Magna Scientia Advanced Research and Reviews

eISSN: 2582-9394

Cross Ref DOI: 10.30574/msarr

Journal homepage: https://magnascientiapub.com/journals/msarr/

(SHORT COMMUNICATION)

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Colorimetric Reverse Transcription-Loop-Mediated Isothermal Amplification (RT-LAMP) in rapid detection of SARS-CoV-2

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Magna Scientia Advanced Research and Reviews, 2022, 04(02), 001–004

Publication history: Received on 11 January 2022; revised on 15 February 2022; accepted on 15 February 2022

Article DOI: https://doi.org/10.30574/msarr.2022.4.2.0026

Abstract

Diagnosis by RT-LAMP (Reverse Transcription–Loop-Mediated Isothermal Amplification) is relatively simple, fast and requires inexpensive equipment. The result is visualized with the naked eye, and therefore, has the potential to become popular among molecular tests for viral detection. This work aimed to test and validate different RT-LAMP protocols for diagnostics of SARS-CoV-2. To this end, we used 20 samples that had been tested at the Laboratory of Molecular Diagnostics for SARS-CoV-2 by RT-PCR, with results ranging from "detected to undetected". The colorimetric RT-LAMP results were compatible with those obtained by RT-PCR in 95% of the cases. The results show good sensitivity and fidelity of the test. Its applicability detecting SARS-CoV-2 can be important for massive testing.

Keywords: Alternative diagnostics; Covid-19; Viral detection; Molecular diagnostics; RT-qPCR

1 Introduction

Massive testing is one of the best alternatives for a government to improve its strategy to combat COVID-19, such as defining periods and intensity of quarantine, reducing negative impacts on the economy and reducing the transmission rate of the virus [1]. Based on this, it is important to develop simpler and faster diagnostic methods that make it possible to make more tests available to the population.

RT-LAMP (Reverse Transcription–Loop-Mediated isothermal amplification) is a fast and simple technique used in diagnostics in several areas [2], and it is a promising approach in SARS-CoV-2 detection [3]. The amplification occurs under isothermal conditions, which can be provided, for example, by water bath and heating blocks. The result of the reaction can be observed from the analysis of fluorescence using ultraviolet light [4], of the turbidity of the medium [5] or even through the use of dyes that vary in color from according to the presence of by-products generated from DNA/RNA amplification [4, 6]. Changing the color of the reaction can also be performed using a pH indicator [7], which takes advantage of the pH change resulting from amplification reactions by proton production. In addition, there is the possibility of doing together with other procedures in addition to the use of purified RNA, aiming to reduce costs and time demanded by this process [3].

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The objective of this work was to test different reaction conditions of the RT-LAMP protocol for the diagnosis of SARS-CoV-2 compared to the standard procedure by RT-qPCR, to validate it for the diagnosis of viral infection in samples of the swab.

2 Material and methods

The Laboratory of Molecular Diagnostics receives suspect samples collected by health professionals from the Regional Health Superintendence of Patos de Minas, Minas Gerais, Brazil, just for SARS-CoV-2 diagnosis since April 2020. To validate the RT-LAMP results, we have chosen 20 samples already with known results (10 detected and 10 undetected) by RT-qPCR in the months of August and September, 2020.

2.1 RNA Isolation

We performed the standard extraction using magnetic beads according to the manufacturer's instructions (MagMAX™ Viral RNA Isolation Kit, Thermo Fisher Scientific, in an automatized extractor (Extracta 32, Loccus). This RNA extraction method was the same used for diagnosis by RT-qPCR.

We also tested other methods of RNA isolation to validate a cheaper and faster way to detect the virus in association with the RT-LAMP technique. We tested the QuickExtract reagent (Lucigen) by applying 20 µl of reagent and 20 µl of the sample, followed by incubation at 65°C for 6 minutes, then another 3 min at 95°C. We also tested the reactions with heat treatment only, incubating the samples for 3 min at 98°C and then cooling them to 4°C for 5 min.

2.2 RT-LAMP reactions

For the RT-LAMP reactions, we used the "WarmStart Colorimetric LAMP 2X Master Mix kit" (New England Biolabs). We tested two sets of primers available [8, 9] (Table 1). Each reaction contained 12.5 µL of master mix, 2.5 µL of primer set (10x), 3 µL of extracted RNA and water to make up 25 µL. We incubated the samples at 65°C for 30 minutes. We performed the same procedure for each RNA extraction method tested.

Table 1 Set of primers for RT-LAMP used in the present work

	Lamb et al. [8]	**Yu et al. [9]**	**Final concentration used (µM)**
FIP	AGAGCAGCAGAAGTGGCACAGGT GATTGTGAGGAAGAAGAG	AGGTGAGGGTTTTCTACATCACTAT ATTGGAACAAGCAAATTCTATGG	1.6
BIP	TCAACCTGAAGAAGAGCAAGAACT GATTGTCCTCACTGCC	ATGGGTTGGGATTATCCTAAATGTG TGCGAGCAAGAACAAGTG	1.6
F3	TCCAGATGAGGATGAAGAAGA	CCACTAGAGGAGCTACTGTA	0.2
B3	AGTCTGAACAACTGGTGTAAG	TGACAAGCTACAACACGT	0.2
LF	CTCATATTGAGTTGATGGCTCA	CAGTTTTTAACATGTTGTGCCAACC	0.4
LB	ACAAACTGTTGGTCAACAAGAC		0.4
LB4		TAGAGCCATGCCTAACATGCT	0.4

3 Results and discussion

Alternative RNA preparation methodologies were not efficient for RT-LAMP reactions, so we are presenting the first 20 assays with RNA isolated by the standard method. In these samples, only one of them had an incongruent result between that obtained by RT-qPCR and by RT-LAMP, which suggests 95% consistency (Figure 1).

A positive sample by RT-qPCR was negative on RT-LAMP. This divergence may have occurred due to an insufficient amount of viral RNA copies for detection [3]. The mean Ct of this sample in RT-PCR was 33.39, suggesting a low amount of viral RNA copies. Despite this, Lamb et al. [8] verified in their work that the set of primers designed by them proved to be quite sensitive in serial dilution tests, with the detection limit estimated at 0.08 fg, which is approximately equal to 304 viral copies. This concentration represents an average Ct equivalent to 30.7 in their RT-PCR assays. The sensitivity tests of the Yu set [9] showed that it could detect up to ten copies of the ORF1ab gene, however, when tested

in samples, there were indications that a concentration below 60 copies per µl was the detection limit since samples from patients with mean Ct greater than 35 showed random color changes in replicates. Concerning tests carried out with samples not extracted and samples that underwent rapid manual extraction, their results were probably not useful due to the presence of impurities in the medium that inhibited the pH change or the reaction itself, and this can be studied to improve the procedure.

Figure 1 Illustrative RT-LAMP results for 20 samples using Lamb [8] (A and B lines) and Yu [9] (C and D lines) set of primers. The colors indicate Negative (rose) and positive (yellow) diagnostics for the presence of the SARS-CoV-2 virus. Sample #1 has divergent results between the primer sets, meanwhile, sample #4 (lines A and C) has a distinct result from RT-qPCR (supposed to be positive)

4 Conclusion

Based on the results obtained in the tests, it can be said that there was good sensitivity and fidelity of the test and its applicability in the diagnosis of SARS-CoV-2, depending on the quality of the RNA used in the reaction and the number of RNA copies virus present in the sample, or even the improvement of the method. This would be advantageous due to the reduction of time and resources spent on the molecular diagnosis of COVID-19, allowing a massive testing of the population.

Compliance with ethical standards

Acknowledgments

The authors would like to thank all the team at Laboratory of Molecular Diagnosis of UFV, campus Rio Paranaíba, for their support when conducting the diagnostic tests. The experiment was conducted thanks to the grant by the Federal University of Viçosa.

Disclosure of conflict of interest

There is no conflict of interest.

Statement of ethical approval

The university's institutional review board approved the analysis and issued a waiver of consent (Ethics Committee Ruling number – CAAE: 33446820.8.0000.5153).

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