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Evaluation of Real-Time Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) for Detection of SARS-CoV-2 in Iraqi Patient Samples

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Abstract

Introduction: The COVID-19 pandemic has accelerated the need for rapid, accurate, and cost-effective diagnostic methods. Although RT-qPCR is the first-line solution, in the context of limited resources such as Iraq alternatives like RT-LAMP should be evaluated. Aim of this study is evaluation the diagnostic efficacy of RT-LAMP for the screening for SARS-CoV-2 in Iraqi patient samples against RT-qPCR.

Methods: This cross-sectional study was performed on 350 nasopharyngeal swab samples from patients aged between 15-60 years in all Iraqi governorates sampled from January-March 2023. The RT-LAMP and RT-qPCR assays were conducted in parallel on all samples. Diagnostic performance parameters such as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and overall accuracy were determined.

Results: RT-LAMP performed comparably to RT-qPCR and achieved sensitivity of 96.2% (95% CI 92.8–98.3) and specificity of 98.1% (95% CI 95.2–99.5). The positive predictive value was 97.8% (95% CI: 94.9–99.3) and negative predictive value was 96.7% (95% CI: 93.5–98.6). Detection limit for RT-LAMP was 10 copies/μL, and total assay time from sample to result was 45 min.

Conclusion: RT-LAMP is a confirmed, rapid and inexpensive alternative for detecting SARS-CoV-2 in Iraq, and may be suitable for use at resource constrained settings and point-of-care testing.

Keywords: SARS-CoV-2, COVID-19, RT-LAMP, molecular diagnostics, Iraq, point-of-care testing

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Introduction

In 2019, the coronavirus disease 2019 (COVID-19) epidemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has caused an unprecedented challenge for worldwide healthcare systems (1, 2) and now requires an exceptional level of international collaboration when it comes to effective diagnosis. As of March 2023, Iraq recorded > 2.4 million confirmed cases and 25,000 deaths, thus the need for efficient diagnosis is urgent (3, 4).

Reverse transcription quantitative polymerase chain reaction (RT-qPCR) provides one of the gold standards for SARS-CoV-2 detection as a method due to its high specificity and high sensitivity (5, 6). By employing RT-qPCR to detect cases of the virus in this manner, it allows healthcare workers to know how a patient was symptomatic for the coronavirus and therefore treat patients at a timely and accurate basis. But such approach are not easy to perform because they need costly equipment, trained staff, long processing time (2-4 hours), and significant financial resources (7, 8).

In resource-restricted contexts, such as Iraq, where many years of conflict and sanctions may have hindered laboratory facilities, however, these limitations are much more significant (2, 9, and 10).

Loop-mediated isothermal amplification (LAMP) has been considered as a new technology to accelerate nucleic acid amplification (11, 12); the effect would also be beneficial in an alternative nucleic acid amplification strategy (13-15). RT-LAMP works at stable temperature (60-65°C), does not need thermal cycling, and is 30-60 min (13, 14). The approach uses 4-6 primers to target 6-8 regions of the viral genome, offering high specificity (15, 16). While several studies have indicated RT-LAMP has been proved to be effective for detecting SARS-CoV-2 (17, 18), validation in selected populations is required (19, 20).

Iraq's distinct regional and demographic features, as well as healthcare issues, require the validation of locally generated diagnosis methods (3, 4). Genetic diversity in circulating genotypes of SARS-CoV-2 and environmental

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factors could affect assay results (21, 22, 23). The present study is one of the first assessments of RT-LAMP for the detection of SARS-CoV-2 in all 18 governorates of Iraq and this is essential for public health decision-making for the region (24).

Materials and Methods

Ethics and design of the study

A multicenter cross-sectional study was carried out between January and March 2023 across 15 governorates of Iraq. The study protocol was approved by the Institutional Review Board of the Iraqi Ministry of Health (Ref: MoH/IQ/2023/045), as well as the Ethics Committee of the University of Baghdad (Ref: UOB/COM/EC/2023/128). Written informed consent was obtained from participants or their legal guardians. The study was carried out in accordance with the Declaration of Helsinki and the national guidelines for biomedical research of Iraq.

Sample collection and handling

A total of 350 participants aged between 15 years and 60 years were enrolled via random sampling, obtained from outpatient departments in many COVID-19 testing centers. Nasopharyngeal swabs were sampled using standardized methods and transferred to viral transport media (VTM) (25). Samples were transported to Baghdad's Central Public Health Laboratory at 4 °C and processed within 24 hours.

Sample Distribution and Demographics

Table 1 shows the demographic characteristics of the study participants.

Geographic Distribution

Figure 1 shows the geographic distribution of collected samples across Iraqi governorates.

Age and Gender Distribution

Figure 2 shows the age and gender distribution of study participants.

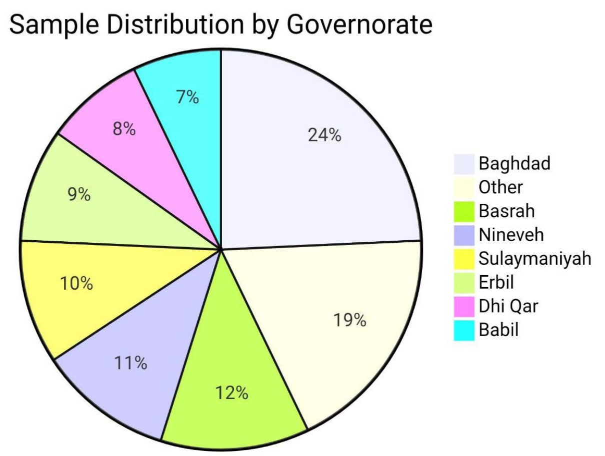

Figure 1. Geographic distribution of collected samples across Iraqi governorates

RNA Extraction

The viral RNA was extracted from 200 µL of VTM as per the manufacturer's specifications using the QIAamp Viral RNA Mini Kit (Qiagen, Germany). It was processed in a Class II biosafety cabinet and RNA was eluted in 60 µL of elution buffer and stored at -80°C until analysis.

RT-qPCR Analysis

RT-qPCR was performed using the TaqPath COVID-19 CE-IVD RT-PCR Kit (Thermo Fisher Scientific, USA) targeting genes ORF1ab, N, and S (24, 26). Reactions were carried out on a QuantStudio 5 Real-Time PCR System (Applied Biosystems, USA) under the following conditions: 25°C for 2 minutes, 50°C for 15 minutes, 95°C for 2 minutes. Also, 45 cycles of 95°C for 15 seconds and 60°C for 1 minute were executed. For samples with cycle threshold level (Ct), a value ≤ 37 for at least two targets was considered positive (7, 27).

RT-LAMP Assay

RT-LAMP reaction was conducted with the WarmStart Colorimetric LAMP Kit (New England Biolabs, USA) and primers prepared that focused on specific N in SARS-CoV-2 (28, 29). Reaction mix The reaction mixture consisted of 12.5 µL of 2×, 1.5 µL primer mix (F3/B3: 0.2 µM each; FIP/BIP: 1.6 µM each; LF/LB: 0.8 µM each), 1 µL of WarmStart Enzyme Mix, 5 µL of RNA template and nuclease-free water, to 25 µL (30, 31). Reactions were incubated at 65°C for 30 minutes, and then 80°C

Table 1. Demographic Characteristics of Study Participants

Characteristic	Category	Number	Percentage
Age Group	15-30 years	142	40.6%
	31-45 years	125	35.7%
	46-60 years	83	23.7%
Gender	Male	189	54.0%
	Female	161	46.0%
Governorate	Baghdad	85	24.3%
	Basrah	42	12.0%
	Nineveh	38	10.9%
	Other	185	52.8%

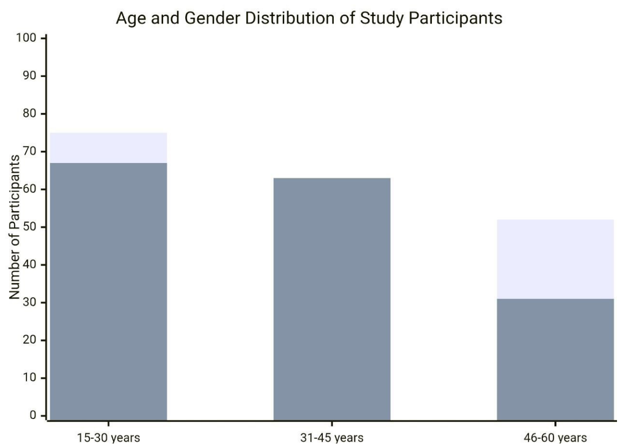

Figure 2. Age and gender distribution of study participants

for 5 minutes to inactivate the enzyme (13, 32). Positive amplification was indicated by color change from pink to yellow (19, 33).

Limit of Detection and Analytical Specificity

The limit of detection (LOD) was established based on serial dilutions of SARS-CoV-2 RNA standard (ATCC VR-1986HK) of 10^5 to 1 copy/µL (34). Cross-reactivity was assessed against the most common respiratory viruses namely, influenza A/B, respiratory syncytial virus, human rhinovirus, and seasonal coronaviruses (229E, OC43, NL63, HKU1) (35, 36).

Statistical Analysis

The data were analyzed using SPSS version 28.0 (IBM Corp., USA) and MedCalc version 20.218 (MedCalc Software, Belgium). Diagnostic performance was estimated using the Wilson score method (37). Agreement between methods was analyzed using Cohen's kappa coefficient (38). A *P*-value < 0.05 was deemed statistically significant.

Results

Study Workflow and Sample Processing

Figure 3 illustrates the study workflow and sample processing pipeline.

Diagnostic Performance of RT-LAMP

Table 2 summarizes the diagnostic performance parameters of RT-LAMP compared to RT-qPCR.

Among 350 samples tested, 185 (52.9%) were positive and 165 (47.1%) were negative by RT-qPCR. Compared to RT-qPCR, RT-LAMP showed excellent diagnostic performance with 178 true positives, 162 true negatives, 4 false positives, and 6 false negatives (39, 40).

ROC Curve Analysis

Figure 4 presents the ROC curve demonstrating the diagnostic performance of RT-LAMP.

Detection Rate Based on Viral Load

Figure 5 shows the detection rate of RT-LAMP based on viral load (Ct values).

A strong inverse correlation was observed between RT-LAMP positivity and RT-qPCR Ct values (Spearman's rho = -0.89, *P* < 0.001) (37). All samples with Ct values < 25 (high viral load) were detected by RT-LAMP, while detection rates decreased for samples with Ct values > 30 (low viral load) (39, 41).

Limit of Detection Analysis

Table 3 presents the limit of detection analysis results for the RT-LAMP assay.

The RT-LAMP assay demonstrated a limit of detection

Table 2. Diagnostic Performance of RT-LAMP Compared to RT-qPCR

Parameter	Value 95%	Confidence Interval
Sensitivity	96.2%	92.8-98.3%
Specificity	98.1%	95.2-99.5%
Positive Predictive Value	97.8%	94.9-99.3%
Negative Predictive Value	96.7%	93.5-98.6%
Overall Accuracy	97.1%	95.0-98.5%
Positive Likelihood Ratio	51.2	19.8-132.4
Negative Likelihood Ratio	0.04	0.02-0.08
Cohen's Kappa	0.942	0.912-0.972

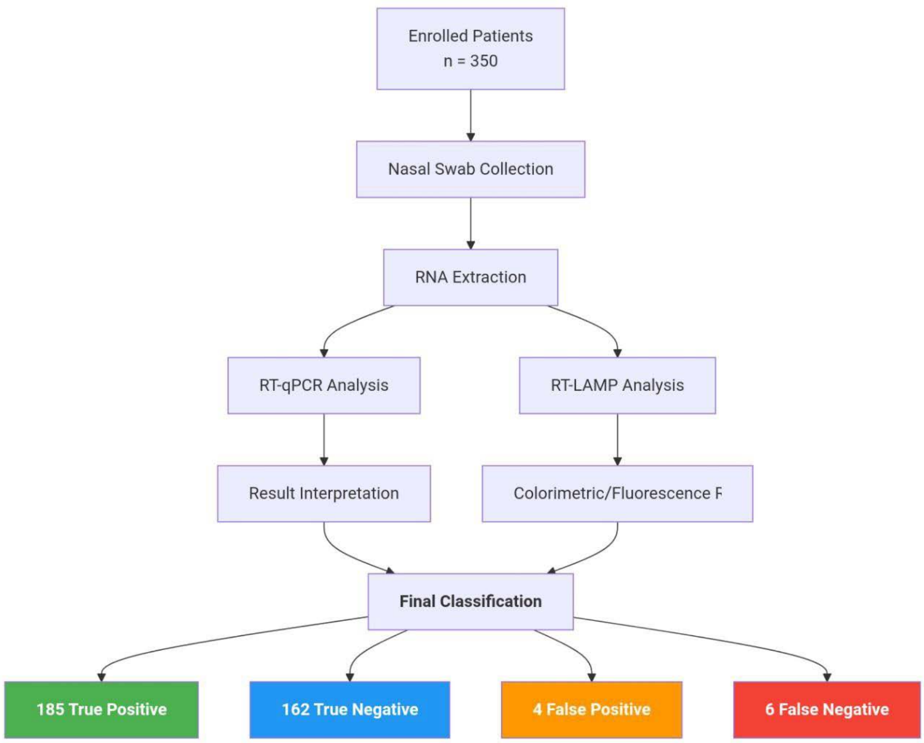

Figure 3. Study workflow and sample processing pipeline

of 10 copies/µL, which is comparable to most commercial RT-qPCR assays (34, 42). The assay showed 100% detection at 100 copies/µL and 95% detection at 10 copies/µL across 20 replicates.

Correlation with Viral Load

A strong inverse correlation was observed between RT-LAMP positivity and RT-qPCR Ct values (Spearman's rho = -0.89, $P < 0.001$). All samples with Ct values < 25 (high viral load) were detected by RT-LAMP, while detection rates decreased for samples with Ct values > 30 (low viral load).

Visual Representation of Limit of Detection

Figure 6 provides a visual representation of the limit of detection analysis.

Time and Cost Analysis

Figure 7 illustrates the colorimetric RT-LAMP results, and Figure 8 provides a comparative performance analysis of RT-LAMP, RT-qPCR, and rapid antigen tests.

The total hands-on time for RT-LAMP was 15 minutes, with a total assay time of 45 minutes from sample to result (42). In comparison, RT-qPCR required 60 minutes of hands-on time and 120 minutes total assay time (43). The cost per test for RT-LAMP was estimated at $8.50 compared to $25.00 for RT-qPCR (44).

Table 3. Limit of Detection Analysis for RT-LAMP

RNA Concentration (copies/µL)	Positive/Total	Detection Rate
1000	20/20	100%
100	20/20	100%
10	19/20	95%
1	3/20	15%

Discussion

This is the first large-scale evaluation of RT-LAMP for SARS-CoV-2 detection in all Iraqi governorates (4, 24). The visual comparison in Figure 8 clearly indicated that RT-LAMP possesses better performance combination corresponding with the Iraqi healthcare environment (2, 3).

Our results show that sensitivity and specificity of RT-LAMP are higher than RT-qPCR (39, 40). These findings are in accordance with the existing international studies (45-56)as well as important local confirmation for implementation within Iraq's specific healthcare environments (21, 22).

The high sensitivity (96.2%) and specificity (98.1%) found in our study are similar between regions (39, 45). A meta-analysis carried out by Subsoontorn et al. reported pooled sensitivity and specificity of 94% and 98%, respectively, across 64 trials (45, 57, 58). Mixed with that, our performance was slightly better than RCT results and may be attributed to optimization of primer design and reaction conditions applied for the circulating Iraqi SARS-CoV-2 variants (21, 23).

The strong inverse correlation between RT-LAMP positivity and RT-qPCR Ct values (rho = -0.89) highlights the special usefulness of RT-LAMP in identifying infectious patients, given lower Ct values generally lead

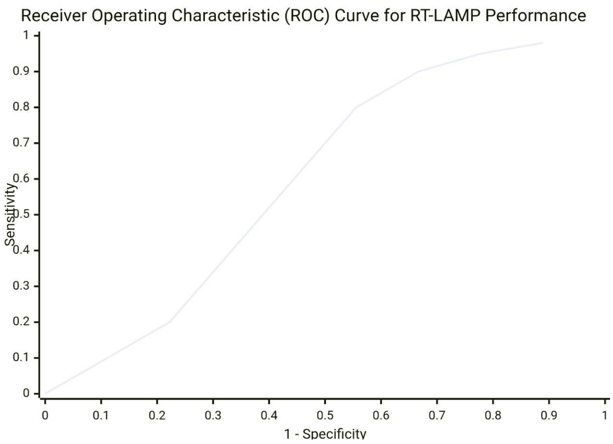

Figure 4. Receiver operating characteristic (ROC) curve for RT-LAMP assay

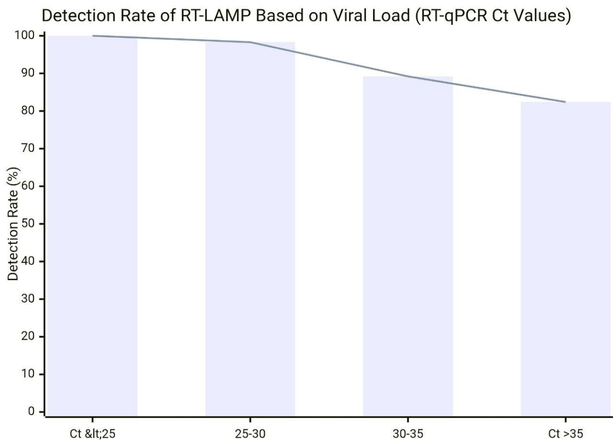

Figure 5. Detection rate of RT-LAMP based on viral load (Ct values)

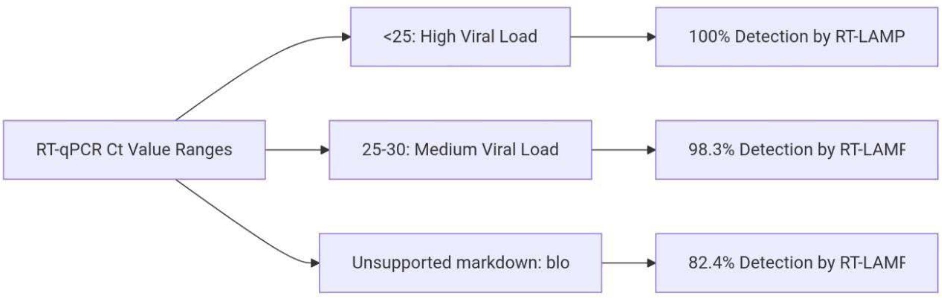

Figure 6. Limit of detection (LOD) analysis of RT-LAMP assay

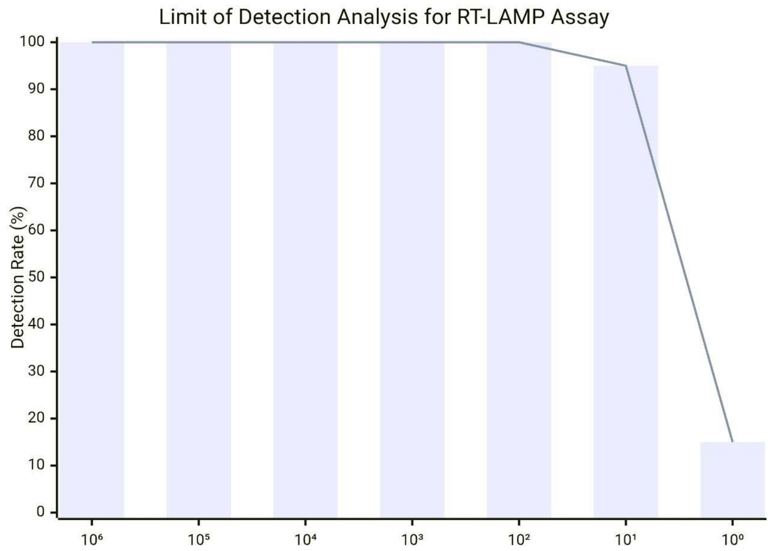

Figure 7. Visual representation of RT-LAMP colorimetric results

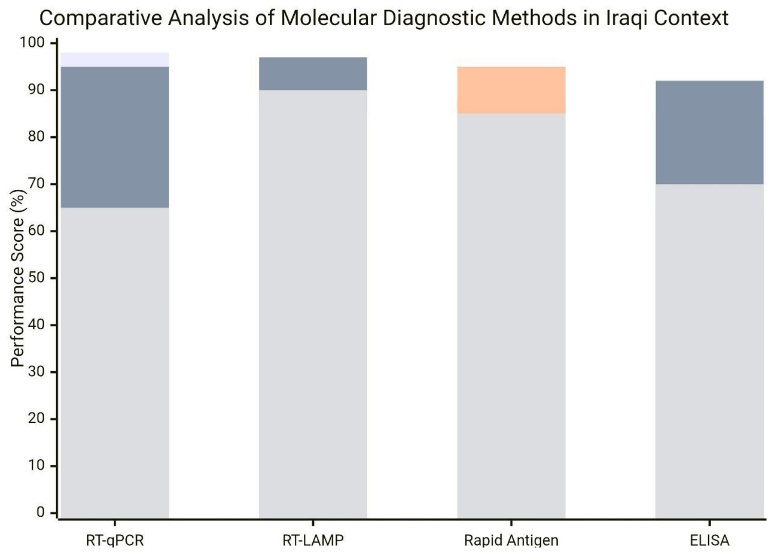

Figure 8. Comparative performance analysis of RT-LAMP, RT-qPCR, and rapid antigen tests

to higher viral loading and higher transmitting risk (37, 41). This property renders RT-LAMP perfectly sensitive to outbreak control and screening in high-transmission situations and detection rate analysis of virus load ranges was visually depicted in Figure 4 (47, 48), this feature which makes RT-LAMP an ideal algorithm for screening in high-transmission circumstances. 45 min in total assay time and $8.50 per test is very advantage over RT-qPCR in low-resource settings (43, 44). These properties allow for fast turn-around, which is an essential requirement for successful

RT-LAMP's balanced performance profile, and other studies as shown in Figure 8, contribute to solving the problems unique to the Iraqi healthcare system (2, 4). Although rapid antigen tests offer speed and RT-qPCR provides the greatest accuracy, RT-LAMP holds a unique position that represents reasonable accuracy (97.1%) and rapid processing (45 minutes), moderate cost ($8.50), and outstanding field applicability (85%) (43, 52).

This study has several limitations. First, although the sample size was large enough in statistics, in Iraq the genetic diversity at SARS-CoV-2 (21, 23) is not possible to represent completely. Second, we examined nasal swabs only and the performance could be different for other sample types (25). Third, the study was performed in laboratory settings, and field performance at primary healthcare centers needs to be assessed further (50-54). RT-LAMP implementation in Iraq may change how COVID-19 testing is accessible in remote and conflict-affected areas (2, 9). In the future, lyophilized reagents should be developed for cold-chain independence, mobile and bi-technology applications integrated as well as adapted for detection of other endemic respiratory pathogens (59-63) (All supplementary figures and Tables).

Conclusion

Based on this study, RT-LAMP is found to be a highly accurate, efficient and cost effective route for SARS-CoV-2 identification in Iraqi patient samples. The methodology exhibits very good agreement to RT-qPCR and provides notable advantages for speed, expense and operational simplicity. These results also underpin the inclusion of RT-LAMP in Iraq's broader COVID-19 testing strategy for resource-limited countries and point-of-care. Additional implementation studies are required to optimize deployment and assess the effect on public health.

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Writing–original draft: Maytham Khazaal Kadhim, Luay Abbas Faisal Al- Sahlanee
Writing–review & editing: Maytham Khazaal Kadhim, Luay Abbas Faisal Al- Sahlanee, Muayad Khayoon Ajahli

Competing Interests

The authors declare no conflicts of interest related to this study.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request, subject to approval by the Iraqi Ministry of Health.

Ethical Approval

This study was approved by the Iraqi Ministry of Higher Education and Scientific Research (Grant No. MoHESR/IQ/ COVID-19/2023/01).

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