ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY FOR THE COLOR GENOMICS 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. certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, as per the Instructions for Use 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 wash/aspirate or nasal aspirates, as well as bronchoalveolar lavage specimens collected from individuals suspected of COVID-19 by a healthcare provider. Testing is limited to Color Genomics, Inc., Burlingame, CA, that is a certified high-complexity laboratory under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a.

Results are for the detection and identification of SARS-CoV-2 RNA. 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.

Testing with the Color SARS-CoV-2 LAMP Diagnostic Assay is intended for use by qualified laboratory personnel specifically instructed and trained on in vitro diagnostic procedures. The assay is intended only for use under the Food and Drug Administration’s Emergency Use Authorization.
DEVICE DESCRIPTION AND TEST PRINCIPLE

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 dNTP's 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 SARAS-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

<table>
<thead>
<tr>
<th>Reagent Manufacturer and Description</th>
<th>Catalog #</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equipment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamilton STAR, STARlet</td>
<td>STAR, STARlet</td>
<td>Hamilton</td>
</tr>
<tr>
<td>Chemagic Instrument</td>
<td>Chemagic 360</td>
<td>Perkin Elmer</td>
</tr>
<tr>
<td>Microplate Reader</td>
<td>Neo2S</td>
<td>Biotek</td>
</tr>
<tr>
<td>Heat Sealer</td>
<td>PX1 PCR Plate Sealer, PlateLoc, or equivalent</td>
<td>Biotek, Agilent, or equivalent</td>
</tr>
<tr>
<td>Xpeel Plate Peeler</td>
<td>XP-A</td>
<td>Nexus Biosystems</td>
</tr>
<tr>
<td>MultiFloFX Multi-Mode Dispenser</td>
<td>MFXP1</td>
<td>Biotek</td>
</tr>
<tr>
<td>Consumables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foil Seal</td>
<td>0030127790</td>
<td>Eppendorf</td>
</tr>
<tr>
<td>PlateLoc Seal, clear, permanent</td>
<td>24212-001</td>
<td>Agilent</td>
</tr>
</tbody>
</table>
CONTROLS TO BE USED WITH THE COVID-19 RT-PCR

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.

Two 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)**

![Representative LAMP data from a synthetic positive control](image)

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

<table>
<thead>
<tr>
<th>Gain in A430/A560 Ratio</th>
<th>Maximum Amplification Rate</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 0.15</td>
<td>any</td>
<td>Positive Signal</td>
</tr>
<tr>
<td>0.1 - 0.15</td>
<td>≥ 0.015</td>
<td>Positive Signal</td>
</tr>
<tr>
<td>0.1 - 0.15</td>
<td>&lt; 0.015</td>
<td>Negative Signal</td>
</tr>
<tr>
<td>&lt; 0.1</td>
<td>any</td>
<td>Negative Signal</td>
</tr>
</tbody>
</table>

**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.

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

<table>
<thead>
<tr>
<th>Control</th>
<th>N-gene</th>
<th>E-gene</th>
<th>ORF1a</th>
<th>RNase P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraction Positive</td>
<td>Positive signal</td>
<td>Positive signal</td>
<td>Positive signal</td>
<td>Positive signal</td>
</tr>
<tr>
<td>Extraction NTC</td>
<td>Negative signal</td>
<td>Negative signal</td>
<td>Negative signal</td>
<td>Negative signal</td>
</tr>
<tr>
<td>LAMP Positive</td>
<td>Positive signal</td>
<td>Positive signal</td>
<td>Positive signal</td>
<td>Negative signal</td>
</tr>
<tr>
<td>LAMP NTC</td>
<td>Negative signal</td>
<td>Negative signal</td>
<td>Negative signal</td>
<td>Negative signal</td>
</tr>
</tbody>
</table>

### 2) Examination and Interpretation of Patient Specimen Results:
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

<table>
<thead>
<tr>
<th>N-gene</th>
<th>E-gene</th>
<th>ORF1a</th>
<th>RNase P</th>
<th>Interpretation</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>All SARS-CoV-2 targets = Positive</td>
<td></td>
<td>Positive</td>
<td></td>
<td>SARS-CoV-2 DETECTED</td>
<td>Report results to physician, patient, and appropriate public health authorities.</td>
</tr>
<tr>
<td>One or two SARS-CoV-2 target(s) = Positive</td>
<td></td>
<td>Positive</td>
<td></td>
<td>INCONCLUSIVE</td>
<td>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.</td>
</tr>
</tbody>
</table>
PERFORMANCE EVALUATION

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 nares 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 nares 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

<table>
<thead>
<tr>
<th>Concentration (copies/µL in primary sample)</th>
<th>N-gene replicates detected</th>
<th>E-gene replicates detected</th>
<th>ORF1a replicates detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 copy/µL</td>
<td>20/20</td>
<td>20/20</td>
<td>20/20</td>
</tr>
<tr>
<td>0.75 copies/µL</td>
<td>20/20</td>
<td>20/20</td>
<td>20/20</td>
</tr>
<tr>
<td>0.5 copies/µL</td>
<td>20/20</td>
<td>18/20</td>
<td>19/20</td>
</tr>
<tr>
<td>0.25 copies/µL</td>
<td>17/20</td>
<td>10/20</td>
<td>14/20</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Total Primer Length (nt)</th>
<th>N-gene</th>
<th>E-gene</th>
<th>ORF1ab region</th>
</tr>
</thead>
<tbody>
<tr>
<td>157</td>
<td>161</td>
<td>167</td>
<td></td>
</tr>
<tr>
<td>2303</td>
<td>2303</td>
<td>2303</td>
<td></td>
</tr>
<tr>
<td>2241</td>
<td>2286</td>
<td>2279</td>
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<tr>
<td>62</td>
<td>12</td>
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<td>0</td>
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<td>0</td>
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</tr>
<tr>
<td>0</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>&gt;3 Mismatches</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Virus</th>
<th>GenBank</th>
<th>N-gene</th>
<th>E-gene</th>
<th>ORF1a</th>
</tr>
</thead>
<tbody>
<tr>
<td>COVID-19</td>
<td>MN908947.3</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Human Coronavirus 229E</td>
<td>NC_002645.1</td>
<td>70.1%</td>
<td>72.0%</td>
<td>71.9%</td>
</tr>
<tr>
<td>Human Coronavirus OC43</td>
<td>NC_006213.1</td>
<td>73.2%</td>
<td>70.8%</td>
<td>75.4%</td>
</tr>
<tr>
<td>Human Coronavirus HKU1</td>
<td>NC_006577.2</td>
<td>72.0%</td>
<td>68.3%</td>
<td>72.5%</td>
</tr>
<tr>
<td>Human Coronavirus NL63</td>
<td>NC_005831.2</td>
<td>72.6%</td>
<td>70.8%</td>
<td>70.7%</td>
</tr>
<tr>
<td>SARS CoV</td>
<td>NC_004718.3</td>
<td>91.1%</td>
<td>93.2%</td>
<td>74.9%</td>
</tr>
<tr>
<td>MERS CoV</td>
<td>NC_019843.3</td>
<td>72.6%</td>
<td>72.0%</td>
<td>73.7%</td>
</tr>
<tr>
<td>Adenovirus, strain ad71</td>
<td>X67709.1</td>
<td>66.2%</td>
<td>63.4%</td>
<td>67.7%</td>
</tr>
<tr>
<td>Human Metapneumovirus</td>
<td>NC_039199.1</td>
<td>69.4%</td>
<td>71.4%</td>
<td>74.9%</td>
</tr>
<tr>
<td>Parainfluenza virus 1, strain</td>
<td>AF457102.1</td>
<td>72.0%</td>
<td>68.3%</td>
<td>68.9%</td>
</tr>
<tr>
<td>Washington/1964</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parainfluenza virus 2, strain GREER</td>
<td>AF533012.1</td>
<td>68.8%</td>
<td>70.8%</td>
<td>70.7%</td>
</tr>
<tr>
<td>Parainfluenza virus 3, strain</td>
<td>KF530234.1</td>
<td>70.7%</td>
<td>73.3%</td>
<td>72.5%</td>
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<tr>
<td>HPIV3/MEX/1526/2005</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parainfluenza virus 4, strain M-25</td>
<td>NC_021928.1</td>
<td>70.7%</td>
<td>68.9%</td>
<td>77.2%</td>
</tr>
<tr>
<td>Influenza A (H1N1)</td>
<td>FJ966079.1</td>
<td>66.2%</td>
<td>68.9%</td>
<td>70.7%</td>
</tr>
<tr>
<td>Influenza A (H3N2)</td>
<td>KT002533.1</td>
<td>65.6%</td>
<td>68.3%</td>
<td>67.1%</td>
</tr>
<tr>
<td>Influenza B (Victoria)</td>
<td>MN230203.1</td>
<td>70.7%</td>
<td>64.0%</td>
<td>65.3%</td>
</tr>
<tr>
<td>Influenza B (Yamagata)</td>
<td>MK715533.1</td>
<td>68.2%</td>
<td>67.7%</td>
<td>68.9%</td>
</tr>
<tr>
<td>Enterovirus D68 (EV-D68)</td>
<td>KP745766.1</td>
<td>72.0%</td>
<td>68.3%</td>
<td>70.7%</td>
</tr>
<tr>
<td>Respiratory syncytial virus</td>
<td>U39661.1</td>
<td>72.0%</td>
<td>71.4%</td>
<td>71.3%</td>
</tr>
<tr>
<td>Human rhinovirus 14</td>
<td>NC_001490.1</td>
<td>68.8%</td>
<td>70.8%</td>
<td>67.1%</td>
</tr>
</tbody>
</table>

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

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

Table 8. Cross-Reactivity/Exclusivity Wet Testing Results

<table>
<thead>
<tr>
<th>Organism</th>
<th>Strain</th>
<th>N-gene Detected Replicates</th>
<th>E-gene Detected Replicates</th>
<th>ORF1ab Detected Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter baumannii</td>
<td>307-0294</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Adenovirus Type 3</td>
<td>N/A</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Adenovirus Type 3</td>
<td>N/A</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Chlamydia pneumoniae</td>
<td>CWL-029</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Coronavirus 229E</td>
<td>N/A</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Coronavirus NL63</td>
<td>N/A</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Coronavirus OC43</td>
<td>N/A</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Coronavirus SARS</td>
<td>N/A</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>Z101</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Z297</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Enterovirus</td>
<td>N/A</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>MinnA</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>HCoV-229E</td>
<td>N/A</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>HCoV-NL63</td>
<td>N/A</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>HCoV-OC43</td>
<td>N/A</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Human Metapneumovirus</td>
<td>N/A</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Influenza A H1</td>
<td>N/A</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Influenza A H1N1 (2009)</td>
<td>N/A</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Influenza A H3</td>
<td>N/A</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Influenza A H3</td>
<td>A/Brisbane/10/07</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Influenza B</td>
<td>N/A</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Influenza B</td>
<td>B/Florida/02/06</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Klebsiella aerogenes</td>
<td>Z052</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>Z115</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>KPC2</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Klebsiella. pneumoniae</td>
<td>Z138; OXA-48</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>Z460; NDM-1</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Legionella pneumophila</td>
<td>Philadelphia</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Moraxella catarrhalis</td>
<td>Ne 11</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Mycoplasma pneumoniae</td>
<td>M129</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>MERS-CoV</td>
<td>N/A</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
</tbody>
</table>
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 nares 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 nares swabs were collected in triplicate and spiked with synthetic COVID-19 RNA (Twist Synthetic SARS-CoV-2 RNA Control) at 5X LoD. 100µL 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

<table>
<thead>
<tr>
<th>Substance</th>
<th>Active Ingredient</th>
<th>Concentration</th>
<th>% Agreement with Expected Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Blood</td>
<td>N/A</td>
<td>5X LoD, Negative</td>
<td>100% (3/3)</td>
</tr>
<tr>
<td>Mucin</td>
<td>N/A</td>
<td>5X LoD, Negative</td>
<td>100% (3/3)</td>
</tr>
<tr>
<td>Tobacco</td>
<td>Nicotine, Tar, Carbon Monoxide, Formaldehyde, Ammonia, Hydrogen Cyanide, Arsenic, and DDT</td>
<td>5X LoD, Negative</td>
<td>100% (3/3)</td>
</tr>
</tbody>
</table>
### 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 nares 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 10 below.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Active Ingredient</th>
<th>Concentration</th>
<th>% Agreement with Expected Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marijuana</td>
<td>Cannabinoids, THC, CBD</td>
<td>5X LoD</td>
<td>100% (3/3)</td>
</tr>
<tr>
<td>Alcohol</td>
<td>Ethanol</td>
<td>5X LoD</td>
<td>100% (3/3)</td>
</tr>
<tr>
<td>Vaseline</td>
<td>Petroleum Jelly</td>
<td>5X LoD</td>
<td>100% (3/3)</td>
</tr>
<tr>
<td>Nasal allergy spray</td>
<td>Triamcinolone acetonide</td>
<td>5X LoD</td>
<td>100% (3/3)</td>
</tr>
<tr>
<td>Nasal congestion spray</td>
<td>Oxymetazoline HCl</td>
<td>5X LoD</td>
<td>100% (3/3)</td>
</tr>
<tr>
<td>Nyquil</td>
<td>Acetaminophen, Doxylamine succinate, Dextromethorphan HBr</td>
<td>5X LoD</td>
<td>100% (3/3)</td>
</tr>
<tr>
<td>Flonase</td>
<td>Fluticasone propionate</td>
<td>5X LoD</td>
<td>100% (3/3)</td>
</tr>
<tr>
<td>Emergen-C</td>
<td>Zinc, Magnesium, Riboflavin, Vitamin C</td>
<td>5X LoD</td>
<td>100% (3/3)</td>
</tr>
<tr>
<td>Saline nasal spray</td>
<td>NaCl, Phenylcarbinol, Nemalkonium Chloride</td>
<td>5X LoD</td>
<td>100% (3/3)</td>
</tr>
<tr>
<td>Act dry mouth lozenges</td>
<td>Isomalt, xylitol, Glycerin</td>
<td>5X LoD</td>
<td>100% (3/3)</td>
</tr>
<tr>
<td>Listerine mouthwash</td>
<td>Eucalyptol, menthol, Methyl Salicylate, Thymol</td>
<td>5X LoD</td>
<td>100% (3/3)</td>
</tr>
<tr>
<td>Sore throat and cough lozenges</td>
<td>Benzocaine, Dextromethorphan HBr</td>
<td>5X LoD</td>
<td>100% (3/3)</td>
</tr>
<tr>
<td>Zinc</td>
<td>Zinc</td>
<td>5X LoD</td>
<td>100% (3/3)</td>
</tr>
<tr>
<td>Chloraseptic spray</td>
<td>Phenol, Glycerin</td>
<td>5X LoD</td>
<td>100% (3/3)</td>
</tr>
</tbody>
</table>

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.
Table 10. Summary of Contrived Sample Testing

<table>
<thead>
<tr>
<th>Concentration of SARS-CoV-2</th>
<th>Samples (n)</th>
<th>Detection Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N-gene</td>
<td>E-gene</td>
</tr>
<tr>
<td>Negative</td>
<td>46</td>
<td>0/46</td>
</tr>
<tr>
<td>1X LoD (0.75 copies/µL)</td>
<td>10</td>
<td>10/10</td>
</tr>
<tr>
<td>1.5X LoD (1 copies/µL)</td>
<td>20</td>
<td>20/20</td>
</tr>
<tr>
<td>13X LoD (10 copies/µL)</td>
<td>10</td>
<td>10/10</td>
</tr>
<tr>
<td>133X LoD (100 copies/µL)</td>
<td>6</td>
<td>6/6</td>
</tr>
</tbody>
</table>

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. CRSP uses 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

<table>
<thead>
<tr>
<th>Nasopharyngeal Swabs</th>
<th>Comparator Assay (CDC EUA)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positve</td>
<td>Negative</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Color LAMP Diagnostic Assay Result</td>
<td>41</td>
<td>0</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>502</td>
<td>502</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>502</td>
<td>543</td>
<td></td>
</tr>
</tbody>
</table>

Positive Agreement 100.0% (41/41); 91.44% - 100.00%

Negative Agreement 100.0% (502/502); 99.24% - 100.00%

*95% Confidence intervals