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

For *in vitro* diagnostic use
Rx only
For use under Emergency Use Authorization (EUA) only

(The Color SARS-CoV-2 LAMP Diagnostic Assay will be performed at Color Genomics, Inc., a Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a certified high-complexity laboratory, as per the Standard Operating Procedures that were reviewed by the FDA under this EUA.)

INTENDED USE

The Color SARS-CoV-2 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 nares 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.

This test is also for use with nasal swab specimens that are self-collected at home or in a healthcare setting by individuals using an authorized home-collection kit specified in this EUA's authorized labeling when determined to be appropriate by a healthcare provider.

Testing is limited to Color Genomics, 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 positive results to the appropriate public health authorities.

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

The Color SARS-CoV-2 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 LAMP Diagnostic Assay and the Color COVID-19

Test Unmonitored Collection Kit are only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

Color COVID-19 Test Unmonitored Collection Kit

The Color COVID-19 Test Unmonitored Collection Kit enables the self-collection of an anterior nasal swab sample that is transported in dry conditions in a sterile collection container to Color Genomics, Inc. (Color) for processing with the Color SARS-CoV-2 LAMP Diagnostic Assay when determined to be appropriate by a healthcare provider. Healthcare providers (HCP) at specific institutions use a COVID-19 eligibility questionnaire that is based on current CDC testing guidelines. Collection kits can be provided at a designated on-site collection location or can be ordered through Color's website. Results of the Color LAMP assay are communicated to the ordering physician. If the ordering physician directs Color to do so, patients will also receive a notification via email or text message containing a link to Color's online HIPAA-compliant post-test portal to access their results.

The Color COVID-19 Test Unmonitored Collection Kit consists of a sterile packaged spun polyester swab, collection tube, biohazard bag, cardboard box, barcode card, instructions for use, and a FedEx bag with a prepaid return label. Each Unmonitored Collection Kit is intended to be returned via 48-hour shipping (or same day shipping via a courier for those collections completed on-site) at ambient conditions. Specimens received for testing at Color will undergo review and accessioning prior to acceptance for testing with the Color SARS-CoV-2 LAMP Diagnostic Assay.

Color SARS-CoV-2 LAMP Diagnostic Assay

The Color SARS-CoV-2 LAMP Diagnostic Assay is a high-throughput, automated method utilizing loop-mediated isothermal amplification (LAMP) technology to detect SARS-CoV-2 viral RNA. The test uses three SARS-CoV-2 specific primer sets, designed to uniquely detect SARS-CoV-2 RNA.

RNA is isolated from upper respiratory specimens and BALs 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 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 LAMP procedure using four different primer sets; one targeting the SARS-CoV-2 N gene, one targeting the SARS-CoV-2 envelope gene (E), one targeting the SARS-CoV-2 ORF1a region, and one targeting 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 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 NEO microplate reader. Reactions displaying a color shift indicate that the target sequence is present.

INSTRUMENTS USED WITH TEST

The Color SARS-CoV-2 LAMP Diagnostic Assay 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

Materials Included in the Unmonitored Collection Kit

Shipping Box – Cardboard box
FedEx bag with prepaid return label
Specimen biohazard bag
Sterile packaged spun polyester swab
Sterile collection tube
Barcode card
Instructions for self-collection

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

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, and ORF1ab gene)	3126565	Integrated DNA Technologies

Reagent Manufacturer and Description	Catalog #	Manufacturer
Equipment		
100 mM dUTP	N0459B	New England Biolabs (NEB)
1U/uL UDG	M0372B	New England Biolabs (NEB)

COLLECTION KITS USED WITH THIS TEST

The Color SARS-CoV-2 LAMP Diagnostic Assay can be used with anterior nasal swabs (dry spun polyester swabs) collected using the Color COVID-19 Test Unmonitored Collection Kit.

MEDICAL OVERSIGHT AND PROCESS TO BE USED FOR UNMONITORED NASAL SWAB COLLECTION

On-Site Unmonitored Collection Workflow

1. At the physician's discretion, the patient or physician completes the eligibility questionnaire via the Color website (http://home.color.com/covid/check) which adheres to the CDC COVID-19 screening guidelines. A healthcare provider (HCP) at specific institutions authenticates the information and determines patient suitability for the unmonitored anterior nasal swab collection kit.
2. The patient collects their own anterior nasal sample following the instructions provided with the kit and returns the completed kit to the on-site collection bin.
3. All samples collected on-site are delivered to Color's laboratory within 72 hours for processing.
4. Test results are communicated back to the patient and the ordering physician. Results are returned electronically or by fax to the ordering provider. If the ordering physician directs Color to do so, patients will also receive a notification via email or text message containing a link to Color's online HIPAA-compliant post-test portal to access their results.
5. Results are automatically shared with local Department of Public Health registries.

At-Home Unmonitored Collection Workflow

1. At the physician's discretion, the patient completes the eligibility questionnaire via the Color website (http://home.color.com/covid/check) which adheres to the CDC COVID-19 screening guidelines. A healthcare provider (HCP) at specific institutions authenticates the information and determines patient suitability for the unmonitored anterior nasal swab collection kit.
2. If the patient is determined to be suitable to receive the unmonitored collection kit, the physician places the order for the patient via Color's HIPAA-compliant platform or by signed documentation from the ordering provider.
    a. The patient will be asked to provide their shipping details through Color's online portal.
3. Color will ship the unmonitored collection kit to the patient's home via 2-day shipping.
4. The patient collects their own anterior nasal swab sample following the instructions provided with the kit and ships the completed kit to Color's laboratory using a prepaid FedEx shipping pack.

5. Test results are communicated back to the patient and the ordering physician. Results are returned electronically or by fax to the ordering provider. If the ordering physician directs Color to do so, patients will also receive a notification via email or text message containing a link to Color's online HIPAA-compliant post-test portal to access their results.
6. Results are automatically shared with local Department of Public Health registries.

In addition, the Color Unmonitored Collection Kit will be provided to specific institutions to help reduce overhead and exposure to clinicians (e.g. state and local public health efforts), or institutions whose staff are at high risk of serving populations in congregate settings (e.g., skilled nursing and correctional facilities).

INSPECTION OF SPECIMENS

Sample Acceptance Criteria for Dry Swabs

In order for Color to perform testing, the samples shall 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
- Accession date is within 72 hours of the collection date/time

For dry swab samples, LIMS will check that the sample is approved by a physician, 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 LAMP DIAGNOSTIC ASSAY

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

- A positive control is used and consists of DNA/RNA Shield media 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 media. This control is processed through the entire end-to-end testing protocol.

The following additional controls are added into each LAMP plate:

- A positive control is used and consists of synthetic viral RNA (Twist Synthetic SARS-CoV-2 RNA Control 1 (MT007544.1) at 5X LoD.
- A no template control (NTC) is used and consists of nuclease-free water.
- An endogenous RNase P internal control should be present in each clinical sample.

INTERPRETATION OF RESULTS

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

1) COVID-19 Test Controls – Positive, Negative, and Internal:

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)

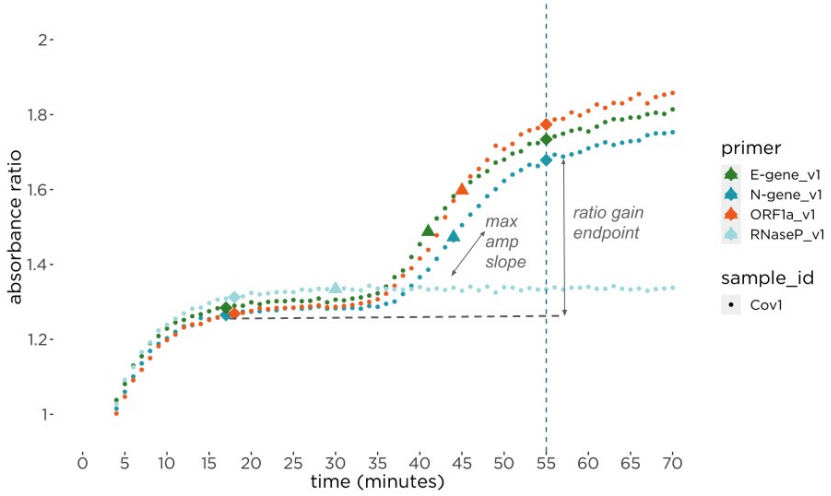

Table 1. Ratio Gain Interpretation for Each Primer

Gain in A430/A560 Ratio	Maximum Amplification Rate	Interpretation
≥ 0.15	any	Positive Signal
0.1 - 0.15	≥ 0.015	Positive Signal
0.1 - 0.15	< 0.015	Negative Signal
< 0.1	any	Negative Signal

Extraction Controls (See Table 2)

- The positive extraction control should exhibit positive signal for all three 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 should not produce positive signal for any SARS-CoV-2 targets or the internal RNase P target. Amplification would indicate that there was contamination during extraction and/or with the LAMP reagents.

LAMP Controls (See Table 2)

- The positive LAMP reaction control should show positive signal for all three SARS-CoV-2 specific targets and no signal for RNase P. A lack of amplification of the SARS-CoV-2 targets would indicate reagent or process failure during LAMP.
- The no template LAMP control should not produce positive signal for any of SARS-CoV-2 targets or the internal RNase P target.
- RNase P should yield positive signal in every clinical specimen in order 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 LAMP Diagnostic Assay

Control	N-gene	E-gene	ORF1ab	RNase P
Extraction Positive	Positive signal	Positive signal	Positive signal	Positive signal
Extraction NTC	Negative signal	Negative signal	Negative signal	Negative signal
LAMP Positive	Positive signal	Positive signal	Positive signal	Negative signal
LAMP NTC	Negative signal	Negative signal	Negative signal	Negative signal

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

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted. Please see the table below (Table 3) for guidance on interpretation and reporting of patient results.

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

N-gene	E-gene	ORF1ab	RNase P	Interpretation	Action
All SARS-CoV-2 targets = Positive			Positive	SARS-CoV-2 DETECTED	Report results to physician, patient, and appropriate public health authorities.
One or two SARS-CoV-2 target (s) = Positive			Positive	INCONCLUSIVE	Re-extract from residual sample, and repeat LAMP. If the repeated result remains inconclusive, report result to ordering physician and appropriate public health authorities. Report indicates that a new sample should be collected.
All SARS-CoV-2 targets = Negative			Positive	SARS-CoV-2 NOT-DETECTED	Report results to physician, patient, and appropriate public health authorities.
All SARS-CoV-2 targets = Negative or Positive			Negative	FAILED	Re-extract from residual sample and repeat LAMP. If result remains FAILED, report to ordering physician and appropriate public health authorities. Report indicates that a new sample should be collected.

All result interpretations will be reported to both the patient and the ordering physician. Results are returned electronically or by fax to the ordering provider. If the ordering physician directs Color to do so, patients will also receive a notification via email or text message containing a link to Color's online HIPAA-compliant post-test portal to access their results.

PERFORMANCE EVALUATION

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

1) Analytical Sensitivity:

Limit of Detection (LoD):

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 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 media. 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 SARS-CoV-2 Assay.

The initial LoD determination of the SARS-CoV-2 Assay was 0.5 copies/µL, which was the lowest concentration of SARS-CoV-2 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 spiked with DNA/RNA shield media at 1 copy/µL, 0.75 copies/µL, 0.5 copies/µL, and 0.25 copies/µL. Samples were spiked with RNA prior to extraction with the Chemagic 360 protocol and instrument. The LoD of the Color SARS-CoV-2 LAMP Diagnostic Assay was determined to be 0.75 copies/µL.

The results of the LoD confirmatory study are summarized below in Table 4.

Table 4. LoD Verification Study Results

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

2) Analytical Inclusivity/Specificity:

Inclusivity *In Silico* Analysis of LAMP Primer Sets:
An *in silico* inclusivity analysis was performed by aligning all primer sequences against SARS-CoV-2 sequences deposited at GISAID on April 2, 2020. This data set included 2,303 SARS-CoV-2 completed sequences that were annotated as high coverage. All three primer sets (N, E, ORF1ab) had a 100% match with the vast majority of COVID-19 strains: 97.3% for N-gene, 99.3% for E-gene and 99.0% for ORF1a. Due to the large number of mutations SARS-CoV-2 is undergoing, each primer set has 1 mismatch for 0.5-2.7% of the strains deposited in GISAID (Table 5). However, previous work on MERS-CoV has demonstrated that a single nucleotide mismatch in one of the primers typically has no impact on the limit of detection of LAMP assays (PMID 25103205).

Table 5. *In silico* Inclusivity Analysis

Characteristic	N-gene	E-gene	ORF1ab region
Total Primer Length (nt)	157	161	167
Total # of Strains Evaluated	2303	2303	2303
100% Match	2241	2286	2279
1 Mismatch	62	12	23

Characteristic	N-gene	E-gene	ORF1ab region
2 Mismatches	0	0	1
3 Mismatches	0	0	0
>3 Mismatches	0	5	0

One strain has 2 mismatches that overlap the binding site of the BIP primer of ORF1a. In addition, 5 strains have incompletely characterized regions of the E-gene that overlap the LAMP primers and therefore, it is uncertain if there is variation that could affect the binding of those specific LAMP primers. It is possible that multiple mismatches could impact the amplification yield of the E target, which could result in an "inconclusive" test result if the sample was truly positive. However, these strains have 100% matches with the E and ORF1ab primer sets targeting SARS-CoV-2.

Cross-Reactivity In Silico Analysis of 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 6 for the organisms assessed *in silico* for potential cross-reactivity to the SARS-CoV-2 LAMP Diagnostic Assay.

Table 6. Cross-Reactivity/Exclusivity *In Silico* Results

Virus	GenBank	N-gene	E-gene	ORF1ab
COVID-19	MN908947.3	100.0%	100.0%	100.0%
Human Coronavirus 229E	NC_002645.1	70.1%	72.0%	71.9%
Human Coronavirus OC43	NC_006213.1	73.2%	70.8%	75.4%
Human Coronavirus HKU1	NC_006577.2	72.0%	68.3%	72.5%
Human Coronavirus NL63	NC_005831.2	72.6%	70.8%	70.7%
SARS CoV	NC_004718.3	91.1%	93.2%	74.9%
MERS CoV	NC_019843.3	72.6%	72.0%	73.7%
Adenovirus, strain ad71	X67709.1	66.2%	63.4%	67.7%
Human Metapneumovirus	NC_039199.1	69.4%	71.4%	74.9%
Parainfluenza virus 1, strain Washington/1964	AF457102.1	72.0%	68.3%	68.9%
Parainfluenza virus 2, strain GREER	AF533012.1	68.8%	70.8%	70.7%
Parainfluenza virus 3, strain HPIV3/MEX/1526/2005	KF530234.1	70.7%	73.3%	72.5%
Parainfluenza virus 4, strain M-25	NC_021928.1	70.7%	68.9%	77.2%
Influenza A (H1N1)	FJ966079.1	66.2%	68.9%	70.7%
Influenza A (H3N2)	KT002533.1	65.6%	68.3%	67.1%
Influenza B (Victoria)	MN230203.1	70.7%	64.0%	65.3%
Influenza B (Yamagata)	MK715533.1	68.2%	67.7%	68.9%
Enterovirus D68 (EV-D68)	KP745766.1	72.0%	68.3%	70.7%
Respiratory syncytial virus	U39661.1	72.0%	71.4%	71.3%
Human rhinovirus 14	NC_001490.1	68.8%	70.8%	67.1%

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, but the ORF1a set matches only 75% in sequence. The likelihood of a false positive is low since amplification of all three SARS-CoV- 2 primer sets is required to interpret a test result as positive (See Table 3 above).

Cross-Reactivity Wet Testing:

In addition to the *in silico* analysis for cross-reactivity, wet testing was also performed to test cross-reactivity/exclusivity with other organisms. Samples were prepared by spiking (inactivated) purified, intact viral particles, cultured RNA, or bacterial cells using those panels/organisms shown in Table 7 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 8) indicating that the SARS-CoV-2 LAMP Diagnostic Assay is designed for the specific detection of SARS-CoV-2, with no expected cross reactivity to other coronaviruses, or human microflora that would predict potential false positive LAMP results.

Table 7. 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-CoV2 SARS	011N-03

Table 8. Cross-Reactivity/Exclusivity Wet Testing Results

Organism	Strain	N-gene Detected Replicates	E-gene Detected Replicates	ORF1ab Detected Replicates
Acinetobacter baumannii	307-0294	0/3	0/3	0/3
Adenovirus Type 3	N/A	0/3	0/3	0/3
Adenovirus Type 3	N/A	0/3	0/3	0/3
Chlamydia pneumoniae	CWL-029	0/3	0/3	0/3
Coronavirus 229E	N/A	0/3	0/3	0/3
Coronavirus NL63	N/A	0/3	0/3	0/3
Coronavirus OC43	N/A	0/3	0/3	0/3
Coronavirus SARS	N/A	0/3	0/3	0/3
Enterobacter cloacae	Z101	0/3	0/3	0/3
Escherichia coli	Z297	0/3	0/3	0/3

Organism	Strain	N-gene Detected Replicates	E-gene Detected Replicates	ORF1ab Detected Replicates
Enterovirus	N/A	0/3	0/3	0/3
Haemophilus influenzae	MinnA	0/3	0/3	0/3
HCoV-229E	N/A	0/3	0/3	0/3
HCoV-Nl63	N/A	0/3	0/3	0/3
HCoV-OC43	N/A	0/3	0/3	0/3
Human Metapneumovirus	N/A	0/3	0/3	0/3
Influenza A H1	N/A	0/3	0/3	0/3
Influenza A H1N1 (2009)	N/A	0/3	0/3	0/3
Influenza A H3	N/A	0/3	0/3	0/3
Influenza A H3	A/Brisbane/10/07	0/3	0/3	0/3
Influenza B	N/A	0/3	0/3	0/3
Influenza B	B/Florida/02/06	0/3	0/3	0/3
Klebsiella aerogenes	Z052	0/3	0/3	0/3
Klebsiella oxytoca	Z115	0/3	0/3	0/3
Klebsiella pneumoniae	KPC2	0/3	0/3	0/3
Klebsiella. pneumoniae	Z138; OXA-48	0/3	0/3	0/3
Klebsiella pneumoniae	Z460; NDM-1	0/3	0/3	0/3
Legionella pneumophila	Philadelphia	0/3	0/3	0/3
Moraxella catarrhalis	Ne 11	0/3	0/3	0/3
Mycoplasma pneumoniae	M129	0/3	0/3	0/3
MERS-CoV	N/A	0/3	0/3	0/3
Metapneumovirus 8	Peru6-2003	0/3	0/3	0/3
Pseudomonas aeruginosa	Z139, VIM-1	0/3	0/3	0/3
Proteus mirabilis	Z050	0/3	0/3	0/3
Parainfluenza virus Type 1	N/A	0/3	0/3	0/3
Parainfluenza virus Type 1	N/A	0/3	0/3	0/3
Parainfluenza virus Type 2	N/A	0/3	0/3	0/3
Parainfluenza virus Type 3	N/A	0/3	0/3	0/3
Respiratory Syncytial Virus A	N/A	0/3	0/3	0/3
Respiratory Syncytial Virus B	N/A	0/3	0/3	0/3
Rhinovirus 1A	N/A	0/3	0/3	0/3
Rhinovirus 1A	N/A	0/3	0/3	0/3
RSV A2	N/A	0/3	0/3	0/3
Streptococcus agalactiae	Z019	0/3	0/3	0/3
Staphylococcus aureus	MRSA, COL	0/3	0/3	0/3
Serratia marcescens	Z053	0/3	0/3	0/3
Streptococcus pneumoniae	Z022	0/3	0/3	0/3
Streptococcus pyogenes	Z018	0/3	0/3	0/3
SARS-CoV	N/A	0/3	0/3	0/3

3) Interfering Substances

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 substances tested inhibited or interfered with the performance of the SARS-CoV-2 LAMP Diagnostic Assay. Swabs both with and without the interfering substance yielded expected results (Table 9).

Table 9. 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)
		Negative	100% (3/3)

4) Clinical Evaluation:

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

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 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 loads: 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 10.

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 SARS-CoV-2 LAMP Diagnostic Assy. Results of the study are summarized in Table 13 below.

Table 10. Summary of Contrived Sample Testing

Concentration of SARS-CoV-2	Samples (n)	Detection Rate			
		N-gene	E-gene	ORF1ab	RNaseP
Negative	46	0/46	0/46	0/46	46/46
1X LoD (0.75 copies/µL)	10	10/10	10/10	10/10	10/10
1.5X LoD (1 copies/µL)	20	20/20	20/20	20/20	20/20
13X LoD (10 copies/µL)	10	10/10	10/10	10/10	10/10
133X LoD (100 copies/µL)	6	6/6	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.

Clinical Study with Previously Confirmed Positive and Negative Samples:

In addition to the contrived clinical study, a total of 543 patient samples (41 positive, 502 negative), were processed through the LAMP Diagnostic Assay and compared against results generated by the CDC EUA authorized assay performed at two different locations:

1) Clinical Research Sequencing Platform (CRSP, Boston)
2) CZI (Chan Zuckerberg Initiative) BioHub (University of California San Francisco)

The composition of the 2 cohorts of samples included the following:

- 509 nasopharyngeal swabs collected by healthcare providers at a San Francisco testing site from patients seeking SARS-CoV-2 testing over a period of approximately 2 weeks and previously tested at CRSP using an implementation of the CDC 2019-nCoV Realtime PCR Test. This cohort contained 7 positive samples and 502 negative samples. All results generated by the Color SARS-CoV-2 LAMP Diagnostic Assay matched those generated by CRSP.
- 34 positive nasopharyngeal swab samples were collected by the University of California, San Francisco and previously tested at the CZI BioHub which uses the CDC 2019-nCoV Realtime PCR Test. All results generated by the Color SARS-CoV-2 LAMP Diagnostic Assay matched those generated by CZI BioHub.

Positive percent agreement (PPA) and negative percent agreement (NPA) were determined by comparing observed results generated by the Color SARS-CoV-2 LAMP Diagnostic Assay with the CDC EUA authorized assay results (Table 11).

Table 11. Performance of Nasopharyngeal Swabs when Compared to the CDC EUA Authorized Assay

Nasopharyngeal Swabs		Comparator Assay (CDC EUA)		
		Positive	**Negative**	**Total**
Color SARS-CoV-2 LAMP Diagnostic Assay Result	**Positive**	41	0	41
	Negative	0	502	502
	Total	41	502	543
Positive Agreement		100.0% (41/41); 91.44% - 100.00%*		
Negative Agreement		100.0% (502/502); 99.24% - 100.00%*		

*95% Confidence intervals

Performance Evaluation for Color COVID-19 Test Unmonitored Collection Kit

1) **Simulated Sample Stability/Shipping Studies:**
Shipping stability of dry spun polyester swabs has been demonstrated by Quantigen Biosciences with support from The Gates Foundation and UnitedHealth Group. The Quantigen study demonstrated 72-hour stability for dry anterior nasal spun polyester swabs. Quantigen Biosciences has granted a right of reference to the stability data to any sponsor, such as Color Genomics pursing an EUA for which a claimed specimen type is dry spun polyester swabs. Therefore, the stability of anterior nasal samples collected using dry spun polyester swabs were not evaluated in the sample stability study.

2) **Dry Swab Resuspension:**
To demonstrate that dry spun polyester swabs were acceptable specimen types for testing with the Color SARS-CoV-2 LAMP Diagnostic Assay, performance of the assay was evaluated using dry swabs resuspended in 1mL 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 from ZeptoMetrix (isolate USAWA1/2020, Cat # 0810587CFHI) into DNA/RNA Shield (Zymo Research, Cat # R1100-250) containing negative clinical anterior nasalswab 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-only DNA/RNA Shield media and lysis buffer). Results are summarized in Table 12. There was 100% agreement with expected results for all positive contrived samples for both swab types. All negative samples were non-reactive for SARS-CoV-2 assay targets for both swab types.

Table 12. Dry Swab Resuspension Study Results Stratified by Assay Target

Swab Type	Concentration	Samples (n)	Detection Rate			
			N-gene	E-gene	ORF1ab	RNase P
Spun Polyester	2X LoD (1.5 copies/µL)	5	5/5	5/5	5/5	5/5
	5X LoD (3.75 copies/µL)	5	5/5	5/5	5/5	5/5
	Negative	5	0/5	0/5	0/5	5/5

3) <u>Self-Collection Validation:</u>

A usability study was conducted to assess user comprehension of the Color COVID-19 Test Unmonitored Collection Kit including both collection and packaging the dry nasal swab for shipment. A demographic question was administered as part of the screening questionnaire to ensure recruitment of a user cohort reflective (or as closely as feasible) to that of the 2019 US population. Participants were also recruited to reflect a variety of ages and education levels, including participants with no high school diploma or equivalent, high school diploma or equivalent, and with higher education. Other demographics including race, marital status, employment status, and geographic location were also documented.

The interviewer observed the participant using the collection kit through videoconferencing with the participant in their home environment. A total of 38 adults completed the study of which 44.7% were ≥51 years of age, 5.3% were between 41 - 50 years, and 26.3% were between 31 - 40 years old, 23.7% were between 18-30; 57.9% of participants were female and 42.1% were male.

Of the 38 kits that were shipped to study participants for self-collection, 37/38 (97%) of the sample kits were received and processed through the Color SARS-CoV-2 LAMP Diagnostic Assay. Of those collection kits received at Color, RNaseP was detected in 35/37 (95%) of samples, indicating successful collection of human biological material that was extracted and amplified. For the two nasal swab samples

that were negative for RNase P, the samples were received past the acceptable shipping stability/accession date of 72 hours (e.g., 6- and 7-days post-collection).

During the actual use testing, staff observed users following the instructions included with the collection kit; however, some participants had difficulty with preparing the biohazard bag. Despite this challenge, no deviations from the Instructions for Use were noted by staff observing the sample collection and furthermore, this task did not affect the ability to properly receive and process the sample with the LAMP assay. As noted previously, one participant did not ship the collected sample to the laboratory for processing, possibly indicating that the participant did not completely understand the process for shipping the collected sample. Answers to the user 10-item questionnaire were also collected for the 37 sample kits that were received at Color for processing. 37/37 participants successfully answered all questions and noted agree/strongly agree for understanding the instructions and finding them easy to follow and locate within the kit. Based on the usability study data and feedback, the collection instructions were understandable, and the kit was easy to use.

Results of the usability testing were analyzed qualitatively to determine if the design of the kit and/or kit instructions need to be modified to reduce the use-related risks to acceptable levels. Cognitive debriefing interviews were conducted following the actual-use testing to gather users' perspectives on each critical task or use scenario. Even though Color staff observed some user's difficulty with placing the collected specimen into the biohazard bag for shipping, the users did not mention or discuss this scenario during the post-collection interview. No changes or modifications to the current instructions needed to be made based in discussions with the participants.

4) **Additional Requirement:**
In addition to validation studies, Color will submit a report to the FDA (within 30 days of authorization) summarizing any testing performed with the Color COVID-19 Test Unmonitored Collection Kit including how many kits were requested, sent for home collection, or used at a collection site or institution. Color will also document the number of kits that were shipped and returned to the laboratory according to the kit instructions, how many specimens were rejected during accessioning and the reasons for rejection, and the positivity rate of the first Color COVID-19 Test Unmonitored Collection Kit lot.

LIMITATION:

- 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.

WARNINGS:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by the authorized laboratory;
- This test has been authorized only for the detection of nucleic acid from SARS CoV-2, not for any other viruses or pathogens; and
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.