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The Potential Role of RT-LAMP in SARS-CoV-2 Diagnosis Based on Limit of Detection

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Abstract

The COVID-19 pandemic, caused by the novel coronavirus SARS-CoV-2, that arose in 2019 underscored the significance of testing and diagnostics in order to combat the rapid spread of the disease. The most common method for SARS-CoV-2 RNA detection has been the RT-qPCR method, which is insufficient in terms of meeting the immense demand for testing due to its relatively high time-consumption. Under these circumstances, the urgent need for a rapid and similarly sensitive testing arised, and the RT-LAMP method was proven to be a promising alternative. This review compares the lowest concentration levels of SARS-CoV-2 RNA that these two mentioned methods can detect, examining several reviews and research. It was found that the detection limit of RT-qPCR is lower in comparison to RT-LAMP, which demonstrates a higher sensitivity, thus, reducing the false negative outcomes. Nonetheless, RT-LAMP is more convenient for being deployed as a point-of-care test for vast amounts of people considering its rapidity in detection and simplicity, albeit it has a higher detection limit compared to RT-qPCR.

Keywords

RT-LAMP; LOD; RT-qPCR; COVID-19 testing

Introduction

The emerging infectious disease COVID-19, caused by the novel coronavirus SARS-CoV-2, was first identified in Wuhan, China, in December 2019, and was later declared a pandemic by the World Health Organization (WHO) as of March of 2020 . The absence of appropriate antiviral drugs or vaccines for the infection has made simple, prompt, and dependable detection crucial for the prevention and control of the COVID-19 pandemic, as well as clinical treatment (Lu et al., 2020). Current researches demonstrate that human-to-human contact is the potential cause of the COVID-19 outbreak. Hence, the isolation of the infected people is essential for minimizing the risk of further COVID-19 spread. Nevertheless, test failures are still a matter of fact, resulting in false negatives which indicate that the person in question is not infected while in fact he is, which can render the pandemic to further spread (Kelly-Cirino et al., 2019).

The neglection of some factors while performing the test could result in relatively large numbers of false negative results (Bahreini et al., 2020). Considering this, the sensitivity and

the diagnostic accuracy of a test becomes the most important factors. One of the measures which affects the reliability of a test is the limit of detection (LOD). In general, LOD is the lowest concentration of a substance in a sample that can be detected in stated circumstances of a test. Although there are many calculation methods of LOD, they all depend on the special set up of the experimental procedure. All conditions in which the assays are carried out affect the lowest amount of analyte detected with high sensitivity and specificity (Shrivastava & Gupta, 2011). In other words, factors such as pH, additional buffers, different kits used in the assay and other primers with different specificity can alter the LOD of the procedure.

With the most commonly used technology for the qualitative and quantitative diagnosis of viruses, Real-time PCR (RT-qPCR) plays a critical role in the laboratory confirmation of SARS-CoV-2 infection (Torretta et al., 2020). With the number of cycles that the sample reaches the critical fluorescence level, this method provides the number of copies of viral DNA that the samples include. The technique utilizes two primers binding different strands of DNA to preserve the starting points of amplification of the DNA segment in question, guiding the DNA polymerase, and a fluorescent dye which fluoresces only when it is bound to double stranded DNA, enabling the monitor of the amplification simultaneously (Maddocks & Jenkins, 2017). That being said, assays of RT-qPCR require advanced equipment and qualified personnel, and is relatively time-consuming, which restrains its ability to meet the demand for detecting the virus in constantly surging number of patients with COVID-19 or suspected infection, or proximate contact with confirmed cases. Thus, simple, rapid, and delicate testing assays are necessary for facilitating the SARS-CoV-2 infection (Thompson & Lei, 2020).

An emerging innovative and cost-efficient detection technique in the field of COVID-19 testing, RT-LAMP (loop-mediated isothermal amplification technique), has been a promising alternative method to RT-qPCR in terms of its rapidity and sensitivity in detection, and independence of sophisticated equipment, which obviates the drawbacks RT-qPCR present. In RT-LAMP, different from RT-qPCR, 4-6 different primers are used. One pair is for the inner part of the amplicon, and the others are for the outer part. Due to the primers' structural differences, the amplified DNA turns into an unusual structure rather than normal double stranded DNA. Moreover, it has a high specificity since it utilizes 4-6 different primers (Thompson & Lei, 2020).

There have been various approaches described for detecting the DNA production in RT-LAMP assays. One approach suggests the use of a pH indicator (e.g., phenol red) and the run of reaction in a weakly buffered medium (Dao Thi et al., 2020). As the chain reaction proceeds, with the release of hydrogen ions as a by-product of the reaction, the pH of the environment decreases, resulting in a salient color change of the pH indicator from red to yellow, which makes it an appealing assay for point-of-care diagnosis. Although RT-LAMP does not provide quantitative information, it is highly useful for diagnostic purposes with its rapid provision of qualitative detection (Tanner et al., 2015).

This review demonstrates and compares both the detection limits of two diagnostic tests, RT-LAMP and RT-qPCR, and their utilities in different conditions.

Discussion & Conclusion

The Coronavirus disease (COVID-19), caused by the Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become a major health burden, and has shown a constant surge of cases around the world since it first emerged. Its rapid spreading capacity demonstrated the importance of early diagnosis of positive cases in order to break this spread and overcome the pandemic eventually. In this sense, the time needed for getting results from a test is critical. Therefore, the urgent need for tests having relatively similar sensitivities and more rapid diagnostic capabilities is unarguable. RT-LAMP emerged as an alternative to RT-qPCR method with its convenience such as no need for sophisticated equipment, cost-efficiency, and rapid detection ability. This review compares these two distinct methods within the frame of limit of detection (LOD).

Based on the investigated studies, it was seen that the LOD of RT-LAMP is higher than that of RT-qPCR. For instance, the research carried out by Minghua Jiang and et al., demonstrated that the LOD of the was 500 copies/ml (0.5 copy/µl) for RT-LAMP, with a high degree of specificity and sensitivity. They subjected the RT-LAMP assay to 260 patients, and only 4 of them were false negative, while solely 1 false positive case was encountered. They attributed the reason behind the false negative and positive outcomes, which are neglectable in such times of pandemic, to the less amount of RNA input in the assay (2 µl) relative to that of RT-qPCR (5 µl) (Jiang et al., 2020).

Another research, in which the researchers developed a new method for the application of RT-LAMP, the LOD of RT-LAMP was demonstrated as 118.6 copies of SARS-CoV-2 RNA per 25 µL reaction (4.7 copies/µl), again exhibiting a high specificity and sensitivity (Lu et al., 2020). The value of LOD is as high as 50 copies per microliter in a research (Ganguli et al., 2020) , yet it is even higher with a value of 80 copies per microlitre, in the research done in April 2020 (Huang et al., 2020). 100 copies per microlitre was the LOD value of RT-LAMP in the research, Rapid point-of-care detection of SARS-CoV-2 using reverse transcription loop-mediated isothermal amplification (RT-LAMP) (Mautner et al., 2020). The LOD value of RT-LAMP was seen as 0.75 copies/µl in the article written in November 2020 (EUA, 2020). Another research demonstrated the LOD value of their assay as 50 copies/µl, which indicates high specificity and sensitivity (Ganguli et al., 2020). The last article investigated stated the LOD value of RT-LAMP as 2.95 copies/µl with 86% sensitivity and 100% specificity (Lalli et al., 2020). All data obtained from the articles discussed in the review can be seen in Table 1.

Table 1: Sample size (n), sensitivity (%), specificity (%), limit of detection (LOD) values of RT-LAMP assays in the articles investigated

	Sample size (n)	Sensitivity (%)	Specificity (%)	Limit of detection (LOD)
Article [1]	260	91.4	99.5	0.5 copy/µl
Article [2]	56	100	100	4.7 copies/µl
Article [3]	16	100	100	80 copies/µl

Article [4]	20	100	100	50 copies/µl
Article [5]	12	100	100	100 copies/µl
Article [6]	20	95	100	0.75 copy/µl
Article [7]	30	86	100	2.95 copies/µl

[1] Development and Validation of a Rapid, Single-Step Reverse Transcriptase Loop-Mediated Isothermal Amplification (RT-LAMP) System Potentially to Be Used for Reliable and High-Throughput Screening of COVID-19 (Jiang et al., 2020).
[2] A novel reverse transcription loop-mediated isothermal amplification method for rapid detection of sars-cov-2 (Lu et al., 2020).
[3] RT‑LAMP for rapid diagnosis of coronavirus SARS‑CoV‑2 (Huang et al., 2020).
[4] Rapid isothermal amplification and portable detection system for SARS-CoV-2.(Ganguli et al., 2020)
[5] Rapid point-of-care detection of SARS-CoV-2 using reverse transcription loop-mediated isothermal amplification (RT-LAMP) (Mautner et al., 2020)
[6] *EMERGENCY USE AUTHORIZATION (EUA) SUMMARY FOR THE COLOR SARS-COV-2 RT-LAMP DIAGNOSTIC ASSAY*.(EUA, 2020)
[7] Rapid and extraction-free detection of SARS-CoV-2 from saliva with colorimetric LAMP (Lalli et al., 2020)

In the articles investigated in this review, it was observed that the detection limits of RT-qPCR for the detection of SARS-COV-2 were relatively lower, in comparison to RT-LAMP assays with LOD values ranging from 0.5 to 100 copies per microlitre. In a review about the essence of LOD of RT-qPCR in SARS-COV-2, it was stated that ”Best-in-class assays demonstrate a limit of detection of ~100 copies of viral RNA per milliliter of transport media” (Arnaout et al., 2020). In terms of microlitre, this accounts for 0.1 copies per microlitre, which is lower than the LOD of RT-LAMP. The relatively lower LOD value of RT-qPCR makes it more reliable, yet, especially in times of a pandemic, the practicality of RT-LAMP can’t be a matter of subject in terms of its other immense benefits.

High detection limits increase the possibility of false negative outcomes. This is a highly perilous situation, as the people that mistakenly tested negative would not be able to isolate themselves, continue spreading the virus. However, the possibility of transmission would be relatively low, since the people with false negative diagnoses would have relatively low viral loads (Jarvis & Kelley, 2020).

Overall, the data obtained by the investigated studies suggest that the detection limit of the RT-LAMP method is relatively higher in comparison with that of RT-qPCR. It is also proven that the sensitivity of RT-qPCR is higher than that of RT-LAMP, resulting in a higher accuracy in the identification of the people with the disease. Notwithstanding the fact that RT-LAMP does not have the qualitative aspect of the RT-qPCR and its sensitivity requires advancements, the benefits of the RT-LAMP technique are immense to offset the downsides, albeit false results yet exist. First of all, a typical RT-LAMP procedure occurs at a faster rate by a wide margin compared to RT-qPCR, as a result of omitting the denaturation and integration of the detection and amplification step. Currently, this holds a vast significance given the urgent need to build the global testing capacity up to 100-fold above the present conditions. Furthermore, the isothermal property of RT-LAMP contributes to the simplicity

and cost-efficiency of its equipment, while also rendering a high-efficient amplification in terms of time-consumption by omitting the relatively time-consuming thermal cycle step that facilitates DNA amplification in the conventional RT-qPCR. Lastly, turbidity and pH dye serving as indicators of amplification, which are visible by the naked eye, makes the readout of RT-LAMP results also relatively simple. The simplicity, rapidity, and cost-effectiveness of RT-LAMP makes it a logical and potential candidate for monitoring the COVID-19 pandemic, while rendering vast amounts of people to use it swiftly with relative ease. On account of the aforementioned benefits, this assay holds a great potential to be deployed as a point-of-care test, playing a vital role in tracking the spread of the SARS-CoV-2 virus (Thompson & Lei, 2020).

Due to its easy detection procedure, RT-LAMP, being an innovative and promising detection method, shows a great feasibility for allowing COVID-19 detection at residential homes with unlimited detection capacity. Such at-home testing methodology shows the potential for screening a large population in a short time, without the need of advanced equipment or well-trained personnel, providing an unlimited testing capacity. This simple home-based diagnosis concept could also facilitate the rapid detection of further infectious diseases which could play a crucial role in combating the outbreaks. The use of RT-LAMP assays would not only increase the detection capacity, but also significantly lower the associated cost of diagnosis, thus helping in the fight against infections, such as COVID-19.

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