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Recommended validation procedures for iAMP-COVID-19 Detection Kit

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IMPORTANT NOTICE

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

INTENDED USE

The recommended procedures are intended for validation studies for Atila iAMP COVID-19 Detection Kit **using the same components provided with the kit**. It serves for new customers who need to validate the iAMP COVID-19 Detection Kit using isolated RNA samples, reference standards, or swab specimens in transport medium, before moving on to dry swabs that are the specimen type for iAMP COVID-19 Detection Kit.

The validation procedures are intended for use by trained laboratory personnel specifically instructed and trained in the techniques of real-time nucleic acid amplification and laboratory research procedures.

KIT COMPONENTS

Please refer to the IFU enclosed in the iAMP COVID-19 Detection Kit

EQUIPMENTS & MATERIALS REQUIRED BUT NOT SUPPLIED with the kit

Please refer to the IFU enclosed in the iAMP COVID-19 Detection Kit

KIT STORAGE INFORMATION

Please refer to the IFU enclosed in the iAMP COVID-19 Detection Kit

SPECIMENS

Biosafety Precautions

Please refer to the IFU enclosed in the iAMP COVID-19 Detection Kit

Acceptable Specimen types for validation studies:

- Direct nasal, nasopharyngeal or oropharyngeal dry swabs collected with Atila Sample Collection tube. Alternatively, synthetic fiber swabs with plastic shafts and 1.5mL collection tubes from other vendors can be used.
- Nasal, sasopharyngeal, or oropharyngeal swabs stored in storage solution (saline, PBS, HBSS, or Copan UTM).
- Isolated RNA from respiratory specimens (swabs, sputum, BAL, etc.)

Specimen Handling and Storage

- Use freshly collected specimens for optimal test performance.
- Dry swab specimens can be stored at room temperature for up to 6 hours or at 4°C for up to 48 hours after collection and before sample processing. If a delay in sample processing

is expected, store dry swab specimens at -70°C or lower. Avoid freeze-thaw cycles of the specimens.

- Swabs stored in saline, PBS, or HBSS need to be stored at 4°C and tested the same day after sample collection.
- Swabs specimens stored in Copan UTM should be stored at 2-25 °C and tested within 48 hours after sample collection.
- Isolated RNA samples should be stored at -70°C after RNA extraction. Avoid freeze-thaw cycles of the RNA samples.
- Sample processed in iAMP Sample Buffer Mix (processed sample) need to be tested within 2 hours. If a delay in sample testing is expected, store processed specimens at 4 °C for up to 12 hours.
- **Do not freeze processed specimens.**

Specimen Rejection criteria

- Specimens not kept as instructed.
- Incomplete specimen labeling or documentation
- Inappropriate specimen type.
- Insufficient sample volume

QUALITY CONTROL

Please refer to the IFU enclosed in the iAMP COVID-19 Detection Kit

TEST PROCEDURE

Procedure Overview Based On Different Sample Types

Specimen type	Specimen processing and testing overview
A. Nasal, nasopharyngeal, or oropharyngeal dry swabs	Dry swab + 350µL 1X Sample Buffer Mix for sample processing 3 µL processed sample + 22uL Reaction Master Mix
B. Isolated viral RNA or reference standard spiked in negative swab matrix	Negative dry swab + 350µL 1X Sample Buffer Mix for sample processing Make contrived sample: 3 µL processed negative sample + 10 µL RNA/standard + 2 µL 6X SBA 15 µL contrived sample + 10 µL Reaction Master Mix "L"
C. Nasal, nasopharyngeal, or oropharyngeal swabs stored in storage solutions*	Option C1: Centrifugation method Pellet + 55µL 1X Sample Buffer Mix for sample processing 15 µL processed sample + 10uL Reaction Master Mix "L" Option C2: Direct addition of liquid specimen 3µL liquid specimen + 12 µL 1X Sample Buffer Mix for sample processing 15 µL processed sample + 10uL Reaction Master Mix "L"
D. Isolated viral RNA from respiratory specimens (swabs, sputum, BAL)	12.5 µL isolated RNA + 2.5uL 6X Sample Buffer A 15 µL processed sample + 10uL Reaction Master Mix "L"

*For swab storage solutions (type C), Copan UTM, PBS, Saline, and HBSS have been validated for both procedural options. If other types of storage solution are used, self-validation is required before sample testing.

Detailed Sample Processing Procedure and Reaction Assembly

Preparation:

1. From the kit, take 6X iAMP COVID-19 Sample Buffer A (COVID-6XSBA) and 30X iAMP COVID-19 Sample Buffer B (COVID-30XSBB) out if using specimen type A (dry swab) or type B (swab in storage solution). Each tube contains enough volume to process 20 dry swabs or more than 100 liquid specimens. Only thaw the number of COVID-6XSBA and COVID-30XSBB tubes that will be enough for each round of sample processing.

2. Take Buffer Mix (COVIDBM), Primer Mix (COVIDPM), Negative Control (COVIDNC) and Positive Control (COVIDPC) out.

   Thoroughly thaw all reagents, vortex briefly, and put on ice.

A. Nasal, nasopharyngeal, or oropharyngeal dry swabs

1. Make 1X **Sample Buffer Mix** by mixing the following components in a 1.5mL centrifuge tube:

COVID-6xSBA	60 x N uL
COVID-30xSBB	12 x N uL
H2O	288 x N uL

   N represents the number of dry swab specimens to process.
   Vortex briefly and do a quick spin to bring liquid to the bottom.
2. Load **350 µL** prepared 1X Sample Buffer Mix into each sample tube with dry swab already inside. Seal the tube cap securely and vortex briefly.
3. Place the sample tube on the bench for **15 min**. Do not leave the processed sample at room temperature for more than 2 hours.
4. Take N+2 PCR tubes. Then make **reaction master mix** by adding the following components in a 1.5 mL centrifuge tube:

COVIDPM	5.2 x (N+2) uL
COVIDBM	5.2 x (N+2) uL
COVID-6xSBA	2.08 x (N+2) uL
H2O	10.4 x (N+2) uL

   Gently vortex and spin, and add **22 µL** reaction master mix to the bottom of each of the PCR tubes.
5. Briefly spin the tubes to bring down the liquid to the bottom of the sample tubes. Add **3µL** of processed specimen samples (Sample #1 to #N) from step of **"Specimen**

Processing" to corresponding reaction PCR tubes from above **Step 1**. For negative control, add **3µL** of Negative Control Template into reaction tube #(N+1). For positive control, add **3µL** of Positive Control Template into reaction tube #(N+2).

B. Isolated viral RNA or reference standards spiked in negative swab matrices

1. Take a fresh nasal, nasopharyngeal, or oropharyngeal dry swab from a healthy individual.
2. Make 1X **Sample Buffer Mix** by mixing the following components in a 1.5mL centrifuge tube:

COVID-6xSBA	60 x N uL
COVID-30xSBB	12 x N uL
H2O	288 x N uL

N represents the number of dry swab specimens to process.
Vortex briefly.

3. Load **350 µL** prepared 1X Sample Buffer Mix into the sample tube with dry swab already inside. Seal the tube cap securely and vortex briefly.
4. Place the sample tube on the bench for **15 min**. This processed negative swab specimen will serve as negative swab matrices.
5. Dilute isolated RNA to desired concentrations for testing using nuclease-free H2O.
6. Take N PCR tubes. Make each contrived sample directly in the PCR tubes (from tube #1 to #N) by mixing:

Negative processed specimen	3µL
COVID-6XSBA	2µL
RNA at desired concentration	10 µL

7. Make **reaction master mix "L"** by adding the following components in a 1.5 mL centrifuge tube:

COVIDPM	5.2 x (N+2) uL
COVIDBM	5.2 x (N+2) uL

Gently vortex and spin. Add **10µL** reaction master mix to the PCR tube from above step 3 (#1 to #N).

Note: Change pipette tip for every loading of reaction mastermix, otherwise contamination will happen.

8. Take 2 more PCR tubes (#N+1 and #N+2). Load **10µL** reaction master mix "L" to both tubes. For negative control, add 15 µL of Negative Control Template into reaction tube #(N+1). For positive control, add 15 µL of Positive Control Template into reaction tube #(N+2).

C. Nasal, nasopharyngeal, or oropharyngeal swabs stored in storage solution

Note: For swab storage solutions, Copan UTM, PBS, Saline, and HBSS have been validated for both procedural options**. If other types of storage solution are used, self-validation is required before sample testing.**

Procedure option 1 and/or 2 can be chosen depending on specific type of storage solutions and equipment availability. Side-by-side test for both options is highly recommended for initial validation of the kit.

Option c1. Centrifugation method

Note: For storage solution that is not listed above, self-validation is needed. In general, if the storage solution is known to have major lysing effect, this method is not ideal to choose.

1. Take 300-1000 uL of each tested specimen, centrifuge at 13,000rpm for 30 min if using a 4°C centrifugation instrument. If 4°C centrifugation instrument is not available, centrifuge at 13,000 rpm for 15min at room temperature.

2. Carefully remove and discard **ALL** the supernatant using a pipette. Do not leave over 10uL residual medium.

3. Make 1X iAMP COVID-19 Sample Buffer Mix (**1X SBM**) by mixing:

COVID-6xSBA	10 x N uL
COVID-30xSBB	2 x N uL
H2O	48 x N uL

N represent the total number of tested specimen.

4. Resuspend the pellet of each specimen using 55uL 1X SBM by pipetting up and down. Leave the sample tubes at room temperature for 15min.

5. Take N+2 PCR tubes (N represents number of specimen samples to be tested). Then make **reaction master mix "L"** by adding

COVIDPM	5.2 x (N+2) uL
COVIDBM	5.2 x (N+2) uL

Gently vortex and spin, and add **10 µL** reaction master mix "L" to the bottom of each of the PCR tubes.

6. Add **15µL** of processed specimen samples (Sample #1 to #N) from above step 4 to corresponding reaction PCR tubes from above **Step 5**. For negative control, add 15µL of Negative Control Template into reaction tube #(N+1). For positive control, add 15µL of Positive Control Template into reaction tube #(N+2).

Option c2. Direct raw solution testing

Note: For storage solution that is not listed above, self-validation is needed.

1. Make 1X iAMP COVID-19 Sample Buffer Mix (**1X SBM**) by mixing

COVID-6xSBA	2.5 x N uL
COVID-30xSBB	0.5 x N uL
H2O	12 x N uL

N represent the total number of tested specimen.

2. Take N PCR tubes. For initial validation test, mix 12uL 1X SBM with 3uL specimen solution in the PCR tube (#1 to #N), and pipette up and down to mix the sample. Leave PCR tubes at room temperature for 15min.

Note: depending on the result from initial test, if significant interference is observed (huge speed delay, impacted sensitivity, and/or strange amplification signals, etc.), mix 14uL 1X SBM with 1uL specimen solutions for the next round of validation test.

3. Make **reaction master mix "L"** by mixing the following components in a 1.5 mL centrifuge tube.

COVIDPM	5.2 x (N+2) uL
COVIDBM	5.2 x (N+2) uL

Gently vortex and spin, and add **10μL** reaction master mix "L" to the PCR tube from above step 2 (#1 to #N).

Note: Change pipette tip for every loading of reaction mastermix, otherwise contamination will happen.

4. Take 2 more PCR tubes (#N+1 and #N+2). Load **10μL** reaction master mix "L" to both tubes. For negative control, add 15 μL of Negative Control Template into reaction tube #(N+1). For positive control, add 15 μL of Positive Control Template into reaction tube #(N+2).

D. Isolated RNA from respiratory specimens

1. Take N+2 PCR tubes (N represents number of specimen samples to be tested). Transfer 12.5 μL purified RNA into each PCR tube (from #1 to #N), and mix each sample with 2.5 μL 6X iAMP COVID-19 Sample Buffer **A (COVID-6XSBA)**.
2. Make **reaction master mix "L"** by adding the following components in a 1.5 mL centrifuge tube,

COVIDPM	5.2 x (N+2) uL
COVIDBM	5.2 x (N+2) uL

Gently vortex and spin, and add **10μL** reaction master mix "L" to the PCR tube from above step 1 (#1 to #N).

Note: Change pipette tip for every loading of reaction mastermix, otherwise contamination will happen.

3. 4. Take 2 more PCR tubes (#N+1 and #N+2). Load **10µL** reaction master mix "L" to both tubes. For negative control, add 15 µL of Negative Control Template into reaction tube #(N+1). For positive control, add 15 µL of Positive Control Template into reaction tube #(N+2).

Isothermal amplification and signal detection using a compatible real-time PCR instrument

1. Cap all the tubes securely. Gently vortex the tubes to mix all the reagents.
2. Briefly spin the tubes in a centrifuge to bring down all the liquid to the bottom of the wells.
3. Set the reaction condition using a compatible real-time PCR machine (All real-time PCR instruments capable of measuring fluorescence in FAM/HEX channel in real-time. Such instruments include but not limited to: Atila PowerGene 9600 Plus Real-Time System, BioRad CFX96 Real-Time PCR Detection System, Roche LightCycler 480 Real-Time PCR System, Applied Biosystems 7500 Real-Time PCR System, etc.)

   Open a new PCR program and do the following edits:

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

   Fluorescence reading was taken at the **FAM/HEX** channels at the end of each cycle.

4. Put the reaction tubes into the sample holder and bring into the real-time PCR machine, close the lid, and start the reaction run.
5. After the run, take out the sample plate and discard them immediately.

To avoid contamination, **DO NOT OPEN THE REACTION TUBE AFTER THE REACTION**.

AMPLIFICATION RESULT INTERPRETATION

iAMP COVID-19 Detection Kit Controls – Positive, Negative and Internal Controls

- Quality control of a test- every instrument run should include:
  a) **Negative Control Template** - serves to verify that analyte contamination does not occur during reaction setup. There should be NO exponential amplification curve shown in any channel in negative control template, otherwise the test is invalid.
  b) **Positive Control Template** - serves as a control for amplification and detection of SARS-CoV-2 RNA (ORF1ab and/or N). It should show exponential curves in both channels, and Ct in each channel should be less than 30, otherwise the test is invalid.

Quality control of an instrument run should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

- **Internal Control in each specimen** - also 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. Internal control is measured in HEX channel in this assay kit. If a sample shows no exponential amplification curve in HEX channel but an exponential curve in 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.

Examination and Interpretation of Patient Specimen Results:

- **Sample test result interpretation**- an exponential amplification curve showing up at any of the two channels **(FAM/HEX)** indicates the presence of corresponding assayed analyte as indicated below:

	Analyte
FAM Channel	ORF1ab and/or N
HEX Channel	Sample internal control

A summary of sample test result interpretation is shown as below.

	FAM	HEX	Result	Action
Case A	-	-	Invalid	Repeat test. If result remains invalid, re-sampling is needed
Case B	-	+	SARS-CoV-2 not detected	Report results to healthcare provider. Consider test for other viruses that cause similar symptoms
Case C	+	+/-	SARS-CoV-2 positive	Report results to healthcare provider and appropriate public health authorities

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