# EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
# Predicine SARS-CoV-2 RT-PCR TEST
# (PREDICINE Inc.)

For *in vitro* Diagnostic Use
Rx only
For use under Emergency Use Authorization (EUA) only

**(The Predicine SARS-CoV-2 RT-PCR Test will be used together with the Predicine COVID-19 Self-Collection Kit. The test will be performed at the laboratories designated by Predicine, Inc., which are certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a and meet the requirements to perform high complexity tests, as described in the laboratory procedures reviewed by FDA under this EUA.)**

## INTENDED USE

The Predicine SARS-CoV-2 RT-PCR Test is an extraction free nucleic acid real-time reverse transcription polymerase chain reaction (RT-PCR) test for the qualitative detection of nucleic acid from SARS-CoV-2 in anterior nasal specimens collected dry from any individual, including individuals without symptoms or other reasons to suspect COVID-19 using the Predicine COVID-19 Self-Collection Kit.

This test is also for use with individual anterior nasal swab specimens collected dry at home (which includes in a community-based setting) from individuals aged 18 years and older (self-collected), 14 years and older (self-collected under adult supervision), or 2 years and older (collected with adult assistance) using the Predicine COVID-19 Self-Collection Kit, when determined to be appropriate by a healthcare provider.

The Predicine SARS-CoV-2 RT-PCR Test is also for the qualitative detection of nucleic acid from SARS-CoV-2 in pooled samples, containing up to eight individual anterior nasal swab specimens, that are collected dry from any individuals, including individuals with or without symptoms or other reasons to suspect SARS-CoV-2 infection using the Predicine COVID-19 Self-Collection Kit. Negative results from pooled testing should not be treated as definitive. If a patient's clinical signs and symptoms are inconsistent with a negative result or results are necessary for patient management, then the patient should be considered for individual testing. Specimens included in pools with a positive or invalid result must be tested individually prior to reporting a result. Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.

Testing is limited to laboratories designated by Predicine, Inc., which are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, and meet the requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in anterior nasal swab 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. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all 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 Predicine SARS-CoV-2 RT-PCR Test is only intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time RT-PCR and *in vitro* diagnostic procedures. The Predicine SARS-CoV-2 RT-PCR Test and Predicine COVID-19 Self-Collection Kit are only for use under the Food and Drug Administration's Emergency Use Authorization.

## DEVICE DESCRIPTION AND TEST PRINCIPLE

### *1) Predicine COVID-19 Self-Collection Kit*

The Predicine COVID-19 Self-Collection Kit is a prescription use only product for individuals aged 2 years or older, including individuals with or without symptoms or other reasons to suspect SARS-CoV-2 infection. The Predicine COVID-19 Self-Collection Kit is available through individual single use or bulk use collection and is provided as in three kit versions: bulk use (for collection in a testing program in a community-based setting), individual single use home collection, and individual single use on-site collection. The contents of each kit version are as follows:

**Predicine COVID-19 Self-Collection Kit (Bulk use collection)**

| Name | Description | Quantity | Vendor | Catalog #/Part Number |
|---|---|---|---|---|
| Nasal Swab | 150 mm Flocked Swab, polyester, sterile | 1 | Medico Technology Co., Ltd | MFS-98000KQ (OEM Type) |
| Collection tube | 1.4 mL Screw-capped tube with barcode or 1.0 mL fluidX tubes with barcode | 1 | Micronic or Azenta | MP32074-Z20 or 68-1003-11 |
| Biohazard bag | Specimen transport bag | 1 | Medline | DYND30261 |
| Instructions For Use | Instruction sheet (Instructions for Bulk Kit)* | 1 | Predicine | PSC000101 |

* Instructions are identical to the instructions for home collection for individuals, except specimens are not shipped by courier, but are dropped off at a designation dropoff location specified by the testing program.

**Predicine COVID-19 Self-Collection Kit (Individual single use home collection)**

| Name | Description | Quantity | Vendor | Catalog #/Part Number |
|---|---|---|---|---|
| Nasal Swab | 150 mm Flocked Swab, polyester, sterile | 1 | Medico Technology Co., Ltd | MFS-98000KQ (OEM Type) |
| Collection tube | 1.4 mL Screw-capped tube with barcode or 1.0 mL fluidX tubes with barcode | 1 | Micronic or Azenta | MP32074-Z20 or 68-1003-11 |
| Biohazard bag | Specimen transport bag | 1 | Medline | DYND30261 |
| Instructions For Use | Instruction sheet (Instructions for Individuals) | 1 | Predicine | PSC000101 |
| UN3373 Envelope | Courier envelope - used to ship temperature sensitive specimens designated as Biological Substance, Category B (UN 3373) | 1 | FedEx or alternate courier | |
| Courier prepaid Return Label | Courier pre-paid return label | 1 | FedEx or alternate courier | 163034 |

**Predicine COVID-19 Self-Collection Kit (Individual single use on-site collection)**

| Name | Description | Quantity | Vendor | Catalog #/Part Number |
|---|---|---|---|---|
| Nasal Swab | 150 mm Flocked Swab, polyester, sterile | 1 | Medico Technology Co., Ltd | MFS-98000KQ (OEM Type) |
| Collection tube | 1.4 mL Screw-capped tube with barcode or 1.0 mL fluidX tubes with barcode | 1 | Micronic or Azenta | MP32074-Z20 or 68-1003-11 |
| Biohazard bag | Specimen transport bag | 1 | Medline | DYND30261 |

## Ordering and Sample Collection

i. Bulk Use Collection

An entity such as a school, government agency, company, is designated by Predicine and operates a testing program that includes collection kit supply pick-up locations and specimen drop-off locations overseen by trained Predicine front-line staff. Bulk collection kits are sent to the designated entity where they are distributed to patients at the pick-up location by trained Predicine front-line staff.

To obtain a Predicine COVID-19 Self-Collection Kit (Bulk Use) from the entity, individuals (or the parents/guardians of minors) can enroll in the screening program that is sponsored by the entity. For individuals who enroll in the screening program, a standing order prescription from a physician will be established for each separate screening program.

Individuals (or their parents/guardians of minors) in each entity, will collect the specimen according to the provided Predicine COVID-19 Self-Collection Kit (Bulk Use) "Instructions for Use (IFU)" and return the specimen to the Predicine trained front-line staff at the drop-off location, and the specimen is transported at ambient temperature to the Predicine laboratory via FedEx, UPS, or a 3rd party courier within the same day of sample collection.

ii. Individual Single Use Collection

a) Home Collection

Individuals who are to request the Predicine COVID-19 Self-Collection Kit through the online portal, he/she must verify he/she is 18 years or older for self-use or for use on a minor (individuals younger than 18) and complete a screening questionnaire at the Predicine website (https://covid19-test.predicine.com/covid-19/pre-screening). The questionnaire is designed based on CDC recommendations and will be reviewed by a physician, and if determined to be appropriate, a prescription is issued, and the Predicine COVID-19 Self-Collection Kit (Home Collection) is then shipped to the patient for home collection.

Individuals may also request the Predicine COVID-19 Self-Collection Kit through their healthcare provider (HCP). When the HCP determines a test is required and is appropriate, he/she issues the prescription, orders the Predicine SARS-CoV-2 RT-PCR Test for the patient and provides the name and address of the patient to Predicine. The Predicine COVID-19 Self-Collection Kit (Home Collection) is then shipped to the patient for home collection.

The specimen collection and shipping instructions, the "Predicine COVID-19 Self-Collection Kit (Single Use)" are included in the kit and instruct the user on how to register their kit, collect the specimen, and package the specimen for shipment. During kit registration, the patient registers his/her personal information and the sample barcode through the Predicine web portal. Without registration, samples will be rejected during the accessioning step. After the kit is registered, the patient collects the anterior nasal specimen packages it and ships the specimen via FedEx (or similar alternate 3rd party courier) at ambient temperature to the Predicine designated laboratory within the same day of sample collection.

b) On-site Collection

An HCP trained on how to appropriately collect the nasal swab specimen issues the prescription, orders the test, collects the sample for the patient using the Predicine COVID-19 Self-Collection Kit (Single Use) instructions, places it in the collection tube, properly packages the specimen and brings the specimen back to the designated laboratory. All nasal samples collected by the HCP are transported back to the Predicine designated laboratory within the same day of sample collection.

**Sample Accessioning**

Samples received at the Predicine designated clinical laboratory via the Predicine COVID-19 Self-Collection Kit undergo the Predicine sample accessioning process prior to acceptance for testing.

Samples with the following issues will be rejected and may require user follow-up and re-sampling:

| Rejection Reason | Definition |
|---|---|
| Tube damaged | The sample collection tube is cracked. |
| Tube not present | Biohazard bag is empty, or the sample is not returned in the supplied package. |
| Swab issue | Swab is not present in the sample tube, or inserted in the tube improperly (e.g., upside down). |
| Barcode issue | Sample barcode is not registered or damaged (e.g., unreadable). |
| Expired specimen | Specimen received >56 hours post collection |

**Results Reporting**

a) A text message will be sent to the patient if the patient's sample is rejected, or if the test result is either invalid or positive.
b) The patient's test result (as summarized in the table below) will be generated in a pdf file, and a secure link of the result will be sent to patient through text message or email.

| Result | Test Name |
|---|---|
| Positive/Detected | Predicine SARS-CoV-2 RT-PCR Test for Covid-19 (RT-PCR/NAAT) single sample testing |
| Negative/Not Detected | Predicine SARS-CoV-2 RT-PCR Test for Covid-19 (RT-PCR/NAAT) single sample testing |
| Negative/Not Detected | Predicine SARS-CoV-2 RT-PCR Test for Covid-19 (RT-PCR/NAAT) pooled sample testing |
| Indeterminate/invalid | Predicine SARS-CoV-2 RT-PCR Test for Covid-19 (RT-PCR/NAAT) single sample testing |

### *2) Predicine SARS-CoV-2 RT-PCR Test*

The Predicine SARS-CoV-2 RT-PCR Test targets specific genomic regions of the SARS-CoV-2 nucleocapsid (N) gene and Envelope (E) gene. The probes for the two genes are both labeled with FAM to generate target-specific signal. The test includes an RNA internal control (IC, human RNase P (RP)) to monitor the processes from sample collection to fluorescence detection. The IC probe is labeled with Atto fluorescent dye to differentiate its fluorescent signal from SARS-CoV-2 targets.

Sample processing and PCR plate set-up steps are automated using the MGISP-960 instrument. Dry anterior nasal swab specimens are rehydrated and lysed by heat-inactivation to release the viral RNA. PCR plates are prepared and then subjected to reverse transcription and amplification using the Powergene 9600 qPCR. During the amplification process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the bound probe, causing the reporter dye

to separate from the quencher dye, generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle by the Powergene 9600 qPCR instrument.

The Predicine SARS-CoV-2 RT-PCR Test targets specific genomic regions of the SARS-CoV-2 nucleocapsid (N) gene and Envelope (E) gene. The N1 and E primer and probe sequences are the same as the target sequences included in the CDC 2019 nCoV Real-Time RT-PCR Diagnostic Panel (https://www.fda.gov/media/134922/download) and WHO protocol (https://www.who.int/docs/default-source/coronaviruse/real-time-rt-pcr-assays-for-the-detection-of-sars-cov-2-institut-pasteur-paris.pdf?sfvrsn=3662fcb6_2 ), respectively.

The Predicine SARS-CoV-2 RT-PCR Test has been validated for pooling with up to 8 samples using a media pooling strategy (dry anterior nasal swab specimens are rehydrated in PBS and lysed by heat-inactivation to release the viral RNA before pooling). Based on the prevalence of SARS-CoV-2 observed in the clinical specimens (<1%), up to eight sample pooling has been evaluated. Based on sample pool monitoring, the pool size will be adjusted for different positive rates in the intended use population. A negative result indicates that all samples in the pool are negative, and a positive result indicates at least one sample within the pool is positive. Thus, individual specimens from positive pools are retested to determine which individual sample is positive.

## INSTRUMENTS AND REAGENTS USED WITH THE TEST

The Predicine SARS-CoV-2 RT-PCR Test is an RNA-extraction free (heat-inactivation) assay. It is to be used with a MGISP-960 High-throughput Automated Sample Preparation System and the PowerGene 9600 Plus Real-time PCR system (Atila BioSystems) for nucleic acid amplification and detection.

### Predicine SARS-CoV-2 RT-PCR Test

| Component or Function | Name | Vendor | Catalog#/Part # |
|---|---|---|---|
| RT-PCR equipment | Powergene 9600 plus real time PCR system using Software “linegene9600” FQD-96a v1.0.0.1 RC 20200327 or higher | Atila BioSystem | Power96-1 |
| Liquid Handler | MGISP-960 High-throughput Automated Sample Preparation System using software v1.4.2.201 or higher | MGI Tech Co., Ltd. | MGISP-960RS V7 |
| PCR enzyme | 4X UltraPlex 1-Step ToughMix | QuantaBio | 95166-01K |
| PCR Primer/Probe Mix | COVID-19 N1-F Primer | IDT | 10006830 |
| | COVID-19 N1-R Primer | IDT | 10006831 |
| | COVID-19 N1-Probe | IDT | 10006832 |
| | COVID-19 E-F Primer | IDT | 10006889 |
| | COVID-19 E-R Primer | IDT | 10006891 |
| | COVID-19 E-Probe | IDT | 10006893 |
| | RP-F Primer | IDT | 10006836 |
| | RP-R Primer | IDT | 10006837 |
| | RP Probe | IDT | 10007062 |
| PC | COVID-19 N Positive Control* | IDT | 10006625 |

| | | | |
|---|---|---|---|
| | Hs_RPP30 Internal Extraction Control | IDT | 10006626 |
| NC | PBS, pH 7.4 (1X) | Gibco | 10010-31 |
| Resuspension Buffer | PBS, pH 7.4 (1X) | Gibco | 10010-31 |

*The concentration is estimated based on the concentration provided by the vendor. The COVID-19 N Positive Control is tested at ~4xLoD.

## CONTROLS

Test controls are tested concurrently with all test samples in each instrumental run.

1) PC - positive control. The positive control contains a diluted equal molar mix of 2019-nCoV_N_Positive control (IDT, Cat#: 10006625;) and Hs_RPP30 Positive Control (IDT, Cat3: 10006626). It was used during the RT-PCR process to serve as a control for amplification and detection of SARS-CoV-2 RNA and human RNase P RNA.
2) NC - negative control. The negative control is 1X PBS. It serves to verify that analyte contamination does not occur during reaction setup. There should be NO exponential amplification curve shown in any channel (FAM or CY5) for the template, otherwise the test is invalid, and the results cannot be used for diagnosis.
3) IC - internal control. The internal control is a non-covid nucleic acid in each reaction and is co-amplified with the target nucleic acid. The internal control gene RNase P serves as the IC control for sample collection, amplification and detection in every reaction, reagent integrity, equipment function, and monitors for the presence of inhibitors.

## INTERPRETATION OF RESULTS

The criteria for interpretation of the results obtained with the assay controls are shown in the table below. All test controls must produce the expected results in order to interpret the results from patient samples.

1) Interpretation of results for assay controls

| | IC (CY5) | SARS-CoV-2 (FAM) | Interpretation |
|---|---|---|---|
| NC | No Ct or no sigmoidal amplification curve | No Ct or no sigmoidal amplification curve | Pass |
| | Sigmoidal amplification curve and Ct value is ≤40 | No Ct or no sigmoidal amplification curve | Fail |
| | No Ct or no sigmoidal amplification curve | Sigmoidal amplification curve and Ct value is ≤40 | Fail |
| | Sigmoidal amplification curve and Ct value is ≤40 | Sigmoidal amplification curve and Ct value is ≤40 | Fail |
| PC | No Ct or no sigmoidal amplification curve | Sigmoidal amplification curve and Ct value is ≤40 | Fail |
| | No Ct or no sigmoidal amplification curve | No Ct or no sigmoidal amplification curve | Fail |

| | | | |
|---|---|---|---|
| | Sigmoidal amplification curve and Ct value is ≤40 | No Ct or no sigmoidal amplification curve | Fail |
| | Sigmoidal amplification curve and Ct value is ≤40 | Sigmoidal amplification curve and Ct value is ≤40 | Pass |

2) Interpretation of results for patient individual samples

| Example | IC (CY5) Observation | SARS-CoV-2 (FAM) Observation | Interpretation |
|---|---|---|---|
| Sample 1 | Sigmoidal amplification curve and Ct value is ≤40 | Sigmoidal amplification curve and Ct value is ≤40 | **Positive for SARS-CoV-2 RNA** |
| Sample 2 | No Ct or no sigmoidal amplification curve | Sigmoidal amplification curve and Ct value is ≤40 | **Positive for SARS-CoV-2 RNA** |
| Sample 3 | Sigmoidal amplification curve and Ct value is ≤40 | No Ct or no sigmoidal amplification curve | **Negative for SARS-CoV-2 RNA** |
| Sample 4 | No Ct or no sigmoidal amplification curve | No Ct or no sigmoidal amplification curve | **Invalid Test, Retest Required*** |

* The assay needs to be repeated with the same specimen. If the test fails again, a new specimen needs to be collected from the patient and tested.

3) Interpretation of results for pooled patient samples

| Example | IC (CY5) Observation | SARS-CoV-2 (FAM) Observation | Interpretation |
|---|---|---|---|
| Pool 1 | Sigmoidal amplification curve and Ct value is ≤40 | Sigmoidal amplification curve and Ct value is ≤40 | **Positive for SARS-CoV-2 RNA, individual samples in the pool need to be tested** |
| Pool 2 | No Ct or no sigmoidal amplification curve | Sigmoidal amplification curve and Ct value is ≤40 | **Positive for SARS-CoV-2 RNA, individual samples in the pool need to be tested** |
| Pool 3 | Sigmoidal amplification curve and Ct value is ≤40 | No Ct or no sigmoidal amplification curve | **Negative for SARS-CoV-2 RNA, all individual samples in the pool are negative*** |

| | | | |
|---|---|---|---|
| Pool 4 | No Ct or no sigmoidal amplification curve | No Ct or no sigmoidal amplification curve | **Invalid Test, Retest Required*** |

* If the pool has an invalid result, the assay needs to be repeated on individual samples in the pool. If a test on the individual sample is also invalid, a new specimen needs to be collected from the patient and re-tested.

## PERFORMANCE EVALUATION

### *1) Limit of Detection (LoD)-Analytical Sensitivity*

Preliminary LoD:

The preliminary LoD of the Predicine SARS-CoV-2 RT-PCR Test was evaluated using samples prepared with heat-inactivated SARS-CoV-2 (ATCC VR-1986HK). A preliminary LoD was established by testing 5 replicate swabs each of 2-fold dilutions of ATCC VR-1986HK on SARS-CoV-2 negative dry anterior nasal swabs (swabs collected from individuals who tested negative for SARS-CoV-2), targeting the final swab elution concentration of 100 copies/µL, 50 copies/µL, 25 copies/µL, 12.5 copies/µL, 6.25 copies/µL, 3.125 copies/µL, and 1.56 copies/µL, 0.78 copies/µL, 0.39 copies/µL. Each swab was spiked with 20 µL of diluted SARS-CoV-2 virus and allowed to dry for ~5 min prior to resuspension in the PBS buffer. The preliminary LoD was estimated to be 273 copies/swab (Table 1).

**Table 1. Preliminary LoD estimation**

| Copies/µL (After Rehydration in PBS) | Copies/Swab | Total # | Positive # | Mean Ct |
|---|---|---|---|---|
| 100 | 35,000 | 5 | 5 | 25.57 |
| 50 | 17,500 | 5 | 5 | 26.8 |
| 25 | 8,750 | 5 | 5 | 27.62 |
| 12.5 | 4,375 | 5 | 5 | 28.88 |
| 6.25 | 2187.5 | 5 | 5 | 30.14 |
| 3.125 | 1093.75 | 5 | 5 | 31.34 |
| 1.56 | 546 | 5 | 5 | 32.29 |
| **0.78** | **273** | **5** | **5** | **33.67** |
| 0.39 | 136.5 | 5 | 3 | 34.72 |

Confirmation of the LoD:

The LoD was confirmed by testing an additional 20 independent replicate swabs at each of the six target levels around the estimated value as shown in Table 2. The confirmed LoD, defined as the lowest level at which ≥ 95% of replicates were reported positive, was 1.56 copies/µL (546 copies per swab) for the nasal matrix.

**Table 2. LoD confirmation**

| Copies/µL (After Rehydration in PBS) | Copies/Swab | Total # | Positive # | Mean Ct |
|---|---|---|---|---|
| 25 | 8,750 | 20 | 20 | 27.98 |
| 12.5 | 4,375 | 20 | 20 | 29.08 |
| 6.25 | 2187.5 | 20 | 20 | 30.12 |
| 3.125 | 1093.75 | 20 | 20 | 31.27 |
| **1.56** | **546** | **20** | **20** | **32.18** |
| 0.78 | 273 | 20 | 13 | 34.52 |

### *2) Inclusivity (Analytical Sensitivity)*

The Predicine SARS-CoV-2 RT-PCR Test targets specific genomic regions of the SARS-CoV-2 nucleocapsid (N) gene and Envelope (E) gene. The N1 and E primer and probe sequences are the same target sequences that are included in the CDC 2019 nCoV Real-Time RT-PCR Diagnostic Panel (https://www.fda.gov/media/134922/download) and WHO protocol (https://www.who.int/docs/default-source/coronaviruse/real-time-rt-pcr-assays-for-the-detection-of-sars-cov-2-institut-pasteur-paris.pdf?sfvrsn=3662fcb6_2 ), respectively.

Inclusivity was demonstrated by performing BLAST alignment of the primers/probes of both N1 and E genes against the NCBI SARS-CoV-2 data set. A total of 674,677 SARS-CoV-2 complete sequences were downloaded from NCBI (https://www.ncbi.nlm.nih.gov/labs/virus/vssi/) as of Apr 28, 2022. Sequences shorter than 28,000 nt or containing ambiguous bases were filtered out, with 510,957 high quality sequences remaining for further analysis. Among the 510,957 SARS-CoV-2 complete sequences, 302,385 sequences have 100% homology with both the N1 and E gene primer/probe sets. 20,355 sequences have 100% homology with either the N1 or E gene primer/probe sets. 188,217 sequences have mismatches with both N1 and E gene primer/probe sets. Detailed analysis revealed that these mismatches are observed in both Omicron BA.1 and Omicron BA.2, which harbor two mutations causing mismatch with N gene probe and E gene forward primer. Both one base mismatches are located at the 5' ends of the primer or probe and are not predicted to affect assay performance.

The *in silico* inclusivity analysis of new emerging variants demonstrates that no impact on the performance of the assay is expected in terms of detecting recently emerging variants (Alpha variant, lineage B.1.1.7; Beta variant, lineage B.1.351; Gamma variant, lineage P.1; Delta variant (including Delta plus variant), lineage B.1.617.2; and Lambda variant, lineage C.37) as the primers and probes do not overlap with the specific mutations of the variant sequences. For Omicron variants BA.1 (lineage B.1.1.529.1) and BA.2 (lineage B.1.1.529.2), one mismatch in the E Fwd Primer and one mismatch in the N probe are found. Therefore, lab testing using the synthetic Omicron variant BA.2 RNA (Twist, Cat# 104529) was conducted at the 1X LoD (546 copies/swab) concentration. This variant was successfully detected in all 20 replicates. Thus, the performance of the assay to detect

new emerging variants including Omicron BA.1 and BA.2 is not predicted to be affected.

### *3) Cross Reactivity (Analytical Specificity)*

An *in silico* analysis was conducted to assess the potential for cross-reactivity with the Predicine SARS-CoV-2 RT-PCR Test primer and probes.

BLAST analysis queries of the N1 and the E primer and probe sets were performed against the human genome and 47,899 virus sequences (after excluding SARS-CoV-2 sequences) from the NCBI GenBank database (https://www.ncbi.nlm.nih.gov/labs/virus/vssi/) as of Jan 3, 2022. The primer and probe sets were also evaluated using BLAST against the complete genome sequences for a panel of representative bacterial pathogens. The *in silico* analysis demonstrated <80% homology for the majority of primers/probes for each virus and microorganism evaluated. For the few organisms/viruses with >80% homology, only one primer or probe showed the homology, and therefore amplification is not predicted. For SARS-CoV-1, >80% homology was observed for both the E gene (both primers and probe) and N1 gene targets (Reverse primer and probe); however the potential cross-reactivity with this virus is low risk because it is not currently circulating in humans. In summary, the *in silico* data support that the assay is not expected to cross react with any of the clinically-relevant organisms/viral strains evaluated.

Wet testing was also performed for organisms listed in Table 3. Each organism/virus was tested in triplicate for potential cross-reactivity by spiking the organism/virus into nasal matrix at the final concentrations listed in the table with or without presence of the SARS-CoV-2 virus. No cross-reactivity was observed for any organisms and viruses tested.

**Table 3. Wet lab cross-reactivity testing**

| Sample Name | Specimen Info | Spiking concentration (equivalent copies/mL) | Positive Samples Results (#Pos/Total)* | Negative Samples Results (#Pos/Total) |
|---|---|---|---|---|
| MER-COV | ATCC VR-3248SD | 1*10^5 | 3/3 | 0/3 |
| HCoV-HKU1 | ATCC VR-3262SD | 1*10^6 | 3/3 | 0/3 |
| HCoV-NL63 | ATCC VT-3263SD | 1*10^6 | 3/3 | 0/3 |
| Adenovirus 11 | ATCC VR-12D | 1*10^6 | 3/3 | 0/3 |
| Adenovirus 5 | ATCC VR-5D | 1*10^6 | 3/3 | 0/3 |
| Influenza A | Twist Synthetic influenza H1N1 | 1*10^6 | 3/3 | 0/3 |
| Influenza B | ATCC VR-1735D | 1*10^6 | 3/3 | 0/3 |
| Human Coronavirus OC43 | Twist Synthetic | 1*10^6 | 3/3 | 0/3 |

*Positive samples were generated by spiking the SARS-CoV-2 virus into anterior nasal matrix at 3x LoD** containing the above organism at the final concentration listed in the table.

** Nasal matrix is prepared by rehydration of the dry anterior nasal swabs from SARS-CoV-2 negative patients with the 1XPBS buffer, 350 µL per dry anterior nasal swab.

### *4) Endogenous Interference*

The potential impact of interfering substances on Predicine SARS-CoV-2 RT-PCR Test performance was evaluated. Anterior nasal swabs collected from healthy individuals were rehydrated with the 1X PBS sample buffer containing the interference listed in the Table 4. Then the heat-inactivated SARS-CoV-2 virus (ATCC VR-1986HK) was spiked into the negative swab specimens at the concentration of 3X LoD (1638 copies/swab). The potentially interfering substances indicated in Table 4 was added to the contrived positive samples at the indicated concentration. Each substance was tested in triplicate for positive contrived samples and triplicate for negative swab specimens.

Results indicate that Predicine SARS-CoV-2 RT-PCR Test performance is not compromised by the presence of any of the substances at the concentrations evaluated. Neither false positives nor false negatives were observed.

**Table 4. Interference Testing**

| Substance | Concentration | SARS-CoV-2 Concentration | Positive Sample Results (#Pos/Total) | Negative Sample Results (#Pos/Total) |
|---|---|---|---|---|
| Afrin Original Nasal Spray | 15% v/v | 3X LoD | 3/3 | 0/3 |
| Basic Care Allergy Relief Nasal Spray (Glucocorticoid) | 5% v/v | 3X LoD | 3/3 | 0/3 |
| NeilMed Nasal Gel | 1.25% v/v | 3X LoD | 3/3 | 0/3 |
| GoodSense All Day Allergy, Cetirizine HCl Tablets 10 mg | 1 mg/mL | 3X LoD | 3/3 | 0/3 |
| Cepacol Sore Throat (benzocaine/menthol lozenges) | 5 mg/mL | 3X LoD | 3/3 | 0/3 |
| Blood (human) | 2.5% v/v | 3X LoD | 3/3 | 0/3 |
| Mucin: bovine submaxillary gland, type I-S | 2.5 mg/mL | 3X LoD | 3/3 | 0/3 |

### *5) Clinical Evaluation for Patients Suspected of COVID-19*

Clinical performance of the Predicine SARS-CoV-2 RT-PCR Test was evaluated by testing a total of 60 paired anterior nasal swab samples (one dry swab [rehydrated in PBS] and one swab collected in VTM) collected from patients suspected of COVID-19 by a health care provider. One of the paired nasal swabs was collected into 3 mL VTM and tested with a highly-sensitive FDA-authorized molecular SARS-CoV-2 RT-PCR assay. Among these specimens, 30 were positive and 30 were negative as determined by the comparator method. After testing the paired de-identified dry swabs rehydrated in PBS buffer with the Predicine SARS-CoV-2 RT-PCR Test, the positive percent agreement between the two methods was 96.7% (29/30) and the negative percent agreement was 100% (30/30). Based on the Ct values obtained with the comparator method, at least 25% of the positive samples have Ct values within 3 cycles of the average Ct at the LoD of the comparator assay and were considered as “weak positive”. The results of this study support the use of the Predicine

SARS-CoV-2 RT-PCR Test for SARS-CoV-2 testing for individuals suspected of COVID infection and are presented in Table 5

**Table 5. Clinical evaluation results for patients suspected of COVID-19**

| | | EUA Authorized SARS-CoV-2 RT-PCR Assay | | |
|---|---|---|---|---|
| | | **Positive*** | **Negative** | **Total** |
| Predicine SARS-CoV-2 RT-PCR Test | Positive | 29 | 0 | 29 |
| | Negative | 1** | 30 | 31 |
| | Total | 30 | 30 | 60 |

* At least 25% of the positive samples are weak positive based on the comparator assay.
**The one discordant sample was tested with an additional FDA-authorized assay and confirmed to be positive.

Positive percent agreement = 29/30 = 96.7% (95% CI: 82.8% - 99.9%)
Negative percent agreement = 30/30 = 100% (95% CI: 88.4% - 100%)

### *6) Clinical Evaluation for Individuals Without Symptoms or Other Reasons to Suspect COVID-19*

To evaluate the clinical performance of Predicine SARS-CoV-2 RT-PCR test among individuals without symptoms or other reasons to suspect COVID-19 infection, paired anterior nasal swab samples (one dry swab [rehydrated in PBS] and one swab in VTM) were prospectively collected from asymptomatic individuals enrolled either in a school or work environment. Individuals who had symptoms or were close contacts of individuals with COVID-19 were excluded. One of the paired anterior nasal swabs was collected into 3 mL VTM and tested with a highly-sensitive FDA-authorized molecular SARS-CoV-2 RT-PCR assay. For the first 25 consecutive positive and 100 consecutive negative anterior nasal swab samples with the comparator assay, testing was conducted with the paired swabs in PBS buffer using the Predicine SARS-CoV-2 RT-PCR Test. Based on the Ct values obtained with the comparator method, at least 25% of the positive samples had Ct values within 3 cycles of the average Ct at the LoD and were considered as “weak positive”. Results from tests are presented in Table 6.

**Table 6. Clinical evaluation results for patients without symptoms or other reasons to suspect COVID-19**

| | | EUA Authorized SARS-CoV-2 RT-PCR Assay | | |
|---|---|---|---|---|
| | | **Positive*** | **Negative** | **Total** |
| Predicine SARS-CoV-2 RT-PCR Test | Positive | 25 | 0 | 25 |
| | Negative | 0 | 100 | 100 |
| | Total | 25 | 100 | 125 |

* At least 25% of the positive samples are weak positive based on the comparator assay.

Positive percent agreement = 25/25 = 100% (95% CI: 86.3% - 100%)
Negative percent agreement = 100/100 = 100% (95% CI: 96.4% - 100%)

### *7) Fresh vs Frozen Study*

A Fresh vs Frozen study was performed to evaluate the performance of the Predicine SARS-CoV-2 RT-PCR Test with fresh and frozen anterior nasal samples in the PBS buffer. Eighty clinical samples including 40 positive and 40 negative specimens were initially tested fresh with the Predicine SARS-CoV-2 RT-PCR Test as the test results for "Fresh Samples". The remnant of these samples was stored at -80ºC freezer and then re-run using the same assay to produce the "Frozen Samples" data. The results from these 80 clinical samples are summarized below. Twelve of the 40 positive samples (30%) were weak positive samples with Ct value above 30.0 with the Predicine SARS-CoV-2 RT-PCR Test. Study results are presented in Table 7.

**Table 7. Summary Fresh vs Frozen results from anterior nasal swab samples**

| | **Results** | **Fresh Sample** | |
|---|---|---|---|
| | | **Positive** | **Negative** |
| Frozen Sample | Positive | 37 | 0 |
| | Negative | 3* | 40 |

Positive percent agreement = 37/40 = 92.5%* (95% CI: 79.6%-98.4%)
Negative percent agreement = 40/40 = 100% (95% CI: 91.2%-100%)
* Three false negatives were only tested in weak positive samples that have Ct values (Ct=37.9, 36.7 or 36.5) above that at LoD (Ct=32.1).

### *8) Sample Stability*

The shipping stability of dry swabs has been demonstrated by Quantigen Biosciences, Inc. with support from The Gates Foundation and UnitedHealth Group. The Quantigen study demonstrated 56-hour stability for dry anterior nasal polyester swabs when subjected to both summer and winter thermal excursions. Quantigen Biosciences, Inc. has granted a right of the stability data to any sponsor, such as Predicine, Inc. for the purposes of obtaining an EUA for at-home swab collection to support SARS-CoV-2 testing. Therefore, the stability of anterior nasal samples collected using dry polyester swabs was not evaluated in a separate sample stability study.

### *9) Dry Swab Rehydration Validation*

To demonstrate that dry swabs were acceptable specimen types for SARS-CoV-2 testing, a validation study was performed on swabs reconstituted in PBS following dry storage. Twenty contrived positive specimens at 3X LoD (1638 copies/swab) were prepared by spiking inactivated SARS-CoV-2 (ATCC VR-1986HK) directly onto the polyester swabs with natural clinical matrix (anterior nasal swabs collected from healthy donors). Meanwhile, 30 negative dry anterior nasal swab specimens were also collected from healthy individuals. Following storage of the swabs at ambient temperatures for 24 hours, the dry swabs were reconstituted in 350 µL of 1X PBS and then lysed for 30 min in a 65ºC degree oven. After lysis, the samples were tested with the Predicine SARS-CoV-2 RT-PCR Test. Results are summarized below. There was 100% agreement with expected results for all positive contrived samples and negative samples (Table 8).

**Table 8. Summary of the rehydration validation results**

| | Results | Expected results | |
|---|---|---|---|
| | | Positive | Negative |
| Samples Tested Individually | Positive | 20 | 0 |
| | Negative | 0 | 30 |

### *10) Human Usability Study*

This study was conducted to evaluate the usability of the Predicine COVID-19 Self-Collection Kit, including evaluation of individuals ability to properly follow the instructions to appropriately collect, package, and transport a self-collected anterior nasal swab specimen to the Predicine Laboratory for testing. The study was completed in a home-use environment.

1) Human usability study for adults aged 18 and over

Consented participants meeting the study inclusion criteria were sent a Predicine COVID-19 Self-Collection Kit (Home collection) and observed via a video conferencing platform for self-collection of anterior nasal swab specimens. After the entire process, each participant was given a questionnaire to indicate the ease of use of the collection kit.

A total of 53 participants were included in this study. The participants all completed the entire process of registering patient information, obtaining an anterior nasal swab specimen, returning it for testing (shipping by FedEx) and completing the usability questionnaire. All participants were included in the final study dataset. None of the participants had prior laboratory experience or experience with specimen home collection. Out of the 53 participants, 100% had no difficulty in identifying the kit components, following the instruction, or collecting the nasal sample. All adults indicated that no discomfort was experienced and that no help was needed. All participants successfully registered their kits. The observers did not note any significant deviations from the instructions in the way the participants collected nasal specimens using the Predicine COVID-19 Self-Collection Kit (Home collection). The study included ordering, registering, specimen collection, packaging, and shipping the home collection kit by FedEx.

The participants' age ranges from 18-87 years old (Table 9). All samples received by the laboratory met acceptance criteria and were tested. Positive Human RNase P signals were detected in all the samples when tested with the Predicine SARS-CoV-2 RT-PCR Test, indicating all participants were successful in self-collecting the anterior nasal swab specimens.

**Table 9. Characteristics of the study population**

| Age | Number of Participants |
|---|---|
| 18-39 years | 15 |
| 40-49 years | 23 |
| 50-59 years | 5 |

| | |
|---|---|
| >60 years | 10 |
| **Gender** | **Number of Participants** |
| Male | 22 |
| Female | 31 |
| **Education Level** | **Number of Participants** |
| Grade 12 or less | 27 |
| Bachelor or above | 26 |

2) Human usability study for minors aged 2-17

An additional usability study was performed to validate collection of anterior nasal samples from 65 minors: 15 (14-17 years old) via self-collection under the guidance and supervision of an adult, and 50 (2-13 years old) with anterior nasal swab specimen collected by an adult. Anterior nasal samples were collected at the participants' homes and observed by a medical assistant using online video conferencing. Participant demographics are presented in Table 10.

**Table 10. Characteristics of the study population**

| **Minors aged 14-17 years old** | | **Number of Participants** | **Minors aged 2-13 years old (adults paired with minors)** | | **Number of Participants** |
|---|---|---|---|---|---|
| **Gender** | Female | 8 | **Gender** | Female | 35 |
| | Male | 7 | | Male | 15 |
| **Education Level** | Grade 12 or less | 15 | **Education Level** | Grade 12 or less | 27 |
| | Bachelor or above | 0 | | Bachelor or above | 23 |
| **Age (years)** | 14 | 5 | **Age (years)** | 2 | 1 |
| | 15 | 7 | | 3 | 4 |
| | 16 | 1 | | 4 | 4 |
| | 17 | 2 | | 5 | 2 |
| | | | | 6 | 8 |
| | | | | 7 | 5 |
| | | | | 8 | 2 |
| | | | | 9 | 5 |
| | | | | 10 | 5 |
| | | | | 11 | 2 |
| | | | | 12 | 7 |
| | | | | 13 | 5 |

Of the 65 anterior nasal swab specimens collected from minors aged 2-17, all samples (100%) were received in acceptable condition and showed detection of human RNase P (100%) when tested with the Predicine SARS-CoV-2 RT-PCR Test (Table 11).

**Table 11. RNase P Detection Results**

| Age group | # of samples | # of samples detected | Ct range | Mean Ct | Detection Rate (%) |
|---|---|---|---|---|---|
| ≥18 | 53 | 53 | 20.37-34.47 | 24.8 | 100% |
| 2-17 | 65 | 65 | 18.92-33.37 | 24.6 | 100% |

3) Observer assessment and outcome post participation questionnaire

During the usability study, the observer did not note difficulties regarding the usability of the Predicine COVID-19 Self-Collection Kit (Home collection).

Following sample collection, participants (>18 years old, minors aged 14-17 years old, and parents of the 2-13 years old) completed the questionnaire. Favorable or neutral responses were given to the majority of questions regarding ease of identifying the kit components, following the instruction, collecting the nasal sample, packaging and shipping the sample.

A total of 13/65 minors (20%) reported sneezing, coughing, or tearing during the sample collection. Therefore, an additional warning was incorporated in the instructions to emphasize the possibility of feeling discomfort during the procedure. The following warnings were included in the instructions:

- You may feel sneezing, coughing or tearing during the sample collection
- For young children, do not insert swab greater than 0.5 inches.

Based on survey feedback from the usability study, the following additional changes were implemented in the IFU: 1) The kit registration procedure was clarified and 2) phone number and a website link (for Predicine FAQ pages) were added.

All 118 individuals included in the usability study were informed of the test result within 24 hours of sample accessioning and none reported any issues after viewing the test results. In summary, results from the Usability study support that the Predicine COVID-19 Self-Collection Kit can appropriately be used for collection of anterior nasal swab specimens from individuals aged 18 years and older (self-collected), 14-17 years, (self-collected under adult supervision), or 2-13 (collected with adult assistance).

### *11) Sample Pooling Evaluation*

Preliminary Clinical Sample Pooling Validation Study:

Based on the low prevalence (<1%) of SARS-CoV-2 in the intended use population, the decision to implement a pooling approach was reached as pooling 8 individual samples for 1 test. The test is validated for an 8-sample media pooling strategy and can be performed for any n ≤8 sample pools.

The pool size is re-considered by monitoring the positivity rate periodically in the intended population.

All clinical specimens chosen for the pooling evaluation were selected based on the detection results from a highly-sensitive FDA EUA authorized SARS-CoV-2 RT-PCR assay. To assess sample pooling with clinical specimens, 180 individual clinical nasal swab specimens (160 negative clinical specimens and 20 positive clinical specimens) were combined into pools of N=8. Based on the Ct values obtained with the comparator method, at least 25% of the positive individual samples have Ct values within 3 cycles of the average Ct at the LoD and were considered as "weak positive". All the 20 positive samples were de-identified and individually tested by both the Predicine SARS-CoV-2 RT-PCR test and the comparator assay, and remain 100% positive (PPA, $\text{Predicine}_{\text{individual}}$ vs $\text{Comparator}_{\text{individual}}$ = 100%). The positive pools were prepared by combining equal volumes of one positive sample and seven randomly selected negative samples into 20 pools. The negative sample pools were created by individually pooling 160 negative clinical samples into 20 pools of N=8.

The positive percent agreement (PPA) between the pooled samples using the Predicine SARS-CoV-2 RT-PCR test and individual samples using the comparator assay is 90% (PPA, $\text{Comparator}_{\text{individual}}$ vs $\text{Predicine}_{\text{pool}}$ = 90%, 95% CI: 68.3%-98.8%). All the negative samples remain negative in 8-sample pools. The results from the study are presented in Table 12.

**Table 12. Individual and Pooled Specimens Agreement with a Pool Size of 8.**

| | | Individual Test Result (Comparator assay) | | |
|---|---|---|---|---|
| | | **Positive** | **Negative** | **Total** |
| **Samples Tested in 8-Sample Pool (Predicine SARS-CoV-2 RT-PCR Test)** | **Positive** | 18 | 0 | 18 |
| | **Negative** | 2 | 20 | 22 |
| | **Total** | 20 | 20 | 40 |

Note: At least 25% of the positive individual samples are weak positives based on the comparator assay.

Positive percent agreement =18/20 = 90% (95% CI: 68.3% - 98.8%)
Negative percent agreement = 20/20 = 100% (95% CI: 83.2% - 100%)

Clinical sample pooling validation study with samples from three geographically diverse US site:

To evaluate the potential effect of 8-sample pooling on clinical performance with the Predicine SARS-CoV-2 test, *in silico* analysis was conducted using historical data collected from 3 geographically diverse US sites. In this analysis, a mean Ct shift of 3.54, calculated based on data generated from the preliminary clinical sample pooling validation study and additional wet testing of dilutions of a positive clinical sample in replicates was applied to the individual positive results from the historical dataset to determine the percentage of positive results that would be expected to remain positive upon 8-sample pooling. A total of 300 historical positive results from 3 geographically US locations were used in this analysis. The final PPA ($\text{Predicine}_{\text{pool}}$ vs $\text{Predicine}_{\text{individual}}$) for the 3 geographically sites are calculated as 97.23%, 96.20% and 96.17%, respectively.

## WARNINGS

- For Emergency Use Authorization (EUA) only.
- For *in vitro* diagnostic use.
- For prescription use only.
- This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by the authorized laboratories;
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of SARS-CoV-2 under Section 564(b)(1) of the Federal Food, Drug and Cosmetics Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated, or authorization is revoked sooner.

## LIMITATIONS

- Samples should only be pooled when testing volume (demand) exceeds laboratory capacity and/or when testing reagents are in short supply.
- Laboratories are required to report all negative and positive results to the appropriate public health authorities.
- Primers and probes for the Predicine SARS-CoV-2 RT PCR Test target highly conserved regions within the genome of SARS-CoV-2. Mutations rarely occur in these highly conserved regions, but if a mutation did occur in these regions, SARS-CoV-2 RNA could become undetectable.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.