color

Diagnostic Testing

Version 1.2 – Updated 05.20.20

Executive Summary

Diagnostic testing identifies the presence of the SARS-CoV-2 virus during infection.

The viral RNA is detected using nucleic acid amplification. There are currently two nucleic acid amplification testing methods with Food and Drug Administration (FDA) approval for diagnostic testing: real time reverse transcription polymerase chain reaction (RT-PCR) and loop-mediated isothermal amplification (LAMP).

Color's SARS-CoV-2 assay utilizes LAMP technology to detect the presence of the virus in a patient sample.

The Color LAMP assay showed 100% positive predictive agreement and negative predictive agreement with established RT-PCR assays across 543 clinical samples.

AT Assay Technology

Diagnostic nucleic acid amplification testing (NAAT) relies on amplification of the viral RNA genome. Briefly, RNA is reverse transcribed into DNA, and then specific regions of the genome are targeted and amplified. Currently most diagnostic assays use real time reverse transcription polymerase chain reaction (RT-PCR), however, the Color assay uses loop-mediated isothermal amplification (LAMP).

LAMP demonstrates similar validity as RT-PCR ***without***

1. the need for reagents and supplies that are currently in high demand
2. the PCR infrastructure required to accommodate high sample throughput [1]

RT-PCR

In the United States, the first assay available to detect the presence of SARS-CoV-2 viral RNA, which causes the disease COVID-19, was an RT-PCR assay developed by the Centers for Disease Control and Prevention (CDC). This assay uses a set of primers and probes specific for the amplicons generated during PCR. These probes have a 3` quencher that, due to its proximity (~ 20 base pairs or less) to the 5` fluorophore on each probe, prevents fluorescence when excited by the appropriate wavelength of light. During PCR, the endonuclease activity of the polymerase cleaves the fluorophore from the probe, separating it from the quencher, thus making the light emitted from the excited fluorophore detectable. After each cycle of PCR, the amount of newly generated template is estimated by the change in light intensity emitted by the reaction.

LAMP

Color's SARS-CoV-2 assay is a high-throughput, automated method utilizing colorimetric LAMP technology to detect SARS-CoV-2 viral RNA. LAMP is similar to RT-PCR in that it detects the presence of viral RNA but there are a few key differences. Unlike RT-PCR, LAMP occurs at a single temperature and thus does not require sophisticated thermocycling instrumentation. Instead, isothermal amplification is carried out through a set of primers that create loop-structures for self-priming exponential amplification.

Figure 1:
LAMP plate after incubation. The color change of the pH sensitive dye from red to yellow indicates amplification of the target.

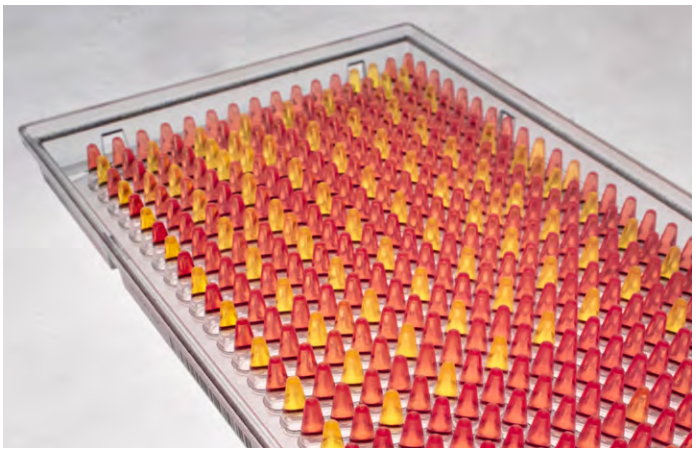
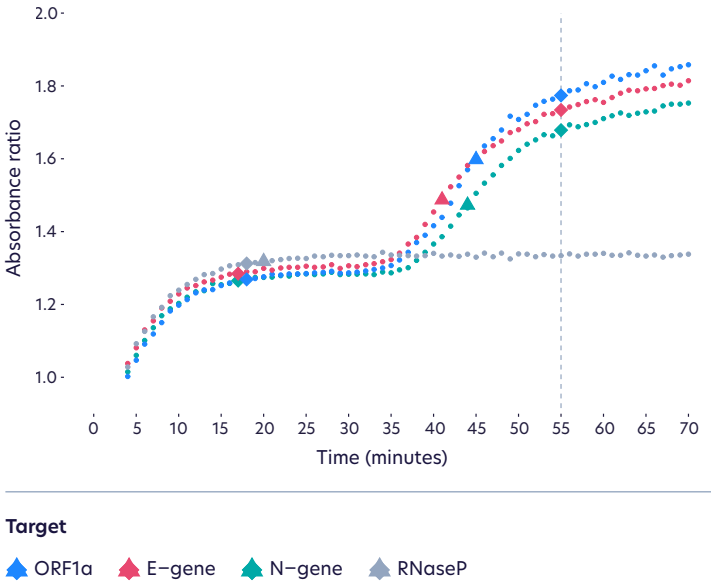


Figure 2:
An example trace of the absorbance ratio change during LAMP for a positive control sample.



The Color LAMP assay uses three SARS-CoV-2 specific primer sets, designed to uniquely detect SARS-CoV-2 viral RNA. The first primer set targets the SARS-CoV-2 nucleocapsid gene (N), the second primer set targets the envelope gene (E), and the third primer set targets the ORF1a region. In addition, a fourth primer set that targets the human RNaseP (RP) transcript is used as an internal control. The Color assay uses LAMP chemistry modified from New England BioLabs system (NEB, E1700). Primer extension by the polymerase releases protons, changing the color of a pH sensitive dye (Figure 1). Reaction performance is measured by the change in the ratio of light absorbed at 430 and 550 nm. Each of the four primer reactions are monitored at 60 second intervals (Figure 2). Quantification of absorbance change is performed by determining the reaction's baseline ratio (between 10 and 25 minutes), the amplification slope (between 25 and 55 minutes), and the ratio gain between baseline and endpoint (55 minutes).

LAMP analytical sensitivity

To compare the analytical sensitivity of the Color LAMP assay to RT-PCR-based assays, 543 patient samples (41 positive and 502 negative) were processed through the Color assay and compared against external results from RT-PCR-based assays. These samples consisted of two different cohorts:

509 nasopharyngeal swabs

collected by healthcare providers at a San Francisco, California testing site from patients seeking SARS-CoV-2 testing over a period of approximately two weeks. These samples had been previously tested at the Clinical Research Sequencing Platform (CRSP) at the Broad Institute of MIT and Harvard and contained seven positive and 502 negative samples.

BROAD INSTITUTE

34 nasopharyngeal swabs

collected by the University of California, San Francisco and previously tested at Chan Zuckerberg BioHub in San Francisco, California.

UCSF | Chan Zuckerberg Initiative

All results generated by the Color assay matched previously assay results (Table 1), yielding a positive predictive agreement and negative predictive agreement of 100%.

Table 1:
Comparison of RT-PCR and LAMP protocol tested on 543 clinical samples.

		Previous assay result		
		Positive	**Negative**	**Total**
Color assay result	**Positive**	41	0	41
	Negative	0	502	502
	Total	41	502	543
Positive agreement		100% (41/41)		
Negative agreement		100% (502/502)		

Furthermore, Color's LAMP assay has the ability to detect SARS-CoV-2 RNA in concentrations as low as 0.75 viral copies per µl of primary specimen. This is similar to the level of detection (LoD) of current FDA Emergency Use Authorization (EUA) approved RT-PCR tests, which vary from approximately 0.5 to 80 copies per µl.[2] No cross reactivity with other respiratory viruses, including other human coronaviruses, was found. Cross-reactivity/exclusivity analysis was performed in two stages:

A. *In silico* analysis was performed by aligning the LAMP primer sequences against sequences of 15 common viruses as well as four coronaviruses related to SARS-CoV-2 RNA. None of the viruses included in the cross-reactivity analysis had a match greater than the recommended threshold of 80% across the total length of the primer sequences.

B. The Color LAMP assay was used to test cross-reactivity/exclusivity with 41 other organisms. Samples were prepared by spiking (inactivated) purified, intact viral particles, cultured RNA, and bacterial cells into negative buccal swab matrix and processed in triplicate through the assay. All results were negative.

Conclusion

Diagnostic testing is used to identify individuals with COVID-19 and can detect the presence of SARS-CoV-2 virus in respiratory samples. The clinical sensitivity of diagnostic assays varies over the course of the disease. To date the two equivalent technologies used for diagnostic nucleic acid amplification testing are RT-PCR and LAMP. Color has developed a LAMP-based assay that detects SARS-CoV2 with 100% positive and negative predictive agreement to previous test results on RT-PCR-based assays.

References

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