



The detection of SARS-CoV-2 using Reverse Transcription Loop–Mediated Isothermal Amplification (RT–LAMP) in developing country

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Abstract: The severe acute respiratory syndrome coronavirus (SARS-CoV-2) has infected the human system resulting in Covid-19, and has spread rapidly worldwide. Therefore, a fast, simple, cost-effective, and accurate detecting tool is required. The standard diagnostic tool of the World Health Organization is the reverse transcription–polymerase chain reaction (RT–PCR). This method detects the presence of viral genetic material in the human body with accurate results. However, it has several limitations in terms of equipment, personnel, duration, and cost. Therefore, a fast, simple, and sensitive alternative detection, is required, one of which is the reverse transcription loop–mediated isothermal amplification (RT–LAMP) that functions under isothermal conditions. This method is battery-driven, hence, easy to move closer to the patient. Conclusively, the RT–LAMP test for SARS-CoV-2 diagnosis produces comparable sensitivity to a standard RT–PCR and is more suitable for resource-poor settings, such as rural areas of developing countries.

Keywords: sars-cov-2, rt-lamp, reverse transcription loop–mediated isothermal amplification

Introduction

At the end of 2019, a new type of virus that infects the human system was discovered and named Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) by the World Health Organization, and the associated disease was named coronavirus disease 2019 (Covid-19) [1]. The first case was reported in Wuhan, China, and became a pandemic with a broad spectrum of impact in health, geopolitics, economy, and socio-culture. This virus infects the human respiratory system and causes mild symptoms or severe lung infections, and even death. They attack various populations such as geriatrics, adults, children, and infants, as well as pregnant women and breastfeeding mothers. Individuals with weak immunity or who possess a comorbid disease such as diabetes and hypertension are more vulnerable to the attacks [2].

SARS-CoV-2 has spread worldwide at an astonishing speed, consequently, the World Health Organization declared this a global pandemic. On the other hand, Indonesia has declared Covid-19

as a public health emergency that needs to be prevented and controlled. The country's Taskforce for the Acceleration of Handling Covid-19 reported 2.8 million confirmed cases until mid-July 2021, with a death toll of 73,582 people. Furthermore, the world's number of confirmed positive cases until mid-July 2021 is 191 million people, with a death toll of 4.1 million people. This implies that the virus is highly contagious and has spread to nearly all countries, including Indonesia. The virus is transmitted through small droplets from the nose or mouth when coughing or sneezing [3].

Methods

This study was a narrative review, which adopted PRISMA-P (Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols) method [4]. The inclusion criteria include articles that discussed RT–LAMP or RT–PCR as a diagnostic for SARS-CoV-2. The exclusion criteria are non-English articles published before 2019, and the results were organized using the Sciwheel application.

Results and discussion

Reverse Transcription–Polymerase Chain Reaction (RT–PCR)

The reverse transcription–polymerase chain reaction is a laboratory technique used to detect the presence of genetic material present in cells and bacteria, including SARS-CoV-2. The material genetic of SARS-CoV-2 is a type of RNA virus, therefore, it needs to be converted into DNA using the reverse transcriptase enzyme into cDNA, using the reverse transcription process. The RT–PCR is known to detect the existence of RNA viral genetic material in the human body with accurate results [5].

Currently, the RT–PCR tests for Covid-19 generally use a sample of mucus, phlegm, or nasopharynx fluid (the passage between nose and throat) and oropharynx (the passage between mouth and throat). The swab sample is placed into a plastic tube and sent to the laboratory for examination. During mobilization, viral RNA can be degraded under inappropriate storage conditions. Afterward, the sample will be processed and converted into cDNA and was amplified using a thermocycler, with *in vitro* replication principle at a temperature of 50°C to 96°C. Furthermore, the examination of RT–PCR is highly dependent on trained personnel, and when PCR detects virus-specific sequences through the attachment of certain primers, then the test result is positive [5].

The Rutgers Clinical Genomics Laboratory developed the RT–PCR test (TaqPath COVID-19 Combo kit) from saliva samples collected from patients. This type of sampling is faster, less painful, reduces transmission risk for healthcare providers, and enables more sample tests [6]. The requirement for saliva-based sample includes patient who does not eat, drink, brush their teeth 30 minutes before sampling, and gargle with mouthwash in the previous two hours [7].

Reverse Transcription Loop–Mediated Isothermal Amplification (RT–LAMP)

RT–LAMP is a fast, simple, and sensitive RNA detection method performed under isothermal conditions by taking nasal swab samples [5]. Several studies have also used samples in the form of serum, stool, or eye secretions [8–10]. Furthermore, the RT–LAMP method requires a constant temperature of 60°C for 30–60 minutes using simple equipment such as an incubator, water bath, or heat block. Both

the positive and negative indicators are visible to the naked eye from the discoloration in the sample [11].

The RT–LAMP test overcomes the high cost associated with the RT–PCR test without losing the function of detecting nucleic acids from pathogens and also has several other advantages. A well-optimized LAMP test shows comparable sensitivity to PCR and due to its high amplification efficiency, the changes in the reaction mixture components allow the detection of the result by colorimetric methods. This indicates that high throughput tests can be performed using unrefined specimens combined with a non-instrumental detection such as colorimetry [12].

RT–PCR vs RT–LAMP

The RT–PCR method shows a high degree of accuracy, sensitivity, specificity, and reliability. Compared to the RT–LAMP, the method has been used for a long time, therefore, sufficient information regarding usage optimization for various types of diagnostic needs is available. However, this method is difficult to apply widely because of equipment, personnel, duration, and cost constraints [6,11]. Furthermore, the RT–PCR test requires a thermocycler that uses stable electrical power and a long testing time, about 1.5 to 2 hours, when performed with sophisticated equipment and by highly trained personnel to detect viral RNA viruses [12]. One of the problems associated with this method for developing countries is the unavailability of reagents, which must be imported from other countries.

Conversely, the RT–LAMP method does not require a viral RNA extraction step to diagnose the SARS-CoV-2 virus. The advantage of the RT–LAMP method is the use of a simple tool, does not require stable electrical power, and even uses batteries, which makes it easier to use. Furthermore, the potential risk of viral RNA degradation is minimal because the sample does not need to be transported to the laboratory [5]. The RT–LAMP method also has simple operation and low technical requirements for operators [13]. This method can reach areas with minimum infrastructure and help accelerate the tracing and testing stages of the SARS-CoV-2 examination.

The colorimetric detection method can be used to further improve the RT–LAMP reaction reliability. Consequently, a test was performed at 63°C temperature for 50 minutes on a thermocycler or in water bath, and monitored by using fluorescent dyes [12]. In this case, the discoloration of dye from red to orange-yellow

Table 1. Comparison of RT-LAMP and RT-PCR methods

RT-PCR	RT-LAMP
High level of accuracy and WHO gold standard diagnostic	A comparable level of accuracy
Established technology, has an adequate research background	Lack of sufficient data and research background due to relatively new technology
Samples are subjected to rapidly changing temperatures, requiring a thermocycler, laboratory-grade facility with a stable supply of electricity	Samples are subjected to constant temperatures, requiring only simple tools such as an incubator, water bath, or heat block
Expensive equipment	Affordable equipment
Take a long time to get the result	The result is obtained within one hour
Relatively high cost per test	Relatively low cost per test
Require highly skilled personnel to perform the test properly	Does not require highly skilled personnel
Traditional reagents dependencies (from overseas) which often in short supply during pandemics	No dependency on traditional reagents
Difficult to apply in low-income developing countries	Easy to apply in low-income developing countries

indicates a positive reaction. The main mixture of a pH-sensitive indicator dye (cresol red) was evaluated for the detection of RT-LAMP-based SARS-CoV-2 [14]. The RT-LAMP does not rely on complex reagent kits [15], therefore it can be supplied from within the country, enabling easy application in developing countries.

The disadvantage of the RT-LAMP method for detecting SARS-CoV-2 is the change in color or cloudiness of clinical samples, which are sometimes unclear. In contrast, RT-PCR has quantitative values expressed in CT values. Therefore, its disadvantage can be resolved by using an application to read color intensity, which is converted into more quantitative data. RT-LAMP method is a relatively new technology compared to RT-PCR, hence, the research data in the diagnostic application is still insufficient. Furthermore, this method is in the clinical trial stage and needs further research development. Conclusively, early detection of SARS-CoV-2 is an effective countermeasure to prevent the transmission of the virus. Therefore, developing a fast, simple, cost-effective, and accurate RT-LAMP test for the diagnosis of SARS-CoV-2 is a top priority.

Conclusion

The RT-LAMP method can be used to detect the SARS-CoV-2 using simple equipment, with a comparable high level of accuracy to the RT-PCR. It also possesses the potential to be applied in low resources health facilities.

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Author contributions

MRW: conceptualization, investigation, resource, and data curation. EH: curation of analysis and writing-editing, supervision, S: resource, investigation, analysis the data, finishing the original draft, YN: the idea, conceptualization, investigation, analysis the data, editing the original draft. All authors have read the final manuscript and agreed for this submission.

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