Ezplex SARS-CoV-2 G Kit

For Emergency Use Authorization (EUA) only.
For *in vitro* diagnostic use only.
For prescription use only.

Instructions for Use (IFU)

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Proprietary Name: Ezplex SARS-CoV-2 G Kit

I. Intended Use

The Ezplex SARS-CoV-2 G Kit is a real-time RT-PCR *in vitro* diagnostic test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs, oropharyngeal swabs, and sputum specimens collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

This test is also for the qualitative detection of nucleic acid from the SARS-CoV-2 in pooled specimens containing up to five individual nasopharyngeal or oropharyngeal swabs where each specimen is collected by a healthcare provider using individual vials containing transport media from individuals suspected of COVID-19 by their health care provider. 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 if results are necessary for patient management, then the patient should be considered for individual testing. Specimens included in pools with a positive, inconclusive, or invalid result must be tested individually prior to reporting a result. Specimens with low viral loads may not be detected in specimen pools due to the decreased sensitivity of pooled testing.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. 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 Ezplex SARS-CoV-2 G Kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The Ezplex SARS-CoV-2 G Kit is only for use in the United States under the Food and Drug Administration's Emergency Use Authorization.

II. Summary and Explanation

Coronaviruses are a large family of viruses which 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 Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). The most recently discovered coronavirus, SARS-CoV-2, causes the associated coronavirus disease COVID-19. This new virus and disease were unknown before the outbreak began in Wuhan, China, in December 2019.[1]

The most common symptoms of COVID-19 are fever, tiredness, and dry cough. Some patients may have aches and pains, nasal congestion, runny nose, sore throat, new loss of taste or smell, or diarrhea. These symptoms are usually mild and begin gradually. Some people become infected but don't develop any symptoms and don't feel unwell. The disease can spread through respiratory droplets produced when an infected person coughs or sneezes. These droplets can land in the mouths or noses of people who are nearby or possibly be inhaled into the lungs.[2] These droplets also can land on objects and surfaces around the person.[3] Other people may acquire SARS-CoV-2 by touching these objects or surfaces, then touching their eyes, nose, or mouth.

The virus that causes COVID-19 is infecting people and spreading easily from person to person. On March 11, 2020, the COVID-19 outbreak was characterized as a pandemic by the World Health Organization (WHO).[4,5]

III. Principles of the Procedure

The Ezplex SARS-CoV-2 G Kit uses TaqMan-based real-time reverse transcription polymerase chain techniques to conduct in vitro reverse transcription of SARs-CoV-2 RNA, DNA amplification and fluorescence detection. The assay targets specific genomic regions of the SARS-Co-V-2 RdRP and N genes. Nucleic acid is isolated and purified from respiratory specimens using the Qiagen QIAamp® DSP Viral RNA Mini Kit. The purified nucleic acid is then reverse transcribed into cDNA using the Ezplex SARS-CoV-2 G kit. The cDNA is then subsequently amplified using either the CFX96 Dx Real-time PCR instrument (Bio-Rad) or the Applied Biosystems 7500 Real-time PCR instrument (ThermoFisher Scientific). During this 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 probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle.

The negative control included in the kit serves as a general control for exogenous nucleic acid contamination and is used to monitor cross-contamination during the RNA extraction and PCR reaction setup steps. It should be run with each batch of tests.

The positive control included in the kit consists of synthesized plasmid DNA for each gene target and is used to monitor for the presence of inhibitors and the efficiency of the polymerase chain reaction. It should be run with each batch of tests.

An internal control is utilized to ensure that clinical specimens are successfully amplified and detected. This control consists of plasmid DNA that was synthesized to include a portion of the human BCR activator of RhoGEF and GTPase (BCR) gene. This control is added to the reaction master mixture during PCR preparation procedure.

The Ezplex SARS-CoV-2 G Kit does not include an internal control for RNA extraction/recovery or transcription. A known SARS-CoV-2 positive specimen or specimen
containing SARS-CoV-2 RNA (i.e., in vitro transcript or pseudovirus) must be tested with every batch of patient specimens to monitor the integrity of these process steps.

IV. Kit Components and Packaging Specifications

Catalog Number: GNT2011-1 100 tests/kit

No.	Component Name*	Volume	Main Ingredients
1	RQ Mixture	1 vial, 1000uL	DNA Taq polymerase, Reverse Transcriptase, dNTPs with dUTP, Magnesium Chloride, Potassium, Uracil N-glycosylase
2	P+P	1 vial, 500uL	Tris-HCl, EDTA, Oligonucleotide primers specific for SARS-CoV-2 virus, Fluorescent-labeled oligonucleotide probe specific for SARS-CoV-2 virus
3	Positive Control (PC)	1 vial, 50uL	Tris-HCl, EDTA, Synthesized DNA control specific for SARS-CoV-2 virus
4	Negative Control (NC)	1 vial, 50uL	Double Distilled Water
5	Internal Control (IC)	1 vial, 20uL	Tris-HCl, EDTA, Synthesized DNA internal control

***RQ Mixture: Real-time Quantitative PCR Mixture; P+P : Probe + Primer**

Catalog Number: GNT-2011-2 200 tests/kit

No.	Kit Component*	Volume	Main Ingredients
1	RQ Mixture	2 vials, 1000uL	DNA Taq polymerase, Reverse Transcriptase, dNTPs with dUTP, Magnesium Chloride, Potassium, Uracil N-glycosylase
2	P+P	2 vials, 500uL	Tris-HCl, EDTA, Oligonucleotide primers specific for SARS-CoV-2 virus, Fluorescent-labeled oligonucleotide probe specific for SARS-CoV-2 virus
3	Positive Control (PC)	2 vials, 50uL	Tris-HCl, EDTA, Synthesized DNA control specific for SARS-CoV-2 virus
4	Negative Control (NC)	2 vials, 50uL	Double Distilled Water
5	Internal Control)IC)	2 vials, 20uL	Tris-HCl, EDTA, Synthesized DNA internal control

***RQ Mixture: Real-time Quantitative PCR Mixture; P+P : Probe + Primer**

Optional Materials Provided:

- Genetree Viewer Software (CAT No. GNT2011-3)

V. Materials Required But Not Provided

No.	Name	CAT No.	Company
1	For Bio-Rad instrument: any applicable 96 well PCR plate plastics for CFX96 Dx system	MLL9651	Bio-Rad
2	For Bio-Rad instrument: any applicable PCR plate sealing film for CFX96 Dx system	MSB1001	Bio-Rad
3	For ThermoFisher Scientific instrument: microAmp Optical Adhesive Film	4311971	ThermoFisher Scientific
4	For ThermoFisher Scientific instrument: microAmp Optical 96-well Reaction Plate	N8010560	ThermoFisher Scientific
5	For ThermoFisher Scientific instrument: microAmp Optical 8-tube strip(0.2mL)	431567	ThermoFisher Scientific
6	For ThermoFisher Scientific instrument: microAmp Optical 8-cap Strip	4323032	ThermoFisher Scientific
7	Qiagen QIAamp® DSP Viral RNA Mini Kit	61904	Qiagen
8	Known SARS-CoV-2 positive specimen or specimen containing SARS-CoV-2 RNA(in vitro transcript or pseudovirus) to control for extraction/recovery or transcription	MBC137-R (Recommended)	Vircell
9	Computer for installation of Genetree Viewer Analysis Software	1) Microprocessor: Intel(R) i3 3.5 GHz or above 2) Memory: 4 GB or larger; 3) Microsoft Windows 7 or above; 4) more than 1 USB port	

VI. Instruments

PCR Instruments validated for use with the Ezplex SARS-CoV-2 Kit:

- CFX96 Dx Real-time PCR Instrument with 1.6 or 3.1 or later versions of CFX Manager (Bio-Rad) **OR**
- ABI 7500 Real-time PCR Instrument with 2.3 or later versions of 7500 software (ThermoFisher Scientific)

VII. Storage and Handling Conditions

- All kit materials should be stored at -20 °C opened and unopened.
- Use the reagents before the expiration date shown on the labeling.
- Completely thaw the reagents before use.
- Repeated thawing and freezing should be avoided. It should not exceed 5 freeze-thaw cycles.

VIII. Warnings and Precautions

- For *in vitro* diagnostic use. For use under an Emergency Use Authorization (EUA) only.
- This product has not been FDA cleared or approved; but has been authorized by the FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- 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 COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. §360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim
- Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with2019-nCoV. https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html.
- Specimens may be infectious. Use Universal Precautions when performing this assay. Proper handling and disposal methods should be established by the

laboratory director. Only personnel adequately trained in handling infectious materials should be permitted to perform this diagnostic procedure.[6]

- Use appropriate personal protective equipment when collecting and handling specimens from individuals suspected of being infected with SARS-CoV-2 as outlined in CDC Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019 Novel Coronavirus (2019-nCoV).
- Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and reagents. Wash hands thoroughly after handling specimens and reagents.
- Dispose of all material that has come into contact with specimens and reagents in accordance with applicable national, international, and regional regulations.
- Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.
- Avoid cross-contamination during the specimen handling steps. Specimens can contain extremely high levels of virus or other organisms. Ensure that specimen containers do not come in contact with one another, and discard used materials without passing them over any open containers. Change gloves if they come in contact with specimens. It is recommended to use sterile disposable filter-tips to aspirate reagents and specimens.
- Do not use the reagents and controls after the expiration date.
- Do not mix reagents from different lots.
- Since the plasmid DNA in the positive control can degrade, it is recommended that the reagents shall be divided into amounts required for 1-2 tests and stored in a freezer.
- Only the Qiagen QIAamp® DSP Viral RNA Mini Kit can be used with the Ezplex SARS-CoV-2 Kit for nucleic acid extraction.
- Only the Bio-Rad CFX Dx Real-time PCR Instrument and the ThermoFisher Scientific ABI 7500 Real-time PCR Instrument can be used with the Ezplex SARS-CoV-2 Kit. These instruments should be calibrated regularly according to instrument's instructions to eliminate cross-talks between channels.
- The Ezplex SARS-CoV-2 Kit uses PCR-based technology and testing should be conducted in three separate areas: reagent preparation area, specimen preparation area and amplification area. Protective equipment accessories (goggles, work clothes, hats, shoes, gloves, etc.) should be worn during operation and protective equipment accessories should be changed when entering and leaving different work areas. Protective equipment accessories in each work area are not interchangeable.
- Store assay components at the recommended storage condition.
- Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard quality control procedures.

- Contamination may occur if carryover of specimens is not adequately controlled during specimen pool preparation, handling, and processing.
- Testing of pooled specimens may impact the detection capability of the SARS-CoV-2 assay and impact sensitivity.

IX. Collection, Storage and Shipment of Specimens

Only upper respiratory specimens collected in VTM (such as nasopharyngeal and oropharyngeal swabs) and sputum specimens can be used with the test.
Note: Only nasopharyngeal/oropharyngeal swabs in viral transport media (VTM) have been validated for pooling. Ensure that sufficient specimen volumes of any upper respiratory specimen that is utilized for pooling also have enough volume for additional individual testing.

A. Specimen Collection

Use only synthetic fiber swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit PCR testing. Place swabs immediately into sterile tubes containing 3 ml of viral transport media (VTM). For initial testing, nasopharyngeal swab specimens are recommended. Collection of oropharyngeal swabs is a lower priority and is acceptable if other swabs are not available.

- Nasopharyngeal swab (NP): Insert a swab into nostril parallel to the palate. Swab should reach depth equal to distance from nostrils to outer opening of the ear. Leave swab in place for several seconds to absorb secretions. Slowly remove swab while rotating it.
- Oropharyngeal swab (e.g., throat swab, OP): Swab the posterior pharynx, avoiding the tongue.
- Sputum : Educate the patient about the difference between sputum and oral secretions. Have the patient rinse the mouth with water and then expectorate deep cough sputum directly into a sterile screw-cap collection cup or sterile dry container.

B. Specimen Storage

A sample collection device is not a part of the assay kit. Patient samples must be collected according to appropriate laboratory guidelines. All testing for COVID-19 should be conducted in consultation with a healthcare provider. We recommend using CDC guidelines for sample collection of respiratory specimens and sample storage (https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html).
Specimens should be processed within 48 hours from collection and stored at 2-8°C

during that time. If the specimens cannot be tested within 48 hours, samples should be stored frozen at -70ºC or colder.

C. Shipping

Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation External Icon. Store specimens at 2-8°C and ship overnight to the lab on ice pack. If a specimen is frozen at -70°C ship, overnight to the lab on dry ice. Additional useful and detailed information on packing, shipping, and transporting specimens can be found at Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19).

D. For more information, refer to:

Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19)
https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html
Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19)
https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html

E. Ensure that sufficient specimen volumes of any upper respiratory specimen that is utilized for pooling also has enough volume for additional individual testing.

F. Specimen Pooling-Determining Appropriate Strategy for Implementation and Monitoring

When considering specimen pooling, laboratories should evaluate the appropriateness of a pooling strategy based on the positivity rate in the testing population and the efficiency of the pooling workflow. Refer to Appendix A of these instructions for Use for additional information ***prior*** to implementation of specimen pooling.

G. Specimen Preparation

1. Specimens for Individual Testing

- Nucleic acid should be isolated and purified from nasopharyngeal swabs, and oropharyngeal swabs, and a sputum specimens using the QIAamp® DSP Viral RNA Mini Kit.
- Prior to the start of the nucleic acid extraction, an additional negative clinical sample should be prepared and spiked with a known SARS-CoV-2 control. This functions as an extraction control to assess extraction reagent integrity and successful RNA extraction.
- Ensure homogenous mixing of prepared specimens.
- Utilize 140 µL of clinical sample and elute with 50 µL of Buffer AVE from the QIAamp® DSP Viral RNA Mini Kit. If the extracted RNA cannot be used immediately, store at 2 to 8 ° C for up to 24 hours or at -70 ° C for up to 1 month.

- Refer to the QIAamp® DSP Viral RNA Mini Kit Handbook for the protocol for extracting RNA using the QIAamp® DSP Viral RNA Mini Kit.

2. Pooled Specimens

Only upper respiratory specimens collected in VTM, such as nasopharyngeal and oropharyngeal swabs, may be tested with pooled specimens.

- Obtain an empty tube (molecular grade sterile test tube).
- Determine the appropriate volume required from each individual specimen based on the pool size being implemented. The required specimen to extraction ratio in the assay test specimen must be maintained for specimen pooling. The minimum combined volume of individual specimens pooled prior to transferring is 250 µL. The same volume of each specimen included in the pool needs to be used.
- Carefully transfer the determined volume of each individual specimen from the specimen collection container to the empty sterile tube.
- An additional negative clinical sample should be prepared and spiked with known SARS-CoV-2 control. It functions as an extraction control to assess extraction reagent integrity and successful RNA extraction.
- Ensure homogenous mixing of each prepared specimen pool.
- Utilize 140 µL of pooled clinical sample and elute with 50 µL of Buffer AVE from the QIAamp® DSP Viral RNA Mini Kit. If the extracted RNA cannot be used immediately, store at 2 to 8℃ for up to 24
- hours or at -70℃ for up to 1 month.
- Refer to the QIAamp® DSP Viral RNA Mini Kit Handbook for the protocol for extracting RNA using the QIAamp® DSP Viral RNA Mini Kit

NOTE: Retain the individual specimens for additional testing, if required.

X. Test Procedure

A. Nucleic Acid Extraction

The QIAamp® DSP Virus Viral RNA mini Kit (Qiagen GmbH) must be used for RNA extraction and users shall follow the protocol included in the Kit Instructions for Use. After extraction, RNA if not used immediately should be divided into amounts required for 1-2 tests and stored at –70℃ since RNA can degrade.

B. Real-time PCR Amplification

1. Reagent Master Mix Solution Preparation

a. Refer to the table below and prepare the PCR master mix solution according to the number of specimens/controls to be tested.

Component	Volume (uL) per Specimen/Control
RQ Mixture (Real time Quantitative Mixture)	10
P + P (Probe + Primer)	5
Internal control	0.1
Total	15.1

b. Pipette 15uL of PCR master mix solution into each 96-well PCR plate or 8-cap strip. Add 5uL of the extracted RNA specimen into each 96-well PCR plate or 8-cap strip.

c. Also, add 5 uL each of the positive control and negative control into each 96-well PCR plate or 8-cap strip.

d. If the 96-well PCR plate is used for the preparation, seal the top of the plate thoroughly to prevent the liquid from spilling or leaking.

e. If the 8-cap strip is used for the preparation, make sure that every cap is tightly closed on the top of the strips.

f. Centrifuge the 96-well PCR plate or 8-cap strip to make sure that all liquids are placed at the bottom.

g. Each batch of samples tested should include the following controls: positive control, negative control, and extraction/transcription control.

2. PCR Instrument Set Up

a. Set up the Bio-Rad or Applied Biosystems PCR Instrument according to their respective Instrument Reference Guide/Manual using the cycling specifications below:

Step	Temperature / Time	Cycle
Hold	25 ℃ / 2 min	1 Cycle
	50 ℃ / 30 min	
	95 ℃ / 5 min	
Cycle	95 ℃ / 15 sec	40 Cycles
	60 ℃ / 45 sec	

b. Set up fluorescent thresholds for detection targets per the table below.

c. The threshold should be adjusted to fall within the exponential phase of the fluorescence curves and above any background noise signal. The procedure chosen for setting the threshold should be used consistently.

Device	FAM	Cy5	VIC(HEX)
Bio-Rad CFX96 Dx	500	250	500
ThermoFisher Scientific AB7500	0.4	0.1	0.05

d. Add the prepared 96-well PCR plate or 8-cap strips to the PCR instrument and run the Bio-Rad or Applied Biosystems PCR Instrument according to their respective Instrument Reference Guide/Manual.

XI. Quality Control

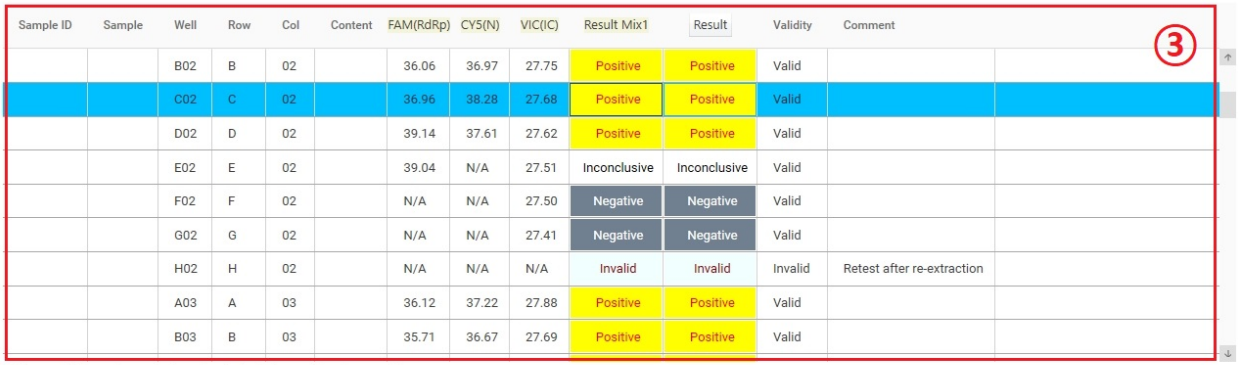
A Positive Control and Negative Control are provided with the Kit and should be run with each batch of specimens. An internal control [plasmid DNA synthesized to include a portion of the human BCR activator of RhoGEF and GTPase (BCR) genes] is provided with the kit and is utilized to ensure that each clinical specimen is successfully amplified and detected. This control is added to the reaction master mix during PCR preparation procedure. The Ezplex SARS-CoV-2 G Kit does not include an internal control for RNA extraction/recovery or transcription. A known SARS-CoV-2 positive specimen or specimen containing SARS-CoV-2 RNA (i.e., in vitro transcript or pseudovirus) must be tested with every batch of patient specimens to monitor the integrity of these process steps.

XII. Genetree Viewer Software Analysis

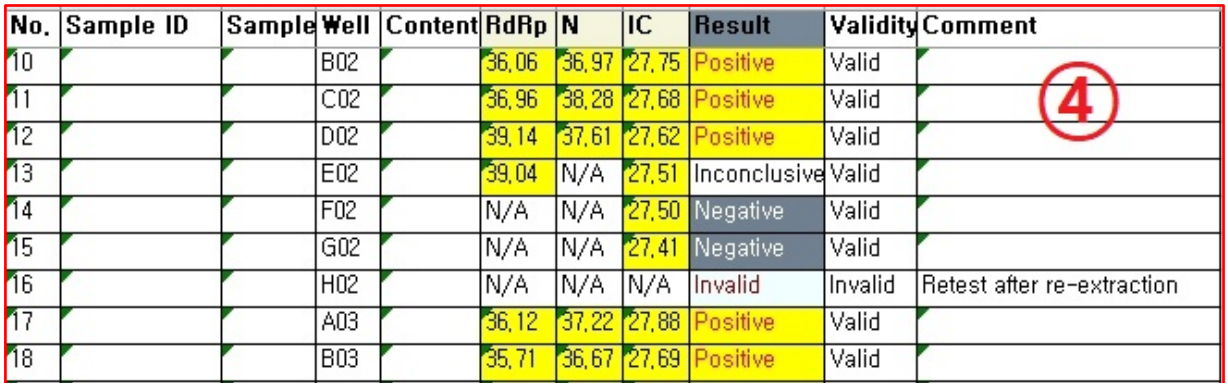
NOTE: Please contact 'technicalsupport@smlgenetree.com' to acquire 'Genetree Viewer' software prior to running the Ezplex SARS-CoV-2 G Test. Please refer to the Genetree Viewer Software Operators Manual for more detailed information.

A. General Description of the Software and Example Screens

No.	Description
①	Positive/Negative results by well are indicated in '+', '-' respectively. Invalid results by well are indicated in '?' respectively.
②	Ct and fluorescent values of the results for each well are plotted on a graph.
③	Ct values of the results for each well are indicated numerically and qualitative results are printed.
④	Analysis results are converted into an excel spreadsheet.



Sample ID	Sample	Well	Row	Col	Content	FAM(RdRp)	CY5(N)	VIC(IC)	Result Mix1	Result	Validity	Comment
		B02	B	02		36.06	36.97	27.75	Positive	Positive	Valid	
		C02	C	02		36.96	38.28	27.68	Positive	Positive	Valid	
		D02	D	02		39.14	37.61	27.62	Positive	Positive	Valid	
		E02	E	02		39.04	N/A	27.51	Inconclusive	Inconclusive	Valid	
		F02	F	02		N/A	N/A	27.50	Negative	Negative	Valid	
		G02	G	02		N/A	N/A	27.41	Negative	Negative	Valid	
		H02	H	02		N/A	N/A	N/A	Invalid	Invalid	Invalid	Retest after re-extraction
		A03	A	03		36.12	37.22	27.88	Positive	Positive	Valid	
		B03	B	03		35.71	36.67	27.69	Positive	Positive	Valid	



No.	Sample ID	Sample	Well	Content	RdRp	N	IC	Result	Validity	Comment
10			B02		36,06	36,97	27,75	Positive	Valid	
11			C02		36,96	38,28	27,68	Positive	Valid	
12			D02		39,14	37,61	27,62	Positive	Valid	
13			E02		39,04	N/A	27,51	Inconclusive	Valid	
14			F02		N/A	N/A	27,50	Negative	Valid	
15			G02		N/A	N/A	27,41	Negative	Valid	
16			H02		N/A	N/A	N/A	Invalid	Invalid	Retest after re-extraction
17			A03		36,12	37,22	27,88	Positive	Valid	
18			B03		35,71	36,67	27,69	Positive	Valid	

XIII. Interpretation of Results

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

a. The table below lists the expected results for the kit with valid positive control and negative control.

FAM Ct (RdRp)	CY5 Ct (N)	VIC/HEX Ct (IC)	Result*	Commet
<40	<40	Any	**Positive**	SARS-CoV-2 RNA is detected
<40	Neg	Any	**Inconclusive**	Further confirmatory testing may be conducted if clinically indicated
Neg	<40	Any	**Inconclusive**	Further confirmatory testing may be conducted if clinically indicated
Neg	Neg	<38	**Negative**	SARS-CoV-2 RNA is not detected
Neg	Neg	≥38 or Neg	**Invalid**	Retest after re-extraction

- The result is judged as Positive only when both the RdRp and N genes are detected.
- Further confirmatory testing may be conducted if clinically indicated if the result is judged as "Inconclusive".
- Retesting after re-extraction is necessary if the result is judged as “Invalid.” If the re-tested result remains invalid, report the invalid result and consider collecting a fresh sample and re-testing if clinically indicated.

b. Interpretation of Results for Pooled Specimens
Negative: For sample pools yielding negative results, all samples making up that pool are presumed to be negative. Negative results from pooled specimen testing should not be treated as definitive. If the patient’s clinical signs and symptoms are inconsistent with a negative result and results are necessary for patient management, then the patient should be considered for individual testing.
The utilization of specimen pooling should be indicated for any specimens with reported negative results.
Positive: Specimens in a positive specimen pool must be tested individually prior to reporting a result. Specimens with low viral loads may not be detected in specimen pools due to the decreased sensitivity of pooled testing.
Invalid: Specimens with an invalid pool result must be tested individually prior to reporting a
result. However, in instances of an invalid run, repeat extraction and testing of the specimen pool may be appropriate depending on the laboratory workflow and required result reporting time.
Inconclusive: Specimens with an inconclusive pool result must be tested individually prior to reporting a result. Specimens with an inconclusive result upon individual testing may need further confirmatory testing if clinically indicated.

XIV. Limitations

- The Ezplex SARS-CoV-2 G Kit has been analytically validated for use with nasopharyngeal and oropharyngeal swabs in VTM and sputum specimens run on the Bio-Rad CFX96 Dx Real-Time PCR and ABI 7500 Real-time PCR Instrument and utilizing the Qiagen QIAamp® DSP Viral RNA Mini Kit for RNA extraction. This assay has been clinically validated for use with nasopharyngeal/oropharyngeal swabs in VTM and sputum specimens run on the Bio-Rad CFX96 Dx Real-Time PCR utilizing the Qiagen QIAamp® DSP Viral RNA Mini Kit for RNA extraction.
- Based on the *in-silico* analysis, SARS-CoV and other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV-2 may cross-react with the N gene in the Ezplex assay. SARS-CoV is not known to be currently circulating in the human population, and therefore is unlikely to be present in patient specimens.
- The procedures in this handbook must be followed, as described. Any deviations may result in assay failure or may cause erroneous results.
- Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.
- Reliable results are dependent on adequate specimen collection, transport, storage, and processing.
- Good laboratory practices are required to ensure the performance of the kit, with care required to prevent contamination of the kit components. Components should be monitored for contamination and any components thought to have become contaminated should be discarded as standard laboratory waste in a sealed pouch or zip-lock plastic bag.
- All specimens should be handled as if they are infectious following proper biosafety precautions.
- False negative results may be caused by:
    - Unsuitable collection, handling and/or storage of specimens
    - Specimen outside of viremic phase
    - Failure to follow procedures in this handbook
    - Use of unauthorized extraction kit or PCR platforms
- False positive results may be caused by:
    - Unsuitable handling of specimens containing high concentration of COVID-19 viral RNA or positive control template
    - Unsuitable handling of amplified product
- All results should be interpreted by a health care professional in the context of patient medical history and clinical symptoms.
- This test cannot rule out diseases caused by other pathogens.
- Negative results do not preclude SARS-CoV-2 infections and should not be used as the sole basis for treatment or other management decisions.
- A positive result indicates the detection of nucleic acid from the relevant virus. Nucleic acid may persist even after the virus is no longer viable.
- Specimen pooling has only been validated using upper respiratory

(nasopharyngeal/oropharyngeal) swabs in VTM.
- Specimens should only be pooled when testing demand exceeds laboratory capacity and/or when testing reagents are in short supply.

XV. Conditions of Authorization for the Laboratory

The Ezplex SARS-CoV-2 G Kit Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas

However, to assist clinical laboratories using the Ezplex SARS-CoV-2 G kit, the relevant Conditions of Authorization are listed below.

1. Authorized laboratories* using the Ezplex SARS-CoV-2 G kit must include with result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

2. Authorized laboratories using specimen pooling strategies when testing patient specimens with the Ezplex SARS-CoV-2 G kit must include with negative test result reports for specific patients whose specimen(s) were the subject of pooling, a notice that pooling was used during testing and that "*Patient specimens with low viral loads may not be detected in specimen pools due to the decreased sensitivity of pooled testing*."

3. Authorized laboratories using the Ezplex SARS-CoV-2 G kit must use the Ezplex SARS-CoV-2 G kit as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to perform the Ezplex SARS-CoV-2 G kit are not permitted.

4. Authorized laboratories implementing pooling strategies for testing patient specimens must use the "Specimen Pooling-Determining Appropriate Strategy for Implementation and Monitoring" available in the authorized labeling to evaluate the appropriateness of continuing to use such strategies based on the recommendations in the protocol.

5. Authorized laboratories that receive the Ezplex SARS-CoV-2 G kit must notify the relevant public health authorities of their intent to run the test prior to initiating testing.

6. Authorized laboratories using the Ezplex SARS-CoV-2 G kit must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

7. Authorized laboratories must collect information on the performance of the test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and SML Genetree (via email: info@genetree.com or genetree@genetree.co.kr) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.

8. All laboratory personnel using the test must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use the test in accordance with the authorized labeling.

9. SML Genetree, its authorized distributor(s) and authorized laboratories using the SARS-CoV-2 G kit must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

10. Authorized laboratories must keep records of specimen pooling strategies implemented including type of strategy, date implemented, and quantities tested, and test result data generated as part of the Protocol for Monitoring of Specimen Pooling Strategies. For the first 12 months from the date of their creation, such records must be made available to FDA within 48 business hours for inspection upon request. After 12 months from the date of their creation, upon FDA's request such records must be made available for inspection within a reasonable time.

* The letter of authorization refers to "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests" as "authorized laboratories."

XVI. Performance Evaluation

A. Analytical Sensitivity (Limit of Detection)

The limit of detection was evaluated for both upper and lower respiratory specimens. Negative clinical nasopharyngeal swabs collected in VTM and negative sputum matrix were used as the background matrices to prepare the samples. SARS-CoV-2 genomic RNA from Vircell (MBC137-R) was spiked into the negative clinical matrices at concentrations of 5, 2.5, 1.25, 0.625, and 0.3125 copies/uL. A total of 20 replicates per dilution were extracted using the QIAamp® DSP Virus Spin Kit and then tested on both the CFX96 Dx and ABI7500 thermocyclers. The preliminary LoD was then confirmed by testing 20 additional extraction replicates. The limit of detection was estimated as the concentration where the overall assay result yielded 95% positivity.

Target	CFX96		ABI 7500	
	Nasopharyngeal	Sputum	Nasopharyngeal	Sputum
RdRp	0.8 copies/uL	0.64 copies/uL	1 copies/uL	1 copies/uL
N	1 copies/uL	1 copies/uL	1 copies/uL	0.64 copies/uL
Overall LOD	1 copies/uL	1 copies/uL	1 copies/uL	1 copies/uL

B. Analytical Sensitivity (Inclusivity)

An in silico analysis comparing the primer and probe sequences from the Ezplex SARS-CoV-2 G Kit against all SARS-CoV-2 sequences deposited in public databases (NCBI and GISAID) as of September 9, 2020 was conducted. The in silico analysis through reference sequences, generated the following data:

Target	Gene	Primer/Probe	Percent Identities
SARS-CoV-2 Virus	RdRp (NCBI, N=5000) (GISAID, N=5204)	Forward Primer	100 %
		Reverse Primer	100 %
		Probe	100 %
	N (NCBI, N=5000) (GISAID, N=5204)	Forward Primer	100 %
		Reverse Primer	100 %
		Probe	100 %

C. Analytical Specificity

1. Cross Reactivity

Nucleic Acid from a total of 24 species of microorganisms that are likely to be found in clinical respiratory specimens were tested with the Ezplex assay. Each species was tested in triplicate at the concentrations indicated in the table below. No cross reactivity was observed.

Microorganism	Concentration	Microorganism	Concentration
Adenovirus	$1X10^4$ copies/uL	*Legionella pneumophila*	$1X10^4$ copies/uL
Human metapneumovirus	$1X10^6$ copies/uL	*Mycobacterium tuberculosis*	$1X10^4$ copies/uL
Human parainfluenza virus 1	$1X10^4$ copies/uL	*Streptococcus pneumoniae*	$1X10^4$ copies/uL
Human parainfluenza virus 2	$1X10^4$ copies/uL	*Bordetella pertussis*	$1X10^4$ copies/uL
Human parainfluenza virus 3	$1X10^4$ copies/uL	*Mycoplasma pneumoniae*	$1X10^4$ copies/uL
Human parainfluenza virus 4	$1X10^4$ copies/uL	*Candida albicans*	$1X10^4$ copies/uL
Influenza A virus	$1X10^4$ copies/uL	*Staphylococcus aureus*	$1X10^4$ copies/uL
Enterovirus 71 type	$1X10^4$ copies/uL	Human coronavirus OC43	$1.41X10^6$ copies/uL
Human respiratory syncytial virus A	$1X10^4$ copies/uL	Human coronavirus NL63	$1X10^6$ copies/uL
Human respiratory syncytial virus B	$1X10^4$ copies/uL	Severe acute respiratory syndrome	$1X10^6$ copies/uL
Human rhinovirus	$1X10^4$ copies/uL	Middle East respiratory syndrome	$1X10^6$ copies/uL
Chlamydophila pneumoniae	$1X10^4$ copies/uL		
Haemophilus influenzae	$1X10^4$ copies/uL		

2. Cross Reactivity (in silico)

A total of 28 organisms were selected to assess the potential for cross reactivity with the primers/probes included in the Ezplex Assay. This analysis was conducted using the NCBI blast database. The percent homology with each primer and probe used in the Ezplex is displayed in the table below:

No.	Organisms	RdRP homology(%)			N homology(%)		
		F primer	Probe	R primer	F primer	Probe	R primer
1	Human coronavirus 229E*	**89%**	None	70%	58.8%	41.6%	50%
2	Human coronavirus OC43*	None	None	40%	**94%**	41.6%	50%
3	Human coronavirus HKU1*	**91.6%**	None	76.6%	52.9%	37.5%	60%
4	Human coronavirus NL63	33.3%	None	40%	52.9%	37.5%	60%
5	SARS-coronavirus **	75%	None	**93.3%**	**100%**	**83.3%**	**80%**
6	MERS-coronavirus*	60%	None	**90%**	58.8%	45.8%	60%
7	Adenovirus 71	37.5%	None	30%	52.9%	41.6%	50%
8	Human Metapneumovirus	45.8%	None	36.6%	58.8%	45.8%	55%
9	Parainfluenza virus 1	33.3%	None	33.3%	52.9%	37.5%	45%
10	Parainfluenza virus 2	None	None	None	None	None	None
11	Parainfluenza virus 3	None	None	None	None	None	None
12	Parainfluenza virus 4	50%	None	36.6%	64.7%	41.6%	50%
13	Influenza A	54.1%	None	43.3%	76.4%	62.5%	60%
14	Enterovirus group	50%	None	46.6%	70.5%	62.5%	70%
15	Respiratory syncytial virus	None	None	None	None	None	None
16	Rhinovirus	50%	None	46.6%	70.5%	62.5%	70%
17	*Chlamydia pneumoniae*	50%	None	43.3%	64.7%	54.1%	60%
18	*Haemophilus influenzae*	58.3%	None	43.3%	76.4%	54.1%	70%
19	*Legionella pneumophila**	62.5%	None	50%	**82.3%**	54.1%	65%
20	*Mycobacterium tuberculosis*	50%	None	None	64.7%	62.5%	60%
21	*Streptococcus pneumoniae*	58.3%	None	43.3%	76.4%	54.1%	70%
22	*Streptococcus pyogenes*	54.1%	None	43.3%	76.4%	50%	65%
23	*Bordetella pertussis*	None	None	None	None	54.1%	70%
24	*Mycoplasma pneumoniae*	50%	None	43.3%	58.8%	45.8%	55%
25	*Pneumocystis jirovecii* (PJP)	62.5%	None	76.6%	64.7%	62.5%	65%
26	*Candida albicans**	62.5%	None	53.3%	**88.2%**	54.1%	70%
27	*Pseudomonas aeruginosa*	62.5%	None	46.6%	76.4%	70.8%	65%
28	*Staphylococcus group**	70.8%	None	50%	**82.3%**	79.1%	70%

1) For the organisms marked with *, relatively high homology was observed (more than 80%) for one oligo in the primer/probe set. However, in order for an amplification product to be generated using the PCR mechanism, both of the primers and the probe must anneal to the target sites on the gene. Therefore, cross-reactivity with these organisms is not expected.

2) For the SARS-coronavirus organism marked with **, more than 80% homology was found for all three oligos for the N gene. However, the interpretation algorithm of Ezplex states that only results where both the RdRp and N genes show amplification within 40 Ct are considered positive. Because >80% homology was not seen in all three primer/probe oligos for the RdRp gene, false positive results are not expected to occur.

3. Interference

The effect of endogenous (Albumin (0.24g/mL), Hemoglobin (0.2g/mL), Bilirubin (0.05mg/mL)) and exogenous (Mupirocin(20mg/mL), Tobramycin (80mg/mL), Zanamivir (250ug/mL)) interfering substances on assay performance was evaluated. Samples containing these substances were tested in triplicate with and without SARS-CoV-2 virus material at a concentration of ~3XLoD. No interference was observed as indicated by a less than 1 mean Ct differential between samples with and without interferent for both gene targets in all cases.

D. In Silico Analysis

In silico analysis for all sequences of SARS-CoV-2, available from NCBI and GISAID databases, was conducted by mapping the individual primers and probes of the Ezplex assay. As of Sep 09, 2020, in silico analysis through GISAID (n =5204) and NCBI (n = 5000) sequences generated data as shown in the table below.

Gene	Primer(Probe)	Homology(%)	
		NCBI(N=5000)	GISAID(N=5204)
RdRp	Probe Primer	100	100
	Forward Primer	100	100
	Reverse Primer	100	100
N	Probe Primer	100	100
	Forward Primer	100	100
	Reverse Primer	100	100

E. Clinical Evaluation

The purpose of this clinical evaluation was to assess the clinical performance of the Ezplex SARS-CoV-2 G Kit against an EUA-authorized comparator assay. In the clinical evaluation study, left-over archived specimens from symptomatic patients suspected of COVID-19 infection were tested. Specimens were previously subjected for SARS-CoV-2 testing and then stored at a clinical laboratory in South Korea prior to including in this study. A total of 30 positive and 30 negative clinical specimens confirmed by an EUA-authorized comparator Assay were tested with the investigational Ezplex assay. These

60 total specimens consisted of 15 positive and 15 negative upper respiratory (nasopharyngeal/oropharyngeal swabs collected in VTM) specimens and 15 positive and 15 negative sputum specimens. For this study, the specimens were extracted using the QIAamp® DSP Viral RNA mini Kit (Qiagen). Real-time RT-PCR was performed using the CFX96 Dx Real-time PCR Instrument (Bio-Rad). The results from testing of individual specimens are shown as below.

Upper Respiratory Specimens		Original Test of Record			Lower Respiratory Specimens		Original Test of Record		
		Positive	Negative	Total			Positive	Negative	Total
Ezplex	Positive	15	0	15	Ezplex	Positive	15	0	15
	Negative	0	15	15		Negative	0	15	15
	Total	15	15	30		Total	15	15	30
Positive agreement (95% CI)		100 % (78.2 ~ 100 %)			Positive agreement (95% CI)		100 % (78.2 ~ 100 %)		
Negative agreement (95% CI)		100 % (78.2 ~ 100 %)			Negative agreement (95% CI)		100 % (78.2 ~ 100 %)		

F. Pooling Verification

For the pooling verification study, a total of 180 upper respiratory specimens were collected and initially tested with Ezplex and the comparator EUA-authorized real-time PCR assays. These specimens consisted of 30 comparator positive and 150 comparator negative specimens. Testing with the Ezplex assay revealed concordant results for all specimens. Each single positive specimen was pooled together with 4 negative specimens (N=5) to create the positive pools. The negative pools were prepared by combining 5 negative specimens(N=5). This created a total of 30 positive and 30 negative pools. These pools were prepared by aliquoting 50uL of each specimen into a sterile tube to make a final volume of 250uL. For this study, extraction was performed using the QIAamp® DSP Viral RNA Mini Kit (Qiagen). Real-time RT-PCR was performed using the CFX96 Dx Real-time PCR Instrument (Bio-Rad). The results from testing of pooled specimens are shown below for each target.

1) RdRp Gene Result

SARS-CoV-2 Virus(RdRp)			Individual Testing				
			Expected Positive			Negative	Total
			37< Ct ≤40	34< Ct ≤37	Ct ≤34		
Pooled Testing	Positive	37< Ct ≤40	1	4	-	0	30
		34< Ct ≤37	1	2	2		
		Ct ≤34	-	-	20		
	Negative		0	0	0	30	30
	Total		30			30	60
Positive agreement (95% CI)			100 % (88.4 ~ 100 %)				
Negative agreement (95% CI)			100 % (88.4 ~ 100 %)				

2) N Gene Result

SARS-CoV-2 Virus(N)			Individual Testing				
			Expected Positive			Negative	Total
			37< Ct ≤40	34< Ct ≤37	Ct ≤34		
Pooled Testing	Positive	37< Ct ≤40	4	1	-	0	30
		34< Ct ≤37	-	3	1		
		Ct ≤34	-	-	21		
	Negative		0	0	0	30	30
	Total		30			30	60
Positive agreement (95% CI)			100 % (88.4 ~ 100 %)				
Negative agreement (95% CI)			100 % (88.4 ~ 100 %)				

These results indicate that all individually positive specimens were also detected when present in a 5-sample pool.
Among the 30 positive specimens, 8 weakly positive specimens were included in the verification. The individual Ct values for these specimens were compared to the Ct value where the same specimen was pooled with 4 negative specimens, as indicated below.

Individual Result			
No.	RdRp(FAM)	N(Cy5)	IC(HEX)
1	38.42	38.09	30.82
2	35.65	35.16	30.81
3	35.64	37.11	31.13
4	38.63	38.25	31.37
5	35.29	36.13	30.60
6	36.56	37.19	30.52
7	35.43	36.33	30.69
8	36.63	35.59	30.64

Pooled Result		
RdRp(FAM)	N(Cy5)	IC(HEX)
36.90	39.09	31.28
36.72	36.35	31.30
37.57	37.66	31.52
38.72	38.94	31.25
38.06	36.44	31.49
37.73	39.19	31.37
37.15	36.95	31.23
36.81	39.92	31.23

These data indicate that low positive samples are able to be detected when present in 5 sample pools.

XVII. FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded specimen testing was used to establish specificity and to confirm the LoD. The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded specimens and a standard testing protocol provided by the FDA. The extraction method and amplification instrument used were the QIAamp® DSP Viral RNA Mini Kit (62904) and the CFX96™ Dx Systems (1845097-IVD) respectively. The results are summarized in the Table below.

Table: Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal Swab in VTM	1,200 NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not detected

Appendix A: Specimen Pooling-Determining Appropriate Strategy for Implementation and Monitoring

Sample Pooling Monitoring (Laboratory Monitoring Part A)

Before a pooling strategy is implemented, a laboratory should determine the appropriate pool size based on percent positivity rate in the testing population and pooling testing efficiency (Table 1). The Ezplex SARS-CoV-2 assay has been validated for n-sample pool sizes up to five samples per pool.

Table 1. Efficiency of pooling based on the positivity of SARS-CoV-2 RNA in individual samples (as an example)

P, percent of positive subjects in the tested population	$n_{maxefficiency}$ (n corresponding to the maximal efficiency)	Efficiency of n-sample pooling corresponding to $n_{maxefficiency}$ (a maximum increase in the number of tested patients when Dorfman n- pooling strategy used)
5%	5	2.35
6%	5	2.15
7%	4	1.99
8%	4	1.87
9%	4	1.77
10%	4	1.68
11%	4	1.61
12%	4	1.54
13%	3	1.48
14%	3	1.43
15%	3	1.39
16%	3	1.35
17%	3	1.31
18%	3	1.28
19%	3	1.25
20%	3	1.22
21%	3	1.19
22%	3	1.16
23%	3	1.14
24%	3	1.12
25%	3	1.10

A.1 If Historical Data for Individual Specimens is Available

A.1.1 Positivity Rate of Individual Testing

- Estimate positivity rate ($P_{individual}$) in the laboratory based on individual sample testing. For this consider the 7-10 previous days and calculate the number of patients tested during those days. $P_{individual}$ is the number of positive results divided by the total number of tested patients during these 7-10 days.

A.1.2 Selection of test developer validated size of sample pools, n

- Use $P_{individual}$ and Table 1 to choose an appropriate validated pool size. Table 1 presents the pool size with the maximum efficiency for the validated pool sizes and positivity rates. If the positivity rate ($P_{individual}$) is in Table 1, choose n from Table 1 which corresponds to the maximum efficiency (F).
- If $P_{individual}$ in your laboratory does not correspond to the largest validated pool size in Table 1, the pool size with maximum efficiency for this positivity rate was not validated and you should choose the maximum n which was validated. For example, for the calculation of efficiency of 5-sample pooling, using formula $F=1/(1+ 1/5-(1-P)^5)$, when $P_{individual}$ is 1%, the efficiency F is 3.46 for n=5. It means that 1,000 tests can cover testing of 3,460 patients on average.
- If $P_{individual}$ is greater than 25%, then pooling patient samples is not efficient and should not be implemented.

A.2 If Historical Individual Data for Individual Specimens is Unavailable

If historical data from the previous 7-10 days is unavailable, the maximum pool size validated in the EUA and any smaller pool sizes can still be implemented, as the EUA test has been validated for the maximum pool size-specimen pooling. However, note that without $P_{individual}$, the laboratory may choose a pooling size that does not maximize pooling efficiency.

Sample Pooling Monitoring (Laboratory Monitoring Part B)

After implementing a n-sample pooling strategy, calculate the percent positivity rate (P_{pool}) based on n sample pooling strategy periodically using the data from pooled samples from the previous 7-10 days*.

B.1 If Historical Data for Individual Specimens is Available

If historical data for individual specimens is available, compare P_{pool} to $P_{individual}$ periodically. If P_{pool} is less than 85% of $P_{individual}$ ($P_{pool} < 0.85 \times P_{individual}$), it is recommended that:

- The pool size be adjusted to maximize pooling efficiency according to Table 1 above and the new n should not be more than the test developer validated maximum n in the EUA.
- If P_{pool} is greater than 25%, pooling of patient samples is not efficient and should be discontinued until the percent positivity rate decreases.

B.2 If Historical Data for Individual Specimens is Unavailable

- After implementing a n-sample pooling strategy, first calculate the positivity rate ($P_{pool\text{-}initial}$) based on n-sample pool size using the data from testing pooled samples from the first 7-10 days*.
  - If $P_{pool\text{-}initial}$ is greater than 25%, pooling of patient specimens is not efficient and should be discontinued until the percent positivity rate decreases.
  - If $P_{pool\text{-}initial}$ is less than or equal to 25%, pooling of patient specimens can be continued.
- Continue to monitor n-sample pooling strategy by calculating the positivity rate among patient samples during n-sample pooling ($P_{pools\text{-}x}$) for subsequent 7-10* day period based on n-sample pool testing. ($P_{pool\text{-}x}$) should be updated daily using a moving average.

Compare $P_{pool\text{-}initial}$ to $P_{pool\text{-}x}$ periodically. If $P_{pool\text{-}x}$ is less than 90% of $P_{pool\text{-}initial}$ ($P_{pool\text{-}x} < 0.90 \times P_{pool\text{-}initial}$), it is recommended that:

- The pool size be adjusted to maximize pooling efficiency, according to the criteria in Table 1 above, and the new n should not be more than the test developer validated maximum n in the EUA.
- If P_{pool} is greater than 25%, pooling of patient samples is not efficient and should be discontinued until the percent positivity rate decreases.

* It is recommended that $P_{individual}$ be calculated from the previous 7-10 days, while P_{pool} and $P_{pool\text{-}x}$ are calculated from data collected during a 7-10 day time frame. However, when determining if 7-10 days is appropriate, take into consideration the laboratory testing volume and percent positivity, among other factors. Note that if the number of individual or pooled positive results collected during a given time frame is less than 10, $P_{individual}$, P_{pools}, and $P_{pool\text{-}x}$ may not be representative of the percent positivity in the testing population and the laboratory may want to consider extending the testing time period to increase the chance of capturing positives.

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XIX. Symbols and Information

Symbol	Meaning	Symbol	Meaning
	Storage Temperature		In-Vitro Diagnostic Medical Devices
	Expiration date		Product User Manual
	Catalogue Number		Manufacturer
	Lot Number		Keep away from sunlight (P+P)
	Contents sufficient for <n> tests		
EC REP Obelis S.A. Bd. Général Wahis 53, B-1030 Brussels, Belgium	Authorized Representative in the European Community		For prescription use only

XX. Technical and Customer Support

For Technical Support, please contact our Genetree Technical Support team. Before contacting Genetree Technical Support, collect the following information:

- Product name
- Lot number
- Software version

- Email for Technical Support: technicalsupport@smlgenetree.com
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- Tel: +82-2-2057-7900
- Fax: +82-70-7425-3950
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