compatible with Applied Biosystems™ 7500 Real-Time PCR Systems.
 - PCR strips (MicroAmp™ Fast Reaction tubes, ABI, 4358293)
 - PCR cap strips (MicroAmp™ Optical 8-Cap Strips, ABI, 4323032)
 - PCR plates (MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL, ABI, 4346906)
 - PCR plate sealing film (MicroAmp™ Optical Adhesive Film, ABI, 4311971)
7. Vortex mixer or equivalent
8. PCR tube/plate holder
9. 1.5 mL and 2 mL microcentrifuge tubes and racks
10. Surface decontaminants
11. Adjustable pipettes with corresponding filter-plugged pipette tips
12. Disposable powder-free gloves and other personal protective equipment

KIT STORAGE INFORMATION

All kit reagents (**COVIDBM, COVIDPM, COVIDRM, COVIDNC, COVIDPC, 6X COVID-SBA, and 30X COVID-SBB**) should be stored at -20°C freezer for long time storage. Shelf life of the kit is currently assigned as 1 year and the open kit stability is 6 months when kit is properly stored.

Swabs and collection tubes can be stored at room temperature until expiration date.

SPECIMENS

Biosafety Precautions

Wear appropriate personal protective equipment (e.g. gowns, gloves, eye protection) when working with clinical specimens. Specimen processing should be performed in a certified class II biological safety cabinet following biosafety level 2 or higher guidelines. For more information, refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation for COVID-19 (<https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html>).

Acceptable Specimens

Nasal Swabs, nasopharyngeal swabs and/or oropharyngeal swabs collected dry with the Atila Sample Collection Device (iAMP-COVID19-SCD; provided in the kit).

After sample collection, immediately insert swab into the collection tube provide with the Atila COVID-19 Sample Collection Device.

Note: Standard precautions should be taken with regard to sample collection, handling, and storage prior to sample processing

Specimen Handling and Storage

- Use freshly collected specimens for optimal test performance.
- Specimens can be stored at room temperature for up to 12 hours, or -20°C for up to 2 days after collection and before sample processing.
- If a delay in sample processing is expected, store **dry swab specimens** at -70°C or lower. Avoid free-thaw cycles of the specimens.
- As soon as specimens are added to the 1x iAMP COVID-19 Sample Buffer Mix (i.e., called “processed specimens”) they need to be tested within 2 hours and may not be stored.

Specimen Rejection criteria

- Specimens not stored properly as instructed.
- Incomplete specimen labeling or documentation.
- Inappropriate specimen type.

WARNING and PRECAUTIONS

- For use under FDA Emergency Use Authorization only based on FDA's review policies.
- Federal Law restricts this device to sale by or on the order of a licensed practitioner.
- For in vitro diagnostic use (IVD).
- This test has not been FDA cleared or approved; the test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet the requirements to perform high complexity tests.
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- Modifications to assay reagents, assay protocol, or instrumentation are not permitted, and are in violation of the product Emergency Use Authorization.
- This test is intended to be used with the Atila sample collection device provided in the kit. Testing of other samples collected with other collection devices may result in inaccurate results.
- Samples and controls should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- Follow necessary precautions when handling specimens. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
- Samples must be collected, transported, and stored using appropriate procedures and conditions.
- Reagents must be stored and handled as specified in these instructions for use. Do not use the kit after the indicated expiry date.
- Do not eat, drink, smoke, or apply cosmetic products in the work areas.
- Dispose of waste in compliance with local, state, and federal regulations.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

QUALITY CONTROL

Due to the sensitivity of iAMP COVID-19 reaction, these assays should be conducted using strict quality control and quality assurance procedures. Following the guidelines below will help minimize chance of false-positive or false-negative amplification.

General Considerations

- Personnel must be familiar with the protocol and equipment/instruments used.
- Maintain separate areas and dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g., microcentrifuge tubes, pipette tips, gowns and gloves) for assay reagent setup and handling of processed samples.
- Workflow must always be from the clean area to the dirty area.

- Wear clean disposable gowns and new powder-free gloves during assay reagent setup and handling of processed samples. Change gloves whenever contamination is suspected.
- Do not use reagents beyond their expiry dates.
- Keep reagent tubes and reactions capped as much as possible.
- Clean and decontaminate surfaces.
- Do not bring processed samples or reaction products into the assay setup area.
- Always use aerosol barrier (filter) pipette tips. Tips that are used must be sterile and free from DNases and RNases.

Assay Controls

Assay controls should be tested concurrently with all test samples in each instrumental run.

- PC - positive template control with an expected Ct value range, serves as a control for amplification and detection of SARS-CoV-2 RNA.
- NC - negative template control, serves to verify that analyte contamination does not occur during reaction setup.

TEST PROCEDURE

Specimen Processing

1. From the iAMP COVID-19 Detection Kit, take 6X iAMP COVID-19 Sample Buffer A (COVID-6XSBA) and 30X iAMP COVID-19 Sample Buffer B (COVID-30XSBB) out. Each tube contains enough volume to process 20 dry swabs. Only thaw the number of COVID-6XSBA and COVID-30XSBB tubes that will be enough for each round of sample processing. After complete thaw, put all reagents on ice.
2. Make 1X iAMP COVID-19 Sample Buffer Mix by mixing

COVID-6XSBA	(60 x N) μ L
COVID-30XSBB	(12 x N) μ L
nuclease-free H ₂ O	(288 x N) μ L

N represents the number of dry swab specimens to be processed in one run
3. Load 350 μ L 1X iAMP COVID-19 Sample Buffer Mix into each sample tube (Atila Sample Collection Tube with the tested swab inside). Seal the tube cap securely and vortex briefly.
4. Place the sample tube on the bench for 15 min.

Note: Make sure to use freshly prepared 1X Sample Buffer Mix for dry swab sample processing.

Reaction assembly

1. From the kit, take Buffer Mix (COVIDBM), Primer Mix (COVIDPM), Reaction Mix (COVIDRM), Positive Control (COVIDPC), and Negative Control (COVIDNC) out. After complete thaw, put all reagents on ice.

2. Prepare iAMP COVID-19 master mix by mixing the following components in a 1.5 mL centrifuge tube:

COVIDBM	5.2 X (N+2) μ L
COVIDPM	5.2 X (N+2) μ L
COVIDRM	12.48 X (N+2) μ L

N represents number of specimen samples to be tested. Gently vortex and spin;

3. Take N+2 PCR tubes (tube strips or PCR plate that are compatible with the instrument used) and add 22 μ L iAMP COVID-19 master mix to the bottom of each of the PCR tubes.
4. Briefly spin the tubes to bring down the liquid to the bottom of the tubes. Add 3 μ L of processed specimen samples (Sample #1 to #N) from step of "Specimen Processing" to corresponding reaction PCR tubes from above Step 3. For the negative control, add 3 μ L of Negative Control into reaction tube #(N+1). For the positive control, add 3 μ L of Positive Control Template into reaction tube #(N+2).
5. Cap all the tubes securely (if using 96-Well PCR Plates, seal the plate using sealing film that is compatible with the PCR plate). Gently vortex the tubes to mix all the reagents.
6. Briefly spin the tubes in a centrifuge to bring down all the liquid to the bottom of the wells.

Run setup

A. Using Biorad CFX96 Manager Software (3.0 or higher) connected to the Biorad CFX96 Real-Time PCR System

1. Open the Biorad CFX Manager, and program the following setting:

Step	Cycle	Temperature	Time
Denaturation	1	61 °C	30 sec
cDNA synthesis, isothermal amplification, and signal detection	50	61 °C	1 min

Table 1. Thermal Profile for cDNA and Isothermal Amplification

Fluorescence reading is taken at the **FAM/HEX** channels at the end of each cycle using the default instrument settings of the Biorad CFX96 instrument.

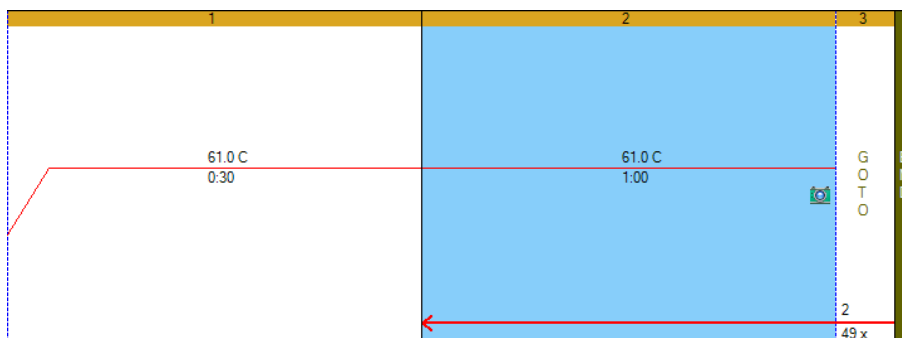


Figure 2. Program set-up using Biorad CFX Manager.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Unk FAM HEX NC											
B	Unk FAM HEX PC											
C	Unk FAM HEX Sample ID 1											
D	Unk FAM HEX Sample ID 2											
E	Unk FAM HEX Sample ID 3											
F	Unk FAM HEX Sample ID 4											
G	Unk FAM HEX Sample ID 5											
H	Unk FAM HEX Sample ID 6											

Figure 3. Selection of fluorophores and typing NC, PC, and sample ID using Plate Editor in Biorad CFX Manager.

- Put the reaction tubes into the sample holder in the real-time PCR machine, and make sure that the tubes are placed according to the plate setup. Then start the reaction run.
- After the run, take out the sample tubes/plate and discard them immediately into a sealable zip bag. To avoid contamination **DO NOT OPEN THE REACTION TUBE AFTER THE REACTION** and do not bring tubes in the sample processing area.

B. Using Roche LightCycler 480 Software Release 1.5.0 connected to the Roche LightCycler® 480 Instrument II Real-Time PCR System

- Open the LightCycler 480 Software Release 1.5.0
- In Detection Format, choose Dual Color Hydrolysis Probe/UPL Probe. And in the Customize window, choose FAM (483-533) and VIC/HEX/Yellow555 (523-568) as shown in Figure 4.

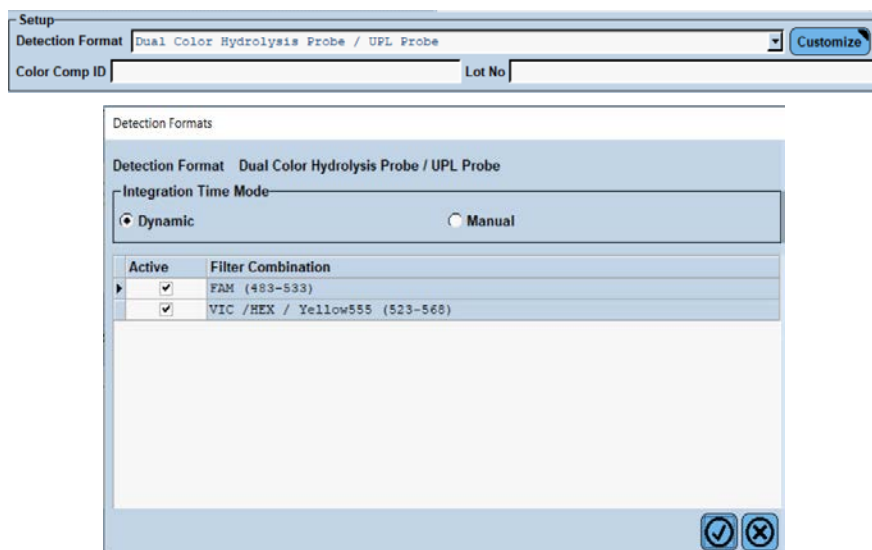


Figure 4. Detection Format setup using LightCycler 480 Software.

3. Setup program according to the following Table 2 and Figure 5.

Step	Cycle	Temperature	Time
Denaturation	1	61 °C	30 sec
cDNA synthesis, isothermal amplification, and signal detection	50	61 °C	1 min

Table 2. Thermal Profile for cDNA and Isothermal Amplification

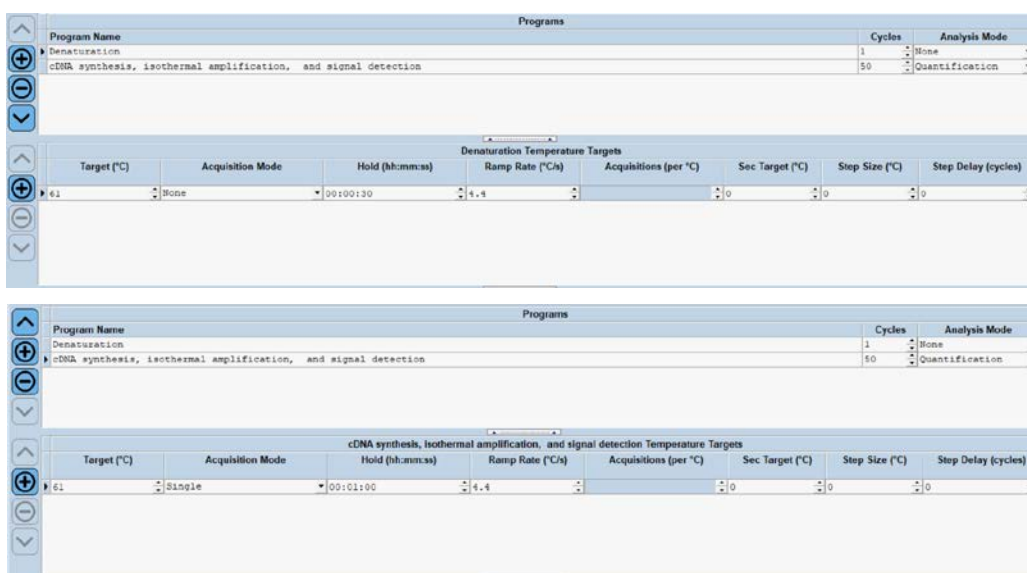


Figure 5. Program set-up using LightCycler 480 Software Release 1.5.0.

- Input sample information as shown in Figure 6, including negative control (NC) and positive control (PC). Assign sample information to the well location on 96 well plate.

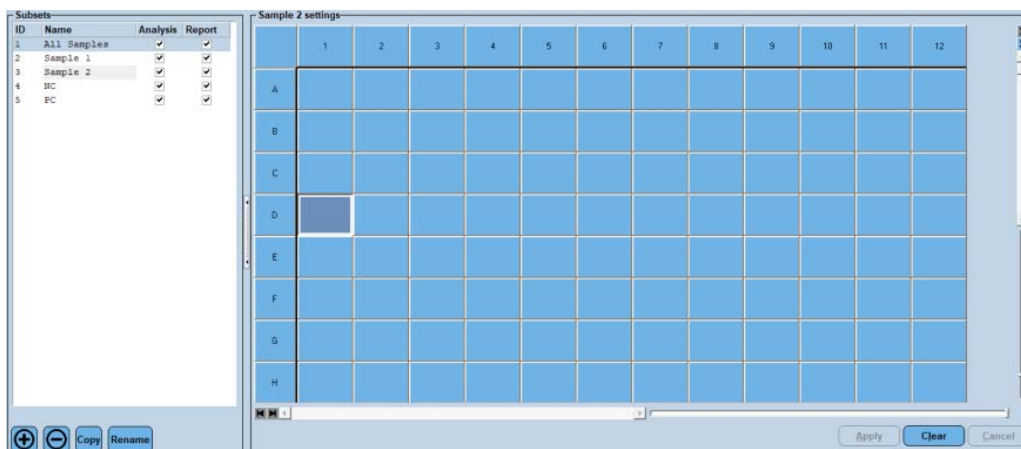


Figure 6. Sample information setup using LightCycler 480 Software Release 1.5.0.

- Put the reaction tubes into the plate loader in the real-time PCR machine, and make sure that the tubes are placed according to the Plate Editor setup.
- Close the plate loader, and start the reaction run by clicking Start Run.
- After the run, take out the sample tubes/plate and discard them immediately into a sealable zip bag. To avoid contamination **DO NOT OPEN THE REACTION TUBE AFTER THE REACTION** and do not bring tubes in the sample processing area.

C. Using PowerGene9600 Software connected to the Atila PowerGene 9600 Plus Real-Time PCR System

- Open the PowerGene9600 Software
- Setup Detector as shown in Figure 7.

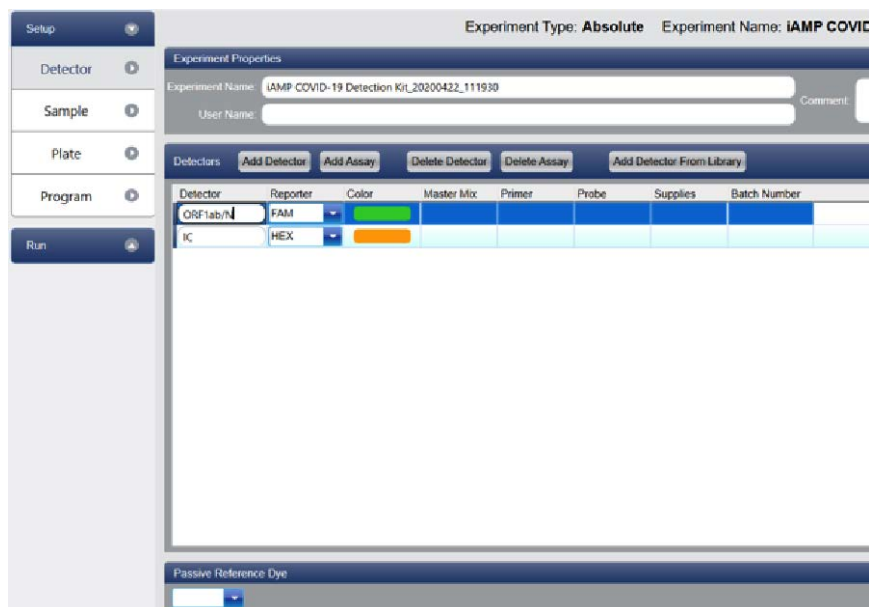


Figure 7. Detector setup.

- Input sample information as shown in Figure 8, including negative control (NC) and positive control (PC).

Figure 8. Sample information setup.

- Assign sample information to the well location on 96 well plate, as shown in Figure 9.

Figure 9. Selection of detectors and NC, PC, and sample information.

- Setup program according to the following Table 3 and Figure 10. Fluorescence reading is taken at the **FAM/HEX** channels at the end of each cycle using the default instrument settings of the PowerGene 9600 Plus instrument.

Step	Cycle	Temperature	Time
Denaturation	1	61 °C	30 sec
cDNA synthesis, isothermal amplification, and signal detection	50	61 °C	1 min

Table 3: Thermal Profile for cDNA and Isothermal Amplification

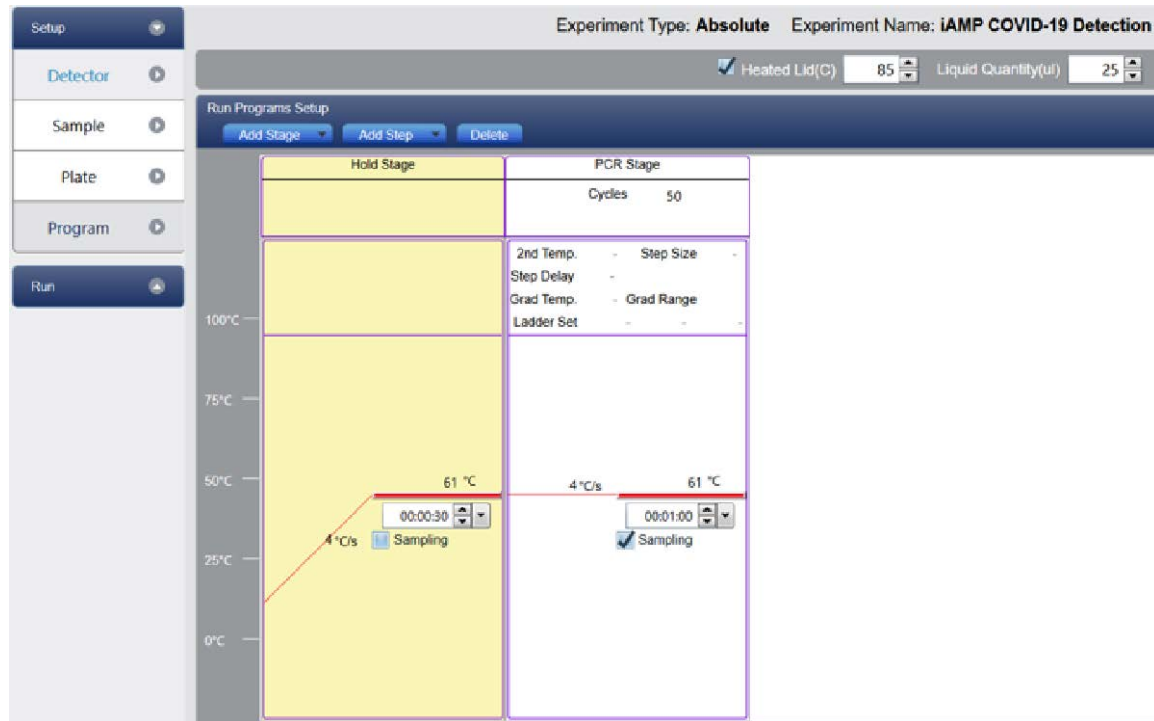


Figure 10. Program set-up using PowerGene 9600 Software.

- Put the reaction tubes into the sample holder in the real-time PCR machine, and make sure that the tubes are placed according to the Plate Editor setup.
- Close the lid, and start the reaction run by clicking Run and Start Run.
- After the run, take out the sample tubes/plate and discard them immediately into a sealable zip bag. To avoid contamination **DO NOT OPEN THE REACTION TUBE AFTER THE REACTION** and do not bring tubes in the sample processing area.

D. Using Applied Biosystems 7500 Software connected to the Applied Biosystems™ 7500 Real-Time PCR Systems

1. Open the 7500 Software. Click “New Experiment”.
2. Setup the Experiment Properties. Name the experiment and select the correct instrument model. For Experiment Type, choose “Quantitation-Standard Curve”. For Reagents, choose “Taqman® Reagents”. For Ramp Speed, choose “Standard”.
3. Setup Plate as shown in Figure 11. Define Targets on target names and reporters. Edit sample information, including negative control (NC) and positive control (PC)

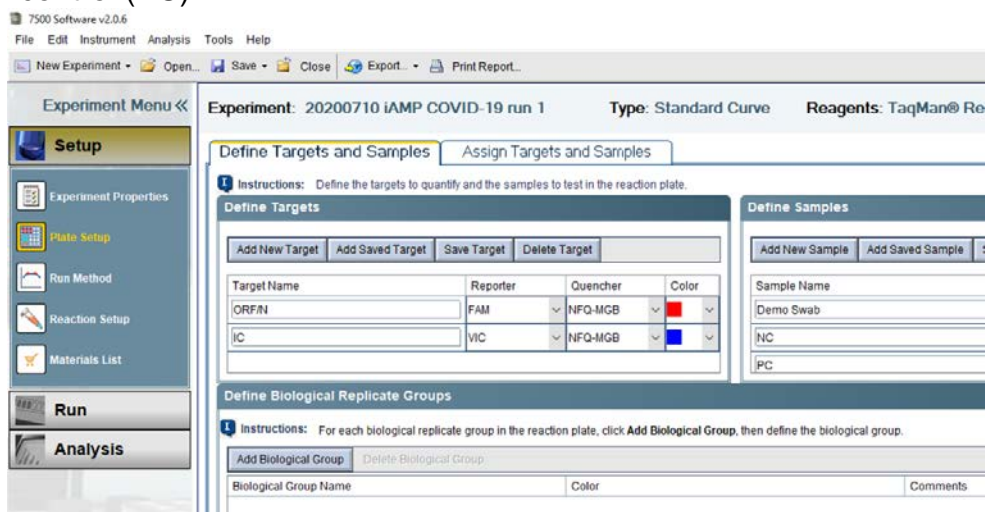


Figure 11. Plate setup.

4. Assign target and sample information to the well location on 96 well plate, as shown in Figure 12. For the passive reference, select “None”. There is no need to define and set up standards.

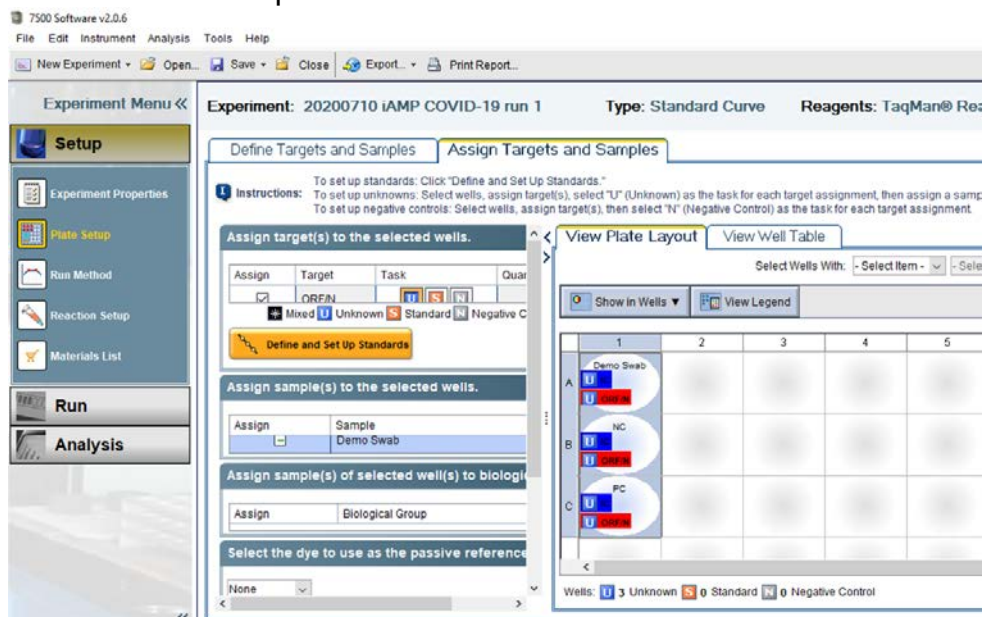


Figure 12. Assign targets and samples.

- Setup Run Method according to the following table 4 and Figure 13.
Fluorescence reading is taken at the end of each cycle. Reaction volume per well is 25µL.

Step	Cycle	Temperature	Time
Denaturation	1	61 °C	30 sec
cDNA synthesis, isothermal amplification, and signal detection	50	61 °C	1 min

Table 4: Thermal Profile for cDNA and Isothermal Amplification

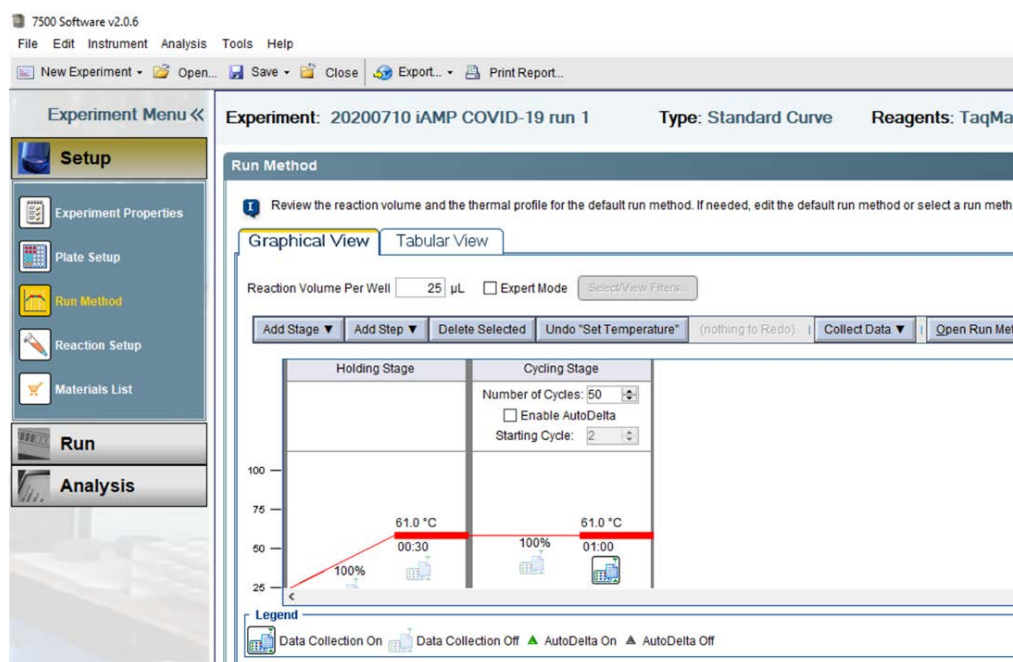


Figure 13. Run method set-up using 7500 Software.

- Put the reaction tubes into the sample holder in the real-time PCR machine, and make sure that the tubes are placed according to the Plate Layout.
- Start the reaction run by clicking Run and Start Run.
After the run, take out the sample tubes/plate and discard them immediately into a sealable zip bag. To avoid contamination **DO NOT OPEN THE REACTION TUBE AFTER THE REACTION** and do not bring tubes in the sample processing area.

RESULT INTERPRETATION

1. INTERPRETATION OF TEST CONTROLS

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

iAMP COVID-19 Detection Kit Controls – Positive, Negative

- a) **Negative Control Template** - serves to verify that analyte contamination does not occur during reaction setup and is used once in each instrument run. There should be NO exponential amplification curve shown in any channel (FAM or HEX) for the negative control template, otherwise the test is invalid, and the results cannot be used for diagnosis.
- b) **Positive Control Template** - serves as a control for amplification and detection of SARS-CoV-2 RNA specific sequences of the N and ORF1ab genes. It should show exponential curves in both channels (FAM and HEX).

Setting up the threshold- Choose linear mode for amplification plot (Δ fluorescence vs cycle). Measure the signal amplitude of Positive Control in FAM and HEX channel at cycle 50 (FAM₅₀ and HEX₅₀). Set the threshold as 10% X FAM₅₀ for FAM channel, and 10% X HEX₅₀ for HEX channel.

Ct of the Positive Control in each channel must be less than 30 for the control to be valid. If a Ct value above 30 is observed in any of the channels the test is invalid, and the results cannot be used for diagnosis.

2. EXAMINATION AND INTERPRETATION OF PATIENT SPECIMEN RESULTS

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

Remain the threshold as set up above (under Positive Control) for FAM and HEX channel.

- **Expected Results of the Internal Control in each specimen** – The internal control serves as a nucleic acid extraction procedural control that validates both the sufficiency of sample collection as well as nucleic extraction procedure and reagent integrity. This control is a human gene present in every test sample, that is amplified with a specific primers and probe set in the kit and measured in the HEX channel. If a sample shows no exponential amplification curve in the HEX channel but an exponential curve in the FAM channel, the sample is still reported as a valid run and will be interpreted following instructions as below. If there is no exponential amplification curve in any channel in a sample, the sample test result is invalid, and a new sample of the patient needs to be collected, processed and re-tested.
- **Sample test result interpretation-** an exponential amplification curve showing up at

any of the two channels (**FAM/HEX**) with Ct<50 indicates the presence of corresponding analyte as indicated below:

FAM (ORF1ab and/or N)	HEX (<i>Internal Control</i>)	RESULT	ACTION
-	-	Invalid	Repeat test. If result remains invalid, re-sampling is needed
-	+	SARS-CoV-2 not detected	Report results to healthcare provider. Consider test for other viruses that cause similar symptoms
+	+/-	SARS-CoV-2 positive	Report results to healthcare provider and appropriate public health authorities

Table 5. Interpretation of patient specimen results.

LIMITATIONS

- The use of this assay is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.
- Performance of the iAMP COVID-19 Detection Kit was established for OP and NP swab specimens only. Other specimen types have not been evaluated.
- Nasal swabs and mid-turbinate swabs are considered acceptable specimen types for use with the iAMP COVID-19 Detection Kit but performance with these specimen types has not been established. Testing of nasal and mid-turbinate nasal swabs (self-collected under supervision of or collected by a healthcare provider) is limited to patients with symptoms of COVID-19. Please refer to FDA's FAQs on Diagnostic testing for SARS-CoV-2 for additional information.
- Improper collection, transport, or storage of specimens may impact the ability of the assay to perform as indicated.
- False-negative results may arise from: Improper sample collection, storage and transport and resulting in degradation of the SARS-CoV-2 RNA, the presence of RT-PCR inhibitors, Mutation in the SARS-CoV-2 virus, and/or failure to follow instructions for use.
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated.
- The iAMP COVID-19 Detection Kit cannot rule out respiratory diseases caused by other bacterial or viral pathogens.
- Negative results do not preclude infection with SARS-CoV-2 virus and should not be the sole basis of a patient management decision.
- Laboratories are required to report results consistent with local, state and federal public health authorities.

Conditions of Authorization for the Laboratory

The iAMP COVID-19 Detection Kit Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: <https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd>.

However, to assist clinical laboratories using the iAMP COVID-19 Detection Kit (“your product” in the conditions below), the relevant Conditions of Authorization are listed below:

- Authorized laboratories¹ using your product will include with result reports of your product, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using your product will use your product as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.
- Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.
- Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Atila BioSystems, Inc. (Covid19_support@atilabiosystems.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.
- All laboratory personnel using your product must be appropriately trained in real-time nucleic acid amplification techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling.
- Atila BioSystems, Inc., authorized distributors, and authorized laboratories using your product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

¹ The letter of authorization refers to, “laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests” as “authorized laboratories.”

PERFORMANCE CHARACTERISTICS

Limit of Detection (LoD) – Analytical Sensitivity

The analytical sensitivity of iAMP COVID-19 Detection Kit was determined in Limit of Detection (LoD) studies using Biorad CFX96 Real-Time System, Roche LightCycler 480 Instrument II, Atila PowerGene 9600 Plus Real-Time PCR System, and Applied Biosystems™ 7500 Real-Time PCR Systems. The iAMP COVID-19 Detection Kit were tested according to the Instructions for Use using Twist Synthetic SARS-CoV-2 RNA Control for a tentative LoD study using Biorad CFX96 Real-Time System, followed by a confirmative LoD study using Accuplex™ SARS-CoV-2 Verification Panel (SeraCare, 0505-0129) in all four instruments. The tables below are a summary of the LoD studies; line data are included in an excel file.

a. Tentative LoD Study

For a tentative LoD study, oropharyngeal swab collected from healthy individuals were treated with 1x iAMP COVID-19 Sample Buffer Mix. Known titer of Twist RNA control was then spiked into negative oropharyngeal swab specimens to mimic clinical samples and the contrived samples were processed following the IFU of iAMP COVID-19 Detection Kit. The tentative LoD of the assay was determined for swab matrix using Biorad CFX96 Real-Time PCR System.

Due to the competition in the multiplex assay, amplification of SARS-COV-2 target may delay or totally suppress the Gapdh internal control signal. Therefore, samples with a positive fluorescence signal for the ORF1ab/N targets are valid positive even in the absence of the internal control.

Table 6. Tentative LoD studies for Biorad CFX96 Real-Time System.

SARS-CoV-2 - Tentative LoD							
Target Level	Valid results	SARS-CoV-2 (N/ORF) Positive		SARS-CoV-2 (N/ORF) Detection Rate	Internal Control Positive		Internal control Detection Rate
		n	Mean Ct		n	Mean Ct	
0 cp/μL	5	0	NA	0%	5	16.71	100%
4 cp/μL	5	3	16.16	60%	5	17.08	100%
10 cp/μL	5	5	14.60	100%	5	17.69	100%
15 cp/μL	5	5	16.48	100%	5	17.87	100%
20 cp/μL	5	5	16.40	100%	5	18.47	100%
30 cp/μL	5	5	14.39	100%	5	18.83	100%
Tentative LoD: 10 cp/μL [lowest target level demonstrating >95% detection rate of SARS-COV-2]							

b. Confirmatory LoD Study

Confirmatory LoD studies for Biorad CFX96 Real-Time System, Roche LightCycler 480 Instrument II, Atila PowerGene 9600 Plus Real-Time PCR System, and Applied Biosystems™ 7500 Real-Time PCR Systems were performed using AccuPlex SARS-CoV-2 Verification Panel from SeraCare (0505-0129). Pseudovirus was spiked into negative nasopharyngeal swab specimen at the concentration of 10 copies/μL sample and processed following IFU of the kit.

Table 7. Confirmatory LoD studies for different Real-Time PCR instruments

SARS-CoV-2 - Confirmatory LoD							
Target Level	Valid results	SARS-CoV-2 (N/ORF) Positive		SARS-CoV-2 (N/ORF) Detection Rate	Internal Control Positive		Internal Control Detection Rate
		n	Mean Ct		n	Mean Ct	
Biorad CFX96 Real-Time System							
10 cp/μL	20	20	15.39	100%	20	29.96	100%
Roche LightCycler Instrument II							
10 cp/μL	20	20	16.65	100%	20	29.49	100%
Atila PowerGene 9600 Plus Real-Time System.							
10 cp/μL	20	20	14.84	100%	20	34.95	100%
Applied Biosystems™ 7500 Real-Time PCR Systems.							
10 cp/μL	20	20	18.44	100%	19	38.78	95%

All 20 Replicates that were tested gave positive results for SARS-CoV-2. While some replicates were undetermined for Gapdh (IC), based on the result interpretation the LoD of iAMP COVID-19 Detection Kit is 10 copies/μL sample for all four PCR instruments (note the result interpretation calls valid positive results in the absence of Gapdh).

Inclusivity

All primer-annealing regions in both Orf1ab and N were analyzed in silico using NCBI nBLAST, and showed 100% match to all of the published SARS-CoV-2 complete genome sequences from Genbank (n=10,000 as of July 12, 2020). The iAMP COVID-19 kit is therefore predicted to detect all currently circulating strains for SARS-CoV-2.

Cross-Reactivity (Analytical Specificity)

The list of organisms shown below has been analyzed *in silico* for potential cross-reactivity with the primers or probe sequences in the iAMP COVID-19 test. There were no primers and probes in the iAMP COVID-19 Detection Kit with homology ≥ 80% and therefore cross reactivity with the organisms below is not expected.

Organisms tested in in Silico Cross-Reactivity analysis			
Pathogen	GenBank Acc#	Pathogen	GenBank Acc#
Human coronavirus 229E	NC_002645.1	Parainfluenza virus 4a isolate HPIV4_DK(459)	KF483663.1
Human coronavirus OC43	NC_006213.1	Parainfluenza virus 4b strain 04-13	JQ241176.1
Human coronavirus HKU1	NC_006577.2	<u>Influenza B</u> B/Illinois/13/2005 (segment 7)	CY019500.1
Human coronavirus NL63	NC_005831.2	Enterovirus 68 isolate NZ-2010-541	JX070222.1
SARS-coronavirus B093	AY686864	Respiratory syncytial virus	NC_001803
MERS-coronavirus NL140422	MG021452.1	Human Rhinovirus B3 strain SC2606	KY967365.1
Human Metapneumovirus (hMPV) isolate 00-1	NC_039199	<i>Chlamydia pneumoniae</i> TW-183	NC_005043.1
Parainfluenza virus 1 isolate NM001	KX639498.1	<i>Bordetella pertussis</i> strain B3921	CP011448.1
Parainfluenza virus 2 isolate VIROAF10	KM190939.1	<i>Pseudomonas aeruginosa</i> UCBPP-PA14	CP000438.1
Parainfluenza virus 3 strain HPIV3/AUS/3/2007	KF530243.1	<i>Streptococcus salivarius</i> CCHSS3	FR873481.1

Table 8. *In silico* cross-reactivity analysis

In addition, the organisms listed in the table below were wet-tested. Oropharyngeal swab collected from healthy individuals were treated with 1x iAMP COVID-19 Sample Buffer Mix. Then purified genome DNA/RNA spiked into the negative oropharyngeal swab specimen at the concentration of 10^5 genome copies/ μ L sample. Samples were processed following the IFU. Three replicates were tested for each organism. No false positive signal was observed in FAM channel (which SARS-CoV-2 is assigned) for any of the replicates when testing with the iAMP COVID-19 Detection Kit, whereas amplification

curves for the internal control in the HEX channel showed up as expected. No cross reactivity was observed with the organisms below at the tested concentration.

Organisms wet-tested in swab matrix	SARS-CoV-2 (N/ORF) Detection Rate
Adenovirus (ATCC VR-1516)	0% (0/3)
Influenza A (H1N1)	0% (0/3)
<i>Haemophilus influenzae</i>	0% (0/3)
<i>Legionella pneumophila</i>	0% (0/3)
<i>Mycobacterium tuberculosis</i>	0% (0/3)
<i>Streptococcus pneumoniae</i>	0% (0/3)
<i>Streptococcus pyogenes</i>	0% (0/3)
<i>Mycoplasma pneumoniae</i>	0% (0/3)
<i>Pneumocystis jirovecii</i> (PJP)	0% (0/3)
<i>Candida albicans</i>	0% (0/3)

Table 9. Cross-reactivity analysis with wet-test

Endogenous Interference Substances Studies

Interfering substances studies were performed for iAMP COVID-19 Detection Kit. Oropharyngeal swab collected from healthy individuals were treated with 1x iAMP COVID-19 Sample Buffer Mix. Then Twist Synthetic SARS-CoV-2 RNA Control was spiked into the negative oropharyngeal swab specimens at the concentration of 2.5X LoD (25 copies/ μ L sample). The potentially interfering substances indicated in the table below were added to the positive contrived samples at the indicated concentration, and the samples were processed following IFU. Each substance was also added to the negative oropharyngeal swab specimen to test potential false positives. After sample processing 3 μ L sample was tested in a 25 μ L reaction according to the IFU. Each substance was tested in three replicates for positive contrived samples and 3 replicates for negative swab specimens. Results indicate that iAMP COVID-19 test can well tolerate all the substances at the concentration equal or lower than the indicated values without significant interference. Neither false positive or false negative was observed.

Potential Interfering Substance	Conc.	Positive Samples		Negative Samples
		Viral Strain Level	Results	Results
Mucin: bovine submaxillary gland, type I-S	2.5 mg/ml	2.5X LoD	3/3	0/3
Blood (human)	2.5% v/v	2.5X LoD	3/3	0/3
Afrin Original nasal spray	15% v/v	2.5X LoD	3/3	0/3
Basic Care allergy relief nasal spray (Gluococorticoid)	5% v/v	2.5X LoD	3/3	0/3
NeilMed Nasal gel	1.25%	2.5X LoD	3/3	0/3
GoodSense All Day Allergy, Cetirizine HCl Tablets 10 mg	1mg/mL	2.5X LoD	3/3	0/3
Cepacol Sore Throat (benzocaine/menthol lozenges)	5 mg/mL	2.5X LoD	3/3	0/3
Zanamivir	3.3 mg/mL	2.5X LoD	3/3	0/3
Tamiflu	2.2 µg/mL	2.5X LoD	3/3	0/3
Mupirocin ointment	5mg/mL	2.5X LoD	3/3	0/3
tobramycin	4ug/mL	2.5X LoD	3/3	0/3

Table 10. Interference studies

Clinical Evaluation

Clinical respiratory samples were tested at two sites. Samples were tested with the Atila SARS-CoV-2 test according to the instructions for use. Sample results for the Atila test were compared to the results of the EUA authorized tests used at each site). Results are summarized in the table below. The specimen matrix of the clinical samples were nasopharyngeal swab specimens at both sites.

Site 1	Comparator Test Positive	Comparator Test Negative	Total
Atila SARS-CoV-2 Positive	8	0	8
Atila SARS-CoV-2 Negative	0	10	10
Total	8	10	18

Table 12. Clinical Evaluation of the Atila SARS-CoV-2 Detection Kit (Site 1: Atila Powergene)

PPA: 8/8 = 100%, (95% CI: 67.6%-100%)

NPA: 10/10 = 100%, (95% CI: 72.3%- 100%)

Site 2	Comparator Test Positive	Comparator Test Negative	Total
Atila SARS-CoV-2 Positive	24	1	25
Atila SARS-CoV-2 Negative	1	32	33
Total	25	33	58

Table 13. Clinical Evaluation of the *Atila SARS-CoV-2 Detection Kit (site 2: ABI7500)*

PPA: 24/25 x 96% = 96.3%, (95% CI: 80.5%-99.3%)

NPA: 58/60 x 100%= 97.0%, (95% CI: 84.7%- 99.5%)

© 2020 Atila BioSystems, Inc. All rights reserved. No part of this publication may be reproduced, transmitted, transcribed, or translated into any language or computer language, in any form or by any means without prior express, written consent of Atila BioSystems, Inc. Atila BioSystems, Inc. reserves the right to modify its products and services at any time. Notifications will be sent to end users regarding changes that impact the use, performance and /or safety and effectiveness of the device. Any modifications to the device will be made in accordance with applicable regulatory requirements. Atila BioSystems, Inc. assumes no liability for any damages resulting from the off-label application or misuse of this information.

For technical Support and adverse event reporting, please contact at 650-968-8848, or email Covid19_support@atilabiosystems.com.



740 Sierra Vista Ave, Suite E
Mountain View, CA 94043, USA
Tel: +1 (650)-968-8848
Email: info@atilabiosystems.com