# 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 Genomics, 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 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 dry nasal swab specimens that are self-collected unsupervised at home or in a healthcare setting, by individuals using the Color COVID-19 Self-Swab Collection Kit when determined to be appropriate by a healthcare provider based on results of a COVID-19 medical questionnaire.

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 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.

## DEVICE DESCRIPTION AND TEST PRINCIPLE

### 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 viral RNA. The test uses two 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 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; one targeting the SARS-CoV-2 N gene, one targeting the SARS-CoV-2 envelope gene (E), 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 RT-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

### 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 |

| | | |
|---|---|---|
| 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 |
| 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 nasal swabs (dry spun polyester swabs) collected using the Color COVID-19 Self-Swab Collection Kit manufactured by Color Genomics, Inc.

## 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 56 hours from the collection date/time, as described in the Color COVID-19 Self-Swab Collection Kit EUA Summary

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 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 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.
    - Depending upon the results of the Extraction NTC, the Control Confirmation Sample (LAMP NTC) will be examined.

**RNase P Endogenous Human Specimen Control**

- 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) Color SARS-CoV-2 RT-LAMP Assay Controls – Extraction Positive, Extraction Negative, and Internal RNase P:

**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)**

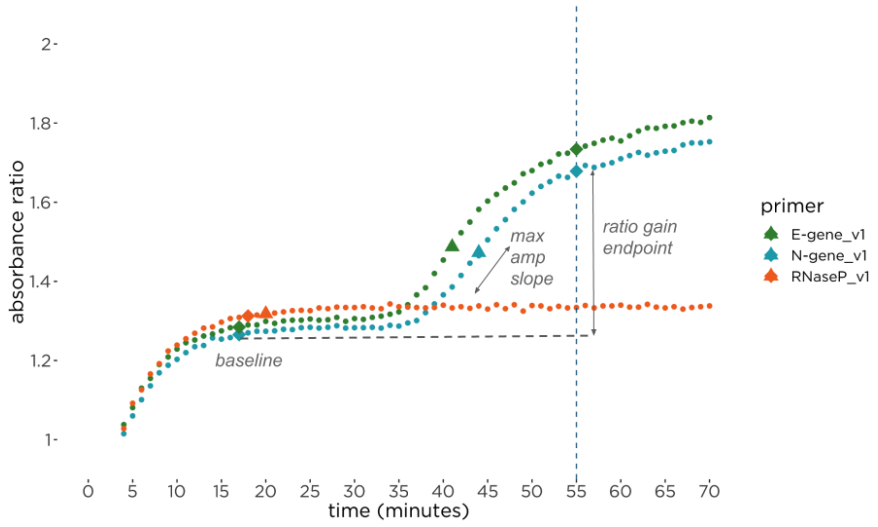


**Table 1. Ratio Gain Interpretation for Each Primer**

| 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 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 must be negative for all 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.
    - If a single viral primer is positive in the Extraction NTC, the results of the Control Confirmation Sample (LAMP NTC) must be considered. If the same viral primer is negative in the Control Confirmation Sample, it suggests random amplification rather than contamination. The results for the NTC are acceptable and the batch results can be reported. If the same viral primer 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 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 RT-LAMP Diagnostic Assay**

| 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 is positive in the NTC, the Control Confirmation Sample (LAMP NTC) should be considered. If the same viral primer is negative in the Control Confirmation Sample, it suggests random amplification rather than contamination. The results for the NTC are acceptable and the batch results can be reported. If the same viral primer is positive in the Control Confirmation Sample, it suggests systemic contamination and the batch is considered failed.

**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.

- 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**

| N-gene | E-gene | RNase P | Interpretation | Action |
|---|---|---|---|---|
| Both SARS-CoV-2 targets = Positive | | Positive | SARS-CoV-2 DETECTED | Report results to physician, patient, and appropriate public health authorities. |

| N-gene | E-gene | RNase P | Interpretation | Action |
|---|---|---|---|---|
| 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 ordering physician and appropriate public health authorities. Report indicates that a new sample should be collected. |
| Both SARS-CoV-2 targets = Negative | | Positive | SARS-CoV-2 NOT-DETECTED | Report results to physician, patient, and appropriate public health authorities. |
| Both 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. |

## PERFORMANCE EVALUATION

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

The initial LoD determination of the Color SARS-CoV-2 RT-LAMP 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 media 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**

| 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 |

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. 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 that had been processed by the Color SARS-CoV-2 RT-LAMP 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 was 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 repeated 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 repeated 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 reporting algorithm.

**2) <u>Dry Swab Resuspension:</u>**

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 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 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-only DNA/RNA Shield media and lysis buffer). Results are summarized in Table 6. 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 6. 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:

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. Both primer sets (N, E) had a 100% match with the vast majority of COVID-19 strains: 97.3% for N-gene and 99.3% for E-gene. 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 7). 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 7. *In silico* Inclusivity Analysis**

| Characteristic | N-gene | E-gene |
|---|---|---|
| **Total Primer Length (nt)** | 157 | 161 |
| **Total # of Strains Evaluated** | 2303 | 2303 |
| **100% Match** | 2241 | 2286 |
| **1 Mismatch** | 62 | 12 |
| **2 Mismatches** | 0 | 0 |
| **3 Mismatches** | 0 | 0 |
| **>3 Mismatches** | 0 | 5 |

Five 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 primer set 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 8 for the organisms assessed *in silico* for potential cross-reactivity to the Color SARS-CoV-2 RT-LAMP Diagnostic Assay.

**Table 8. 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.

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, cultured RNA, or bacterial cells using those panels/organisms shown in Table 9 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 10) 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 that would predict potential false positive LAMP results.

**Table 9. Panels of Organisms Used to Assess Potential Assay Cross-Reactivity Via Wet Testing**

| Vendor | Product | Catalog/Lot |
|---|---|---|
| ZeptoMetrix | NATtrol Pneumonia Panel - Quantifiable Bacteria<br>(no quantification information available) | Ref: NATPPQ-BIO<br>Lot: 323679 |
| ZeptoMetrix | NATtrol Respiratory Validation Panel 3<br>(no quantification information available) | Ref: NATRVP-3<br>Lot: 323354 |
| ZeptoMetrix | NATtrol Pneumonia Panel - Atypical Bacteria & Viruses<br>(no quantification information available) | Ref: NAPPA-BIO<br>Lot: 322617 |
| BEI Resources | HCoV-229E<br>HCov-NL63<br>MERS-CoV<br>HCov-OC43<br>SARS-CoV2<br>SARS | 011N-03 |

**Table 10. 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 |

| Organism | Strain | N-gene Detected Replicates | E-gene Detected Replicates |
|---|---|---|---|
| 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) 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 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 11).

**Table 11. 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) |

| Substance | Active Ingredient | Concentration | % Agreement with Expected Results |
|---|---|---|---|
| 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) |

**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.

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 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 12.

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 12 below.

**Table 12. Summary of Contrived Sample Testing**

| Concentration of SARS-CoV-2 | Samples (n) | Detection Rate | | |
|---|---|---|---|---|
| | | N-gene | E-gene | RNaseP |

| | | | | |
|---|---|---|---|---|
| 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.

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 the EUA authorized assay at the Clinical Research Sequencing Platform (CRSP, Boston, MA). 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 previously tested at CRSP using an implementation of the CDC 2019-nCoV Realtime PCR Test. All results generated by the Color SARS-CoV-2 RT-LAMP Diagnostic Assay were concordant with the CRSP EUA authorized assay.

Positive percent agreement (PPA) and negative percent agreement (NPA) were determined by comparing observed results generated by the Color SARS-CoV-2 RT-LAMP Diagnostic Assay with the EUA authorized assay results (Table 13).

**Table 13. Performance of Nasopharyngeal Swabs when Compared to an EUA Authorized Assay**

| **Nasopharyngeal Swabs** | | **Comparator – EUA Authorized 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%* | | |
| **Negative Percent Agreement** | | 100.0% (502/502); 99.24% - 100.00%* | | |

*Two-sided 95% confidence intervals

**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 Color Genomics, Inc., located at 863 Mitten Road, Burlingame, CA 94010;
- 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 Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or 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 14. Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Pane l***

| Reference Materials Provide d by FDA | Spe cimen Type | Product LoD | Cross-Reactivity |
|---|---|---|---|
| SARS-CoV-2 | Anterior Nares | $1.8x10^4$ 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