



## IntelliPlex™ SARS-CoV-2 Detection Test Kit



**82306 96 Reactions**

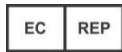


**For In-Vitro Diagnostic Use**



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**IMPORTANT:**

**Read the instructions carefully prior to use**

### 1. INTENDED USE

The IntelliPlex SARS-CoV-2 Detection Test Kit is a real-time RT-PCR assay for the qualitative detection of SARS-CoV-2 virus in patients that may or may not exhibit symptoms of respiratory tract infection (e.g. fever, cough, shortness of breath). The kit detects the presence of ORF1a and N genes of SARS-CoV-2 in RNA extracted from nasopharyngeal swab and saliva samples. Viral RNA needs to be purified before amplification by Real-time RT-PCR (rRT-PCR). The detection of viruses is intended to assist clinicians in the management of infection treatments.

### 2. INTRODUCTION

Coronaviruses are a large family of viruses that may cause illness in animals or humans. In humans, several coronaviruses are known to cause respiratory infections ranging from the common cold to more severe diseases such as Severe Acute Respiratory Syndrome (SARS) and Coronavirus Disease 2019 (COVID-19). The COVID-19 is an infectious disease caused by the most recently discovered coronavirus, SARS-CoV-2. This new virus and disease were unknown before the outbreak began in December 2019. Up to now, COVID-19 has become a pandemic, resulting in over a billion cases. SARS-CoV-2 is a single-stranded RNA virus. Based on real-time RT-PCR, detection of SARS-CoV-2 from the specimen of nasopharyngeal swab and saliva is feasible due to the optimal primer and probe design followed by the technical guidance of the World Health Organization (WHO) announcement.

### 3. TECHNOLOGICAL PRINCIPLES

The procedure is based on processes listed as follows:

- I. Viral RNA purified from nasopharyngeal swab and saliva.
- II. Real-time RT-PCR amplification of viral RNA.
- III. Confirmation of Baseline and Threshold Settings
- IV. Data Analysis

#### 4. WARNINGS AND PRECAUTIONS

- For in-vitro diagnostic use.
- This assay kit should be used by qualified laboratory personnel only.
- Separate, dedicated rooms and equipment for the sample preparation and rRT-PCR process with a unidirectional workflow to avoid any contaminations is required.
- All Pre-PCR steps should be carried out in the laminar flow hood to further reduce contamination risk.
- Do not use a kit or reagent past its expiration date.
- Sample preparation, RT-PCR reaction set up and PCR product analysis should be performed according to approved guidelines such as those published by Clinical And Laboratory Standards Institute; clean all equipment and surface areas regularly (e.g. The workspace including racks and pipettes should be thoroughly cleaned and wiped with 0.5% sodium hypochlorite solution followed by wiping with a 70% ethanol solution).
- Component reagents have been diluted optimally. Further dilution of the component reagents is not recommended.
- All chemicals, biological materials and human origin samples should be considered as potentially hazardous and/or infectious and should be treated accordingly.
- Some reagent contains EDTA and/or Sodium Azide in highly diluted concentration. Follow Good Laboratory Practices and Universal Precautions guidelines to avoid any risk.
- Store assay kits and reagents according to the product label and instructions.
- Do not mix reagents from different lots.
- Dispose of unused reagents, specimens, and waste according to applicable central/federal, state, and local regulations.
- Wear powderless gloves and do not touch and make any markings on the bottom of the plate at any time, as fingerprints and markings would interfere with decoding and signal acquisition. Do not mark the top of the plate.
- General laboratory precautions should be taken:
  - Do not pipette by mouth.
  - Wear protective clothing (e.g., disposable powderless gloves and laboratory coats) and eye protection.
  - Do not eat, drink or smoke in the laboratory.
  - Wash hands thoroughly after handling samples and reagents.
- **Avoid RNase contamination:**
  - **Create an RNase-free working environment.**
  - **Wear gloves during all steps of the procedure.**
  - **Change gloves frequently.**
  - **Use only certified RNase-free sterile, disposable polypropylene tubes and filter strips.**
  - **Keep tubes closed whenever possible during the preparation.**
  - **Use RNase removal cleaning agent product to clean bench surfaces, pipettes and other components used in the experiment.**
- Any serious incident that has occurred with the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.
- Material Safety Data Sheets (SDS) are available upon request from PlexBio Customer Service.

#### 5. PRODUCT USE LIMITATIONS

The recommendations and procedures must be followed to prevent false results and contamination.

- The product can only detect the nucleic acids from SARS-CoV-2, not for detecting any other viruses or pathogens and cannot preclude the infection possibility of any other pathogens.
- The designed primers and probes in this product covered a highly conservative genomic region of the virus. High mutation rate of RNA viruses within the conservative regions still exists, which may lead to false negative results.
- Viruses with mutations/sequences different from the reference sequence may not be identified correctly.
- Improper sampling, transportation, storage, and handling may cause the false-positive or false-negative results.
- Specimen collection, storage, purification, and transport must comply to allow efficient amplification with the RT-PCR assay.

## 6. KIT COMPONENTS

The IntelliPlex SARS-CoV-2 Detection Test Kit provides two parts of kit reagents: Part A is for Extraction and Part B is for Real-Time RT-PCR Detection. The kit is sufficient for 96 reactions and the components supplied are listed as follows.

### IntelliPlex SARS-CoV-2 Detection Test Kit – Part A (Extraction)

- 1. Extraction KIT Silica Magnetic Beads**  
REF: 20581  
**Quantity & Volume:** 1 bottle, 21 mL  
**Description:** For binding with nucleic acids.  
**Contents:** Silica magnetic beads in deionized water
- 2. Extraction KIT Lysis Buffer**  
REF: 20580  
**Quantity & Volume:** 1 bottle, 44 mL; **Add 26 mL Absolute Isopropanol before use.**  
**Description:** Lyse cells  
**Contents:** Guanidinium salts, surfactants in Tris buffer
- 3. Extraction KIT Wash Buffer A**  
REF: 20582  
**Quantity & Volume:** 1 bottle, 32 mL; **Add 41 mL Absolute Ethanol before use.**  
**Description:** Remove impurities. Provided as a concentrate.  
**Contents:** Guanidinium salts in Tris buffer.
- 4. Extraction KIT Wash Buffer B**  
REF: 20583  
**Quantity & Volume:** 1 bottle, 22 mL; **Add 52 mL Absolute Ethanol before use.**  
**Description:** Remove impurities. Provided as a concentrate.  
**Contents:** Saline
- 5. Extraction KIT Elution Buffer**  
REF: 20584  
**Quantity & Volume:** 1 bottle, 12 mL  
**Description:** Elution of nucleic acids  
**Contents:** TE buffer

### IntelliPlex SARS-CoV-2 Detection Test Kit – Part B (Real-Time RT-PCR Detection)

- 1. SARS-CoV-2 KIT Reagent Mix**  
REF: 20644  
**Quantity & Volume:** 1 vial, 500 µL/vial  
**Storage:** Store at -25°C to -15°C upon arrival  
**Description:** For RT-PCR amplification  
**Contents:** ~3.5 µM Primer and Probe
- 2. SARS-CoV-2 KIT Enzyme**  
REF: 20643  
**Quantity & Volume:** 1 vial, 500 µL/vial  
**Storage:** Store at -25°C to -15°C upon arrival  
**Description:** For rRT-PCR amplification  
**Contents:** Optimal proportion of reverse transcriptase and DNA polymerase, dNTPs, RNase inhibitor, stabilizers, and passive reference dye (ROX)

**3. NEG Control**

REF: 20549

**Quantity & Volume:** 1 vial, 120 µL/vial**Storage:** Store at -25°C to -15°C upon arrival**Description:** Assay negative control**Contents:** Nuclease-free water**4. SARS-CoV-2 KIT Process Control**

REF: 20623

**Quantity & Volume:** 1 vial, 1 mL/vial**Storage:** Store at -25°C to -15°C upon arrival**Description:** Assay internal control**Contents:** MS2 bacteriophage with RNA sequence serving as an extraction control**7. MATERIALS AND EQUIPMENT REQUIRED BUT NOT SUPPLIED**

※ **POS Control is required for test validity but supplied separately in IntelliPlex™ SARS-CoV-2 Test Control Kit (PlexBio; REF: 82318), which includes:**

- **SARS-CoV-2 Test KIT POS Control** (REF: 20680), 5 vials, 20 µL/vial
- **NEG Control** (REF: 20549), 5 vials, 120 µL/vial

**Please refer to the package insert of IntelliPlex™ SARS-CoV-2 Test Control Kit for more details.**

**Required products for compatibility with IntelliPrep/ IntelliPlex kits:**

- 96-well plate for real-time RT-PCR (Thermo Fisher; MicroAmp Optical 96-well reaction Plate; REF: N8010560)
- Automated Nucleic Acid Extraction System (BioTeke; REF: AU1001-96)
- MTP Accessory Kit (192) (PlexBio; REF: 80048)
- IntelliPlex™ SARS-CoV-2 Test Control Kit (PlexBio; REF: 82318)
- StepOnePlus™ Real-Time PCR System (Applied Biosystems™; REF: 4376600)
- Mini Plate Spinner (Allsheng; Mini-Plate Centrifuge; REF: Mini-P25)
- Optical Sealing Film (StarMoonBio; UltraClear Real Time Pressured Film; REF: SMB-P4F51-C)
- Disposable gloves, powder-less
- Dedicated micropipette\*
- Filter tips for micropipette\*
- Absolute Isopropanol
- Absolute Ethanol
- (Optional) Reagent Reservoir (StarMoonBio; 100 mL Reagent Reservoir; REF: SMB-O1005)

\* Use dedicated pipettes for sample purification, sample preparation, and sample amplification. Do not share equipment between procedures. Pipettes should be accurate within 3% of the stated volume. Aerosol barrier or positive displacement DNA- and RNase-free tips must be used.

**8. STORAGE, STABILITY AND TRANSPORTATION****Storage**

- **IntelliPlex SARS-CoV-2 Detection Test Kit- PART A (Extraction)** should be stored at room temperature.
- **IntelliPlex SARS-CoV-2 Detection Test Kit- PART B (Real-Time RT-PCR Detection)** should be stored at -25°C to -15°C.

**Stability**

Do not use the IntelliPlex SARS-CoV-2 Detection Test Kit when it is expired. All unopened components are guaranteed up to the expiration date on the label if stored under the recommended conditions.

**Transportation**

- **IntelliPlex SARS-CoV-2 Detection Test Kit- PART A (Extraction)** should be transported at room temperature.

- **IntelliPlex SARS-CoV-2 Detection Test Kit- PART B (Real-Time RT-PCR Detection) should be transported at 2°C to 8°C.** The PART B kit components should be stored at -25°C to -15°C upon arrival.

If the kit package or components are incomplete, please contact PlexBio customer service (service@plexbio.com).

## 9. SPECIMENS

### Specimen Collection

Follow specimen collection devices manufacturer instructions for proper collection methods.

- **Nasopharyngeal Swab:** Only synthetic fiber swabs with plastic shafts should be used. Do not use calcium alginate swabs or swabs with wooden shafts (inactivate some viruses and inhibit PCR testing). Place swabs immediately into sterile tubes containing 2-3 ml of viral transport media.
- **Saliva-** Collect 1-5 mL of saliva in a sterile, leak-proof screw cap container either at home or at a testing site under supervision. No preservative is required.

### Specimen Transport

Suspected and confirmed SARS-CoV-2 patient specimens, cultures, or isolates must be packaged and shipped according to UN 3373 Biological Substance, Category B, and in accordance with the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulations. Personnel must be trained to pack and ship according to the regulations and in a manner that corresponds to their function-specific responsibilities.

Store specimens at 2-8°C and ship overnight on the ice pack. If a specimen is frozen at -20°C or lower, ship overnight on dry ice.

### Specimen Storage

Specimens can be stored at 2-8°C for up to 48 hours after collection. If longer storage is expected, store specimens at -20°C or lower.

### Purification and Storage of Extracted RNA

Samples are purified by using Automated Nucleic Acid Extraction System according to the manufacturer's instruction. The specimen volume needed for purification processing is 140 µL, and eluted in 100 µL elution buffer.

Extracted RNA can be stored at 2°C to 8°C for up to 4 hours, or at -15°C to -25°C for up to 7 days. Long-term storage is not recommended.

## 10. BEFORE YOU START

### **Important Handling Instructions:**

*Separate, dedicated areas and equipment for sample purification, sample preparation and sample amplification must be used. Equipment (including lab coats) must not be shared between areas. All equipment and surface areas should be cleaned before and after each run (e.g., using a 0.5 – 1 % Sodium hypochlorite solution or 70% ethanol). All work should be performed according to approved guidelines such as those published by the Clinical and Laboratory Standards Institute.*

- **IntelliPlex SARS-CoV-2 Detection Test KIT – PART A (Extraction)**

Before extraction, please add Absolute Isopropanol and Absolute Ethanol to each buffer according to instructions listed as below.

1. Add 26 mL Absolute Isopropanol to Lysis Buffer and mix well.

### **NOTE:**

- 10 µL SARS-CoV-2 KIT Process Control should be added into each specimen well before extraction. **DO NOT** vortex the **SARS-CoV-2 KIT Process Control**. Please tap the vial gently then centrifuge briefly prior to use.
  - If users run 96 reactions at one time, please add **1 mL SARS-CoV-2 KIT Process Control and 26 mL Absolute Isopropanol** into Lysis Buffer directly.
2. Add 41 mL Absolute Ethanol to Wash Buffer A and mix well.
  3. Add 52 mL Absolute Ethanol to Wash Buffer B and mix well.

## 11. ASSAY PROCEDURE

### Warning:

**Read the instructions carefully and follow every step of the assay protocol correctly.**

### 11.1 RNA Extraction

The automated extraction procedures of SARS-CoV-2 Extraction should be operated and followed correctly on Automated Nucleic Acid Extraction System (BioTeke; REF: AU1001-96). Please refer to the instrument user manual for operation details.

1. Take out the 96 deep well plates from MTP Accessory Kit and mark plates for **Plate 1, Plate 2, Plate 3, Plate 4 and Plate 6**, respectively.
2. Check the Lysis Buffer and Wash Buffer A for salt precipitate before use. If any precipitate is observed, incubate the buffer in an 80°C water bath for 15 minutes or until the precipitate is redissolved.
3. Keep samples on ice.
4. Add 700 µL Wash Buffer A (Absolute Ethanol added) into each well of **Plate 3**.
5. Add 700 µL Wash Buffer B (Absolute Ethanol added) into each well of **Plate 4**.
6. Add 100 µL Elution Buffer into each well of **Plate 6**.
7. Vortex the bead mixture before pipetting. Add 180 µL Silica Magnetic Beads into each well of **Plate 2**.  
Vortex the bead mixture tube every 3 lanes in between dispensing to ensure homogenous beads suspension.
8. Add 670 µL Lysis Buffer (Absolute Isopropanol added) into each well of **Plate 1**.
9. Transfer 10 µL **SARS-CoV-2 Process Control** into each well of **Plate 1**.

**NOTE: (Optional)** If users run 96 reactions at one time, please add **1 mL SARS-CoV-2 KIT Process Control and 26 mL Absolute Isopropanol** into Lysis Buffer directly. And transfer 680 µL **Lysis Buffer** (Absolute Isopropanol and Process Control added). **DO NOT** vortex the **SARS-CoV-2 KIT Process Control** before use.

10. Vortex mix and transfer 140 µL **sample** into each well of **Plate 1**.
11. Place **Plate 1, Plate 2, Plate 3, Plate 4 and Plate 6** into the assigned positions of Automated Nucleic Acid Extraction System.
12. Place Magnetic Rod Cover from MTP Accessory Kit into the assigned position of Automated Nucleic Acid Extraction System.
13. Set up the extraction program conditions on Automated Nucleic Acid Extraction System as listed below. Refer to the instrument user manual for more setting details.

Step	Plate Working Station	Waiting Time (min)	Incubation Time (min)	Incubation Speed	Magnetic Time (sec)	Magnetic Speed	Volume (µL)
1	1	0	2	5	0	1	820
2	2	0	0	1	30	1	180
3	1	0	15	3	30	1	820
4	3	0	1	5	30	1	700
5	4	0	1	5	30	1	700
6	4	1	0	1	0	1	700
7	6	0	5	5	120	1	100
8	4	0	1	5	0	1	700

14. After setting up the program conditions, click the thermometer icon on the bottom left to enter the "Temperature Control" page.
15. Turn on the temperature setting for both Cracking set (Lysis step) and Elution set at 65°C.

- Save the temperature setting to run the Extraction Program automatically on Automated Nucleic Acid Extraction System. Once the program finishes, take out **Plate 6** with eluted nucleic acids inside for following Real-time RT-PCR preparation or seal the **Plate 6** to store the eluates properly.

**NOTE:**

- The minimum elution volume is 100  $\mu\text{L}$  on Automated Nucleic Acid Extraction System.
- Pipet carefully to avoid cross-contamination caused by dropping liquid.
- Ensure the deep well plates are placed correctly and do not reuse the plates.

**11.2 Real-time RT-PCR**

The Real-time RT-PCR procedures of SARS-CoV-2 Detection should be operated and followed assay procedures correctly on StepOnePlus™ Real-Time PCR System. Please refer to the operation instructions of StepOnePlus™ Real-Time PCR System for more details.

- Pipetting mix each extracted sample before use and keep samples on ice.
- Thawing detection kit components completely to prepare the Real-time RT-PCR **Master Mix**.

Master Mix Preparation	For 1 rxn
SARS-CoV-2 KIT Reagent Mix	5 $\mu\text{L}$
SARS-CoV-2 KIT Enzyme	5 $\mu\text{L}$
Total	10 $\mu\text{L}$

**NOTE:**

- The amount of Reagent Mix and Enzyme required for a Master Mix depends on the number of reactions. Always prepare a surplus.
- Transfer 10  $\mu\text{L}$  **Master Mix** into each well of the real-time RT-PCR plate.
  - Add 10  $\mu\text{L}$  extracted RNA into each sample well, 10  $\mu\text{L}$  NEG control into NEG well, and 10  $\mu\text{L}$  POS control into POS well, respectively.

**For each Real-time RT-PCR reaction:**

rRT-PCR Preparation	For 1 rxn
Master Mix	10 $\mu\text{L}$
Extracted RNA/ NEG Control/ POS Control	10 $\mu\text{L}$
Total	20 $\mu\text{L}$

**NOTE:**

- POS Control and NEG Control must be included in each assay run for test validity, and the default well position for **NEG Control** is **H12**, and for **POS Control** is **G12**, respectively.
  - POS Controls and additional NEG Controls could be acquired from IntelliPlex™ SARS-CoV-2 Test Control Kit (PlexBio; REF: 82318).
- Mix by tapping the tubes and spin down before placing the tubes on the StepOnePlus™ Real-Time PCR System.
  - Open the StepOnePlus™ Real-Time PCR System (StepOne™ Software v2.3) and select **“Set Up” & “Template”**.
  - Browse to and open the **IntelliPlex SARS-CoV-2 Detection Test Kit template**.
  - In the **Experiment Properties** tab, enter or confirm the following.
    - Experiment Name:** Enter a unique name (e.g., MMDDYYYY SARS-CoV-2 Test)
    - Instrument type:** StepOnePlus™ Instrument (96 Wells)
    - Experiment type:** Quantitation - Standard Curve
    - Chemistry:** TaqMan™ Reagents
    - Run Mode:** Standard (~ 2 hours to complete a run)

9. In the **Run Method** tab, confirm that the Volume is **20 µL**. Set up the RT-PCR program conditions as shown below:

Step	Temp. (°C)	Time	Cycles
Reverse Transcription	50	10 min	1
Taq Activation	95	10 min	1
Denaturation	95	10 sec	45
Annealing/ Extension	60	30 sec	

10. In the **Plate Setup** tab, click **Define Targets and Samples**

11. In the **Define Targets** table, confirm that the reporter dye and the target pairs are correct.

Reporter Dye	Target Name	Quencher
FAM	ORF1a Gene	None
VIC	N Gene	None
TAMRA	Process Control	None

12. In the **Assign Targets and Samples** pane, assign sample(s) to the selected wells and select the **Passive Reference** to be **ROX**.

13. In the **View Plate Layout**, confirm that the targets, samples as well as controls above are assigned to each well correctly.

**NOTE:** The default well position for **NEG Control** is **H12**, and for **POS Control** is **G12**, respectively.

14. In the **Run** tab, click **Start Run**.

15. Once the assay is completed, save the run file from StepOnePlus™ Real-Time PCR System for data analysis.

**11.3 Real-time RT-PCR Data Analysis**

1. In the **Analysis** tab, select all sample wells from the plate layout. All the amplification plots should be displayed as Fig. 1.



Fig. 1. Displayed Amplification Plot

2. To confirm the baseline setting, select **Linear** from **Graph Type** menu. Click **Baseline** checkbox to show the baseline range, which normally starts from 3-5 cycles and ends at 15-20 cycles.

**NOTE:** The baseline setting is automatically done by instrument. Users could correct the baseline settings manually as needed.

3. To confirm the threshold setting by plot configuration as follows. Click **Threshold** checkbox to show the threshold which should be set in the exponential phase of the amplification curve.

- **Plot Type:** ΔRn vs Cycle
- **Graph Type:** Log
- **Target:** ORF1a, N or Process Control

**NOTE:** Each target has its dedicated threshold setting and the threshold setting could vary between different instruments due to the signal intensities deviation.

- Repeat step 2-3 to analyze results of each target (ORF1a, N Gene and Process Control).
- Click **Analyze** to analyze data after changes of settings. Once the analysis is completed, open the **Export** tab to export data file.
- In the **Export** tab, enter **Export File Name**, **Export File Location**, choose all items from **select data to export** and select **One File** to export all data in one file. Click **Start Export** to export data file.

## 12. DISCLAIMERS

### Negative Test Result

A negative test result means the IntelliPlex SARS-CoV-2 Detection Test Kit does not detect the virus in the sample. It does not preclude the possibility that the specimen does contain the virus. Only samples with detectable amounts of the virus matching the reference sequences are detectable; false-negative test results might occur due to experimental errors or other causes. Interpretation of the results should consider these possibilities.

### Positive Test Result

A positive test result means that the IntelliPlex SARS-CoV-2 Detection Test Kit detects SARS-CoV-2 in the sample. False-positive test results may be caused by experimental errors or other causes. Interpretation of the results should consider these possibilities.

## 13. INTERPRETATION OF RESULTS

Additional external NEG Control and POS Control wells must be run for each batch of rRT-PCR assay. The NEG Control and POS Control wells must pass the acceptance criteria that batch of assays to be considered valid.

POS Control	Channel	Ct	Cutoff
ORF1a Gene	FAM	< 37	37
N Gene	VIC	< 40	40
Process Control	TAMRA	Any Value	-
NEG Control	Channel	Ct	Cutoff
ORF1a Gene	FAM	>37	37
N Gene	VIC	>40	40
Process Control	TAMRA	>35	35

### Result Interpretation:

Orf1a Gene	N Gene	Process Control	Interpretation
Positive (Ct < 37)	Positive (Ct < 40)	Ct : Any value	Valid Result SARS-CoV-2 Detected
Positive (Ct < 37)	Negative (Ct > 40 or Undetermined)	Ct : Any value	Valid Result SARS-CoV-2 Detected
Negative (Ct > 37 or Undetermined)	Positive (Ct < 40)	Ct : Any value	Valid Result SARS-CoV-2 Detected
Negative (Ct > 37 or Undetermined)	Negative (Ct > 40 or Undetermined)	Positive (Ct < 35)	Valid Result SARS-CoV-2 Not Detected
Negative (Ct > 37 or Undetermined)	Negative (Ct > 40 or Undetermined)	Negative (Ct > 35 or Undetermined)	Repeat extraction and rRT-PCR. If the repeated result remains invalid, please collect a new fresh specimen from the patient

## 14. ANALYTICAL PERFORMANCE

### Limit of Blank

Limit of Blank (LoB) was determined by performing 60 saliva and 60 nasopharyngeal swab specimens from confirmed negative individuals. Each specimen was tested triplicate on 2 reagent lots and one set of instruments. The cutoff values of each targeted gene were determined by the measured maximum Ct value values, respectively.

Only “No Detected” results were observed in these SARS-CoV-2 negative samples.

### Limit of Detection (Analytical Sensitivity)

Limit of Detection (LoD) studies determine the lowest detectable concentration of a pathogen at which greater than or equal to 95% of all replicates test positive.

To determine the LoDs of the IntelliPlex™ SARS-CoV-2 Detection Test Kit, contrived samples were prepared by spiking AccuPlex SARS-CoV-2 Verification Panel (Material Number: 0505-0168, SeraCare) at different defined concentrations into two different clinical matrixes, respectively. The clinical matrixes are pooled saliva from individuals tested negative for SARS-CoV-2 and the confirmed negative for SARS-CoV-2 from nasopharyngeal (NP) swabs in universal transport media (UTM).

Two reagent lots of the LoD concentration for Orf1a and N genes were calculated by the Probit program at a 95% hit rate. The LoDs of first tested lot were 1.42E+02 copies/ml for Orf1a gene and 2.34E+02 copies/ml for N gene. The LoDs results for second lot were 1.28E+02 copies/ml for Orf1a and 2.32E+02 copies/ml for N gene.

First Lot	Total	Hit Rate	
		Orf1a Gene	N Gene
500 copies / ml	22	100.00%	100.00%
350 copies / ml	22	100.00%	100.00%
250 copies / ml	22	100.00%	95.50%
200 copies / ml	22	95.50%	86.40%
150 copies / ml	22	95.50%	90.90%
100 copies / ml	22	90.90%	72.70%
50 copies / ml	22	63.60%	45.50%
0 copies / ml	22	0.00%	0.00%

Second Lot	Total	Hit Rate	
		Orf1a Gene	N Gene
500 copies / ml	22	100.00%	100.00%
350 copies / ml	22	100.00%	100.00%
250 copies / ml	22	100.00%	100.00%
200 copies / ml	22	95.45%	86.36%
150 copies / ml	22	95.45%	81.82%
100 copies / ml	22	95.45%	50.00%
50 copies / ml	22	72.73%	18.18%
0 copies / ml	22	0.00%	0.00%

The confirmatory LoD testing was performed by additional 20 replicates spiked AccuPlex SARS-CoV-2 Verification Panel into saliva and nasopharyngeal swab specimens at 2.34E+02 copies/ml. The hit rate of contrived 1xLoD saliva samples for Orf1a gene and N gene are both 95%. And the hit rate of contrived 1xLoD nasopharyngeal swab samples for Orf1a gene and N gene are both 95%, respectively.

<b>1xLoD of Contrived Positive Saliva Specimens (234 copies / ml)</b>	<b>Detected</b>	<b>Not Detected</b>	<b>Total</b>	<b>Hit Rate</b>
<b>Orf1a Gene</b>	19	1	20	95%
<b>N Gene</b>	19	1	20	95%

<b>1xLoD of Contrived Positive Nasal Swab Specimens (234 copies / ml)</b>	<b>Detected</b>	<b>Not Detected</b>	<b>Total</b>	<b>Hit Rate</b>
<b>Orf1a Gene</b>	19	1	20	95%
<b>N Gene</b>	19	1	20	95%

### Repeatability and Reproducibility

The repeatability and reproducibility of the IntelliPlex™ SARS-CoV-2 Detection Test Kit were evaluated across two reagent lots, three sites, six operators, three instruments and five non-consecutive testing days. Two operators were at one site and one operator performed one run per day for 5 runs to have a total of 10 runs at one site. Repeatability and reproducibility were demonstrated with low-positive concentration (1x LoD) and high-positive concentration (6x LoD) AccuPlex SARS-CoV-2 Verification Panel spiked in human saliva and nasopharyngeal swab which is SARS-CoV-2 negative confirmed. Each concentration had 90 replicates and the correct call of all testing level of each target gene was 100% (90/90) across all variance combined (i.e., site/instrument, operator, and day). Across all variance components (i.e., site/instrument, operator, and day), the overall coefficient of variation is less than 5% across all panel members.

### Inclusivity (Analytical Sensitivity)

#### SARS-CoV-2

The inclusivity of the IntelliPlex™ SARS-CoV-2 Detection Test Kit for the detection of SARS-CoV-2 was analyzed in silico. Sequences were obtained from the Global Initiative on Sharing All Influenza Data (GISAID; <https://www.gisaid.org/>). Only full-length or near full-length sequences containing less than 20% N in their sequences were utilized in the analysis; a total of 242,258 sequences were included in the analysis, covering representative sequences from all strains (as of February 28<sup>th</sup> 2021).

The conducted in silico study concluded a SARS-CoV-2 sequence as “detected” if:

- The primer pair can bind to the target (allowing up to 1 mismatch) and the distance between both binding sides is less than 200 nucleotides  
and
- The probe can bind (allowing up to 1 mismatch) to the sequence between both primer pairs.

The results are summarized below:

Primer Pair	probe	Target	#Detected	% Detected
CoV-2/ORF-F + CoV-2/ORF-R	CoV-2/ORF probe	SARS-CoV-2 ORF1a gene amplicon	235,696	96.90%
CoV-2/N-F + CoV- 2/N-R	CoV-2/N probe	SARS-CoV-2 N gene amplicon	226,567	93.50%
<b>Total Hits</b>			241,713	99.76%
<b>#Lost Detection</b>			572	0.23%
<b>Total Sequences</b>			242,285	100%

The inclusivity *in silico* study revealed that at least 99.76% of sequences available on GISAID (as of 02/28/2021) can be detected by the IntelliPlex™ SARS-CoV-2 Detection Test Kit. The sequences that fall into the 0.23% that were not predicted to be detected may still be detected. Any sequences published on GISAID that contain base N (not determined; could be any of A, T, C, G) at any position in the primer/probe binding sequence are assumed to be a mismatch. If more than one mismatch between primer and target sequence is identified, the sequence is assigned to be a “not detected” (independent if the mismatches are at the 5’ or 3’ end of the primer). Thus, sequences predicted to be missed in this analysis may still be detectable with IntelliPlex™ SARS-CoV-2 Detection Test Kit.

## Cross-Reactivity

### In-silico analysis

Cross-reactivity of the IntelliPlex™ SARS-CoV-2 Detection Test Kit was evaluated by *in silico* analysis. The BLASTn analysis queries of the IntelliPlex™ SARS-CoV-2 Detection Test Kit primers and probes were performed against the sequences of the pathogens listed in the table below.

Pathogens	Orf1a	Orf1a	Orf1a	N	N	N	MS2	MS2	MS2
	Forward	Reverse	Probe	Forward	Reverse	Probe	Forward	Reverse	Probe
Human coronavirus HKU1	50%	60%	40%	55%	41%	42%	50%	38%	36%
Human coronavirus OC43	55%	45%	40%	55%	41%	42%	45%	38%	40%
Human coronavirus NL63	45%	45%	36%	45%	41%	38%	36%	38%	36%
Human coronavirus 229E	50%	70%	48%	50%	45%	42%	50%	50%	40%
SARS-coronavirus	100%	100%	100%	100%	100%	100%	0%	0%	0%
MERS-coronavirus	50%	50%	44%	55%	55%	46%	55%	50%	36%
Influenza A	60%	60%	64%	70%	68%	54%	55%	50%	48%
Influenza B	65%	55%	44%	70%	50%	0%	45%	42%	0%
Adenovirus TYPE 1	50%	50%	40%	50%	41%	38%	41%	38%	36%
Adenovirus TYPE 7	60%	55%	36%	55%	41%	38%	41%	42%	36%
Candida albicans	60%	70%	60%	75%	55%	58%	73%	63%	48%
Cytomegalovirus	75%	55%	52%	80%	59%	58%	59%	50%	52%
Enterovirus (e.g., EV68)	45%	55%	48%	55%	45%	46%	50%	38%	40%

Pathogens	Orf1a Forward	Orf1a Reverse	Orf1a Probe	N Forward	N Reverse	N Probe	MS2 Forward	MS2 Reverse	MS2 Probe
Epstein Barr virus	50%	55%	56%	60%	55%	50%	55%	63%	56%
Parainfluenza virus 1	50%	50%	40%	45%	41%	42%	45%	38%	32%
Parainfluenza virus 2	50%	45%	44%	50%	45%	46%	50%	50%	28%
Parainfluenza virus 3	65%	45%	56%	60%	55%	38%	45%	38%	40%
Parainfluenza virus 4	45%	45%	52%	55%	50%	50%	55%	38%	36%
Measles	50%	55%	56%	50%	45%	54%	50%	42%	40%
Human Metapneumovirus (hMPV)	50%	60%	48%	65%	50%	50%	41%	46%	36%
Human parvovirus	45%	50%	44%	45%	45%	38%	45%	38%	32%
Mumps virus	45%	60%	44%	55%	50%	38%	55%	42%	40%
Respiratory syncytial virus type b	50%	65%	44%	50%	41%	42%	45%	38%	36%
Rhinovirus	80%	55%	44%	50%	50%	58%	50%	50%	44%
Bordetella pertussis	80%	60%	52%	0%	0%	54%	55%	0%	0%
Chlamydia pneumoniae	55%	60%	44%	60%	50%	54%	59%	50%	44%
Corynebacterium sp.	80%	70%	64%	85%	64%	67%	68%	67%	64%
Escherichia coli	70%	90%	60%	75%	64%	58%	77%	63%	60%
Haemophilus influenzae	75%	65%	64%	70%	55%	50%	55%	50%	48%
Lactobacillus sp.	80%	50%	60%	90%	59%	58%	64%	54%	64%
Legionella	50%	75%	76%	70%	64%	75%	68%	58%	52%
Legionella pneumophila	0%	0%	0%	0%	0%	0%	0%	0%	0%
Moraxella catarrhalis	60%	65%	48%	60%	64%	54%	64%	54%	44%
Mycobacterium tuberculosis	75%	60%	60%	60%	55%	54%	55%	50%	48%
Mycoplasma pneumoniae	65%	50%	56%	55%	50%	58%	50%	54%	40%
Neisseria meningitidis	0%	0%	0%	0%	0%	0%	0%	0%	0%
Pseudomonas aeruginosa	70%	65%	60%	65%	59%	63%	73%	63%	52%
Pneumocystis jirovecii (PJP)	60%	60%	64%	80%	50%	54%	50%	46%	48%
Staphylococcus aureus	0%	0%	0%	0%	0%	0%	0%	0%	0%
Staphylococcus epidermidis (taxid:1282)	55%	60%	52%	65%	59%	58%	50%	50%	44%
Streptococcus pneumoniae	55%	65%	60%	70%	50%	54%	59%	54%	64%
Streptococcus pyogenes	50%	65%	52%	65%	64%	67%	59%	54%	52%
Streptococcus salivarius	65%	65%	48%	65%	55%	50%	59%	54%	48%
HSV1	55%	50%	44%	75%	50%	42%	50%	46%	52%
HSV2	50%	50%	48%	50%	0%	46%	45%	42%	48%

Pathogens	Orf1a Forward	Orf1a Reverse	Orf1a Probe	N Forward	N Reverse	N Probe	MS2 Forward	MS2 Reverse	MS2 Probe
HPEV	40%	50%	40%	50%	41%	38%	41%	38%	44%
VZV	55%	55%	48%	55%	45%	46%	45%	42%	44%

### Wet Testing

The IntelliPlex™ SARS-CoV-2 Detection Test Kit was tested to evaluate cross-reactivity between SARS-CoV-2 virus and SARS-coronavirus.

The nucleic acid of the organism listed in the table below was extracted with IntelliPlex SARS-CoV-2 Detection Test Kit-Part A and Automated Nucleic Acid Extraction System, used as template in the rRT-PCR reaction (pathogens > 10<sup>6</sup> copies/ rRT-PCR reaction; all tests were performed in triplicates)

Pathogen (strain)	Source	Copy number	Result
SARS-coronavirus	vircell (AmpliRun MBC136-R)	>10 <sup>6</sup> copies/ rRT-PCR	0/3 SARS-CoV-2 Not Detected

### Interference

To exclude inhibitory effects of substances encountered in saliva and nasopharyngeal swab specimens, the impact of potentially interfering substances that may pre-exist in the specimen was assessed.

Testing was conducted by spiking SARS-CoV-2 at a concentration of 2 x LoD into saliva derived from pooled saliva, sample number 1 to 10, or, nasopharyngeal swab, sample number 11 to 19, from individuals negative for SARS-CoV-2. RNAs utilized were obtained from AccuPlex SARS-CoV-2 Verification Panel (Material Number: 0505-0168, SeraCare). To mimic the “worst-case scenario”, interfering substances listed in the table below were blended with saliva and nasopharyngeal swab at a particular concentration (to ensure the potentially interfering substances are present in the specimen at a high concentration).

Sample Number	Main compound	Brand Name	Concentration
1	Toothpaste	Sensodyne	5% v/v
2	Mouthwash	day and night	5% v/v
3	Cough Syrup	Robitussin	1% v/v
4	Cough Syrup	Root chemical	1% v/v
5	Whole blood	NA	2.5% v/v
6	Menthol lozenges	Pulmoll	5mg/mL
7	Aspirin™	Bokey	1% w/v
8	Nicotine	Nicorette	0.03 mg/mL
9	Human genomic DNA	A549 cell culture	10 ng/μL
10	Mupirocin	Shiteh Organic Pharmaceutical	500 ng/mL
11	Mometasone Furoate	Merck Sharp & Dohme	50 μg/dose
12	Beclomethasone Dipropionate	HWANG'S Pharmaceutical	100 μg/dose
13	Xylometazoline	Otrivin	1mg/mL
14	Budesonide Micronized	Synmosa	100 μg/dose
15	Triamcinolone acetonide	Sanofi	55 μg/dose
16	Azelastine Hydrochloride	SIU GUAN CHEMICAL INDUSTRIAL	0.14mg/dose
17	Azelastine Hydrochloride	Synmosa	140 μg/dose

18	Naphazoline Hydrochloride	KASHIN MEDICINES	0.5mg/mL
19	Sodium Chloride and Sodium Bicarbonate	Neil Med	10 mg/mL

All contrived samples were tested using the IntelliPlex™ SARS-CoV-2 Detection Test Kit on the Automated Nucleic Acid Extraction System. All testings were performed as described following the User Manuals of the assay kits and instruments. None of the tested compounds impacted the performance of the IntelliPlex™ SARS-CoV-2 Detection Test Kit.

## 15. CLINICAL EVALUATION

A total of 60 contrived nasopharyngeal specimens (20 of 2xLoD positive nasal swab specimens, 10 of 3xLoD positive nasal swab specimens, 30 negative specimens) were used to evaluate the clinical performance of the IntelliPlex™ SARS-CoV-2 Detection Test Kit on nasal swab specimens. The hit rate of 2X and 3X LoD contrived positive nasal swab specimens were 100% (20/20) and 100% (10/10) and none of the negative specimens were detected positive (0/30).

A total of 60 contrived saliva specimens (20 of 2xLoD positive saliva specimens, 10 of 3xLoD positive saliva specimens, 30 negative samples) were also used to evaluate the clinical performance of the IntelliPlex™ SARS-CoV-2 Detection Test Kit on saliva specimens. The hit rate of 2X and 3X LoD contrived positive saliva specimens were 100% (20/20) and 100% (10/10) and none of the negative specimens were detected positive (0/30).

A clinical evaluation study of the IntelliPlex™ SARS-CoV-2 Detection Test Kit was conducted with de-identified natural leftover clinical saliva specimens, and all specimens were evaluated with an FDA emergency use authorized comparator assay. A total of 130 clinical specimens, including 120 clinical nasal swab specimens (60 of SARS-CoV-2 tested positive, 60 SARS-CoV-2 tested negative) and 10 clinical saliva specimens (5 of SARS-CoV-2 tested positive, 5 SARS-CoV-2 tested negative) were conducted in the study, respectively.













All specimens were blind-tested with the IntelliPlex™ SARS-CoV-2 Detection Test Kit and an FDA emergency use authorized comparator assay. All assay procedures were followed by the instructions within the instruments and assay kit user manuals. The SARS-CoV-2 tested results by IntelliPlex™ SARS-CoV-2 Detection Test Kit were reported with 61 positive clinical nasal swab specimens (one false-positive) and 60 negative clinical nasal swab specimens. The **60/60 (100%)** Positive Percent Agreement (PPA) and **59/60 (98.33%)** Negative Percent Agreement (NPA) on nasal swab specimens were exhibited. The clinical saliva specimens were reported with 5 positive and 5 negative which demonstrated the **5/5 (100%)** Positive Percent Agreement (PPA) and **5/5 (100%)** Negative Percent Agreement (NPA), respectively.

Clinical Nasal Swab Specimens		FDA Emergency Use Authorized Comparator Assay		
		Positive	Negative	Total
IntelliPlex™ SARS-CoV-2 Detection Test Kit	Positive	60	1	61
	Negative	0	59	59
	Total	60	60	120
Positive Percent Agreement, PPA		60/60; 100% (94.04%-100%) <sup>1</sup>		
Negative Percent Agreement, NPA		60/60; 98.33% (91.06%-99.96%) <sup>1</sup>		


Clinical Saliva Specimens		FDA Emergency Use Authorized Comparator Assay		
		Positive	Negative	Total
IntelliPlex™ SARS-CoV-2 Detection Test Kit	Positive	5	0	5
	Negative	0	5	5
	Total	5	5	10
Positive Percent Agreement, PPA		5/5; 100% (47.82%-100%) <sup>1</sup>		
Negative Percent Agreement, NPA		5/5; 100% (47.82%-100%) <sup>1</sup>		

<sup>1</sup>Two-sided 95% score confidence intervals.

**16. SYMBOLS**

Symbol	Explanation	Symbol	Explanation
	In-vitro diagnostic use		Catalog number
	Batch number		Consult instructions for use
	Manufacturer		Use by Date
	Temperature limitation		Caution
	Contains sufficient for <n> tests		Date of Manufacture
	European Union Conformity		European Authorized Representative

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