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SHORT COMMUNICATION

Reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) as a user-friendly system to detect SARS-CoV-2 infection: a multicentric study

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SUMMARY

Although reverse transcriptase quantitative PCR remains the gold standard to perform viral detection, reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) is already used to perform diagnosis of various infections. This work reports the results of a multicentric study performed in Sicily to evaluate the diagnostic power of an RT-LAMP kit for the diagnosis of SARS-CoV-2 infection on a total of 551 samples collected in January and February 2021, revealing sensitivity, specificity, accuracy, positive and negative predictive values ≥95%. Our results suggest the potential employment of this kit as a screening test to be used where fast and reliable results are demanded without the need for expensive instruments and highly-skilled personnel.

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The novel SARS-CoV-2 that emerged in Wuhan in late 2019, recognized as the etiological agent of a new Severe Acute Respiratory Syndrome named COVID-19, has rapidly become one of the most threatening pandemic events of the modern era due to its ability to spread easily worldwide. Quantitative reverse transcription PCR (RT-qPCR) of nasopharyngeal swabs is considered the gold standard for viral detection, but more rapid and affordable diagnostic tests are required to identify the infection, especially in hospital emergency-rooms, where the risk of crowding is high. Real-Time loop-mediated isothermal amplification coupled with reverse transcription (Real-Time RT-LAMP) is a quicker, cheaper, and easier method for COVID-19 diagnostic testing (Falzone *et al*., 2012; Jiang *et al*., 2020) that has some benefits compared to RT-qPCR, including limited dependency on expensive instruments (Garcia-Venzor *et al*., 2021), fast processing time (Rödel *et al*., 2020), and user-friendly interpretation of results (Huang *et al*., 2020). Moreover, RT-LAMP has proved capable of detecting very low copies of the virus (de Oliveira Coelho *et al*., 2021) These advantages make this methodology convenient to reply with fast and reliable responses.

Herein, we report on the accuracy of an Italian Real-Time RT-LAMP diagnostic kit for SARS-CoV-2 diagnosis developed by Enbiotech SRL (Palermo, Italy) through the analysis of the data collected in the first Sicilian multicentric study comparing Real-Time RT-LAMP and RT-qPCR results.

For this purpose, 551 nasopharyngeal specimens collected from patients admitted to the Cannizzaro and Garibaldi hospitals in Catania and the Papardo hospital in Messina in January and February 2021 were processed by Real-Time RT-LAMP and RT-qPCR in their microbiological analysis laboratory and in a private laboratory. All materials needed for the study were provided to the participants by Enbiotech SRL to limit the variability related to swabs and reagents.

Key words:
RT-LAMP, COVID-19, diagnostic power, molecular tests, multicentric study.

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To minimize any possible bias, the amount of universal transport medium buffer required for the analyses with both systems was taken from the same tube. Because no patient had to be enrolled, ethics committee approval was not strictly necessary, but the ethics committee of each involved hospital was informed of the study and the study obtained formal consent.

All samples were tested by using the SARS-CoV-2 POC kit (Enbiotech, Cat. EBT 102-48) - with a Limit of Detection (LoD) of 50 viral genome copies for reaction or 1 copy/µl as reported by the manufacturer - on the ICGENE Health (Enbiotech SRL, Cat. EBT 806) or the ICGENE Plus (Enbiotech, Cat. EBT 804 Plus_D) and the commercially available systems used in each laboratory, i.e., the Allplex SARS-CoV-2 Assay (Seegene, Cat. RV10248X – Seul, South Korea), the MOLgen SARS-CoV-2 Real Time RT-PCR Kit (Adaltis, Cat. MESARS-CoV-2 - Guidonia Montecelio, Roma, Italy), and the CFX96 Touch Real-Time PCR Detection System (Bio-rad - Hercules, California, USA), according to the manufacturer's protocols. All the listed systems are recognized as medical devices by the Italian Ministry of Health with the following codes: 1973997; 1973993; 2034346; 1954317; 1945495.

Samples were representative of the different analyses requested from the laboratories during this pandemic: 218 were nasopharyngeal specimens from patients at their first hospital access, 105 were requested as antigen test confirmation, whereas 333 samples were collected as a control of infection progression in formerly positive patients.

Each tested sample was recorded on a datasheet providing the following information: ID, date and aim of the test, Real-Time RT-LAMP results reporting the amplification curve timepoint, RT-qPCR system name and results reporting the threshold cycle (Ct) values for each of the three amplified genes, and overall result (positive or negative) for both Real-Time RT-LAMP and RT-qPCR.

Statistical analyses were performed by calculating sensitivity, specificity, accuracy, and positive and negative predictive values comparing SARS-CoV-2 POC kit by Real-Time RT-LAMP with RT-qPCR results according to the following formulas:

- Sensitivity=(True Positives)/(True Positives+False Negatives).
- Specificity = (True Negatives)/(True Negatives+-False Positves).
- Accuracy = (True Positives+True Negatives)/(Total).
- Positive Predictive Value = (True Positives)/(True Positives+False Postives).
- Negative Predictive Value = (True Negatives)/(True Negatives+False Negatives).

As reported in *table 1*, 39.9% (220/551) and 41.1% (227/551) of samples tested positive for SARS-CoV-2 by Real-Time RT-LAMP and RT-qPCR analysis, respectively.

This small discrepancy was statistically investigated as reported in table 2 by calculating the sensitivity, specificity, accuracy, and positive and negative predictive value of the SARS-CoV-2 POC kit by Real-Time RT-LAMP compared with the data obtained with RT-qPCR, considering this latter as a reference. These analyses revealed accuracy, sensitivity and specificity of ≥95% and strong positive and negative predictive values (98% and 97%, respectively), indicating the reliability of the method. These observations are in line with data reported in other studies (Hu *et al.*, 2020).

Starting from these values, a McNemar's test and a Chi-square test were performed to reveal if the different results between Real-Time RT-LAMP and RT-qPCR were statistically significant [Tests performed using the GraphPad QuickCalcs Web site: http://www.graphpad.com/quickcalcs/ConfInterval1.cfm (accessed October 2021)].

McNemar's test gave a p-value of 0.1213, whilst Chi squared was 0.367 with a p-value of 0.5446. By conventional criteria, these values mean that the differences are not considered statistically significant.

The robustness of our results is supported by the number of samples analyzed, suggesting that Real-Time RT-LAMP could be a valid diagnostic alternative to detect SARS-CoV-2 positive patients where and when there is a need for rapid yet reliable tests that can be performed without the use of expensive instruments and with reduction of any potential user-dependent biases. In fact, Real-Time RT-LAMP does not require different RNA extraction steps but only heat treatment of samples for 10 minutes; all reagents needed for the assay (RT and LAMP) are already lyophilized inside the tubes provided with the kit and the operator only has to add the buffer taken from the sample tube after the heat treatment. Moreover, the RT-qPCR kits used as comparison have three target genes and sometimes interpretation of the results could be unclear, with major differences between Ct and the possibility of only two out of three genes being positive, whereas the Real-Time RT-LAMP kit, according to the World Health Organization and the Centers for Disease Control and Prevention guidelines, has two target genes - i.e., the SARS-CoV-2 N and S genes - and its output is only

Table 1 - *Positive and negative results of RT-LAMP and RT-qPCR analyses of the total sample collection.*

	RT-LAMP	*RT-qPCR*
Positive	220	227
Negative	331	324
Total	551	551

The table reports the sample size and the small differences in the results of the tests performed on each sample by using RT-LAMP and RT-qPCR.

Table 2 - *Sensitivity, specificity and predictive values of RT-LAMP assay by comparison with gold standard RT-qPCR.*

RT-LAMP	Positive	216 (TP)	4 (FP)	PPV: 216/(216+4)=0.98
	Negative	11 (FN)	320 (TN)	NPV: 320/(320+11)=0.97
		Sensitivity: 216/(216+11)=0.95	Specificity: 320/(320+4)=0.99	Accuracy: (216+320)/551=0.97

Statistical analyses of our results revealed the high diagnostic power of RT-LAMP.
TP: true positives; FP: false positives; FN: false negatives; TN: true negatives; PPV: positive predictive value; NPV: negative predictive value.

one curve that it is analyzed by the software, easily indicating the positive or negative result to the operator. Furthermore, the overall time needed for an RT-qPCR experiment is more than two hours, whereas Real-Time RT-LAMP requires less than 90 minutes from sampling to results. Considering the cost per sample of both methodologies, even if raw costs are similar, Real-Time RT-LAMP is more affordable due to the "one-step" approach that avoids the need for other kits and instruments to perform RNA extraction and reverse transcription.

Although Real-Time RT-LAMP requires less-skilled operators compared to RT-qPCR, during our data sampling it clearly emerged that nucleic acid contamination should be avoided and all good laboratory practices should be followed when performing this assay, as reported in the manufacturer's protocol, to ensure that the results obtained do not represent false negatives or false positives.

The data presented in this work highlight that Real-Time RT-LAMP has a good diagnostic power even when compared to gold standard RT-qPCR, and may be useful for the implementation of a Point of Care in settings requiring rapidity and reliability without using expensive instruments and highly-skilled personnel.

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Conflicts of Interest

the authors declare no conflict of interest. The SARS-CoV-2 POC kits were kindly provided by Enbiotech SRL (Palermo, Italy), this study was supported by Fundaccio La Marato de TV3 (UPB20725142040).

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