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

(Color Health, Inc.)

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

(The Color SARS-CoV-2 RT-LAMP Diagnostic Assay DTC will be performed at Color Health, Inc., located at 863 Mitten Road, Burlingame, CA 94010, 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 DTC is a direct to consumer product for testing of anterior nasal swab specimens self-collected at home (which includes in a community-based setting), using the Color COVID-19 Self-Swab Collection Kit DTC by any individuals, 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 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 viral RNA. SARS-CoV-2 RNA is generally detectable in anterior nasal swab specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities. Negative results do not preclude SARS-CoV-2 infection.

The Color SARS-CoV-2 RT-LAMP Diagnostic Assay DTC is not a substitute for visits to a healthcare provider. The information provided by this product should not be used to start, stop, or change any course of treatment unless advised by your healthcare provider.

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

SPECIAL CONDITIONS FOR USE STATEMENTS

For Emergency Use Authorization (EUA) Only
For *In vitro* Diagnostic Use
For Use by People 18 Years of Age or Older

The Color SARS-CoV-2 RT-LAMP Diagnostic Assay DTC is a direct to consumer product for testing of anterior nasal swab specimens self-collected at home or at a community-based distribution site using the Color COVID-19 Self-Swab Collection Kit DTC by any individual, 18 years or older, including individuals without symptoms or other reasons to suspect COVID-19.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Color SARS-CoV-2 RT-LAMP Diagnostic Assay DTC 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, designed to uniquely detect SARS-CoV-2 RNA.

RNA is isolated from anterior nasal swab specimens using a bead-based RNA extraction kit (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. Incubation and data collection is performed on the Biotek NEO2 microplate reader. The plate is incubated at 65°C for 70 minutes. During this isothermal reaction, reverse transcription and loop-mediated amplification occur.

Extracted RNA is processed through the colorimetric RT-LAMP procedure using three different primer sets targeting the SARS-CoV-2 N gene, the SARS-CoV-2 envelope gene (E), and the human RNaseP (RP) gene. 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 using a strand-displacing polymerase. 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 DTC is to be used with the following instrumentation:

- Hamilton STAR/STARlet automated liquid handler with Venus 4 software
- 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 DTC

Reagent Manufacturer and Description	Catalog #	Manufacturer
Equipment		
Hamilton STAR, STARlet	STAR, STARlet	Hamilton
Chemagic Instrument	Chemagic 360	Perkin Elmer
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
Consumables		
Foil Seal	0030127790	Eppendorf
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 Kit	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
10 µmol desalted, custom synthesized primer set (RNaseP, N-gene, E-gene,)	3126565	Integrated DNA Technologies
100 mM dUTP	N0459B	New England Biolabs (NEB)
1U/µL UDG	M0372B	New England Biolabs (NEB)

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

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) 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.
    - Depending upon the results of the Extraction NTC, the Control Confirmation Sample (LAMP NTC) will be examined. The LAMP NTC functions as a negative amplification control and is not subjected to the extraction procedure.

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 must be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted (Refer to Table 1 for a summary of control results).

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

Interpretation protocol for 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 LAMP data from a synthetic positive control (Twist Synthetic SARS-CoV-2 RNA Control 1)

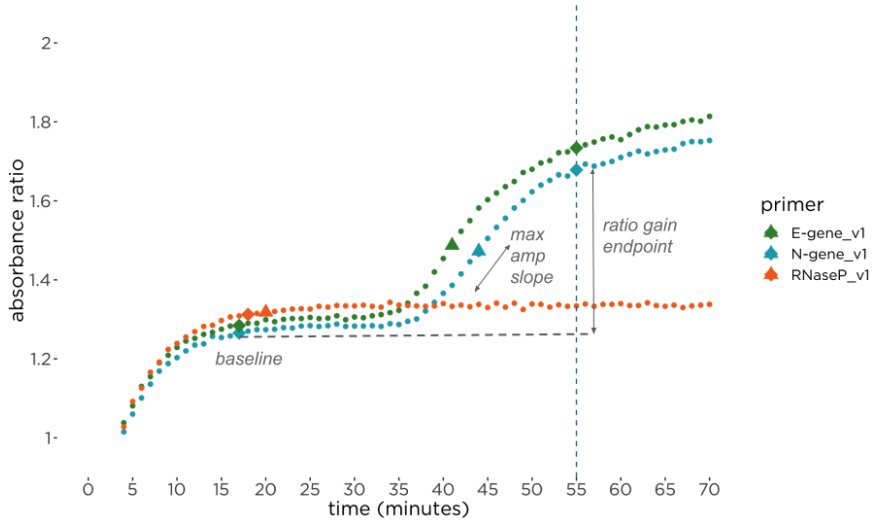

Table 1. Ratio Gain Interpretation for Each Primer

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

Extraction Controls (See Table 2)

- The positive extraction control must exhibit positive signal for both SARS-CoV-2 targets and the internal RNase P control. A lack of amplification would indicate that there was reagent or process failure during extraction or LAMP.
- The no template extraction control must be negative for both SARS-CoV-2 targets and the internal RNase P target. Amplification would indicate that there was contamination during extraction and/or with the LAMP reagents.
    - All samples within a plate are considered failed if the Extraction NTC exhibits a positive signal for both viral primers and/or RNase P.
    - If a single viral primer set is positive in the Extraction NTC, the results of the Control Confirmation Sample (LAMP NTC) must be considered. If the same viral primer set is negative in the Control Confirmation Sample, it suggests random amplification rather than contamination, and the results for the Extraction NTC are acceptable and the batch results can be reported. If the same viral primer set is positive in the Control Confirmation Sample, it suggests systemic contamination and the batch is considered failed.

RNase P Endogenous Human Specimen Control

- RNase P must yield positive signal in every clinical specimen for the run to be valid. Failure to detect RNase P in one specimen would invalidate that specific specimen and indicate extraction failure for that sample.

Table 2. Expected Results of Controls Used in the Color SARS-CoV-2 RT-LAMP Diagnostic Assay DTC

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

- *For troubleshooting purposes, if a single viral primer set is positive in the Extraction NTC, the Control Confirmation Sample (LAMP NTC) must be considered. The Control Confirmation Sample (LAMP NTC) serves as a negative amplification control only and is not subjected to the extraction process. If the same viral primer set is negative in the Control Confirmation Sample, it suggests random amplification rather than contamination, and the results for the Extraction NTC are acceptable and the batch results can be reported. If the same viral primer set is positive in the Control Confirmation Sample, it suggests systemic contamination and the batch is considered failed.
- Note that if RNase P generates signal in the Extraction NTC, the batch is considered failed.
- -; Results are not examined unless the N-gene or E-gene primer set is positive in the Extraction NTC.

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. If the controls are not valid, the patient results cannot be interpreted. Please see the table below (Table 3) for guidance on interpretation and reporting of patient results.

- If both SARS-CoV-2 assay targets are positive (positive signal) and the RNase P target is also positive (positive signal), the patient sample is reported as positive for SARS-CoV-2 RNA.
- If only one SARS-CoV-2 assay target is positive (positive signal), and the RNase P result is positive (positive signal), the result is inconclusive. Repeat the LAMP test using re-extracted nucleic acid from the residual patient specimen. If the repeat result is the same, report the result as inconclusive and indicate that a new patient sample should be collected.
- If both SARS-CoV-2 assay targets are negative (negative signal), and the RNase P result is positive (positive signal), the patient sample is reported as negative for SARS-CoV-2 RNA.
- Regardless of whether the SARS-CoV-2 assay targets are positive or negative, 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 LAMP test. If the repeat result is invalid/failed (negative for all markers), collection of a new patient sample should be considered.

Table 3. Interpretation of Patient Results Using the Color SARS-CoV-2 RT-LAMP Diagnostic Assay DTC

N-gene	E-gene	RNase P	Interpretation	Action
Both SARS-CoV-2 targets = Positive		Positive	SARS-CoV-2 DETECTED	Report results to appropriate public health authorities and individual.*
One SARS-CoV-2 target = Positive		Positive	INCONCLUSIVE	Re-extract from residual sample, and repeat LAMP. If the repeated result remains inconclusive, report result to appropriate public health authorities and individual. Request a new specimen from the individual.*
Both SARS-CoV-2 targets = Negative		Positive	SARS-CoV-2 NOT-DETECTED	Report results to appropriate public health authorities and individual.*
Both SARS-CoV-2 targets = Negative or Positive		Negative	FAILED	Re-extract from residual sample and repeat LAMP. If result remains FAILED, report to appropriate public health authorities and individual. Request a new specimen from the individual.*

* For at home collection or collection from a community-based distribution site, reporting will be done via Color's online portal. Individuals with positive or inconclusive SARS-CoV-2 results will receive a follow-up phone call from a physician via a contracted entity. For details on this process, please refer to the EUA summary for the Color COVID-19 Self-Swab Collection Kit DTC.

INSPECTION OF SPECIMENS

Specimens received at the clinical laboratory for testing with Color SARS-CoV-2 RT-LAMP Diagnostic Assay DTC for the detection of SARS-CoV-2 RNA that is indicated for use with dry anterior nasal swab specimens collected with the Color COVID-19 Self-

Swab Collection Kit DTC will undergo the sample accessioning by the laboratory prior to acceptance for testing, using the "Specimen Receipt and Handling SOP."

PERFORMANCE EVALUATION
(The Color SARS-CoV-2 RT-LAMP Diagnostic Assay DTC is the same RT-LAMP assay as the EUA authorized prescription use only Color SARS-COV-2 RT-LAMP Diagnostic Assay. The performance evaluation of the Color SARS-CoV-2 RT-LAMP Diagnostic Assay DTC described below is the same data used to support the authorization of the prescription use only Color SARS-CoV-2 RT-LAMP Diagnostic Assay. For clarity the "Color SARS-CoV-2 RT-LAMP Diagnostic Assay" name is maintained in the summary of the performed studies).

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 4.

Table 4. 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
0.5 copies/µL	20/20	18/20	19/20
0.25 copies/µL	17/20	10/20	14/20

b. Removal of 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. 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 5.

Table 5. Validation Study Results Using 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, failed and inconclusive sample runs would result in 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 RNaseP 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. <u>Limit of Detection (LoD) Using Heat-Inactivated Whole SARS-CoV-2:</u>
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 6.

Table 6. 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%)

2) Dry Swab Resuspension:

To demonstrate that dry spun polyester swabs collected using the Color COVID-19 Self-Swab Collection Kit were acceptable specimen types for testing with the Color SARS-CoV-2 RT-LAMP Diagnostic Assay, performance of the assay was evaluated using dry swabs resuspended in 1 mL of lysis buffer included in the Chemagic Viral DNA/RNA Kit that is used to perform extraction on the automated Chemagic platform. Eluates underwent gentle shaking on an orbital shaker for 20 minutes at ambient conditions.

Contrived positive specimens at 2X and 5X LoD 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 7. 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 7. Dry Swab Resuspension Study 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

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 8.

Table 8. *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%)
>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 98 for the organisms assessed *in silico* for potential cross-reactivity to the Color SARS-CoV-2 RT-LAMP Diagnostic Assay.

Table 9. 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%
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 10 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 11) 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 LAMP results.

Table 10. 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 11. 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
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

Organism	Strain	N-gene Detected Replicates	E-gene Detected Replicates
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 12).

Table 12. 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)

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

5) Clinical Evaluation:

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:

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 13.

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 13 below.

Table 13. 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:
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 14).

Table 14. Performance of the Color SARS-CoV-2 RT-LAMP Diagnostic Assay with Nasopharyngeal Swabs when Compared to an EUA Authorized Molecular Assay

Nasopharyngeal Swabs		**Comparator – EUA Authorized Molecular**		
		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

6) Clinical Validation for Testing of Asymptomatic Samples:

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 the FDA authorized EUA assay are shown in Tables 15-16. Positive and Negative Percent Agreement (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 15. Performance of the Color SARS-CoV-2 RT-LAMP Diagnostic Assay in comparison to an EUA Authorized Molecular Comparator Assay for Asymptomatic Samples

Asymptomatic Samples		EUA Authorized Molecular 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 16. Performance of the Color SARS-CoV-2 RT-LAMP Diagnostic Assay in comparison to an EUA Authorized Molecular Comparator Assay for Symptomatic Samples

Symptomatic Samples		EUA Authorized Molecular 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

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:

- For In Vitro Diagnostic Use
- For Use Under an Emergency Use Authorization (EUA) Only
- This product has not been FDA cleared or approved, but has been authorized by FDA under an Emergency Use Authorization (EUA) for use by Color Health, Inc., that is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets requirements to perform high complexity tests;
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; 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.

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded 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 17. 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

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