EMERGENCY USE AUTHORIZATION (EUA) SUMMARY

SynergyDx SARS-CoV-2 RNA Test DTC

For In vitro Diagnostic Use
For use under Emergency Use Authorization (EUA) only
For individuals 18 years of age or older

Direct to consumer (DTC) home self-collected nasal swabs collected by individuals 18 years of age or older (unobserved) with the SynergyDx Home Collection Kit for COVID-19 DTC will be sent to laboratories designated by SynergyDx Diagnostic Laboratory and tested with the SynergyDx SARS-CoV-2 RNA Test DTC. Synergy Diagnostic Laboratory designated laboratories are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a to perform high complexity tests.

INTENDED USE

The SynergyDx SARS-CoV-2 RNA Test DTC is a direct-to-consumer product for testing of individual anterior nasal swab specimens self-collected at home using the SynergyDx Home Collection Kit for Covid-19 DTC by any individual, age 18 years or older, including individuals without symptoms or other reasons to suspect COVID-19. Testing of self-collected anterior nasal swab specimens is limited to laboratories designated by Synergy Diagnostic Laboratory, Inc., which are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C.§263, and meet the requirements to perform high-complexity tests.

All test results are delivered to the user via secure email. Additionally, individuals with positive or invalid results will be contacted by a healthcare provider. The direct-to-consumer home collection system is intended to enable users to access information about their COVID-19 infection status that could aid with determining if self-isolation or quarantine is appropriate and to assist with healthcare decisions after discussion with a healthcare provider.

Results are for the qualitative detection of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in anterior nasal swab specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with medical history and other diagnostic information is necessary to determine 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.

The SynergyDx SARS-CoV-2 RNA Test DTC is not a substitute for visits to a healthcare provider. The information provided by this kit should not be used to start, stop, or change any course of treatment unless advised by your healthcare provider.

The SynergyDx SARS-CoV-2 RNA Test DTC is only intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time RT-PCR and in vitro diagnostic procedures. The SynergyDx SARS-Cov-2 RNA Test DTC is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

1. Test Principle

The SynergyDx SARS-CoV-2 RNA Test DTC utilizes the PerkinElmer New Coronavirus Nucleic Acid Detection kit (catalog number: 2019-nCoV-PCR-AUS) following its Instructions For Use (IFU), which targets specific genomic regions of SARS-CoV-2: nucleocapsid (N) gene and ORF1ab. The TaqMan probes for the two amplicons are labeled with FAM and ROX fluorescent dyes respectively to generate target-specific signal. The assay includes an RNA internal control (IC, bacteriophage MS2) to monitor the processes from nucleic acid extraction to fluorescence detection. The IC probe is labeled with HEX fluorescent dye to differentiate its fluorescent signal from SARS- CoV-2 targets. The assay also uses a dUTP/UNG carryover prevention system to avoid contamination of PCR products and subsequent false positive results. The RNA isolated from nasal swabs is reverse transcribed to cDNA and subsequently amplified using the ThermoFisher Applied Biosystems AB 7500 Standard Real-Time PCR System for qualitative detection with fluorescent-based PCR chemistries.

The test is performed following the IFU of the PerkinElmer New Coronavirus Nucleic Acid Detection kit (30µL reaction). Anterior nasal swabs should be collected, transported and stored according to instructions provided in the SynergyDx Home Collection Kit for COVID-19 DTC. RNA extraction is performed using the PerkinElmer Chemagic 360 automated sample processing system with the Chemagic Viral DNA/RNA 300 Kit H96. The input sample volume is 300µL, the elution volume is 60µL, and the PCR reaction volume is 30µL. The final PCR reaction is composed of 20µl of extracted sample and 10ul of master mix.

In addition to the SynergyDx SARS-CoV-2 RNA Test DTC, a separate single Plex RNaseP control reaction is performed to ensure adequate sample collection for self-collected samples. The test uses one primer and probe set to detect human RNaseP (RP) in a clinical sample. The input volume of extracted RNA sample from the SynergyDx SARS-CoV-2 RNA Test DTC is 10.0 µL combined with 15.0 µL of Reaction Mix. Real-time PCR reactions are performed on ThermoFisher Applied Biosystems AB 7500 Standard Real-Time PCR System.

During the amplification process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the bound probe causing the reported dye to separate from the quencher dye, generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle by the AB 7500 Standard.

2. Collection Device Description

The SynergyDx Home Collection Kit for COVID-19 DTC includes sample registration instructions, sample collection instructions, sample collection and shipping instructions, an individually wrapped anterior nasal swab, prelabeled 10 ml sterile collection tube containing 3 ml viral transport medium solution, return shipping box/envelope, prelabeled biohazard bag with absorbent for sample, alcohol prep pad, return shipping label and UN3373 Lab Pak Bag. Individuals may request the SynergyDx Home Collection Kit for COVID-19 DTC online via the SynergyDx.com website.

The SynergyDx Home Collection Kit for COVID-19 DTC collects and stabilizes SARS-CoV-2 RNA from anterior nasal swab specimen; it can also be used for over-night transportation, or 48-hour shipping of a collected sample, using a drop box. The SynergyDx Home Collection Kit for COVID-19 DTC is used for collecting SARS-CoV-2 RNA by/from individuals 18 years of age and older for in vitro

diagnostic testing in the CLIA high complexity Synergy Diagnostic Laboratory using the SynergyDx SARS-CoV-2 RNA Test DTC.

3. Ordering and Registration

The SynergyDx Home Collection Kit for COVID-19 DTC is a Direct-to-Consumer product and does not require a prescription. Individuals 18 years and older may request the SynergyDx Home Collection Kit for COVID-19 DTC collection device online via the SynergyDx.com website. When purchasing the collection kit online, the CDC Survey Questions are asked prior to the purchase of the home collection kit with an alert when a customer selects "severe symptoms" to seek immediate medical care. This alert will prevent the individual from proceeding with the ordering.

4. Self-collection and Shipping

The individual using SynergyDx Home Collection Kit for COVID-19 DTC to collect anterior nasal (nasal) swab specimen performs the following steps to collect the initial specimen. Using the swab, provided in the home collection kit, insert the swab at least 1 cm (0.5 inch) inside the nostril (naris) and firmly sample the nasal membrane by rotating the swab and leaving in place for 10 to 15 seconds. Sample both nostrils with the same swab. After the nasal swab specimen is collected, the nasal swab is inserted into the Dasky Collection tube, swab side down, and prepared for shipping.

For sample shipping, the individual must collect the nasal swab specimen and properly package the biological sample for shipment back to the laboratory in a pre-labeled FedEx or UPS UN 3373 LabPak Bag. Each SynergyDx Home Collection Kit for COVID-19 DTC is intended to be returned via overnight courier service on the same day or the following day of sample collection in accordance with the transport guidance of suspected COVID-19 samples from the CDC and the WHO.

5. Sample Accessioning

Samples received at the clinical laboratory via the SynergyDx Home Collection Kit for COVID-19 DTC undergo the following receiving process prior to acceptance for testing.

i. Sterilize the exterior of the UN3373 Lab Pak bag.
ii. Inspection of proper labeling of sample
    a. Label affixed to the outside of the Biohazard Bag
    b. Registration Code Label affixed to the collection tube
iii. The laboratory retrieves the sample information by entering a customer's information in the LIS and retrieving the electronic record associated with the shipped collection kit.
iv. Sample Inspection:
    a. Correctly returned in supplied packaging
    b. Sample correctly returned in Biohazard Bag
    c. Sample correctly in collection device/tube
    d. Sample has adequate volume sufficient for test.
    e. Leakage/Tube breakage
v. The sample is processed if it has sufficient volume and fully labeled without errors

The following errors require rejection of the sample or resolution:

i. Improper return of sample packaging
    a. Sample not returned in supplied packaging
    b. Sample not returned in Biohazard Bag
    c. Sample not in correct collection device/tube
    d. Sample shows signs of Quantity Not Sufficient to Test.

ii. No Registration: Sample was not registered by the customer on SynergyDx registration page
iii. Quantity Not Sufficient (QNS): sample swab was completely dry, without VTM
iv. Incorrect Name: name on the order/electronic record does not match
v. Invalid DOB: Date of Birth on order/electronic record does not match
vi. Invalid Collection Date: date of collection either in the future or exceeded expiration.
vii. Wrong Lab: in error the customer mixed up return labels and sample arrived at the incorrect lab for processing.
viii. Replacement Materials: customer received a replacement kit and the information on the label does not match the new Kit ID.
ix. Miscellaneous Error: any error that requires SynergyDx review, including but not limited to rare events often associated with other extenuating factors.

INSTRUMENT REQUIREMENTS

The SynergyDx SARS-CoV-2 RNA Test DTC is for use with the ThermoFisher Applied Biosystems AB 7500 Standard Real-Time PCR System equipped with Design and Analysis Software 2.4, the PreNAT II (SY61) (software version 1.00.06), and the Perkin Elmer Chemagic 360 (2024-0020) with Chemagic Rod Head Set 96 (CMG-371) along with the Chemagic MSM I software version 6.1.0.5.

REAGENTS AND MATERIALS

Components of SynergyDx Home Collection Kit for COVID-19 DTC

Name	Description
Nasal Swab	Sterile Flocked or Foam Tipped Applicator Nasal Swab
Collection Tube *	Disposable sampling tube (non-inactivated) with 3ml of viral transport medium
Tube Label	Label for customer to label tube with name, date of birth and collection date
Alcohol Prep Pad	Pad with alcohol on it
Biohazard bag with Absorbent Sheet	Biohazard bag with zipper closure that contains and absorbent sheet
Poly mailer	Poly mailer with UN3373 label and return label
Sample shipping box	Sample shipping box
Instructions for Use	Document with instruction for use, help, and contact information
Fact Sheet for Individuals	Fact Sheet for Individuals that describes the potential test results.

***Collection Kit Storage and Stability**: The kit is stable if stored sealed at 5-25ºC for two years according to the expiration dating provided by the manufacturer of the collection tube.

Reagents and Materials of the SynergyDx SARS-CoV-2 RNA Test DTC

Reagent	Manufacturer	Catalogue #
Chemagic Viral DNA/RNA 300 Kit H96	PerkinElmer	CMG-1033-S
96 well Deep Well Plates	PerkinElmer	43001-0120
PerkinElmer New Coronavirus Nucleic Acid Detection Kit	Perkin Elmer	2019-nCoV-PCR-AUS
MicroAmp™ Optical 96-Well Reaction Plate	ThermoFisher Scientific	4316813 or 4326659
MicroAmp™ Optical adhesive PCR plate cover	ThermoFisher Scientific	4311971
Nuclease-free water	--	--
Ethanol (96-100%)	--	--
TaqMan™ SARS-CoV-2 RNase P Assay Kit	ThermoFisher Scientific	A49564

QUALITY CONTROLS

The SynergyDx SARS-CoV-2 RNA Test DTC incorporates the following controls that are run in a 30 µL reaction volume:

1. A Negative Control (TE buffer) is used to monitor for contamination of reagents and carryover during RNA extraction and RT-PCR.
2. A Positive Control (SARS-CoV-2 RNA fragments capsulated in bacteriophage) is used to monitor the integrity of the RT-PCR reagents. The positive control is included at the RT-PCR step.
3. A bacteriophage MS2 internal control (IC; TE buffer with bacteriophage MS2) is used to monitor the integrity of extraction and RT-PCR reagents as well as that RNA of sufficient quality and amount and quality is extracted.
4. The endogenous human RNase P mRNA in properly collected nasal swab samples serves as an additional internal control to determine if sufficient sample was collected by the user.

INTERPRETATION OF RESULTS

Interpretation of Control Results

All test controls are run in a 30 µL reaction (like the samples). Controls must be examined prior to interpretation of customer results. Positive control, negative control, internal control in positive and negative controls, and RNaseP in negative control should meet the requirements listed below to ensure valid results. If the controls are not valid, the customer results cannot be interpreted.

- Negative Control: both ORF1ab and N of SARS-CoV-2 must be not detected, and the Ct value of the internal control must be ≤40.
- Positive Control: both ORF1ab and N of SARS-CoV-2 must be detected, and their Ct values should be ≤36, the Ct value of the internal control does not have to be ≤40 for positive control.
- RNaseP: RNase P should not be detected in either the negative or the positive control because the controls are formulated in buffer without human derived clinical matrix.

Control type	Ct Value (30µL reaction)			
	RNaseP (VIC)	**N (FAM)**	**ORF1ab (ROX)**	**IC (HEX)**
Negative Control	Undet or >42	Undet or > 42	Undet or > 42	Ct ≤ 40
Positive Control	Undet or > 42	≤ 36	≤ 36	/

Interpretation of Clinical Sample Results:

Assessment of clinical sample test results must be performed after the positive and negative controls have been examined and confirmed to be valid and acceptable. If the controls are not valid, the customer results cannot be interpreted, and a root cause investigation should be performed. Once the root case has been eliminated sample testing should be repeated. The interpretation of results is described in the table below.

Interpretation of Clinical Sample Results

ORF1ab POS: ≤ 42 NEG: Undet or >42	**N** POS: ≤ 42 NEG: Undet or >42	**IC-MS2** POS: ≤ 40 NEG: Undet or >40	**RNaseP** POS: ≤ 33 NEG: Undet or >33	**Status**	**Result**	**Action**
NEG or POS	NEG or POS	NEG or POS	NEG	INVALID	NA	Repeat test by re-extracting the original sample and repeating the RT-PCR. If the repeat result remains invalid, consider requesting a new specimen from the individual. After retesting one time, report results to the healthcare provider[3], appropriate public health authorities, and individual. IMPORTANT! Samples with a result of SARS-CoV-2 Invalid shall be retested one time.
NEG	NEG	NEG	POS	INVALID	NA	
NEG	NEG	POS	POS	VALID	SARS-CoV-2 Not Detected[1]	Report results to the healthcare provider, appropriate public health authorities and individual
NEG	POS	NEG or POS*	POS	VALID	SARS-CoV-2 Detected[2]	Report results to the healthcare provider, appropriate public health authorities and to the individual.
POS	NEG					
POS	POS					

Undet: Undetermined

*: No requirements on the Ct value.

1: If the result for a sample is SARS-CoV-2 RNA Not Detected/Negative, the Ct value of the internal control must be ≤40, or the result of the sample is invalid.

2: If the result for a sample is SARS-CoV-2 RNA Detected/Positive, the Ct value of the internal control is NOT required for the result to be considered valid.

3. For this EUA, a healthcare provider includes any health professional with prescribing abilities, including, but is not limited to, physicians, nurses, pharmacists, technologists, laboratory directors, and epidemiologists.

RESULT REPORTING

- Positive Results: Customers will receive a phone call from the Healthcare Provider to discuss the SARS-CoV-2 test results and next steps as well as an email with a password protected pdf of the results.
- Invalid Results: Customers with an invalid result will receive a phone call from the Healthcare Provider and will be receiving a second test kit.
- Negative Results: Customers will be notified via an email with a password protected pdf of the results.

All Results will be reported by Synergy Diagnostics Laboratory Inc. to public health agencies as required.

PERFORMANCE EVALUATION

1. Analytical Sensitivity (LoD)

For the preliminary Limit of Detection (LoD) study, negative clinical matrix was prepared by pooling residual nasal swab matrix collected from 10 individuals in the Dasky Collection tube used in the SynergyDx Home Collection Kit for COVID-19 DTC. The pooled nasal swab matrix was tested using the SynergyDx Home Collection Kit for COVID-19 DTC, confirmed to be negative, and was then used in preparing dilutions of SARS-CoV-2 inactivated virus (Isolate USA-WA1/2020/NR-52350). A total of ten preliminary dilutions of known concentrations were prepared by spiking inactivated SARS-CoV-2 inactivated virus in negative clinical matrix. The results show a 100% detection rate using nasal swabs spiked at 4.75 copies/mL.

For the LoD confirmation study, twenty replicates of inactivated SARS-Related Coronavirus 2 (Isolate USA-WA1/2020/NR-52350) were spiked in negative clinical matrix at concentrations of 4.75 copies/mL, 20.8copies/mL, and 30.6copies/mL. LoD was determined as the lowest concentration of SARS-CoV-2 at which the assay can detect at a ≥95% positivity rate. The final LoD for the SynergyDx SARS-CoV-2 RNA Test DTC was determined to be 20.8 copies/mL on the AB 7500 Standard PCR System.

Summary of the Confirmatory LoD Study

Concentration (Copies/mL)	# of Valid Results	Overall Detection Rate	N (FAM) Gene		ORF1ab (ROX) Gene		IC (HEX/VIC) Gene	
			Detection Rate (%)	Mean Ct (SD)	Detection Rate (%)	Mean Ct (SD)	Detection Rate (%)	Mean Ct (SD)
30.6	20	20/20 100%	20/20 (100)	35.44 (0.9)	18/20 (90)	37.52 (1.1)	20/20 (100)	31.50 (0.7)
20.8	20	20/20 100%	20/20 (100)	36.46 (0.8)	18/20 (90)	38.22 (0.8)	20/20 (100)	31.45 (0.4)
4.75	20	7/20 35%	2/20 (10)	39.90 (0.8)	7/20 (35)	39.11 (0.8)	20/20 (100)	31.44 (0.8)

2. Analytical Reactivity (Inclusivity), Analytical Specificity (Cross-Reactivity, Interfering Substances)

The SynergyDx SARS-CoV-2 RNA Test DTC utilizes the PerkinElmer New Coronavirus Nucleic Acid Detection kit following its Instructions For Use (IFU). The analytical reactivity (inclusivity), analytical specificity (cross-reactivity) and interfering substance testing of the PerkinElmer New Coronavirus Nucleic Acid Detection kit has been demonstrated by PerkinElmer, Inc. in the EUA submission authorized on 02/05/2021. Details of the performance can be found here: https://www.fda.gov/media/136410/download. PerkinElmer, Inc. granted Right of Reference to SynergyDx for the authorized PerkinElmer New Coronavirus Nucleic Acid Detection kit.

3. Clinical Performance

The SynergyDx SARS-CoV-2 RNA Test DTC utilizes the PerkinElmer New Coronavirus Nucleic Acid Detection kit following its Instructions For Use (IFU). The clinical performance of the PerkinElmer New Coronavirus Nucleic Acid Detection kit has been demonstrated by PerkinElmer, Inc. in the EUA submission authorized on 02/05/2021. Details of the performance can be found here: https://www.fda.gov/media/136410/download. PerkinElmer, Inc. granted Right of Reference to SynergyDx for the authorized PerkinElmer New Coronavirus Nucleic Acid Detection kit.

Two clinical studies were performed by SynergyDx to support performance of the SynergyDx SARS-CoV-2 RNA Test DTC with specimens collected from individuals without symptoms or other reasons to suspect COVID-19 infection. In the first study, a total of 227 anterior nasal swab samples were collected by a healthcare provider during a community testing event, and were initially tested with the SynergyDx SARS-CoV-2 RNA Test DTC, generating 31 positive and 196 negative results. Among the 31 positive samples, 17 were from asymptomatic individuals who did not report any symptoms in the last 5 days, and the remaining 14 were from individuals with symptoms that were consistent with a potential COVID-19 infection. All samples were stored at -70°C after collection. Fifteen (15) out of 17 asymptomatic positive samples, and the first 100 negative samples from individuals without symptoms were de-identified and sent to comparator testing with an EUA authorized high sensitivity comparator method. A summary of the results is shown in the table below, which demonstrates 100% positive agreement and 100% negative agreement. Eight (8) of the 15 asymptomatic positive sample are low positive (within 3 Cts of the average Ct at LoD concentration with the comparator method).

Summary of Clinical Evaluation of Individuals Without Symptoms or Other Reasons to Suspect COVID-19*

Anterior Nasal Swab Specimens		**EUA Authorized Comparator**		
		Positive	**Negative**	**Total**
SynergyDx SARS-CoV-2 RNA Test DTC	**Positive**	15	0	15
	Negative	0	100	100
	Total	15	100	115
Positive Percent Agreement (95% CI)		100% (83.9-100%)		
Negative Percent Agreement (95% CI)		100% (96.3-100%)		

*15 of 17 SynergyDx tested asymptomatic positive samples and 100 of 196 SynergyDx tested asymptomatic negative samples were tested with the comparator method

Because not all positive and negative samples were tested by the comparator assay, there is verification bias. To address this, the clinical evaluation data was adjusted and then used to calculate performance estimates. Tables with the adjusted clinical data and performance estimates are provided below:

Adjusted Summary of Clinical Evaluation of Individuals Without Symptoms or Other Reasons to Suspect COVID-19

Anterior Nasal Swab Specimens		EUA Authorized Comparator		
		Positive	Negative	Total
SynergyDx SARS-CoV-2 RNA Test DTC	Positive	17	0	17
	Negative	0	196	196
	Total	17	196	213

Performance Estimates for Individuals Without Symptoms or Other Reasons to Suspect COVID-19

	Performance Estimate	95%CI
PPA	100% (17/17)	(69.9%; 100%)*
NPA	100% (196/196)	(98.1%; 100%)*
PPV	100% (15/15)	(79.6%; 100%)
NPV	100% (100/100)	(96.3%; 100%)

*95%CI for PPA and NPA were adjusted because only 15 out 17 SynergyDx tested asymptomatic positive samples and 100 out of 196 SynergyDx tested asymptomatic negative samples had the comparator test results.

In the second study, a total of 106 anterior nasal swab samples were collected by a healthcare provider during a community testing event, and were initially tested with the SynergyDx SARS-CoV-2 RNA Test DTC, generating 5 positive and 101 negative results. All samples were from asymptomatic individuals who did not report any symptoms in the last 5 days. The samples were stored at -70°C after collection. All samples were deidentified and sent to comparator testing with an EUA authorized high sensitivity comparator method. A summary of the results is shown in the table below, which demonstrates 100% positive agreement and 100% negative agreement. Three (3) of the 5 asymptomatic positive sample are low positive (within 3 Cts of the average Ct at LoD concentration with the comparator method).

Summary of Clinical Evaluation of Individuals Without Symptoms or Other Reasons to Suspect COVID-19

Anterior Nasal Swab Specimens		EUA Authorized Comparator		
		Positive	Negative	Total
SynergyDx SARS-CoV-2 RNA Test DTC	Positive	5	0	5
	Negative	0	101	101
	Total	5	101	106
Positive Percent Agreement (95% CI)		100% (56.6-100%)		
Negative Percent Agreement (95% CI)		100% (96.3-100%)		

4. Simulated Sample Shipping Stability Study

To support home use of the SynergyDx Home Test Kit for COVID-19 DTC, a Simulated Shipping Study was designed to evaluate the effect of temperature variation on the stability of SARS-CoV-2 RNA during transport of collected nasal swab sample. The study was conducted using residual clinical sample that had previously been reported as SARS-CoV-2 positive or negative using SynergyDx SARS-CoV-2 RNA Test DTC, and which were stored at -80C until the start of the study. The SARS-CoV-2 positive sample were selected based on the Ct values obtained upon initial testing and covered the spectrum of Ct values observed with the assay. Low positive samples are ~1x LoD based on the Ct values from the LoD study.

To perform the study, the samples were prepared separately, thawed and then incubated in individual vials with the thermal profiles outlined in the table below, which were designed to simulate the experience a shipment of sample may have during the freeze thaw cycles of both the summer and winter.

Temperature Profile of the Simulated Shipping Study

Temperature	Cycle Period	Cycle Period Hours	Total Time Hours
Summer			
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
Winter			
-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

Testing was performed using the SynergyDx SARS-CoV-2 RNA Test DTC. The results of the temperature excursion validation were as expected for both temperature profiles. The 30 residual positives were detected after 56 hours of cycling in and out of high & low temperature. Similarly, 10 negatives were negative for both targets at all time points with internal control, Ct values <40. No apparent degradation of signal was observed over the temperature excursion time course as observed by no increase in Ct value at 56 hours. The acceptance criterion for the study was a 95% agreement or greater for positives samples. Both temperature profiles Winter & Summer have met the acceptance criterion and support sample shipping stability for over-night transportation, or 48-hour shipping of a collected sample, using a drop box.

Summary of the Simulated Shipping Study

Sample Group	Test Point	n	Mean Ct (Standard Deviation)			Detection Rate Positive (%)
			N Gene	ORF1ab Gene	MS2	
Low Positive	T=0	20	37.2 (1.9)	38.0 (0.8)	31.3 (1.0)	20/20 (100)
	Summer	20	36.6 (0.8)	37.8 (0.8)	31.6 (0.3)	20/20 (100)
	Winter	20	36.5 (0.8)	37.9 (0.8)	31.4 (0.4)	20/20 (100)

High Positive	T=0	10	33.9 (4.9)	34.7 (5.0)	33.7 (1.1)	10/10 (100)
	Summer	10	33.7 (4.3)	34.3 (4.7)	29.7 (3.8)	10/10 (100)
	Winter	10	31.1 (4.4)	32.4 (4.3)	36.0 (3.2)	10/10 (100)

NA: Not Applicable

5. Nasal Swab Collection Kit Usability Study

A usability study was conducted using the SynergyDx Home Test Kit user instructions. Each participant received either the SynergyDx Home Test Kit and followed the instructions in the kit to register the sample, collect the sample, fill out the survey and prepare to ship the sample to the laboratory. The sample was received in the lab, it was inspected for packing and shipping appropriateness according to the Accessioning Criteria outlined for acceptance and rejection of samples received via overnight delivery. If the sample met the criteria it was tested in the lab. If a sample was rejected, it was recorded in the results. The samples collected during the study were tested for sample adequacy using the SynergyDx SARS-CoV-2 RNA Test DTC per Synergy Diagnostic Laboratory Inc.

30 participants that met the following criteria for inclusion were included in the study, participants who met the exclusion criteria did not participate.

Inclusion criteria:

- Willingness to receive and perform test
- Willingness to fill out survey

Exclusion Criteria:

- Healthcare worker
- Previous or current at home collection experience
- Previous or current Healthcare experience
- Previous or current Laboratory experience
- Unwillingness to complete the test and corresponding documentation

Of the 30 participants, seven (23%) were between 51 and 65, twelve (40%) were between 41 and 50, five (17%) were between 18 and 40, four (13%) were between 15 and 18, and two (7%) were below 15 years of age. Participants had various levels of schooling, ranging from grade school, middle school, high school graduate, associate degree, and bachelor's degree.

Summary of Participants' Demographics

#	Age	Level of Schooling Completed	#	Age	Level of Schooling Completed
1	43	BA	16	52	AA
2	62	HS	17	64	HS
3	35	BA	18	31	BA
4	58	AA	19	31	BA
5	16	MS	20	49	HS
6	50	HS	21	50	HS
7	50	HS	22	45	MS
8	41	MS	23	11	GS
9	38	MS	24	43	BA
10	46	HS	25	16	MS
11	17	HS	26	65	HS

12	42	BA	27	55	BA
13	49	HS	28	25	BA
14	50	HS	29	9	GS
15	52	AA	30	15	MS

GS Grade School
MS Middle School
HS High School
AA Associates Degree
BA Bachelor's Degree

a. Summary of RNaseP Results

The study evaluated participants for proper collection of a nasal swab sample and a simulated shipment of the sample to the laboratory. All samples that were received by the laboratory met acceptance criteria for testing with acceptable Ct values for RNaseP as shown below.

RNase P Values of Self-Collected Samples in the Usability Study

# of samples	# of valid samples	Average Ct of RNaseP	SD Ct of RNaseP	Percentage of valid samples
30	30	30.12	0.95	100%

b. Summary of Study Observations

The study observer noted that most participants were able to collect their sample without direct assistance and 30/30 were confident that they collected a good sample. The observer noticed two participants within the age group of 9-12 experienced some level of confusion based around why they were doing this, and they did not take the time to read the directions, therefore they were noted as failing to complete the key task of reading instructions. These two participants were meant to focus on the reading of instructions, but in the end needed assistance from their parents to complete the collection successfully. One participant in their 60s had some trouble with opening the packaging and asked for assistance. One participant in their 20s found the process very easy.

Overall, the survey results demonstrate that lay users can adequately perform the sample self-collection at home following the instructions provided in the SynergyDx Home Collection Kit for Covid-19 DTC. Further supporting the users' ability to collect adequate sample, all of the samples tested positive for RNaseP Internal Control substantiating the validity of the sample collection.

WARNINGS:

- For Emergency Use Authorization (EUA) only.
- For in vitro diagnostic use.
- This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- 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 Cosmetics Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
- The solution in the collection tube contains hazardous ingredients. if the solution contacts the skin or eye, flush with plenty of water.

TEST LMITATIONS:

1. The use of this assay as an *in vitro* diagnostic under FDA Emergency Use Authorization (EUA) is limited to laboratories designated by Synergy Diagnostic Laboratory, Inc., which are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C.§263, and meet the requirements to perform high-complexity tests.
2. This kit is used for the qualitative detection of SARS-CoV-2 RNA from human nasal swab samples. The results cannot directly reflect the viral load in the original sample.
3. The SynergyDx SARS-CoV-2 RNA Test DTC performance has only been established with the sample types described in the Intended Use section. Testing other types of samples may cause inaccurate results.
4. The samples to be tested shall be collected, processed, stored and transported in accordance with the instructions. Inappropriate sample preparation and operation may lead to inaccurate results
5. Extraction and amplification of nucleic acid from clinical samples must be performed as specified in the methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.
6. Amplification and detection of SARS-CoV-2 RNA with the SynergyDx SARS-CoV-2 RNA Test DTC has only been validated with the Applied Biosystems 7500 Real-Time PCR instrument. Use of other instrument systems may cause inaccurate results.
7. The Limit of Detection (LoD) is determined based on a 95% confidence of detection. When SARS-CoV-2 RNA presents at or above the LoD concentration in the test sample, there will be a low probability that SARS-CoV-2 RNA is not detected. When SARS-CoV-2 RNA presents below the LoD concentration in the test sample, there will also be a certain probability that the SARS-CoV-2 RNA can be detected.
8. Negative results do not preclude SARS-CoV-2 infections and should not be used as the sole basis for treatment or other management decisions.
9. The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutics, or immunosuppressant drugs have not been evaluated.
10. Laboratories are required to report all negatives and positive results to the appropriate public health authorities.
11. Primers and probes for the SynergyDx SARS-CoV-2 RNA Test DTC target highly conserved regions within the genome of SARS-CoV-2. Mutations rarely occur in these highly conserved regions, but if a mutation did occur this may result in RNA being undetectable.
12. The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.