EMERGENCY USE AUTHORIZATION (EUA) SUMMARY FOR THE COLOR SARS-COV-2 RT-LAMP DIAGNOSTIC ASSAY

For *In vitro* Diagnostic Use
Rx Only
For Use Under Emergency Use Authorization (EUA) Only

(The Color SARS-CoV-2 RT-LAMP Diagnostic Assay will be performed at Color Health Inc., located at 863 Mitten Road, Burlingame, CA 94010, 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 described in the Standard Operating Procedures that were reviewed by the FDA under this EUA.)

INTENDED USE

The Color SARS-CoV-2 RT-LAMP Diagnostic Assay is a loop-mediated isothermal amplification (LAMP) assay intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs, oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasopharyngeal washes/aspirates or nasal aspirates, as well as bronchoalveolar lavage specimens collected from individuals suspected of COVID-19 by a healthcare provider, and anterior nasal swabs collected from any individual, including from individuals without symptoms or other reasons to suspect COVID-19 infection.

The test is also for use with anterior nasal swab specimens that are collected using either the Color COVID-19 Self-Swab Collection Kit or the Color COVID-19 Self-Swab Collection Kit with Saline when used consistent with their authorizations.

Testing is limited to Color Health, Inc., located at 863 Mitten Road, Burlingame, CA 94010, 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.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in 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 Color SARS-CoV-2 RT-LAMP Diagnostic Assay is intended for use by qualified laboratory personnel specifically instructed and trained in LAMP and *in vitro* diagnostic procedures. The Color SARS-CoV-2 RT-LAMP Diagnostic Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

SPECIAL CONDITIONS FOR USE STATEMENTS

For Emergency Use Authorization (EUA) Only
For Prescription Use Only
For *In vitro* Diagnostic Use

The Color SARS-CoV-2 RT-LAMP Diagnostic Assay is authorized for use with dry anterior nasal swab specimens or anterior nasal swab specimens in saline that are collected using the Color COVID-19 Self-Swab Collection Kit or the Color COVID-19 Self-Swab Collection Kit with Saline, respectively, when used consistent with their authorizations.

DEVICE DESCRIPTION AND TEST PRINCIPLE

Overview of the Color SARS-CoV-2 RT-LAMP Diagnostic Assay

The Color SARS-CoV-2 RT-LAMP Diagnostic Assay is a high-throughput, automated method utilizing loop-mediated isothermal amplification (LAMP) technology to detect SARS-CoV-2 RNA. The test uses two SARS-CoV-2 specific primer sets and a third primer set targeting the human RNase P (RP) gene. Samples are initially tested using the primer sets targeting the SARS-CoV-2 specific nucleocapsid gene (N) and the human RNase P gene. Any SARS-CoV-2 positive samples identified during the screening run (run #1) are considered "candidate" positives and are re-tested with a reflex testing protocol (run #2), together with any samples that produce invalid (i.e., failed) test result due to the inability to detect the endogenous RNase P target. RNA from all "candidate" positives and samples with invalid results is re-extracted from the residual clinical samples and tested with all three primer sets targeting the SARS-CoV-2 N-gene, the SARS-CoV-2 envelop gene (E), and the human RNase P gene. The modified workflow (screening + reflex testing) replaces the originally authorized version (N, E, and RNase P primer sets run simultaneously).

Nucleic Acid Extraction

RNA is isolated from upper respiratory specimens and BALs using a bead-based RNA extraction kit (Chemagic Viral DNA/RNA 200 Kit H96) and an automated protocol on the Chemagic 360 instrument platform. Extracted RNA is transferred from the extraction elution plate to a 384-well plate, and the RT-LAMP reaction is set up, using the automated Hamilton STARlet system or the Agilent Bravo liquid handler.

RT-LAMP

Extracted RNA is initially processed through the colorimetric RT-LAMP procedure using two different primer sets targeting the (SARS-CoV-2 nucleocapsid (N) gene) and the human RNase P (RP) gene, followed by reflex testing (run #2) on "candidate" positives and samples with invalid results identified by the screening assay (run #1). Each primer set is comprised of 6 individual primers, targeting specific regions of viral or human

RNA which are amplified in separate wells during isothermal incubation at 65°C using a strand-displacing polymerase. During this isothermal reaction, reverse transcription and loop-mediated amplification occur. The incorporation of dNTPs during amplification causes a pH change in the reaction which is visually detectable with pH-sensitive dyes. The reaction color change initiated by amplification is measured spectrophotometrically over a period of 70 minutes using the Biotek NEO2 microplate reader. Reactions displaying a color shift indicate that the target sequence is present.

INSTRUMENTS USED WITH TEST

The Color SARS-CoV-2 RT-LAMP Diagnostic Assay is to be used with the following instrumentation:

- Hamilton STAR/STARlet automated liquid handler with Venus 4 software
- Agilent Bravo automated liquid handling platform with VWorks software version 13.1.0.1366
- Perkin Elmer Chemagic 360 extraction instrument platform and Chemagic software v6.3.0.3
- Biotek Synergy NEO2 multi-mode microplate reader with Gen5 software v3.9

REAGENTS AND MATERIALS

Reagents Used to Perform the Color SARS-CoV-2 RT-LAMP Diagnostic Assay

Reagent Manufacturer and Description	Catalog #	Manufacturer
Equipment		
Hamilton STAR, STARlet	STAR, STARlet	Hamilton
Agilent Bravo	Bravo	Agilent
Chemagic Instrument	Chemagic 360	Perkin Elmer
Plate Centrifuge	5910R	Eppendorf
Microplate Reader	Neo2S	Biotek
Heat Sealer	PX1 PCR Plate Sealer, PlateLoc, or equivalent	Biotek, Agilent, or equivalent
Xpeel Plate Peeler	XP-A	Nexus Biosystems
MultiFloFX Multi-Mode Dispenser	MFXP1	Biotek
Thermomixer, 384 block, ThermoTop	5382000023, 5307000000 and 5308000003	Eppendorf
Consumables		
Foil Seal	0030127790	Eppendorf
Microseal “B” Optical Seal	MSB-1001	Bio-Rad
Universal Plate Lid	3098	Corning
PlateLoc Seal, Clear, Permanent	24212-001	Agilent
384-Well Plate	HSP3901	Bio-Rad
96-Well, Hardshell PCR Plate	HSP9641, HSP9631	Bio-Rad
Reagents		
Chemagic Viral DNA/RNA 200 Kit H96	CMG-1033	Perkin Elmer
Nuclease Free Water	SH30538LS	Hyclone
Total Human RNA	4307281	Thermo Fisher Scientific
DNA/RNA Shield + Collection Swab	R1100-250	Zymo Research
WarmStart Colorimetric LAMP 2X master mix	M1800B-1L	New England Biolabs (NEB)
SARS-CoV-2 RNA Control 1	102019	Twist Bioscience
ATCC SARS-CoV-2 genomic RNA	ATCC VR1986-D	ATCC

10 µmol Desalted, Custom Synthesized Primer Set (RNase P, N-gene, E-gene)	3126565	Integrated DNA Technologies
100 mM dUTP	N0459B	New England Biolabs (NEB)
1U/µL UDG	M0372B	New England Biolabs (NEB)

COLLECTION KITS USED WITH THIS TEST

The Color SARS-CoV-2 RT-LAMP Diagnostic Assay can be used with anterior nasal swabs (dry spun polyester swabs) that are collected using the Color COVID-19 Self-Swab Collection Kit which is manufactured by Color Health, Inc.

The Color SARS-CoV-2 RT-LAMP Diagnostic Assay can also be used with anterior nasal swabs that are collected using the Color COVID-19 Self-Swab Collection Kit with Saline which is manufactured by Color Health, Inc.

INSPECTION OF SPECIMENS

Sample Acceptance Criteria for Dry Anterior Nasal Swabs

For Color to perform testing, samples must meet the following criteria:

- Sample collection tube must be intact and not visibly damaged.
- The tube barcode label must be present and readable by a barcode scanner.
- The tube cap must be properly secured onto the tube (i.e., push top closure).
- The collection tube contains exactly one swab.
- The swab is oriented correctly with the bud facing the bottom of the tube.
- The expiration date on the kit is not exceeded.
- Accession date/time is within 56 hours from the collection date/time, as described in the Color COVID-19 Self-Swab Collection Kit EUA Summary.

For dry anterior nasal swab samples, LIMS will check that there is a physician order for the sample, a consent form is present, and that the collection kit has been activated via the on-line portal within the last 56 hours.

Sample Acceptance Criteria for Anterior Nasal Swabs in Saline

For Color to perform testing, samples must meet the following criteria:

- Sample collection tube must be intact and not visibly damaged.
- There are no signs of leakage in the biohazard bag.
- The tube barcode label must be present and readable by a barcode scanner.
- The tube cap must be properly secured onto the tube (i.e., screw cap closure).
- The medium must be in a liquid state and not congealed.
- The collection tube with saline contains exactly one swab.
- The swab is oriented correctly with the bud facing the bottom of the tube.
- The expiration date on the kit is not exceeded.
- Accession date/time is within 48 hours of the collection date/time, as described in the Color COVID-19 Self-Swab Collection Kit with Saline EUA Summary.

For anterior nasal swab samples collected in saline, LIMS will check that there is a physician order for the sample, a consent form is present, and that the collection kit has been activated via the on-line portal within the last 48 hours.

CONTROLS TO BE USED WITH THE COLOR SARS-COV-2 RT-LAMP DIAGNOSTIC ASSAY

Two controls are included in each extraction batch, and carried through the full process:

Extraction Controls

- A positive control is used and consists of DNA/RNA Shield medium spiked with human total extracted nucleic acid and synthetic viral SARS-CoV-2 RNA (Twist Synthetic SARS-CoV-2 RNA Control 1 (MT007544.1) or ATCC SARS-CoV-2 genomic RNA (ATCC VR1986-D) at 5X LoD.
- A no template control (NTC) is used and consists of DNA/RNA Shield medium. This control is processed through the entire end-to-end testing protocol.

RNase P Endogenous Human Specimen Control

- An endogenous RNase P internal control must be present in each clinical sample.

INTERPRETATION OF RESULTS

All test controls on the initial screening plate (run #1) must be examined prior to interpretation of patient results and disposition of samples as "candidate" positive, negative or invalid. If the controls are not valid, the patient results cannot be interpreted. If reflex testing is needed, all controls with assay run #2 must be valid prior to interpretation and reporting of patient results.

1) **<u>Color SARS-CoV-2 RT-LAMP Diagnostic Assay Controls – Extraction Positive, Extraction NTC, and Internal RNase P:</u>**

   Interpretation protocol for RT-LAMP reactions

   Visible light absorbance in each well is measured once per minute, from time t=0 to t=70 minutes and the absorbance ratio (A430/A560) at each point is calculated. Three points are identified: the absorbance ratio at baseline, the absorbance ratio at the endpoint, and the maximum rate of amplification (Figure 1, Table 1):

   - The derivative of the absorbance ratio is calculated, and this curve is smoothed using a rolling average of 9 adjacent data points. The baseline time point is identified as the first point that the slope of the curve between drops below 0.005. If this point has not been identified in the time window between 5-25 minutes with absorbance ratios between 1.2-1.6, the baseline assessment is set to "failed". The baseline time point is used to calculate the baseline ratio, which is the average of 5 adjacent data points.
   - For the endpoint set at 55 minutes the absorbance ratio is quantified using a rolling average of 5 adjacent data points. The ratio gain is defined as the difference between the absorbance ratios of the end point and baseline point.

- The maximum amplification rate is calculated as the maximum slope achieved between 20 minutes and the endpoint, using a rolling average.

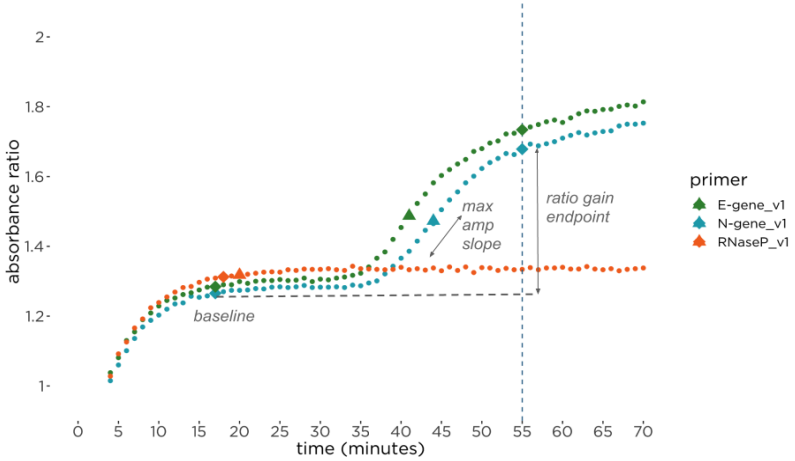

Figure 1 Representative RT-LAMP Data From a Synthetic Positive Control (Twist Synthetic SARS-CoV-2 RNA Control 1)

Table 1. Ratio Gain Interpretation for Each Primer

Gain in A430/A560 Ratio	Maximum Amplification Rate	Interpretation
≥ 0.25	any	Positive Signal
< 0.25	any	Negative Signal

Extraction Control Results for Screening Plate (Run #1) (See Table 2)

- The positive extraction control must exhibit positive signal for both the SARS-CoV-2 specific N1 target and the internal RNase P target. A lack of amplification would indicate that there was reagent or process failure during extraction or RT-LAMP.
- The no template extraction control must be negative for both the SARS-CoV-2 specific N1 target and the internal RNase P target. Amplification would indicate that there was contamination during extraction and/or with the RT-LAMP reagents.
    - All samples within a plate are considered failed if the Extraction NTC exhibits a positive signal for the N-gene viral primer set and/or RNase P.

RNase P Endogenous Human Specimen Control

- RNase P must yield positive signal in every clinical specimen for the run (screening or reflex testing) to be valid. Failure to detect RNase P in a specimen would invalidate that specific specimen.

Table 2. Expected Results of Controls for Screening Plate (Run #1) Used in the Color SARS-CoV-2 RT-LAMP Diagnostic Assay

Control	N-gene	RNase P
Extraction Positive	Positive signal	Positive signal

Extraction NTC	Negative signal	Negative signal

Extraction Control Results for Reflex Testing on Candidate Positives or Invalid Samples (Run #2) (See Table 3)

- The positive extraction control must exhibit positive signal for both the SARS-CoV-2 specific targets (N and E) and the internal RNase P control. A lack of amplification would indicate that there was reagent or process failure during extraction or RT-LAMP.
- The no template extraction control must be negative for both SARS-CoV-2 specific targets (N and E) and the internal RNase P control. Amplification would indicate that there was contamination during extraction and/or with the RT-LAMP reagents.
    - All samples within a plate are considered failed if the Extraction NTC exhibits a positive signal for either viral primer set and/or RNase P.

Table 3. Expected Results of Controls for Run #2-Reflex Testing (Only Applicable if there are Invalid Samples or Candidate SARS-CoV-2 Positive Clinical Samples Detected in Run #1)

Control	N-gene	E-gene	RNase P
Extraction Positive	Positive signal	Positive signal	Positive signal
Extraction NTC	Negative signal	Negative signal	Negative signal

2) <u>Examination and Interpretation of Patient Specimen Results:</u>

Assessment of clinical specimen test results must be performed after the positive and negative controls have been examined and determined to be valid on the screening plate (run #1). If the controls are not valid, the patient results cannot be interpreted. Valid, negative results from the screening plate can be reported, and reflex testing can be completed for samples with "candidate" positive and invalid screening results. Interpretation of clinical specimen test results from reflex testing (run #2) cannot be performed until the controls shown in Table 3 above are determined to be valid. Please see Table 4 for guidance on interpretation and reporting of patient results based on the screening and reflex testing procedures.

- If the N1 SARS-CoV-2 target is negative (negative signal), and the RNase P result is positive (positive signal) on the screening plate (run #1), the sample is reported as negative for SARS-CoV-2 RNA. No reflex testing is completed.
- Regardless of whether the SARS-CoV-2 target is positive or negative on the initial screening plate, if RNase P is negative (negative signal), the assay is invalid/failed. The user is instructed to re-extract nucleic acid from residual clinical sample and repeat the RT-LAMP assay using the reflex testing protocol (run #2) with both the N and E-gene viral primers and RNase P.
    - If the repeat result is invalid/failed (negative for all markers), collection of a new sample is recommended.
    - If both viral primer sets and RNase P are positive upon reflex testing, the sample is reported as positive for SARS-CoV-2 RNA.

  - If both viral primer sets are negative and RNase P is positive upon reflex testing, the sample is reported as negative for SARS-CoV-2 RNA.
  - If only one viral primer set is positive and RNase P is positive upon reflex testing, the sample is reported as inconclusive and collection of a new sample is recommended.
- If the N1 SARS-CoV-2 target is positive (positive signal) and the RNase P target is also positive (positive signal) on the screening plate (run #1), the clinical sample is considered "Candidate Detected" and the sample is re-extracted and re-tested with both the N and E-gene SARS-CoV-2 specific primer sets and the RNase P primer set following the reflex testing protocol (run #2).
  - If both viral primer sets and RNase P are positive upon reflex testing, the sample is reported as positive for SARS-CoV-2 RNA.
  - If both viral primer sets are negative and RNase P is positive upon reflex testing, the sample is reported as negative for SARS-CoV-2 RNA.
  - If only one viral primer set is positive and RNase P is positive upon reflex testing, the sample is reported as inconclusive and collection of a new sample is recommended.
  - If the screening result is positive and the reflex testing result is invalid/failed (negative signal for RNase P), re-extraction and re-testing is recommended. If insufficient volume remains for further testing, collection of a new sample is recommended.

Table 4. Interpretation of Patient Results Using the Color SARS-CoV-2 RT-LAMP Diagnostic Assay

Screening Plate (Run #1)				Reflex Testing (Run #2)				
N-gene	**RNase P**	**Interpretation #1**	**Action #1**	**N-gene**	**E-gene**	**RNase P**	**Interpretation #2**	**Action #2**
Negative	Positive	SARS-CoV-2 Not Detected	Report results to physician, patient and appropriate public health authorities.	N/A	N/A	N/A	N/A	N/A
Positive or Negative	Negative	FAILED (INVALID)	Re-extract from residual sample and repeat RT-LAMP with two viral primer sets (proceed with Reflex Testing).	Positive or Negative	Positive or Negative	Negative	FAILED (INVALID)	Collection of a new patient sample is recommended, Report results to physician and patient.
				Positive	Positive	Positive	SARS-CoV-2 Detected	Report results to physician, patient, and appropriate public health authorities.
				Negative	Negative	Positive	SARS-CoV-2 Not Detected	Report results to physician, patient, and appropriate public health authorities.

				Positive	Negative	Positive	Inconclusive	Collection of a new patient sample is recommended. Report results to physician and appropriate public health authorities.
				Positive	Negative	Positive		
Positive	Positive	Candidate Detected	Re-extract from residual sample and repeat RT-LAMP with two viral primer sets (proceed with Reflex Testing).	Positive	Positive	Positive	SARS-CoV-2 Detected	Report results to physician, patient, and appropriate public health authorities.
				Negative	Negative	Positive	SARS-CoV-2 Not Detected	Report results to physician, patient, and appropriate public health authorities.
				Positive	Negative	Positive	Inconclusive	Collection of a new patient sample is recommended. Report results to physician, patient and appropriate public health authorities.
				Negative	Positive	Positive		
				Positive or Negative	Positive or Negative	Negative	FAILED (INVALID)	Re-extract a 2nd time from residual sample (volume permitting) and repeat RT-LAMP with two viral primer sets OR send residual sample to designated testing laboratory. If insufficient volume remains, collection of a new patient sample is recommended.

N/A; Testing Scenario Not Applicable

PERFORMANCE EVALUATION

Analytical and Clinical Performance of the Color SARS-CoV-2 RT-LAMP Diagnostic Assay

1) Analytical Sensitivity:

a. Limit of Detection (LoD) Using SARS-CoV-2 Genomic RNA:

Note that the Color SARS-CoV-2 RT-LAMP Diagnostic Assay was originally authorized for the detection of 3 SARS-CoV-2 specific targets including regions of the N-gene, E-gene, and ORF1ab in separate wells. Color removed the ORF1ab target and provided historical data to support this change as described in section 1b immediately below. The LoD study design and data for the 3 target Color SARS-CoV-2 RT-LAMP Diagnostic Assay is presented here.

The limit of detection (LoD) is defined as the lowest concentration at which 19/20 replicates (or approximately 95% of all true positive replicates) are positively detected. The LoD of the Color SARS-CoV-2 RT-LAMP Diagnostic Assay was established using a dilution series of SARS-CoV-2 genomic RNA (ATCC VR-1986D), spiked into negative anterior nasal swab clinical matrix in DNA/RNA Shield medium. A preliminary LoD was determined by testing serial dilutions (100 copies/µL – 0.01 copies/µL) of RNA spiked into pooled clinical negative matrix and tested with five replicates per concentration. Each spiked replicate was processed through the entire assay, beginning with RNA extraction using the Chemagic Viral DNA/RNA Kit on the Chemagic 360 instrument followed by testing with the Color SARS-CoV-2 RT-LAMP Diagnostic Assay.

The initial LoD determination of the Color SARS-CoV-2 RT-LAMP Diagnostic Assay was 0.5 copies/µL, which was the lowest concentration of SARS-CoV-2 RNA at which ≥ 95% of replicates were detected.

The LoD was verified by testing 20 individual extraction replicates consisting of pooled negative clinical anterior nasal swab matrix with DNA/RNA Shield medium at 1 copy/µL, 0.75 copies/µL, 0.5 copies/µL, and 0.25 copies/µL. Samples were spiked with viral genomic RNA prior to extraction with the Chemagic 360 protocol and instrument. The LoD of the Color SARS-CoV-2 RT-LAMP Diagnostic Assay was determined to be 0.75 copies/µL for the N, E, and ORF1ab targets.

The results of the LoD confirmatory study are summarized below in Table 5.

Table 5. LoD Verification Study Results Using SARS-CoV-2 Genomic RNA

Concentration (copies/µL in primary sample)	N-gene replicates detected	E-gene replicates detected	ORF1ab replicates detected
1 copy/µL	20/20	20/20	20/20
0.75 copies/µL	20/20	20/20	20/20

Concentration (copies/µL in primary sample)	N-gene replicates detected	E-gene replicates detected	ORF1ab replicates detected
0.5 copies/µL	20/20	18/20	19/20
0.25 copies/µL	17/20	10/20	14/20

b. Removal of the ORF1ab Target:

To increase assay throughput, an analysis was completed that assessed the impact on assay performance when the SARS-CoV-2 specific primer set, ORF1ab, was removed (i.e., the number of separate amplification reactions per sample was reduced from 4 [N, E, ORF1ab and RNase P] to 3 [N, E and RNase P only]). Additionally, the interpretation protocol was updated to simplify and remove redundant metrics. The updated interpretation protocol includes an increase in the upper threshold of the ratio gain (from 0.15 to 0.25) and the removal of the maximum rate of amplification from analysis.

To validate these changes, a retrospective analysis of 33,363 patient results obtained with the Color SARS-CoV-2 RT-LAMP Diagnostic Assay and reported during the period between May 28 and June 24, 2020 was performed to compare assay results before and after the aforementioned updates. Because separate master mixes are prepared for each assay primer/probe set and oligonucleotides are not multiplexed, this approach of reviewing historical data was considered acceptable. RT-LAMP data generated at the original time of sample processing were re-analyzed using the updated analysis protocol and the results are shown in Table 6.

Table 6. Validation Study Results for Removal of the ORF1ab Target Using an Historic Data Set

Reported Result Using Authorized Algorithm	Validation Study Result Using Updated Algorithm				
	Detected	Failed*	Inconclusive	Not Detected	Total (% of Total Number of Tests Performed)
Detected	304	23	5	2	334 (1.00%)
Inconclusive	6	0	28	19	53 (0.16%)
Not Detected	0	38	0	32,938	32,976 (98.84%)
Total (% of Total Number of Tests Performed)	310 (0.98%)	61 (0.18%)	33 (0.10%)	32,959 (98.79%)	33,363 (100.00%)

*Per the assay SOP at the time of this modification, failed and inconclusive sample runs would lead to the sample being re-tested before reporting a final result.

In total, 99.72% (n = 33,270 samples-darker shaded regions in Table 5) of samples yielded the same result with the updated algorithm, and 0.28% (n = 93) of samples yielded a different result. Of the samples that yielded a different result, the majority of changes (n = 61, 0.18% of total) were due to an increase in failed samples, reflecting a more stringent threshold for human RNase P amplification ratio gain. Per the assay SOP, failed and inconclusive sample runs would result in

the sample being retested using new extracted RNA from residual clinical sample before reporting a final result.

Additional analysis was performed on the two cases in which the result would have changed from "detected" to "not detected". One sample had an aberrantly fast amplification for the N-gene and E-gene, which caused the baseline to be assessed incorrectly. This case was caught and corrected through manual human review during reporting, per the laboratory SOP, and was reported as "detected". The same data review would have captured this case as "detected" with the new analysis thresholds. The second sample had very late amplification with both the N-gene and E-gene primer sets, which was slightly above the original threshold but below the updated threshold.

Additional analysis was also performed on the six cases in which the result would have changed from "inconclusive" to "detected". In all six cases, the ORF1ab primer set did not amplify at all, while the other two SARS-CoV-2 primer/probe sets showed strong amplification signals. Per the authorized interpretation protocol, this resulted in an "inconclusive" report. However, applying the updated algorithm with removal of the ORF1ab oligonucleotides, the results would have been reported as "detected".

Therefore, the results of these analyses indicated that removing the ORF1ab primer/probe set did not have a significant impact on assay performance, and thus this primer set was removed from the assay and the reporting algorithm.

c. Limit of Detection (LoD) Using Heat-Inactivated Whole SARS-CoV-2:
The LoD of the modified Color SARS-COV-2 RT-LAMP Diagnostic Assay (N-gene, E-gene and RNase P primers only) was also established using whole heat-inactivated SARS-CoV-2 (ATCC VR-1986HK) spiked in negative anterior nasal swab clinical matrix in DNA/RNA Shield (Zymo Research, Cat # R1100-250) at various concentrations. Each spiked replicate was processed through the entire assay, beginning with RNA extraction using the Chemagic Viral DNA/RNA Kit on the Chemagic 360 instrument followed by testing with the Color SARS-CoV-2 RT-LAMP Diagnostic Assay. The initial LoD determination was 1.5 copies/µL, which was the lowest concentration of SARS-CoV-2 at which 3/3 replicates (100%) were detected for both the N and E-gene targets. A confirmatory LoD study was performed using 20 independent extraction replicates consisting of whole heat-inactivated SARS-CoV-2 spiked into negative anterior nasal swab matrix in DNA/RNA Shield at two different concentrations. The Color SARS-CoV-2 RT-LAMP Diagnostic Assay was shown to detect ≥95% of replicates at 0.75 copies/µL. The results of the LoD confirmatory study are summarized in Table 7.

Table 7. LoD Verification Study Results Using Heat-Inactivated Whole SARS-CoV-2

Concentration (copies/µL)	N-gene Replicates Detected	E-gene Replicates Detected	RNase P Replicates Detected

1.5	20/20 (100%)	20/20 (100%)	20/20 (100%)
0.75	20/20 (100%)	19/20 (95%)	20/20 (100%)

d. Removal of the E-Gene Target for Creation of the Initial Screening Assay:
The modified version of the Color SARS-CoV-2 RT-LAMP Diagnostic Assay is designed to perform an initial screen for a SARS-CoV-2 specific region of the nucleocapsid (N) gene along with the human endogenous control RNase P target. As described previously, if the screening assay generates a positive SARS-CoV-2 result or an invalid result, follow-up reflex testing is completed using both the N and E-gene viral primer sets and the primers for RNase P. If the N-gene is negative (and RNase P is positive) by the screening assay, no reflex testing is conducted and the result can be reported as negative for SARS-CoV-2 RNA.

The LoD of the updated Color SARS-CoV-2 RT-LAMP Diagnostic Assay was confirmed using heat-inactivated SARS-CoV-2 (ATCC VR-1986HK) spiked into anterior nasal swab clinical matrix in DNA/RNA Shield medium directly into swabs. The LoD confirmatory study was performed by testing 20 independent extraction replicates that were each processed through the entire assay, beginning with RNA extraction using the Chemagic Viral DNA/RNA Kit on the Chemagic 360 instrument followed by testing with the modified Color SARS-CoV-2 RT-LAMP Diagnostic Assay. According to the updated protocol, all spiked samples were screened for SARS-CoV-2 positivity using the N-gene primer set as well as human RNase P to identify candidate positives. Any replicates identified as candidates for reflex testing were re-extracted and re-run using both the N and E-gene primer sets as well as those for the RNase P endogenous control target. The results of the LoD confirmatory study are summarized in Tables 8 and 9 and show that the LoD of both the screening and reflex assays was 0.75 copies/µL.

Table 8. Confirmatory LoD Study Results for the Screening Assay (N-Gene and RNase P)

Concentration (copies/µL)	N-gene Replicates Detected	RNase P Replicates Detected
0.75	20/20 (100%)	20/20 (100%)

Table 9. Confirmatory LoD Study Results for the Reflex Assay (N and E-Genes, RNase P)

Concentration (copies/µL)	N-gene Replicates Detected	E-gene Replicates Detected	RNase P Replicates Detected
0.75	19/19 (100%)*	19/19 (100%)*	19/19 (100%)*

*There was one replicate that was negative for RNase P upon reflex testing (N and E-gene targets were positive [20/20; 100%]). Per the reporting structure, the replicate was re-extracted and re-tested with the N and E-gene primer sets and RNase P and yielded the same result. Therefore, the run was considered failed.

2) Analytical Comparison/Equivalence Studies:

a. Addition of the Agilent Bravo Liquid Handler:

In addition to use of the Hamilton STARlet, the Agilent Bravo liquid handler was validated for use in preparing the reaction plates for RT-LAMP. A total of 96 individual samples containing human and SARS-CoV-2 genomic RNA at 1X LoD and 90 individual NTC reactions were prepared using the Agilent Bravo instrument on two separate plates along with the standard assay controls. There was 100% detection of the samples (96/96) and all controls passed. The performance of the Agilent Bravo liquid handler was therefore considered acceptable.

b. Dry Anterior Nasal Swabs:

To demonstrate that dry spun polyester swabs collected using the Color COVID-19 Self-Swab Collection Kit are acceptable specimen types for testing with the Color SARS-CoV-2 RT-LAMP Diagnostic Assay, the performance of the assay was evaluated using dry swabs resuspended in 1.3 mL of lysis buffer included in the Chemagic Viral DNA/RNA Kit that is used to perform extraction on the automated Chemagic platform. Swabs in lysis buffer underwent gentle shaking on an orbital shaker for 20 minutes at ambient conditions.

Contrived positive specimens at 2X and 5X LoD (based on the LoD of the modified assay obtained with samples in 1X DNA/RNA Shield) were prepared by spiking inactivated SARS-CoV-2 into DNA/RNA Shield containing negative clinical anterior nasal swab matrix followed by spiking the matrix directly onto the spun polyester swabs. Five technical replicates at both 2X and 5X LoD concentrations were tested in addition to 5 negatives (unspiked-negative clinical anterior nasal swab matrix resuspended in lysis buffer). Results are summarized in Table 10. There was 100% agreement with expected results for all positive contrived samples. All negative samples were non-reactive for SARS-CoV-2 assay targets.

Table 10. Summary of Dry Swab Reconstitution Validation Results Stratified by Assay Target

Swab Type	Concentration	Samples (n)	Detection Rate		
			N-gene	E-gene	RNase P
Spun Polyester	2X LoD (1.5 copies/µL)	5	5/5	5/5	5/5
	5X LoD (3.75 copies/µL)	5	5/5	5/5	5/5
	Negative	5	0/5	0/5	5/5

These results indicate that the Color SARS-CoV-2 RT-LAMP Diagnostic Assay generated similar results with dry anterior nasal swab samples (resuspended in lysis buffer) and swabs in DNA/RNA Shield.

c. Anterior Nasal Swabs in Saline:

To demonstrate that anterior nasal swabs collected using the Color COVID-19 Self-Swab Collection Kit with Saline are acceptable for testing with the Color SARS-CoV-2 RT-LAMP Diagnostic Assay, a study was performed with

contrived positive and negative samples that were prepared in parallel using pooled anterior nasal swab matrix in either DNA/RNA Shield or 0.9% saline. Negative anterior nasal swab matrices in DNA/RNA Shield and saline were spiked with inactivated whole SARS-CoV-2 from ATCC. Each spiked replicate was processed through the entire assay, beginning with RNA extraction using the Chemagic Viral DNA/RNA Kit on the Chemagic 360 instrument, followed by testing with the Color SARS-CoV-2 RT-LAMP Assay. Results from the study are shown in Table 11 below.

Table 11. Comparison of Results for Samples in 1X DNA/RNA Shield Medium and 0.9% Sterile Saline

X LoD	Concentration (copies/µL)	DNA/RNA Shield	0.9% Saline
		# of Replicates Detected / Total Replicates Tested	# of Replicates Detected / Total Replicates Tested
2X LoD	1.50	5/5	5/5
3X LoD	2.25	5/5	5/5
5X LoD	3.75	5/5	5/5
Negative	N/A	0/5	0/5

N/A; Not applicable

These studies indicate that the Color SARS-CoV-2 RT-LAMP Diagnostic Assay generated similar results with anterior nasal swab samples prepared in 1X DNA/RNA Shield, 0.9% sterile saline and with dry swabs resuspended in lysis buffer.

3) Analytical Inclusivity/Specificity:

a. Inclusivity *In silico* Analysis of RT-LAMP Primer Sets:

An *in silico* inclusivity analysis was performed by aligning all 6 LAMP primer sequences for each target against SARS-CoV-2 sequences deposited within GenBank at NCBI on February 11, 2021. This data set included SARS-CoV-2 complete whole genome sequences that were annotated as high coverage, and included 40,737 sequences for the N-gene LAMP primers and 41,859 sequences for the E-gene.

Both primer sets (N, E) had a 100% match with the vast majority of SARS-CoV-2 sequences: 96.0% for the N-gene and 97.4% for the E-gene. The total number of mismatches across all 6 LAMP primers for each target are shown below in Table 12.

Table 12. *In silico* Inclusivity Analysis

Characteristic	N-gene	E-gene
Total Primer Length (nt)	157	161
Total # of Strains Evaluated	40,737	41,859
100% Match	39,125 (96.0%)	40,773 (97.4%)
1 Mismatch	1530 (3.8%)	1042 (2.5%)
2 Mismatches	46 (0.11%)	39 (0.09%)
3 Mismatches	36 (0.09%)	3 (< 0.01%)

Characteristic	N-gene	E-gene
>3 Mismatches	0 (0%)	2 (< 0.01%)

Two sequences have > 3 mismatches within the E-gene primers which could impact the amplification yield of the E-gene target, resulting in an "inconclusive" test result if the sample was truly positive. These sequences have 100% matches to the N-gene primer set that is specific for SARS-CoV-2.

In addition, the primer sets used in the Color SARS-CoV-2 RT-LAMP Diagnostic Assay do not overlap any of the variants that have been associated with the UK B.1.1.7 and the South African B.1.351 strains. The *in silico* analysis predicts that these specific variants would be detected by the Color SARS-CoV-2 RT-LAMP Diagnostic Assay.

b. Cross-Reactivity *In silico* Analysis of RT-LAMP Primer Sets:
In silico cross-reactivity analysis was performed by aligning the LAMP primer sequences against sequences of common viruses as well as coronaviruses related to SARS-CoV-2. See Table 13 for the organisms assessed *in silico* for potential cross-reactivity to the Color SARS-CoV-2 RT-LAMP Diagnostic Assay.

Table 13. Cross-Reactivity/Exclusivity *In silico* Results

Virus	GenBank	N-gene	E-gene
COVID-19	MN908947.3	100.0%	100.0%
Human Coronavirus 229E	NC_002645.1	70.1%	72.0%
Human Coronavirus OC43	NC_006213.1	73.2%	70.8%
Human Coronavirus HKU1	NC_006577.2	72.0%	68.3%
Human Coronavirus NL63	NC_005831.2	72.6%	70.8%
SARS CoV	NC_004718.3	91.1%	93.2%
MERS CoV	NC_019843.3	72.6%	72.0%
Adenovirus, strain ad71	X67709.1	66.2%	63.4%
Human Metapneumovirus	NC_039199.1	69.4%	71.4%
Parainfluenza virus 1, strain Washington/1964	AF457102.1	72.0%	68.3%
Parainfluenza virus 2, strain GREER	AF533012.1	68.8%	70.8%
Parainfluenza virus 3, strain HPIV3/MEX/1526/2005	KF530234.1	70.7%	73.3%
Parainfluenza virus 4, strain M-25	NC_021928.1	70.7%	68.9%
Influenza A (H1N1)	FJ966079.1	66.2%	68.9%
Influenza A (H3N2)	KT002533.1	65.6%	68.3%
Influenza B (Victoria)	MN230203.1	70.7%	64.0%
Influenza B (Yamagata)	MK715533.1	68.2%	67.7%
Enterovirus D68 (EV-D68)	KP745766.1	72.0%	68.3%
Respiratory syncytial virus	U39661.1	72.0%	71.4%

Virus	GenBank	N-gene	E-gene
Human rhinovirus 14	NC_001490.1	68.8%	70.8%

With the exception of SARS-CoV, which is closely related to SARS-CoV-2, none of these viruses have a match against the total sequence length of the SARS-CoV-2 primers greater than the recommended threshold of 80%. Both the N-gene and E-gene primer sets have a match >90% with SARS-CoV; however. the likelihood of a false positive is low because there are no known circulating strains of SARS-CoV in the human population.

c. Cross-Reactivity Wet Testing:

In addition to the *in silico* analysis for cross-reactivity, wet testing was also performed to evaluate the potential cross-reactivity/exclusivity of the assay with other organisms. Samples were prepared by spiking (inactivated) purified, intact viral particles, purified RNA, or bacterial cells using those panels/organisms shown in Table 14 into negative buccal swab matrix and processed in triplicate with the assay. Because no quantification information was available for the individual organisms that were wet tested, 50 µL of each stock was spiked into negative clinical matrix and tested. All results of wet bench testing were negative (Table 15) indicating that the Color SARS-CoV-2 RT-LAMP Diagnostic Assay is designed for the specific detection of SARS-CoV-2, with no expected cross reactivity to other coronaviruses, or human microflora tested that would predict potential for false positive RT-LAMP results.

Table 14. Panels of Organisms Used to Assess Potential Assay Cross-Reactivity Via Wet Testing

Vendor	Product	Catalog/Lot
ZeptoMetrix	NATtrol Pneumonia Panel - Quantifiable Bacteria (no quantification information available)	Ref: NATPPQ-BIO Lot: 323679
ZeptoMetrix	NATtrol Respiratory Validation Panel 3 (no quantification information available)	Ref: NATRVP-3 Lot: 323354
ZeptoMetrix	NATtrol Pneumonia Panel - Atypical Bacteria & Viruses (no quantification information available)	Ref: NAPPA-BIO Lot: 322617
BEI Resources	HCoV-229E HCoV-NL63 MERS-CoV HCoV-OC43 SARS-CoV-2 SARS	011N-03

Table 15. Cross-Reactivity/Exclusivity Wet Testing Results

Organism	Strain	N-gene Detected Replicates	E-gene Detected Replicates
Acinetobacter baumannii	307-0294	0/3	0/3
Adenovirus Type 3	N/A	0/3	0/3
Adenovirus Type 3	N/A	0/3	0/3
Chlamydia pneumoniae	CWL-029	0/3	0/3
Coronavirus 229E	N/A	0/3	0/3

Organism	Strain	N-gene Detected Replicates	E-gene Detected Replicates
Coronavirus NL63	N/A	0/3	0/3
Coronavirus OC43	N/A	0/3	0/3
Coronavirus SARS	N/A	0/3	0/3
Enterobacter cloacae	Z101	0/3	0/3
Escherichia coli	Z297	0/3	0/3
Enterovirus	N/A	0/3	0/3
Haemophilus influenzae	MinnA	0/3	0/3
HCoV-229E	N/A	0/3	0/3
HCoV-Nl63	N/A	0/3	0/3
HCoV-OC43	N/A	0/3	0/3
Human Metapneumovirus	N/A	0/3	0/3
Influenza A H1	N/A	0/3	0/3
Influenza A H1N1 (2009)	N/A	0/3	0/3
Influenza A H3	N/A	0/3	0/3
Influenza A H3	A/Brisbane/10/07	0/3	0/3
Influenza B	N/A	0/3	0/3
Influenza B	B/Florida/02/06	0/3	0/3
Klebsiella aerogenes	Z052	0/3	0/3
Klebsiella oxytoca	Z115	0/3	0/3
Klebsiella pneumoniae	KPC2	0/3	0/3
Klebsiella. pneumoniae	Z138; OXA-48	0/3	0/3
Klebsiella pneumoniae	Z460; NDM-1	0/3	0/3
Legionella pneumophila	Philadelphia	0/3	0/3
Moraxella catarrhalis	Ne 11	0/3	0/3
Mycoplasma pneumoniae	M129	0/3	0/3
MERS-CoV	N/A	0/3	0/3
Metapneumovirus 8	Peru6-2003	0/3	0/3
Pseudomonas aeruginosa	Z139, VIM-1	0/3	0/3
Proteus mirabilis	Z050	0/3	0/3
Parainfluenza virus Type 1	N/A	0/3	0/3
Parainfluenza virus Type 1	N/A	0/3	0/3
Parainfluenza virus Type 2	N/A	0/3	0/3
Parainfluenza virus Type 3	N/A	0/3	0/3
Respiratory Syncytial Virus A	N/A	0/3	0/3
Respiratory Syncytial Virus B	N/A	0/3	0/3
Rhinovirus 1A	N/A	0/3	0/3
Rhinovirus 1A	N/A	0/3	0/3
RSV A2	N/A	0/3	0/3
Streptococcus agalactiae	Z019	0/3	0/3
Staphylococcus aureus	MRSA, COL	0/3	0/3
Serratia marcescens	Z053	0/3	0/3
Streptococcus pneumoniae	Z022	0/3	0/3
Streptococcus pyogenes	Z018	0/3	0/3
SARS-CoV	N/A	0/3	0/3

4) <u>Interfering Substances:</u>

Interfering substances which could be found in respiratory samples endogenously or exogenously were tested to evaluate the extent, if any, of potential assay inhibition. Baseline anterior nasal swabs were collected in triplicate from study volunteers as negative control samples (without potential interfering substance). The study

volunteers then used the interfering substances as recommended by the manufacturer of the substance which should represent the relevant dose. Immediately after the substances were used, anterior nasal swabs were collected in triplicate and spiked with synthetic COVID-19 RNA (Twist Synthetic SARS-CoV-2 RNA Control) at 5X LoD. 100 µL of whole blood and mucin were separately added into negative clinical matrix in triplicate and then spiked with synthetic COVID RNA (Twist Synthetic SARS-CoV-2 RNA Control) at 5X LoD. The negative swabs that did not contain potentially interfering substances were also spiked with synthetic RNA at 5X LoD. None of the tested substances inhibited or interfered with the performance of the Color SARS-CoV-2 RT-LAMP Diagnostic Assay. Swabs both with and without the interfering substance yielded expected results (Table 16).

Table 16. Endogenous and Exogenous Substances Evaluated for Potential Assay Interference

Substance	Active Ingredient	Concentration	% Agreement with Expected Results
Whole Blood	N/A	5X LoD	100% (3/3)
		Negative	100% (3/3)
Mucin	N/A	5X LoD	100% (3/3)
		Negative	100% (3/3)
Tobacco	Nicotine, Tar, Carbon Monoxide, Formaldehyde, Ammonia, Hydrogen Cyanide, Arsenic, and DDT	5X LoD	100% (3/3)
		Negative	100% (3/3)
Marijuana	Cannabinoids, THC, CBD	5X LoD	100% (3/3)
		Negative	100% (3/3)
Alcohol	Ethanol	5X LoD	100% (3/3)
		Negative	100% (3/3)
Vaseline	Petroleum Jelly	5X LoD	100% (3/3)
		Negative	100% (3/3)
Nasal allergy spray	Triamcinolone acetonide	5X LoD	100% (3/3)
		Negative	100% (3/3)
Nasal congestion spray	Oxymetazoline HCl	5X LoD	100% (3/3)
		Negative	100% (3/3)
Nyquil	Acetaminophen, Doxylamine succinate, Dextromethorphan HBr	5X LoD	100% (3/3)
		Negative	100% (3/3)
Flonase	Fluticasone propionate	5X LoD	100% (3/3)
		Negative	100% (3/3)
Emergen-C	Zinc, Magnesium, Riboflavin, Vitamin C	5X LoD	100% (3/3)
		Negative	100% (3/3)
Saline nasal spray	NaCL, Phenylcarbinol, Nemalkonium Chloride	5X LoD	100% (3/3)
		Negative	100% (3/3)
Act dry mouth lozenges	Isomalt, xylitol, Glycerin	5X LoD	100% (3/3)
		Negative	100% (3/3)
Listerine mouthwash	Eucalyptol, menthol, Methyl Salicylate, Thymol	5X LoD	100% (3/3)
		Negative	100% (3/3)
Sore throat and cough lozenges	Benzocaine, Dextromethorphan HBr	5X LoD	100% (3/3)
		Negative	100% (3/3)
Zinc	Zinc	5X LoD	100% (3/3)
		Negative	100% (3/3)
Chloraseptic spray	Phenol, Glycerin	5X LoD	100% (3/3)

Substance	Active Ingredient	Concentration	% Agreement with Expected Results
		Negative	100% (3/3)

5) Clinical Evaluation for Testing of Samples from Patients Suspected of COVID-19:

Performance of the Color SARS-CoV-2 RT-LAMP Diagnostic Assay was evaluated using both contrived positive and negative samples as well as confirmed clinical positive and negative nasopharyngeal swabs.

a. Contrived Testing Using the N, E, and RNase P Primer Sets Run Simultaneously:
A total of 46 negative and 46 contrived positive samples were evaluated as part of the clinical evaluation for the Color SARS-CoV-2 RT-LAMP Diagnostic Assay. The 46 contrived positive specimens were spiked with SARS-CoV-2 genomic RNA (ATCC VR-1986D) into individual negative clinical anterior nasal swab matrix in DNA/RNA Shield media to produce the following viral concentrations: 10 samples at 1X LoD, 20 samples at 1.5X LoD, 10 samples at 13X LoD, and 6 samples at 133X LoD as shown in Table 17.

These 92 samples (46 spiked positives, 46 clinical negative samples) were randomized and blinded, and RNA was extracted using the Chemagic System followed by testing with the Color SARS-CoV-2 RT-LAMP Diagnostic Assy. Results of the study are summarized in Table 17 below.

Table 17. Summary of Contrived Sample Testing Using SARS-CoV-2 Genomic RNA

Concentration of SARS-CoV-2	Samples (n)	Detection Rate		
		N-gene	E-gene	RNase P
Negative	46	0/46	0/46	46/46
1X LoD (0.75 copies/µL)	10	10/10	10/10	10/10
1.5X LoD (1 copies/µL)	20	20/20	20/20	20/20
13X LoD (10 copies/µL)	10	10/10	10/10	10/10
133X LoD (100 copies/µL)	6	6/6	6/6	6/6

The results at all tested levels for spiked positives in clinical matrix demonstrated 100% agreement and all negative samples were non-reactive.

b. Clinical Study with Previously Confirmed Positive and Negative Samples Using the N, E, and RNase P Primer Sets Run Simultaneously:
In addition to the contrived clinical study, a total of 539 patient samples were processed through the Color SARS-CoV-2 RT-LAMP Diagnostic Assay and compared against an FDA authorized molecular RT-PCR assay. The cohort of

tested samples included 539 nasopharyngeal swabs (37 positives and 502 negatives) collected by healthcare providers from patients seeking SARS-CoV-2 testing and who were previously tested using the authorized molecular assay. All results generated by the Color SARS-CoV-2 RT-LAMP Diagnostic Assay were concordant with the authorized molecular assay (100% positive and negative percent agreement; Table 18).

Table 18. Performance of the Color SARS-CoV-2 RT-LAMP Diagnostic Assay with Nasopharyngeal Swabs when Compared to an FDA Authorized Molecular RT-PCR Assay

Nasopharyngeal Swabs		FDA Authorized Molecular RT-PCR Comparator Assay		
		Positive	Negative	Total
Color SARS-CoV-2 RT-LAMP Diagnostic Assay Result	Positive	37	0	37
	Negative	0	502	502
	Total	37	502	539
Positive Percent Agreement		100.0% (37/37); 90.59% - 100.00%[1]		
Negative Percent Agreement		100.0% (502/502); 99.24% - 100.00%[1]		

[1] Two-sided 95% confidence interval

c. Clinical Study to Validate the Modified Assay Format of a Screening Assay (N-gene and RNase P Only) Followed by Reflex Testing if Applicable (N and E-gene and RNase P):

To validate the modified assay format, a new clinical study was performed that included a cohort of anterior nasal swab specimens that were tested by both the updated Color SARS-CoV-2 RT-LAMP Diagnostic Assay as well as an FDA authorized molecular RT-PCR assay. All clinical samples evaluated in this study were dry anterior nasal swabs collected either from patients suspected of COVID-19 by a healthcare provider (HCP) or from individuals without symptoms or other reasons to suspect COVID-19 infection when evaluated by an HCP. Dry swabs were resuspended in 1.3 mL of lysis buffer from the Chemagic Viral DNA/RNA Kit, as per Color's validated resuspension protocol. Extraction was performed as authorized using the Chemagic extraction kit on the automated Chemagic 360 instrument followed by RT-LAMP. Residual specimens (anterior nasal swabs resuspended in lysis buffer) were processed using an acceptable FDA authorized molecular RT-PCR assay.

The results of testing the modified Color SARS-CoV-2 RT-LAMP Diagnostic Assay in comparison to an FDA authorized molecular assay are displayed in Table 19 and Table 20. Both the positive and negative percent agreements (PPA and NPA) were 100%.

Table 19. Stratification of the Results of the Updated Color Assay (Screening Assay Followed by Reflex Testing on Candidate Positives and Failed Reactions) in Comparison to an Acceptable Authorized SARS-CoV-2 Molecular RT-PCR Assay

Symptomatic and Asymptomatic Samples	FDA Authorized Molecular RT-PCR Comparator Assay

			Positive	Negative	Total
Color SARS-CoV-2 RT-LAMP Diagnostic Assay (updated format)	Screening Assay (N-gene, RNase P)	Candidate Detected	30	0	30
		Negative	0	30	30
		Failed (Invalid)	0	0	0
		Total	30	30	60
	Reflex Assay (N-gene, E-gene, RNase P)	Positive	30	0	30
		Negative	0	0	0
		Inconclusive	0	0	0
		Failed (Invalid)	0	0	0
		Total	30	0	30

Table 20. Overall Qualitative Results of the Clinical Evaluation Using the Updated Color SARS-CoV-2 RT-LAMP Diagnostic Assay Compared to an Acceptable Authorized SARS-CoV-2 Molecular RT-PCR Assay

Symptomatic and Asymptomatic Samples		FDA Authorized Molecular RT-PCR Comparator Assay		
		Positive	Negative	Total
Color SARS-CoV-2 RT-LAMP Diagnostic Assay	Positive	30	0	30
	Negative	0	30	30
	Total	30	30	60
Positive Percent Agreement		30/30; 100.00% (88.65% - 100.00%)[1]		
Negative Percent Agreement		30/30; 100.00% (88.65% - 100.00%)[1]		

[1]Two-sided 95% confidence interval

The results of the clinical study indicated that removing the E primer/probe set to create an initial screening assay did not have an adverse impact on the performance of the Color SARS-CoV-2 RT-LAMP Diagnostic Assay compared to that of another, highly sensitive FDA-authorized method. The study included a sufficient number of weak clinical positive samples as determined by the comparator and the confirmatory LoD study shown above in section 1 indicated that the LoD was unchanged compared to that established with the N, E, and RNase P primer sets when run simultaneously. These data support the use of the updated Color SARS-CoV-2 RT-LAMP Diagnostic Assay to test samples collected from any individual regardless of symptoms or other reasons to suspect COVID-19 when determined to be appropriate by a healthcare provider.

6) **Clinical Validation for Testing of Asymptomatic Samples** Using the N, E, and RNase P Primer Sets Run Simultaneously**:**

Dry anterior nasal swab samples were collected from individuals, regardless of symptom status, from three different county programs available for walk-up/drive-thru COVID-19 testing. There were a total of three collection sites spanning San Francisco county as part of the testing program. At the conclusion of each day, all specimens were bulk-shipped to Color for testing with the Color SARS-CoV-2 RT-LAMP Diagnostic Assay.

Health history data from consecutive anterior nasal swab specimen collections at the designated county sites were reviewed over a period of three days. Consecutively

collected positive samples for asymptomatic testing validation were selected by reviewing the questionnaires received, of which a total of 104 consecutive anterior nasal swab specimens were collected: 52 were from individuals with no reported symptoms (asymptomatic) and 52 from individuals with reported symptoms (symptomatic). Two hundred and sixteen (216) consecutively collected negative samples were tested: 107 came from individuals with no reported symptoms (asymptomatic) and 109 from individuals with reported symptoms (symptomatic).

The dry swabs were processed according to Color's validated resuspension protocol. All samples were run on both the Color SARS-CoV-2 RT-LAMP Diagnostic Assay and an FDA authorized molecular assay that has been validated for asymptomatic testing.

The results of testing asymptomatic and symptomatic dry anterior nasal swab samples with the Color SARS-CoV-2 RT-LAMP Diagnostic Assay in comparison to an FDA authorized molecular comparator assay are shown in Tables 21-22. The PPA and NPA for asymptomatic subjects were both 100% (52/52 and 107/107, respectively). Low positive samples as determined based on the comparator assay's Ct values were also detected in both the asymptomatic and symptomatic positive data sets.

Table 21. Performance of the Color SARS-CoV-2 RT-LAMP Diagnostic Assay in Comparison to an FDA Authorized Molecular Comparator Assay for Asymptomatic Samples

Asymptomatic Samples		FDA Authorized Molecular RT-PCR Comparator Assay		
		Positive	Negative	Total
Color SARS-CoV-2 RT-LAMP Diagnostic Assay	Positive	52	0	52
	Negative	0	107	107
	Total	52	107	159
Positive Percent Agreement		52/52; 100.00% (93.12% - 100.00%)[1]		
Negative Percent Agreement		107/107; 100.00% (96.54% - 100.00%)[1]		

[1] Two-sided 95% confidence interval

Table 22. Performance of the Color SARS-CoV-2 RT-LAMP Diagnostic Assay in Comparison to an FDA Authorized Molecular Comparator Assay for Symptomatic Samples

Symptomatic Samples		FDA Authorized Molecular RT-PCR Comparator Assay		
		Positive	Negative	Total
Color SARS-CoV-2 RT-LAMP Diagnostic Assay	Positive	50	0	50
	Negative	2	109	111
	Total	52	109	161
Positive Percent Agreement		50/52; 96.15% (87.02% - 98.94%)[1]		
Negative Percent Agreement		109/109; 100.00% (96.60% - 100.00%)[1]		

[1] Two-sided 95% confidence interval

7) 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 using the assay version where the N and E-gene viral primer sets and the RNase P primer set are run simultaneously. Blinded sample testing was used to establish specificity and to confirm the LoD. The extraction method used was the Perkin Elmer Chemagic 360 extraction instrument platform with the Chemagic software v6.3.0.3. The data was collected using the Biotek Synergy NEO2 multi-mode microplate reader with Gen5 software v3.9. The results are summarized in the following table.

Table 23. Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Pane l*

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Anterior Nasal	1.8×10^4 NDU/mL	N/A
MERS-CoV		N/A	ND

*The results were obtained with the device authorized on 8/28/2020 (N-gene, E-gene, and RNase P primer sets run simultaneously)
NDU/mL = RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not detected

LIMITATIONS:

- Detection of RNase P indicates that human nucleic acid is present and implies that human biological material was collected and successfully extracted and amplified. It does not necessarily indicate that the specimen is of appropriate quality to enable detection of SARS-CoV-2.
- 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.
- In the absence of symptoms, it is difficult to determine if asymptomatic individuals have been tested too late or too early. Therefore, negative results in asymptomatic individuals may include individuals who were tested too early and may become positive later, individuals who were tested too late and may have serological evidence of infection, or individuals who were never infected.

WARNINGS:

- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an Emergency Use Authorization (EUA) for use by the authorized laboratory;
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and,
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use

of in vitro diagnostics for detection and/or diagnosis of 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.