color

Diagnostic Testing

Version 2.0 – Updated 09.17.20

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 real time reverse transcription loop-mediated isothermal amplification (RT-LAMP).

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

The clinical sensitivity of diagnostic tests is still under investigation, but appears to decrease as time from symptom onset increases.

The Color RT-LAMP assay showed 100% positive predictive agreement and negative predictive agreement with established RT-PCR assays across 539 clinical samples.

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 real time reverse transcription loop-mediated isothermal amplification (RT-LAMP). The FDA first awarded an Emergency Use Authorization (EUA) for the Color assay on May 19, 2020 (EUA#200539).

RT-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 US Centers for Disease Control and Prevention (CDC). This assay uses a set of primers and probes that specifically target and amplify regions of the viral genome. Amplification is performed by enzymes in the reaction that are activated by temperature changes. By cycling through a series of temperatures, these enzymes first convert the viral RNA into DNA (in a process called reverse transcription) and then create thousands to millions of copies of that DNA. After each cycle of RT-PCR, the amount of newly generated template is estimated in real time by measuring light emitted by fluorescent dyes that are stimulated by the amplification process.

RT-LAMP

Color's SARS-CoV-2 assay is a high-throughput, automated method utilizing colorimetric RT-LAMP technology to detect SARS-CoV-2 viral RNA. RT-LAMP is similar to RT-PCR in that it detects the presence of viral RNA but there are a few key differences. In both procedures, the viral RNA genome is reverse transcribed into DNA, and the DNA is amplified and monitored in real time. However, unlike RT-PCR, RT-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. RT-LAMP plate after incubation. The color change of the pH sensitive dye from red to yellow indicates amplification of the target.

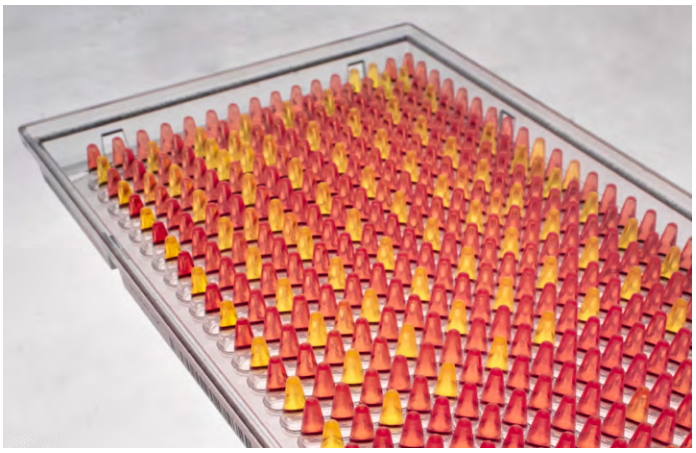
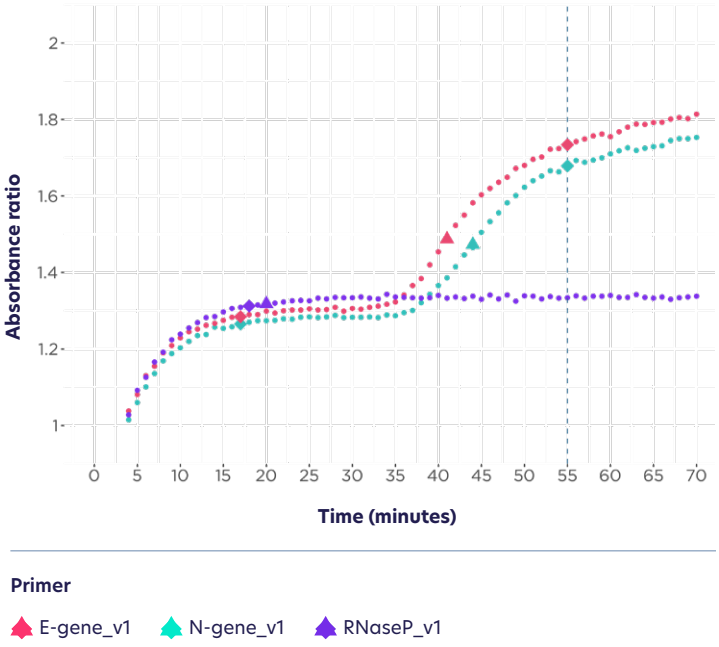


Figure 2. An example trace of the absorbance ratio change during RT-LAMP for a positive control sample.



The Color RT-LAMP assay uses two SARS-CoV-2 specific primer sets, designed to uniquely detect SARS-CoV-2 viral RNA. In addition, a third primer set that targets the human RNaseP (RP) transcript is used as an internal control. The Color assay uses RT-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 three primer reactions are monitored at 60 second intervals (Figure 2).

RT-LAMP analytical sensitivity

To compare the analytical sensitivity of the Color RT-LAMP assay to RT-PCR-based assays, 539 patient samples (37 positive and 502 negative) were processed through the Color assay and compared against external results from an RT-PCR-based assay. These samples consisted of:

539 nasopharyngeal swabs

collected by healthcare providers from patients seeking SARS-CoV-2 testing. These samples had been previously tested at the Clinical Research Sequencing Platform (CRSP) at the Broad Institute of MIT and Harvard and contained 37 positive and 502 negative samples.

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Table 1. Comparison of RT-PCR and RT-LAMP protocol tested on 539 clinical samples.

		Previous assay result		
		Positive	**Negative**	**Total**
Color assay result	**Positive**	**37**	**0**	**37**
	Negative	**0**	**502**	**502**
	Total	**37**	**502**	**539**
Positive agreement		**100% (37/37)**		
Negative agreement		**100% (502/502)**		

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

Furthermore, Color's RT-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 RT-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 RT-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 RT-LAMP. Color has developed an RT-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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