EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
ETHOS LABORATORIES SARS-COV-2 MALDI-TOF ASSAY
(ETHOS LABORATORIES)

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

(The Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay will be performed at Ethos Laboratories, located at 29 E. 6th St., Newport, KY 41071, which is certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, and meets requirements to perform high complexity tests as per the Instructions of Use that were reviewed by the FDA under this EUA.)

INTENDED USE

The Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay is a RT-PCR and MALDI-TOF mass spectrometry assay intended for the qualitative detection of nucleic acid from SARS-CoV-2 RNA in upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab specimens) and bronchoalveolar lavage specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to the Ethos Laboratories, located at 29 E. 6th St., Newport, KY 41071, which is certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets requirements to perform high-complexity tests.

The Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay is also for use with the Ethos Laboratories U-Collect At Home Collections kit for self-collection of nasal swab specimens at home by individuals when determined and prescribed by a healthcare provider to be appropriate based on the results of an online COVID-19 questionnaire.

Results are for the detection and identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens and bronchoalveolar lavage 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 infective 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 positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The Ethos Laboratories SARS-CoV-2 MALDI-TOF assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of RT-PCR and MALDI-TOF mass spectrometry and *in vitro* diagnostic procedures. The Ethos Laboratories SARS-CoV-2 MALDI-TOF assay is only for use under the Food and Drug Administration's Emergency Use Authorization

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay

The Ethos Laboratories SARS-CoV-2 assay uses the Agena SARS-CoV-2 Panel Set (RUO) kit for amplification and detection of SARS-CoV-2 RNA. The Agena assay is a real-time reverse transcription polymerase chain reaction assay that utilizes a RT-PCR reaction to reverse transcribe viral RNA into cDNA and amplify the nucleic acid material in the same reaction from three regions of the SARS-CoV-2 single stranded RNA genome: the N gene, ORF1 gene, and ORF1ab gene.

Nucleic acids are first isolated and purified from upper respiratory specimens using the MagBind Viral DNA/RNA kit and the KingFisher Flex Purification System. The purified nucleic acid is then reverse transcribed into cDNA and subsequently amplified using a multi-step process in a Veriti 96 Fast Thermal Cycler. The first step is the amplification of the genomic DNA using the SARS-CoV-2 PCR primers. Then, short amplicon primer (SAP) treatments are performed to dephosphorylate any remaining free deoxynucleotides. Finally, the primers are extended by one of the terminator nucleotides -A, T, C, and G- in the extension step, which produces allele-specific extension products of different masses.

These products are ionized and analyzed using a MALDI-TOF mass spectrometer where they are separated based on their mass-to-charge ratio through a drift tube. Using the arrival time of the individual ionized DNA analytes, the MassARRAY System determines the mass and displays a mass spectrum identifying the different genetic targets.

Ethos Laboratories U-Collect At Home Collections

The Ethos Laboratories U-Collect At Home Collections collection device consists of a Copan swab, VTM (Hardy Diagnostics or Biomed Diagnostics), specimen bag with absorbent sheet, order form, sample collection and shipping instructions, and a pre-labeled UPS shipping bag. The instructions direct users on the completion of the order, sample collection, sample packaging, storage and shipping requirements.

Home Collection Kit Ordering and Processing:

Individuals may request the Ethos Laboratories U-Collect At Home Collections collection device through the website's online questionnaire, which screens patients based on the CDC

recommendations for testing prioritization. Patients that meet the qualification criteria for COVID-19 testing as determined by an authorized healthcare provider, will then be prescribed for an Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay test. Upon approval submission by the healthcare provider, the website notifies the Ethos Laboratories Telehealth team of the pending order. The order is then processed and an Ethos Laboratories U-Collect At Home Collections kit is prepared. The kit is shipped to the patient's home. The instructions included in the kit are designed to guide users through the entire home collection process to prevent injury to the nasal cavity, rejection of the specimen due to improper packaging, loss of the sample, insufficient sample collection, and cross contamination. Furthermore, those individuals who do not meet the requirements for the Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay test based on the responses from the online questionnaire will also be notified of their denial for testing.

The Ethos Laboratories U-Collect At Home Collections kit collects and stabilizes viral RNA from nasal swab specimens; it can also be used for the transportation and short-term room temperature storage of a sample. The Ethos Laboratories U-Collect At Home Collections kit is a non-invasive alternative for collecting high quality and high quantity viral RNA from individuals who are suspected to be positive for COVID-19 by their healthcare provider.

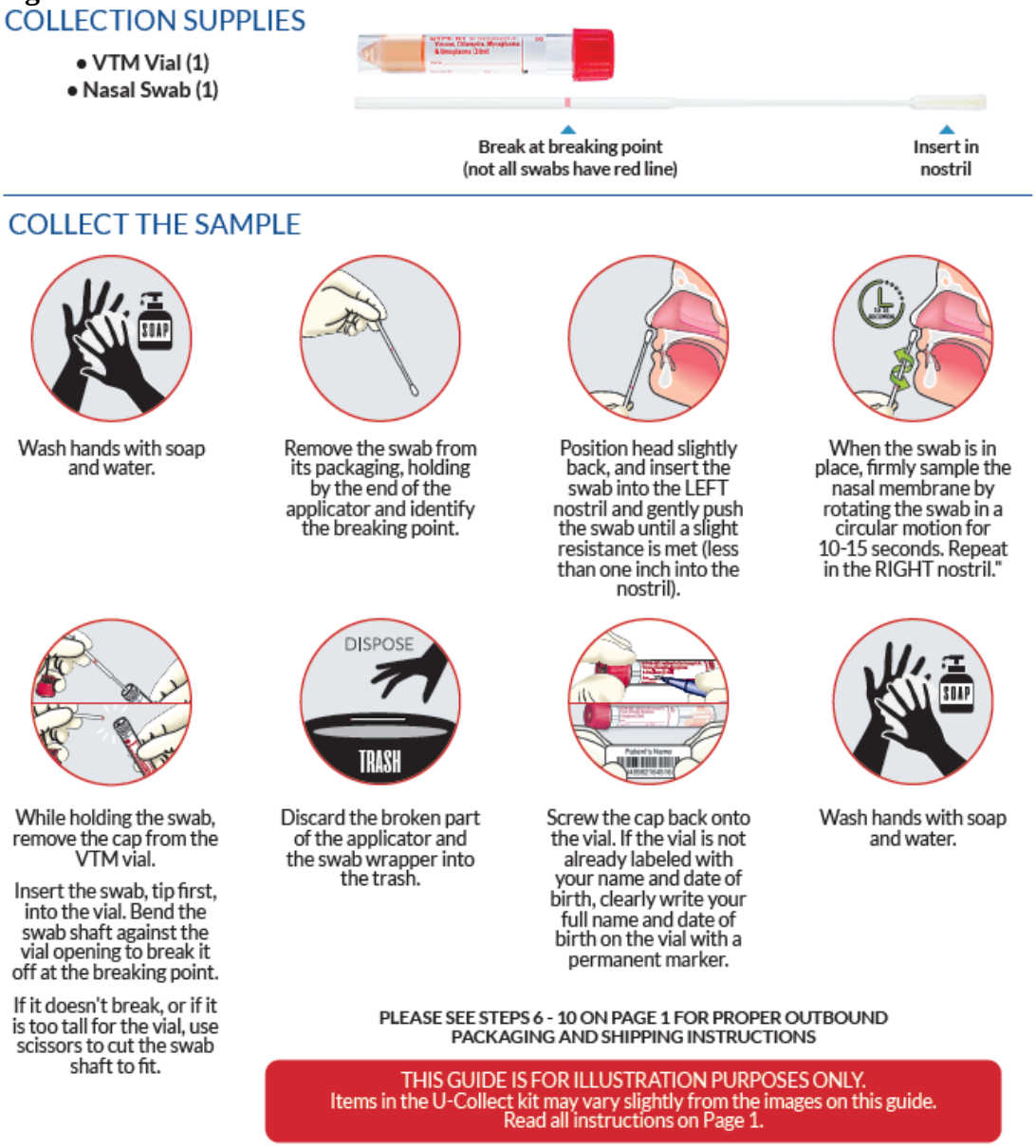
The Ethos Laboratories U-Collect At Home Collections kit consists of a nasal swab and collection tube containing viral transport media. The individual using the Ethos Laboratories U-Collect At Home Collections kit to collect nasal swab specimens performs the following steps to collect the initial specimen. A swab should be removed from the packaging and only held by the end of the applicator. The patient positions their head slightly back and inserts the swab into the left nostril until a slight resistance is met (less than one inch). The patient then rotates the swab in a circular motion for 10-15 seconds and repeats the process on the right nostril. The swab is inserted into the VTM tube and the applicator handle is bent 180 degrees at the breaking point (or cut if necessary) to break off the applicator end. The cap of the VTM tube is screwed back on and securely tightened.

The Viral Transport Medium is manufactured by Hardy Diagnostics or Biomed Diagnostics and consists of a salt solution that is supplemented with animal proteins and sucrose for virus stabilization. The pH is maintained at 7.3 +/-0.2 with a buffer solution and phenol red is used as the pH indicator. Bacterial and fungal contaminants are inhibited with added selective agents and the cryoprotectant medium ensures the stabilization of the virus through freezing and thawing.

For device shipping, the specimen is placed in the specimen collection bag with the paperwork in the outer pocket and prepared for shipping using the pre-labeled UPS shipping bag. Specimens can be placed in a drop box, mailbox or taken to a UPS store, and packages can be stored at ambient temperature while awaiting pickup. All samples will be returned to Ethos Laboratories via the UPS next-day air, early courier service, and tracked by Ethos Laboratories.

Specimens received at the clinical laboratory for testing with the Ethos Laboratories SARS-CoV-2 MALDI-TOF assay undergo the accessioning prior to acceptance for testing.

Figure 1: Ethos Laboratories U-Collect At Home Collections Instructions for Use



Results for the Ethos Laboratories U-Collect At Home Collection kit will include the following comments based on the results reported, which direct the patient to follow up with their HCP. The U-Collect kit results will be reported directly to the patient based on the email they provide during the online registration. When the results are ready and approved by the Medical Director, the patient will receive an email with instructions on how to access their report. The PDF result can be downloaded and printed and shared with their HCP.

SARS-CoV-2 Viral Test	**POSITIVE**	**No Range**	**PCR**

A Positive viral test result means that you are positive for SARS-CoV-2, the virus that causes COVID-19. You should consult with your primary healthcare provider and self-isolate to avoid spreading the virus to others. There is a very small chance that this test can give a positive result that is incorrect (a false positive). Your healthcare provider will work with you to determine how best to care for you based on your test results, medical history, and symptoms.

SARS-CoV-2 Viral Test	Negative	No Range	PCR

A Negative viral test result means that you are negative for SARS-CoV-2, the virus that causes COVID-19. If you are experiencing symptoms, but your result was Negative, this usually means that your illness was not due to COVID-19. However, there is a small chance that this test can give a negative result that is incorrect (a false negative) in some people with COVID-19. Your primary healthcare provider will consider the test result together with your symptoms, in deciding how to care for you.

SARS-CoV-2 Viral Test	**Inconclusive**	**No Range**	**PCR**

An Inconclusive viral test result means that a definitive Positive or Negative result could not be determined. You should consult with your primary healthcare provider to determine if you should be retested to get a definitive result.

INSTRUMENTS USED WITH TEST

The Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay is to be used with the following instruments:

- KingFisher Flex Purification System (BindIt 4.0 Software, Thermo-Fisher, Massachusetts, USA) Catalog#5400630
- Veriti 96 Thermal Cycler with Thermal Cycler Fleet Control Software Version 1.7.1 (Applied Biosystems, California, USA) Catalog#4375786
- MassARRAY System with Typer Software, Version 5.0 (Agena Bioscience, Catalog#10445)

REAGENTS AND MATERIALS

The Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay has been validated using only the components referenced in this submission.

- MagBind Viral DNA/RNA Kit (Omega Bio-tek, Georgia, USA) Catalog# M6246-03
- KingFisher Flex Purification System (BindIt 4.0 Software, Thermo-Fisher, Massachusetts, USA) Catalog#5400630
- SARS-CoV-2 Panel (includes oligonucleotide primers) (Agena Bioscience, California, USA) Catalog#13274F
- MassARRAY System (Agena Bioscience, California, USA) Catalog#10445
- Typer Software, Version 5.0 (Agena Bioscience, California, USA)
- SpectroCHIP CPM Kit (Agena Bioscience, California, USA) Catalog#10600F
- MicroAMP Clear Adhesive Film (Agena Bioscience, California, USA) Catalog#4306311
- Nuclease-Free Water (Thermo-Fisher, Massachusetts, USA) Catalog#AM9932

- Molecular Biology Grade Ethanol (Thermo-Fisher, Massachusetts, USA) Catalog#BP28184
- Molecular Biology Grade Isopropyl Alcohol (VWR, Pennsylvania, USA) Catalog#87000-050
- Single Channel Pipettes, Multi-Channel (8) Pipettes, and Pipette Tips (VWR, Pennsylvania, USA)
    - 1-10µL Catalog#75788-460
    - 1-100µL Catalog#75788-460
    - 20-200µL Catalog#75788-460
    - 100-1250µL Catalog#75788-460
- 96-Well Plate (Thermo-Fisher, Massachusetts, USA) Catalog#4483343
- 96-Well Plate Magnetic Stand (Thermo-Fisher, Massachusetts, USA) Catalog#12331D
- Plate Seals (Thermo-Fisher, Massachusetts, USA) Catalog#AB0558
- Microtubes (MSP, Georgia, USA) Catalog#62-1008-1
- Mini Microcentrifuge (Corning, New York, USA) Catalog#6770
- Centrifuge with Microplate Rotor (Eppendorf, Hamburg, Germany) Catalog#5810
- REDISHIP Purifier Logic Class II A2 Biosafety Cabinet (Labconco, Missouri, USA) Catalog#76317-616
- UVC/T-M-AR General Purpose PCR UV Cabinet (Grant Instruments, Pennsylvania, USA) Catalog#10117-478
- Vortex Genie 2 (Scientific Industries, New York, USA) Catalog#SI-0236
- Veriti 96 Thermal Cycler with Thermal Cycler Fleet Control Software Version 1.7.1 (Applied Biosystems, California, USA) Catalog#4375786
- Human Liver Total RNA (For use as an Extraction Control) (Thermo-Fisher, Massachusetts, USA) Catalog#AM7960
- Pipet Filler (Thermo-Fisher, Massachusetts, USA) Catalog#89204-754
- Serological Pipets, 50 mL (VWR, Pennsylvania, USA) Catalog#75816-088
- Filtered Pipet Tips (VWR, Pennsylvania, USA) Catalog#
    - 1-10µL Catalog#76322-132
    - 1-100µL Catalog#76322-136
    - 20-200µL Catalog#76322-150
    - 100-1250µL Catalog#76322-156
- Synthetic SARS-CoV-2 RNA Control 1 (Twist Bioscience, California, USA) Catalog#102019
- NanoDrop 8000 Microvolume UV-Vis Spectrophotometer with Software Version V2.2.0 (Thermo-Fisher, Massachusetts, USA) Catalog#: ND-8000-GL
- Heat Inactivated SARS-CoV-2 Culture Fluid (ZeptoMetrix, New York, USA) Catalog#: NATRVP-NNS

Components Included with the Ethos Laboratories U-Collect At Home Collections Kit:

The Ethos Laboratories U-Collet At Home Collections kit will be assembled at Ethos Laboratories with the components listed in Table 1 which are manufactured by Hardy Diagnostics/Biomed Diagnostics and Copan. The product will only be distributed by Ethos Laboratories for the sole purpose of self-collections.

Table 1: Ethos Laboratories U-Collect At Home Collections Kit Components

Name	Material Supplier	Description	Cat./ Part #
CLASSIQSwabs Dry Swabs	COPAN	1 Sterile Regular polyester dry swab with plastic applicator	164KS01
Viral Transport Medium (VTM) Vial	Hardy Diagnostics or Biomed Diagnostics	1 Sterile 3 mL fill VTM	R99
Specimen Bag	McKesson	1 Plastic specimen collection bag with Ethos Labs logo	896061
Order Form	Ethos Laboratories	1 Ethos order/ requisition form	N/A
Pre-Labeled UPS Shipping Bag	UPS	1 Laboratory Pak	UN3373
Absorbent Sheet	Therapak	1 3 x 6” sheet	10306
Home Collection Instructions	Ethos Laboratories	1 Ethos Laboratories Home Collection Instructions	N/A
Accessioning Label (pre-applied to vial)	Ethos Laboratories	1 Ethos Laboratories Accessioning Label	N/A

Individuals will activate their kit and complete a personal health record and a suitability questionnaire (per CDC Person Under Investigation Case Report Form: capturing probable exposure, symptoms, pregnancy and existing co-morbidities). In addition, Ethos Laboratories U-Collet At Home Collections Kit Customer Care will be available to answer questions at any stage in the process either by email or by phone.

Individuals will self-collect the nasal swab sample following the Instructions for Use. The nasal swab is inserted into the sample transport tube containing viral transport media, package as instructed, and return the sample to the Ethos Labs (via Next Day Air shipping) for testing using the Return Label provided.

The sample will be received and accessioned at Ethos Labs and processed using the Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay which is approved under the Food and Drug Administration's Emergency Use Authorization per Standard Operating Protocol.

Testing is limited to labs that are Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a certified high-complexity laboratories.

Patient Inclusion/Exclusion Criteria:

These criteria are based on current CDC testing guidelines.

Exclusion:

- Anyone with severe symptoms
- People not meeting any of the eligibility requirements based on the U-Collect online questionnaire.

Inclusion

- People are eligible for a test if they have any of the following symptoms: cough, sore throat, muscle aches, body aches, flu-like symptoms, fever between 100.4F - 102.0F, vomiting, diarrhea, changes in smell or taste and shortness of breath.
- People required to provide a COVID-19 test result as mandated by the employer before returning to work.
- People exposed through close proximity or close contact (within 6 feet) of someone diagnosed with COIVD-19.

Ethos Lab Accessioning Criteria:

All tests arriving at Ethos Laboratory will be checked for the following deficiencies:

- Quantity Not Sufficient (MQNS) – Quantity of specimen provided/received is not enough to complete the testing that was ordered (with no known cause for low sample volume).
- Leaked Specimen Due to Lose Lid (MLID) – Quantity of specimen received is not enough to complete the testing that was ordered; likely due to a loose lid or one that was not placed on vial correctly.
- Leaked Specimen Due to Damaged Container (MDMG) – Quantity of specimen received is not enough to complete the testing that was ordered; likely due to a specimen container that was damaged in shipping.
- Container Not Labeled (MCNL) – The specimen container was not labeled with two acceptable patient-specific identifiers.
    - Acceptable patient-specific identifiers include:
    - Ethos Requisition Number
    - Patient Full Name (First and Last)
    - Patient Date of Birth

- Conflicting ID (MCID) – The identifying information on the specimen container does not match the identifying information on the accompanying requisition or order.
- Not Suitable for Testing (MUNS) – The specimen is unsuitable to complete the ordered testing.
- Missing Test Code (MTCODE) – The specimen was received without ordered tests indicated on the requisition or order. Attempts to recover the ordered tests for the specimen have been unsuccessful.

Swabs and Transport Media:

Ethos Laboratories U-Collect At Home Collections Kit utilizes Hardy Diagnostics/Biomed Diagnostics Viral Transport Media and Copan swabs.

CONTROLS TO BE USED WITH THE ETHOS LABORATORIES SARS-COV-2 MALDI-TOF ASSAY

To ensure that each step in the Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay process from collection to reporting is performed correctly, separate stepwise controls have been included. These controls are at three critical steps throughout the procedure and indicate the success or failure of that specific process:

1. Specimen collection
2. Extraction
3. PCR amplification, extension, and detection.

If at any point a process control fails, the specimen will either need to be recollected or reprocessed accordingly.

1. To verify adequate specimen collection, every patient sample will be subject to sample validity testing prior to extraction. The detection of nucleic material serves as verification that the sample was properly collected by the patient or provider.

   Sample collection tubes containing VTM and collected nasal swabs and BAL will be vortexed briefly on a Vortex Genie 2 (Scientific Industries Inc., New York, USA) to ensure sample homogeneity. A small aliquot (250 µL) of each sample will be placed into the well of a deep 96-well 2 mL polypropylene plate. The plate will be sealed to prevent cross-contamination and floated in a Branson 1510 Ultrasonic bath (Branson Ultrasonics Corporation, Connecticut, USA) operating at 40 KHz for five minutes to ensure thorough lysis of cellular material and release of nucleic material for detection by UV-Vis absorbance.

   The presence of nucleic material will be quantified using a NanoDrop 8000 Microvolume UV-Vis Spectrophotometer and NanoDrop 8000 version 2.2.0 software (Thermo Scientific, Massachusetts, USA). DNA exhibits a strong absorbance peak at 260 nm

wavelength. Absorbance at this wavelength by upper respiratory specimen (such as nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab specimens) and bronchoalveolar lavage lysates confirms the presence of human cellular material. Absorbance values at 260 nm and the fixed path length of the NanoDrop 8000 analyzer are used by the on-board software to calculate sample DNA concentration. Pure VTM containing no human sample will be used as a blank to remove any background absorbance by the transport media. Sample lysates are then spotted onto the NanoDrop 8000 and absorbance values at 260 nm are measured.

2. The positive extraction control in the SARS-CoV-2 assay is ZeptoMetrix Heat Inactivated SARS-CoV-2 Culture Fluid (Catalog #NATRVP-NNS) and is to be used in each sample batch run. One positive extraction control sample near the assay LOD is aliquoted to each extraction plate and processed in the same manner as patient samples according to the Ethos SARS-CoV-2 MALDI-TOF assay. The positive extraction control is used to monitor for failures of the MagBind Viral DNA/RNA Kit (Omega Bio-tek, Georgia, USA, Catalog# M6246-03) used with the KingFisher Flex Purification System (Thermo-Fisher, Massachusetts, USA, Catalog#5400630). All five SARS-CoV-2 gene targets must be detected for the positive extraction control to pass.

3. The PCR amplification, extension, and detection process control is provided in the form of RNAse P. The presence of SARS-CoV-2 genes in positive samples confirms that the amplification and extension steps were successful. However, in the case of negative samples, RNAse P acts as the control showing successful amplification and extension, and that the absence of SARS-CoV-2 genes represents a true negative. An aliquot of Human Liver Total RNA is included in every sample except the negative control (NTC) prior to PCR. Although clinical samples have endogenous RNAse P naturally, there are two reasons for spiking additional material:

    - Natural RNAse P degrades relatively quickly during sample collection, transport, and extraction. Internal stability studies were conducted near the assay's LOD using intact virus spiked into viral transport media and subjected to extreme temperature profiles. Results of this study suggested no significant degradation of any of the five SARS-CoV-2 viral targets. Stability data is provided in the supporting validation study file.

    - The primers for RNAse P have been added to the PCR master mix in limited concentration so as to not "outcompete" a patient sample with a low viral load. Therefore, high concentrations of the SARS-CoV-2 virus in clinical samples can suppress RNAse P amplification, meaning that RNAse P may not be detected for positive clinical samples even when spiked.

      Spiked RNAse P acts as an external control for the PCR process to ensure that amplification and extension proceeded as expected in true negative samples for which no SARS-CoV-2 genes are detected. Detection of two or more SARS-CoV-

2 genes confirms that the reverse transcription, amplification, and extension steps were successful for positive samples even if RNAse P is not detected.

In addition to the above process controls, a negative/no template control (NTC) is provided in the form of molecular-grade, nuclease-free, water and is used to monitor non-specific amplification, cross-contamination during PCR setup, and nucleic acid contamination of PCR reagents. It is to be used in each sample batch run.

INTERPRETATION OF RESULTS

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

1. Interpretation of SARS-CoV2 RT-PCR Controls

    a. *Specimen Collection:* a patient sample that contains ≥2000 ng/mL of DNA is considered to be a valid specimen. For an invalid specimen, recollection is recommended.

    b. *Extraction*: The positive extraction control is ZeptoMetrix Heat Inactivated SARS-CoV-2 Culture Fluid (Catalog #NATRVP-NNS) and is used to monitor for failures of the extraction. The presence of all five genes (N1, N2, N3, ORF1, and ORF1ab) in the positive extraction control indicate the extraction process was successful.

    c. *PCR Amplification, Extension, and Detection*: The PCR amplification, extension, and detection control is in the form of RNAse P in true negative samples, and in the detection of SARS-CoV-2 targets in positive samples.
        i. QC Status: PASS, when the RNAse P control is detected and SARS-CoV-2 targets are detected or not detected.
        ii. QC Status: PASS-Note, when the RNAse P control is not detected and at least one SARS-CoV-2 target is detected.
        iii. QC Status: FAIL, when the RNAse P control is not detected and all SARS-CoV-2 targets are not detected. If the QC Status: FAIL, the test is invalid.

    d. *The NTC (non-template control):* must be negative in order for the test to be valid.

Table 2: Interpretation of Results for Quality Controls

Control Type	External Control Name	Used to Monitor	N1	N2	N3	ORF 1	ORF 1ab	RP
External	RP	Amplification and extension efficiency	(-)	(-)	(-)	(-)	(-)	(+)
Positive Extraction Control	SC2	RT-PCR efficiency	(+)	(+)	(+)	(+)	(+)	(-)
Negative	NTC	Cross-contamination monitoring	(-)	(-)	(-)	(-)	(-)	(-)

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 positive and negative controls are not valid, the patient results cannot be interpreted.

1. Negative: RNase P detected, all SARS-CoV-2 targets not detected
2. Positive: RNase P detected or not detected, two or more SARS-CoV-2 targets detected
3. Inconclusive: RNase P detected or not detected, only one SARS-CoV-2 target detected
4. Invalid: RNase P not detected, all SARS-CoV-2 targets not detected

Table 3: Interpretation of Test Results for the Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay

N1	N2	N3	ORF1	ORF1ab	RP	Result Interpretation	Report
(-)	(-)	(-)	(-)	(-)	(-)	RP is negative and no SARS-CoV-2 targets are detected; the test is invalid	Invalid
(-)	(-)	(-)	(-)	(-)	(+)	The test is valid, and SARS-CoV-2 is not detected	Not Detected
Any 2 or more (+)					(-)	RNAse P is not detected, but two or more SARS-CoV-2 targets are detected; test is valid; SARS-CoV-2 is detected	Detected
Any 1 (+)					(+)	The test is valid and only 1 SARS-CoV-2 gene was detected; the test is inconclusive	Inconclusive

N1	N2	N3	ORF1	ORF1ab	RP	Result Interpretation	Report
Any 1 (+)					(-)	RNAse P is not detected, and only one SARS-CoV-2 target is detected; test is valid; the test is inconclusive	Inconclusive
Any 2 or more (+)					(+)	The test is valid, and 2 or more SARS-CoV-2 genes were detected; SARS-CoV-2 is detected	Detected

LIMITATIONS

- The use of this assay as an *in vitro* diagnostic under the FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.
- Use of this assay is limited to personnel who are trained in the procedure of RT-PCR and mass spectrometry. Failure to follow these instructions may result in erroneous results.
- The Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay can be used with the specimen types listed in the Intended Use statement. Other specimen types have not been evaluated and should not be used with this assay.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.
- Extraction and amplification of nucleic acid from clinical samples must be performed according the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.
- False-negative results may arise from:
  - Improper sample collection
  - Degradation of the viral RNA during shipping/storage
  - Using unauthorized extraction or assay reagents
  - The presence of RT-PCR inhibitors
  - Mutation in the SARS-CoV-2 virus
  - Failure to follow instructions for use
- False-positive results may arise from:
  - Cross contamination during specimen handling or preparation
  - Cross contamination between patient samples
  - Specimen mix-up
  - RNA contamination during product handling

- The effect of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not yet been evaluated.
- Please note, Negative results do not preclude infection of SARS-CoV-2 virus and should not be the sole basis of a patient management decision. A positive result indicates detection of nucleic acid from the relevant virus. Nucleic acid may persist even after the virus is no longer viable.
- Laboratories are required to report all positive results to the appropriate public health authorities.

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

The Limit of Detection (LoD) was determined for the Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay as the lowest detectable concentration of SARS-CoV-2 at which greater or equal to 95% of all replicates test positive.

An initial LoD study was performed to determine the LoD of each target assay in the Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay using heat Inactivated SARS-CoV-2 Culture Fluid (ZeptoMetrix Catalog #NATRVP-NNS) that was spiked in SARS-CoV-2 confirmed-negative nasopharyngeal swab specimens at concentrations ranging from 2 $TCID_{50}$/mL to 0.01 $TCID_{50}$/mL. Nucleic acid was extracted from the contrived samples using the KingFisher Flex Purification System, reverse transcription RT-PCR was performed using the Veriti 96 Thermal Cycler and MALDI-TOF mass spectrometry was performed on the Agena MassARRAY System. The preliminary LoD results are summarized in the Table below:

Table 4: LoD Determination

SARS-CoV-2 Concentration	No. Replicates Positive Overall
2 $TCID_{50}$/mL	20/20
1 $TCID_{50}$/mL	20/20
0.5 $TCID_{50}$/mL	18/20
0.1 $TCID_{50}$/mL	6/20
0.05 $TCID_{50}$/mL	2/20
0.01 $TCID_{50}$/mL	0/20

The LoD was confirmed by spiking 20 replicates of 1 $TCID_{50}$/mL of SARS-CoV-2 Culture Fluid into nasopharyngeal swab matrix previously confirmed to be negative for SARS-CoV-2. Nucleic acid was extracted from the contrived samples using the KingFisher Flex Purification System, reverse transcription RT-PCR was performed using the Veriti 96 Thermal Cycler and MALDI-TOF spectrometry was performed on the Agena MassARRAY System. The LoD results are summarized in the Table below:

Table 5: LoD Confirmation Study Summary

Conc. RNA	No. Replicates Positive Overall
1 $TCID_{50}$/mL	20/20

The LoD of the Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay was confirmed to be 1 $TCID_{50}$/mL.

2) Analytical Inclusivity:

The Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay has been designed using publicly available SARS-CoV-2 viral RNA sequences for the detection of SARS-CoV-2 strains or isolates. 165 NCBI and target sequences were retrieved and aligned to identify conserved regions and specific regions of the SARS-CoV-2 genome, where primers were designed for the assay. Alignments were performed with the designed oligonucleotide primer sequences of Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay with 2,661 SARS-CoV-2 sequences publicly available in Genbank as of May 20, 2020 to demonstrate the estimated inclusivity of the Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay. All the alignments exhibited 100% identity to the available SARS-CoV-2 sequences, suggesting the potential ability of the Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay to detect 100% of all currently sequenced SARS-CoV-2 strains.

3) Cross-Reactivity:

In-silico Analysis:

In-silico analysis for the N1, N2, N3, ORF1, and ORF1ab primer/probe set of the Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay was conducted to assess cross-reactivity against sequences of pathogens potentially present in upper respiratory specimens and/or with genetic similarities to SARS-CoV-2 according to the Recommended List of Organisms to be analyzed in-silico or by direct wet lab testing.

Table 6: List of organisms tested for cross-reactivity by *in silico* analysis

#	Organism	#	Organism
1	Human coronavirus 229E	14	Rhinovirus
2	Human coronavirus OC43	15	Enterovirus
3	Human coronavirus HKU1	16	*Chlamydia pneumoniae*
4	Human coronavirus NL63	17	*Haemophilus influenzae*
5	SARS-coronavirus	18	*Legionella pneumophila*
6	MERS-coronavirus	19	*Mycobacterium tuberculosis*
7	Adenovirus	20	*Streptococcus pneumoniae*
8	Human Metapneumovirus (hMPV)	21	*Streptococcus pyogenes*

#	Organism	#	Organism
9	Parainfluenza virus 1-4	22	*Bordetella pertussis*
10	Influenza A	23	*Candida albicans*
11	Influenza B	24	*Pseudomonas aeruginosa*
12	Enterovirus	25	*Staphylococcus epidermis*
13	Respiratory Syncytial Virus A	26	*Streptococcus salivarius*

The following is an analysis of the cross-reactivity results for the Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay that exhibit > 80% homology in at least one of their components:

Table 7: *In-silico* Analysis of Cross-Reactivity of the Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay

Assay Name	Assay Primer	Highest % Other Species Homology	Highest Other Specie
N1	SC2_N1_For	82	SARS-coronavirus
	SC2_N1_Rev	75	SARS-coronavirus
	SC2_N1_Probe	94	SARS-coronavirus
N2	SC2_N2_For	91	SARS-coronavirus
	SC2_N2_Rev	68	SARS-coronavirus
	SC2_N2_Probe	55	Human Coronavirus NL63
N3	SC2_N3_For	60	MERS-coronavirus
	SC2_N3_Rev	60	MERS- coronavirus
	SC2_N3_Probe	57	Human coronavirus HKU1
ORF1	SC2_ORF1_For	55	Enterovirus F strain BEV-261
	SC2_ORF1_Rev	50	*Staphylococcus* phage tp310-3
	SC2_ORF1_Probe	76	Possum enterovirus W1
ORF1ab	SC2_ORF1ab_For	76	SARS-coronavirus
	SC2_ORF1ab_Rev	68	Enterovirus SEV-gx
	SC2_ORF1ab_Probe	88	SARS-coronavirus

Results of *in silico* analysis demonstrate that there is significant homology between the N1, N2 and ORFlab assay to SARS-coronavirus. All other homologies were not significant for the pair of primers and probes in order to predict a false positive result *in silico.*

Wet Testing Analysis:

To confirm the absence of cross-reactivity of the Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay in wet-testing conditions, a ZeptoMetrix NATtrol Respiratory Verification Panel (ZeptoMetrix, Catalog # NATRVP-NNS, qualitative panel) was evaluated. Samples containing non-target organisms were prepared by spiking each organism into negative nasopharyngeal swab matrix. Nucleic acid was extracted from the contrived samples using the KingFisher Flex Purification System, reverse transcription RT-PCR was performed using the Veriti 96 Thermal Cycler and MALDI-TOF spectrometry was performed on the Agena MassARRAY System. As a result, none of the tested organisms were detected.

Table 8: Organisms Assessed for Potential *in-vitro* Cross-Reactivity

Organism	Strain	N1	N2	N3	ORF1	ORF1ab
Influenza A H1N1	A/New Calcidonia/20/99	(-)	(-)	(-)	(-)	(-)
Influenza A H3	A/Brisbane/10/07	(-)	(-)	(-)	(-)	(-)
Influenza A 2009 H1N1	NY/02/09	(-)	(-)	(-)	(-)	(-)
Influenza B	B/Florida/02/06	(-)	(-)	(-)	(-)	(-)
Metapneumovirus 8	Peru6-2003	(-)	(-)	(-)	(-)	(-)
Respiratory Syncytial Virus A	N/A	(-)	(-)	(-)	(-)	(-)
Rhinovirus 1A	N/A	(-)	(-)	(-)	(-)	(-)
Parainfluenza Virus Type 1	N/A	(-)	(-)	(-)	(-)	(-)
Parainfluenza Virus Type 2	N/A	(-)	(-)	(-)	(-)	(-)
Parainfluenza Virus Type 3	N/A	(-)	(-)	(-)	(-)	(-)
Parainfluenza Virus Type 4	N/A	(-)	(-)	(-)	(-)	(-)
Adenovirus Type 3	N/A	(-)	(-)	(-)	(-)	(-)
Coronavirus NL63	N/A	(-)	(-)	(-)	(-)	(-)
Coronavirus 229E	N/A	(-)	(-)	(-)	(-)	(-)
Coronavirus OC43	N/A	(-)	(-)	(-)	(-)	(-)
Coronavirus HKU-1	N/A	(-)	(-)	(-)	(-)	(-)
M. pneumoniae	M129	(-)	(-)	(-)	(-)	(-)
C. pneumoniae	CWL-029	(-)	(-)	(-)	(-)	(-)
B. pertussis	A639	(-)	(-)	(-)	(-)	(-)

4) Carry-Over/Cross Contamination:

A study was performed to assess the cross-contamination/carry-over of the Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay by testing a series of alternating high positive (30 $TCID_{50}$/mL of ZeptoMetrix Heat Inactivated SARS-CoV-2 Culture Fluid (Catalog #NATRVP-NNS) and negative samples on a 96-well PCR plate.

No false positive results were observed during testing of high positive samples alternating with negative samples; however, 1 positive sample was invalid. Results demonstrate that recommended sample handling and testing practices are effective in preventing false positive results due to carryover or cross-contamination between samples. The results are presented in the Table below:

Table 9: Carryover Study Summary Data:

Sample Group	N	Detected %	Not Detected %	Invalid %
Negative	48	0 (0%)	48 (100%)	0 (0%)
High (30 $TCID_{50}$/mL)	48	47 (98%)	0 (0%)	1 (2%)

5) Clinical Evaluation

Clinical Evaluation of the Ethos Laboratories SARS-CoV-2 assay

A total of 107 clinical nasopharyngeal swab specimens (54 negatives and 53 positives for SARS-CoV-2) were tested with the Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay and the results were compared to results obtained with the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel (EUA200001). Samples were extracted using the KingFisher Flex Purification System, reverse transcription RT-PCR was performed using the Veriti 96 Thermal Cycler and MALDI-TOF spectrometry was performed on the Agena MassARRAY System. The results are summarized in the Tables below.

Table 10: Clinical Evaluation of the Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay

Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay	FDA EUA RT-PCR Assay		Total	% Performance Agreement	95% CI
	Detected	Not Detected			
Detected	52	2	54	PPA= 98.1% (52/53)	90.1-99.7%
Not Detected	1	52	53	NPA= 96.3% (52/54)	87.5-99.0%
Total	53	54	107		

6) Simulated Shipping Study of the Ethos Laboratories U-Collect At Home Collection Kit

Summer Shipping Stability

A simulated shipping study was performed to support the use of the Ethos Laboratories U-Collect At Home Collections device, which evaluated the effects of temperature variation on the stability of SARS-CoV-2 RNA during transport of the nasal swabs. The study was conducted using nasal swab specimens that were determined to be negative for SARS-CoV-2 and contrived with ZeptoMetrix heat Inactivated SARS-CoV-2 Culture Fluid (Catalog # NATRVP-NNS) and placed in tubes containing Hardy Diagnostics' Viral Transport Medium to achieve high concentrations of 2x the LOD (2 $TCID_{50}$/mL) and high concentration samples at 10x the LOD (10 $TCID_{50}$/mL). Specimens were subjected to the thermal profiles outlined in Table 5 which were intended to simulate the extreme temperature conditions that may be experienced in shipment of specimens during the summer. Cycle 1 represents the time delay between collection of the sample and shipment of the sample. Cycles 2 through 5 are intended to simulate shipping conditions during standard 48-hour domestic freight transport. After being subjected to summer temperature simulated shipping conditions, samples were analyzed with the Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay.

Table 11: Summer Temperature Simulated Shipping Conditions

Temperature	Cycle Period	Cycle Period Hours	Cumulative Hours*
40°C	1	8	8
22°C	2	4	12
40°C	3	2	14
30°C	4	36	50
40°C	5	6	56

*Shipping conditions for cycle periods 2 through 5 are modeled after ISTA 7D 2007 shipping standard (48-hour domestic freight transport) where for cycle period 3 and 5 the temperature has been increased from 35°C to 40°C. The cycle period 1 (8 hours) has been included for the time delay between collection of sample and shipment of sample. The remaining time (48 hours) covers the shipment within the continental U.S. Cycle periods are sequential with the "cycle period hours" required per cycle listed in the table. After each cycle period, the "cumulative hours" increments by the number of hours in the cycle period.

The results of the summer temperature shipping conditions are summarized in the Table below. Results demonstrate that the SARS-CoV-2 virus is stable in Ethos Laboratories U-Collect At Home Collections kit when exposed to a broad range of temperature conditions and support the use of these kits for transport and storage of specimens following home nasal swab collections.

Table 12: Summary of Results from the Summer Simulated Shipping Study with the Ethos Laboratories U-Collect At Home Collections Device

Sample Group	N	Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay Detected					Positive* (%)
		N1	N2	N3	ORF1	ORF1ab	
Negative	10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
2x LoD Pos	20	20 (100%)	20 (100%)	20 (100%)	20 (100%)	19 (95%)	20 (100%)
10x LoD Pos	10	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)

** Result Interpretation = Sample is considered positive if 2 of the 5 targets are positive*

Winter Shipping Stability

For winter shipping conditions, the analytical LOD was determined to be 1 TCID50/mL. Low concentration samples at 2x the LOD (2 $TCID_{50}$/mL) and high concentration samples at 10x the LOD (10 $TCID_{50}$/mL) were created and subjected to the thermal profiles outlined in Table 11. This profile was intended to simulate the extreme temperature conditions that may be experienced in shipment of specimens during the winter. Cycle 1 represents the time delay between collection of the sample and shipment of the sample. Cycles 2 through 5 are intended to simulate shipping conditions during standard 48-hour domestic freight transport.

Table 13: Winter Temperature Simulated Shipping Conditions

Temperature	Cycle Period	Cycle Period Hours	Cumulative Hours*
-10°C	1	8	8
18°C	2	4	12
-10°C	3	2	14
10°C	4	36	50
-10°C	5	6	56

*Shipping conditions for cycle periods 2 through 5 are modeled after ISTA 7D 2007 shipping standard (48-hour domestic freight transport) where for cycle period 3 and 5 the temperature has been increased from 35°C to 40°C. The cycle period 1 (8 hours) has been included for the time delay between collection of sample and shipment of sample. The remaining time (48 hours) covers the shipment within the continental U.S. Cycle periods are sequential with the "cycle period hours" required per cycle listed in the table. After each cycle period, the "cumulative hours" increments by the number of hours in the cycle period.

The results of the winter temperature shipping conditions are summarized in the Table below. Results demonstrate that the SARS-CoV-2 virus is stable in Ethos Laboratories U-Collect At Home Collections kit when exposed to a broad range of temperature conditions and support the use of these kits for transport and storage of specimens following home nasal swab collections.

Table 14: Summary of Results from the Winter Simulated Shipping Study with the Ethos Laboratories U-Collect At Home Collections Device

Sample Group	N	Ethos Laboratories SARS-CoV-2 MALDI-TOF Assay Detected					Positive* (%)
		N1	N2	N3	ORF1	ORF1ab	
Negative	10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
2x LoD Pos	20	15 (75%)	15 (75%)	20 (100%)	18 (90%)	0 (0)	19 (95%)
10x LoD Pos	10	9 (90%)	10 (100%)	10 (100%)	10 (100%)	0 (0)	10 (100%)

** Result Interpretation = Sample is considered positive if 2 of the 5 targets are positive*

Human Usability Study:

A human usability study was performed to assess the Ethos Laboratories U-Collect At Home Collections kit. Testing included 31 participants representing varying education levels and ages and took place in users' homes. An Ethos Laboratories Telehealth team member scheduled in person collections with each participant at their home in order for the entire process to be observed and areas of difficulty to be noted. Participants performed the entire collection workflow using the Ethos Laboratories U-Collect At Home Collections kit, and completed a questionnaire that captured a range of information detailed in the Table below.

Table 14: Participant Stratification by Age, Gender & Highest Level of Education

	Male						Female					
Age	**MS**	**HS**	**SC**	**C**	**M**	**D**	**MS**	**HS**	**SC**	**C**	**M**	**D**
<18	2						2					
18-25				1				2	1	2		
26-35				1	1				1	1		
36-45		1	1						1		1	1
46-60			1	2				1		1	1	
>60		1	1		1			1	1	1		
Total	2	2	3	4	2		2	4	4	5	2	1

C=

MS= Middle School; HS= High School; SC= Some College; College; M= Masters; D= Doctorate

Table 15: Overview of Questionnaire Results

Gender		
	Male	42%
	Female	58%
Age Range		
	< 18	13%
	18-25	19%

26-35	13%
36-45	16%
46-60	19%
>60	19%
Highest Level of Education	
Middle School	13%
High School	19%
Some College	23%
College	29%
Masters	13%
Doctorate	3%
Previous Experience w/ Home Collections	
Yes	0%
No	100%
Overall Ease of Kit **(Scale 1-5 where 1= Difficult & 5= Easy)**	**AVERAGE**
Kit Instructions	4.33
Sample Collection Process	4.46
Packaging Sample	4.50
Shipping Sample	4.66
Understood Consequences of Improper Collection	
Yes	94%
No	0%
Unanswered	6%
Comments Section	
Confusion with labelling VTM tube	19%
Keep instructions to 1 page	9%
Match instructions exactly to process	6%
No Comment(s)	70%
Survey Questions **(Scale: Strongly Agree= 5, Agree=4, Neither=3, Disagree=2, Strong Disagree=1)**	**MEAN**
Understood if liquid is spilled from the VTM tube then it can't be used	3.5
Understood should not touch the swab tip or place it down on any surface before or after collecting sample	4.1
Instructions clearly explained how to collect lower nasal swab sample	4.6

	Instructions clearly explained the location from which my lower nasal swab sample should be collected	4.7
	Understood that I should swab both nostrils with the same swab.	4.5
	Understood how to place lower nasal swab sample inside the VTM tube after swabbing nose	4.3
	Confident performed the lower nasal swab sample collection correctly	4.3
	Instructions explained the tip of the swab needs to be covered by the liquid in the VTM tube	3.5
	Understood VTM tube containing swab sample should be sealed inside the specimen bag	4.4
	Understood specimen bag containing the VTM tube should be placed back inside the cardboard test kit box	4.3
	Understood cardboard test kit box containing the VTM tube should be placed inside the provided return shipping bag	4.3
	Confident in packaging swab samples for shipment correctly	4.3
	Shipping method required to be used for sending my sample was clearly indicated	4.4
	Understood that supposed to ship swab sample to the laboratory IMMEDIATELY after collection (or place in the fridge for up to 2 days).	4.4
	Read and understood the warning in the instruction	3.8
	Understood the absorbent pad should not be removed from the specimen bag	3.6

A total of 31 kits were distributed, and all 31 kits were returned by UPS to Ethos Laboratories. Packages and kits were inspected by laboratory personnel upon arrival and packaging errors or issues with acceptability of the sample for testing were noted. The user success rate was calculated by the number of samples received without errors divided by the total number of samples. Ethos Laboratories predefined an acceptable user success rate of 95%, which evaluates shipping/ receiving errors, kit completion errors (proper paperwork & identifiers), and specimen acceptability for testing. The reported overall success rate is 96.7%. The Ethos Laboratories U-Collect At Home Collections kit is reported to have a 100% success rate with shipping, receiving and kit completion, and 96.7% success rate for samples confirmed acceptable for testing. One sample out of the 31 samples received (3.2%) did not have a secure lid and the specimen completely leaked out of the VTM collection vial. This sample was reported as Leaked Specimen Due to Loose Lid (MLID) and was not able to be tested. All pre-defined acceptance criteria were met and any identified areas with potential risk or confusion within the instructions were modified. Refer to the Table below for more detailed information. There were zero (0) reported

deviations from the Instructions for Use observed, but 4 participants were unsure of proper labeling of the specimen tube. This issue was addressed by pre-labeling all specimen VTM tubes with the patient's name and date of birth prior to shipping. The Instructions for Use was updated, which now requires patients to confirm the proper labeling of their VTM tube prior to shipping. All specimens with adequate volume for testing (30 out of the 31 total specimens) were subject to sample validity (adequacy) testing which verified that a sufficient patient sample was collected (described above in the Specimen Collection Control section). The results show that in all cases where a full VTM sample vial was received, each sample contains ≥2000 ng/mL of DNA, which is considered to be a sufficient sample collection.

Table 16: Results of Human Usability Study for the Ethos Laboratories U-Collect At Home Collections Kit

Kit Summary	**N**	**%**
Kits Distributed	31	100
Kits Received for Testing	31	100
Packaging Errors		
Missing Box	0	0
Missing Specimen Bag	0	0
Specimen Bag Not Sealed	0	0
Absorbent Sheet Removed	0	0
Total Packaging Errors Noted	**0**	**0**
No Packaging Errors Noted	**31**	**100**
Errors Noted at Sample Accessioning		
Quantity Not Sufficient	0	0
Leaked Specimen Due to Loose Lid	1	3.2
Leaked Specimen Due to Damaged Container	0	0
Container Not Labeled	0	0
Conflicting ID	0	0
Not Suitable for Testing	0	0
Missing Test Code	0	0
Missing 2 Patient Identifiers	0	0
Missing Proper Paperwork	0	0
Total Accessioning Errors Noted	**1**	**3.2**
No Accessioning Errors Noted	**30**	**96.8**
Sample Validity/ Adequacy (≥2000 ng/mL DNA)		
Acceptable for Testing	30	100

Unacceptable for Testing	0	0

Human Usability Study for <18 Years of Age:

A human usability study was performed to assess the Ethos Laboratories U-Collect At Home Collections kit for use with people under the age of 18. Testing included 30 paired participants of parent and child representing varying ages from 6 to 17 years of age and took place in users' homes (Table below). An Ethos Laboratories Telehealth team member scheduled in person collections with each paired participant at their home in order for the entire process to be observed and areas of difficulty to be noted. The parent(s) of the child determined whether the child was going to collect their sample independently or with the assistance of the parent. The collection workflow was performed using the Ethos Laboratories U-Collect At Home Collections kit, and completed a questionnaire that captured a range of information detailed in the Table below.

Table 17: Participant Stratification by Age & Gender

Age	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
Female	1	0	3	0	3	0	1	1	0	1	1	1
Male	1	0	3	2	4	2	3	1	1	0	1	0
Total	**2**	**0**	**6**	**2**	**7**	**2**	**4**	**2**	**1**	**1**	**2**	**1**

Table 18: Overview of Questionnaire Results

Gender		
	Male	60%
	Female	40%
Age Range		
	6	6%
	7	0%
	8	20%
	9	6%
	10	23%
	11	6%
	12	13%
	13	6%
	14	3%
	15	3%
	16	6%
	17	3%

Parent's Expectations Vs. Child's Capabilities		
	Parents that **expected** their child to be able to complete the sample collection independently	60%
	Parents that **did not expect** their child to be able to complete the sample collection independently	40%
	Children that were **able** to complete the sample collection independently	77%
	Children that were **not able** to complete the sample collection independently	23%
Adverse Reactions		
	Yes	0%
	No	100%
Comments/ Concerns Section		
	No concerns listed	N/A
Survey Questions for Parent and/or Child (Scale: Strongly Agree= 5, Agree=4, Neither=3, Disagree=2, Strong Disagree=1)		**MEAN**
	Understood if liquid is spilled from the VTM tube then it can't be used	4.8
	Understood should not touch the swab tip or place it down on any surface before or after collecting sample	4.9
	Instructions clearly explained how to collect lower nasal swab sample	4.6
	Instructions clearly explained the location from which my lower nasal swab sample should be collected	4.4
	Understood that I should swab both nostrils with the same swab.	4.6
	Understood how to place lower nasal swab sample inside the VTM tube after swabbing nose	4.7
	Confident performed the lower nasal swab sample collection correctly	4.5
	Instructions explained the tip of the swab needs to be covered by the liquid in the VTM tube	4.6
	Understood VTM tube containing swab sample should be sealed inside the specimen bag	4.6
	Understood specimen bag containing the VTM tube should be placed back inside the cardboard test kit box	4.4
	Understood cardboard test kit box containing the VTM tube should be placed inside the provided return shipping bag	4.7
	Confident in packaging swab samples for shipment correctly	4.7
	Shipping method required to be used for sending my sample was clearly indicated	4.5
	Understood that supposed to ship swab sample to the laboratory IMMEDIATELY after collection (or place in the fridge for up to 2 days).	4.5

	Read and understood the warning in the instruction	4.2
	Understood the absorbent pad should not be removed from the specimen bag	4.3
	Parents understood how to collect the sample on the child	4.8
	Parents were comfortable with the procedures and will to perform the collection again	4.8
	Parents would recommend this home collection kit to others	4.8

A total of 30 kits were distributed, and all 30 kits were returned by UPS to Ethos Laboratories. Packages and kits were inspected by laboratory personnel upon arrival and packaging errors or issues with acceptability of the sample for testing were noted. The user success rate was calculated by the number of samples received without errors divided by the total number of samples. Ethos Laboratories predefined an acceptable user success rate of 95%, which evaluates shipping/ receiving errors, kit completion errors (proper paperwork & identifiers), and specimen acceptability for testing. The reported overall success rate is 100%. The Ethos Laboratories U-Collect At Home Collections kit is reported to have a 100% success rate with shipping, receiving and kit completion, and 100% success rate for samples confirmed acceptable for testing. All pre-defined acceptance criteria were met and there were zero (0) reported deviations from the Instructions for Use observed. All specimens were subject to sample validity (adequacy) testing which verified that a sufficient patient sample was collected (described above in the Specimen Collection Control section). The results show that in all cases where a full VTM sample vial was received, each sample contains ≥2000 ng/mL of DNA, which is considered to be a sufficient sample collection. Refer to the Table below for more detailed information

Table 19: Human Usability Study Results

Kit Summary	**N**	**%**
Kits Distributed	30	100
Kits Received for Testing	30	100
Packaging Errors		
Missing Box	0	0
Missing Specimen Bag	0	0
Specimen Bag Not Sealed	0	0
Absorbent Sheet Removed	0	0
Total Packaging Errors Noted	**0**	**0**
No Packaging Errors Noted	**30**	**100**
Errors Noted at Sample Accessioning		
Quantity Not Sufficient	0	0
Leaked Specimen Due to Loose Lid	0	0

Leaked Specimen Due to Damaged Container	0	0
Container Not Labeled	0	0
Conflicting ID	0	0
Not Suitable for Testing	0	0
Missing Test Code	0	0
Missing 2 Patient Identifiers	0	0
Missing Proper Paperwork	0	0
Total Accessioning Errors Noted	**0**	**0**
No Accessioning Errors Noted	**30**	**100**
Sample Validity/ Adequacy (≥2000 ng/mL DNA)		
Acceptable for Testing	30	100
Unacceptable for Testing	0	0

Warnings:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by Ethos laboratory located at 29 E. 6th St., Newport, KY 41071;
- This test has been authorized only for the detection of nucleic acid from SARSCoV-2, not for any other viruses or pathogens; and
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.