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Editorial note

LAMP assay is an auxiliary tool for rapid and sensitive pathogen diagnosis in veterinary areas

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Abstract

Molecular technique for nucleic acid amplification has become more advanced, especially isothermal nucleic acid amplification which can amplify DNA or RNA molecules at constant temperatures. This allows us to be independent of sophisticated equipment such as thermal cyclers or realtime-PCR. The feasibility of adopting molecular techniques for pathogen detection at point-of-care are approaching practical use. At present, there are various isothermal nucleic acid amplification technologies, including rolling cycle amplification (RCA), helicase-dependent amplification (HDA), recombinase polymerase amplification (RPA) and loop-mediated isothermal amplification (LAMP). LAMP is one of the most popular methods used for pathogen diagnosis in animals due to several readout platforms such as turbidity, fluorescence, lateral flow dipstick (LFD) and color change visualizable by naked eye. In addition, LAMP can be integrated with CRISPR-cas which in turn, augments pathogen detection at ultra sensitivity. This letter shows the implementation of LAMP for pathogen detection in veterinary areas with different readouts as well as a new perspective for LAMP adaptation.

Keywords: Nucleic amplification, Isothermal amplification, Pathogen, CRISPR, Point-of-care

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Several infectious agents in animals can evolve and become contagious to humans, by a phenomenon called zoonosis. We are currently experiencing this during the SAR-CoV2 outbreak which is hypothesized to have originated from a coronavirus in bats. It mutated into an infectious virus to humans, causing the death of millions around the world (WHO, 2021). Infectious or neglected agents in animals is one of the biggest concerns for human health and must be seriously studied. Presently, the "One health" initiative has started to emphasize the importance of health interface among human-animal-ecosystem, which is the circulation of infectious diseases within the same ecosystem, which contributes to better understanding the connection of health among humans and animals as well as preventing/attenuating emerging infectious diseases (Destoumieux-Garzón et al., 2018). Rapid diagnosis tests are crucial tools for the surveillance and control of outbreaks of emerging diseases as well as suitable and effective treatment, contributing to breaking down interdisciplinary barriers between human and veterinary medicines.

During this COVID-19 outbreak, in addition to effective vaccines which were urgently needed, fast, cost-effective and portable diagnostic toolkits for SAR-CoV2 detection have been developed numerously and diversely in different platforms including antibody, antigen, and even nucleic acid detection (Böger et al., 2021; Vandenberg et al., 2021). At the moment, many platforms of molecular testing (e.g., PCR-based assay, isothermal nucleic acid amplification, and CRISPR-cas-based assay) are promptly implemented for rapid diagnostic test (Böger et al., 2021). However, most current diagnostic kits for COVID-19 tests distributed in the general market are developed from serological testing (e.g., antigen or antibody) whereas nucleic acid-based detection (PCR-based detection) for diagnostic toolkits has emerged but hardly distributed for practical diagnostic use. Although nucleic acid-based detections are highly specific, sensitive and robust, they have some limitations such as complexity, inconvenience, requirement of expensive instrument and a long process (Böger et al., 2021).

Over the past decade, isothermal nucleic acid amplification has been established in various methods such as rolling cycle amplification (RCA), helicase-dependent amplification (HDA), recombinase polymerase amplification (RPA) and loop-mediated isothermal amplification (LAMP) (Craw et al., 2015). These methods have been highly valuable tools for nucleic acid amplification at constant temperature which are independent of expensive instruments. This is likely to be adapted to become point-of-care testing for pathogen detection not only in humans but also in economically important and companion animals.

LAMP presents high sensitivity and specificity for amplifying DNA targets based on a minimum of four primers which consecutively amplify DNA molecules by non-cyclic and cyclic steps to generate immense numbers of dumbbell-like DNA molecules at 60-65°C of incubation (Notomi et al., 2000; Notomi et al., 2015). The readout of the LAMP product can be done in various ways with different sensitivities and degrees of contamination risks. Typically, LAMP-generated products are ladder-like bands under agarose gel resulting from different sizes of dumbbell-like DNA molecules amplified (Notomi et al., 2000; Dokphut et al., 2021; Pikulkaew and Potibut et al., 2021). In the end-point detection, we can note the turbidity due to the complex of $MgSO_4$ and pyrophosphate which is generated from DNA polymerization. Also, the metal indicators such as hydroxynaphtol blue (HNB) and calcein has been allowed to

detect the positive LAMP reaction by color change under natural light and the mixture of the two dyes facilitates visualization (Goto et al., 2009; Pang et al., 2019). The binding or unbinding state of magnesium/manganese ion to HNB affects the color, changing from violet (bound to Mg2+) to blue (unbound to Mg2+) and binding Mn2+ to calcein leads to quenching fluorescence. In the contrary, Mg2+ binding to calcein affects the fluorescence emitted (Goto et al., 2009; Pang et al., 2019). Moreover, DNA-binding dyes that act as intercalating dyes such as SYBR green I, SYTO9, Gel®Red can be added at the endpoint of the LAMP solution to observe the result by color change under the naked eye or fluorescence (Quyen et al., 2019). Nevertheless, some fluorescent DNA dyes such as SYTO9, SYTO82, SYTO16, SYTO13, and Miami Yellow exhibit no/low inhibitory effect on real-time LAMP which is suitable to determine the quantity of pathogen infected (Quyen et al., 2019). Besides, pH-sensitive dyes (e.g., phenol red and cresol red) have been utilized for monitoring DNA amplification from the acidity resulting from hydrogen proton accumulation derived from DNA polymerization, leading to color change in acidic solutions (Tanner et al., 2015; Buddhachat et al., 2021). In addition to being used as a LAMP readout, it facilitates a closed tube assay, leading to reduction of contamination from carryover. For point-of-care diagnostic platforms, lateral flow dipstick (LFD) has been suitable to accommodate for visualizing the LAMP results on field by naked eye from the notion of band appearance on the paper. LFD is based on immunoglobin technology so the amplified LAMP products would be attached to biotin and fluorescein isothiocyanate (FITC) would be attached on the opposite of the amplified LAMP products to bind to the ligand on LFD. FITC would be labeled on the DNA probe within the range of the inner primer amplification and hybridizes with biotinylated LAMP products (Yang et al., 2016; Posthuma-Trumpie et al., 2009). Afterward, the LAMP solution would be dropped on the LFD to observe the results.

Recently, LAMP has been widely and extensively used for developing rapid diagnosis tests for various pathogens causing diseases in animals, in particular economic animals (pigs, cattle and poultry) and domesticated animals (e.g. dogs, cats and horses). For example, the development of rapid and easy LAMP for the African swine fever virus (ASFV) in pigs based on the VP72 gene, which can be visualized by naked eye due to the color change of HNB, gave sensitivity as low as 368 plasmid DNA copies/µl without cross-reactivity with other swine pathogens (Dokphut et al., 2021). Moreover, in cyprinid fish, LAMP assay was developed for detecting Ichthyophthirius multifiliis, a parasite causing freshwater white spot disease, or ichthyophthiriasis, in freshwater cyprinid fish and other freshwater fish species. The LAMP assay used 18S rRNA, which was observed by addition of SYBR green I at endpoint of LAMP and exhibited limit of detection at 50 ng with amplification for different external parasites commonly infected in cyprinid fish (Pikulkaew and Potibut et al., 2021). In the livestock industry, LAMP combined with lateral flow dipstick (LFD) assay based on cytochrome b has been achieved to detect two blood parasites, including Babesia bovis and Babesia bigemina displaying DNA detectable as low as 0.14 and 0.85 fg, respectively, which was 100-fold higher than a conventional PCR assay and this approach requires only a heater or water bath to maintain the condition temperature and LFD for readout accommodated the results by naked eye (Yang et al., 2016).

Although LAMP offers a high-performance approach for isothermal nucleic acid amplification, regrading ultra-sensitivity, it is always readily

inherent of contamination from carryover of LAMP products in aerosol, frequently leading to false positive results. To reduce contamination in the LAMP reaction, uracil-dependent glycosylase (UDG) and UTP have been added (Hsieh et al., 2014). UTPs are incorporated into new DNA molecules of LAMP products to generate uracil-contained DNA. When uracil-containing LAMP is contaminated in aerosol, it would drop in the LAMP reaction and UDG would degrade uracil-containing DNA at uracil sites. Therefore, uracil-containing LAMP would not be performed as the DNA template in the LAMP reaction afterward, decreasing the false positive result of the assay. Up to date, there are many ways to diminish the contamination from LAMP, such as avoidance of opening the tube of LAMP reaction after completion to prevent the release of LAMP products in the environment. Detecting LAMP reaction by dyes such as pH indicators and SYTO9 with no inhibition of DNA amplification and ease to visualize has been suggested to notice endpoint results (Quyen et al., 2019). DNA isolation is one of the rate-limiting steps which can impede the shortening time of the diagnosis process; therefore, rapid DNA extraction obtained from various tissues (saliva, blood, urine, or feces) as well as providing sufficient amount and quality with no/low inhibitory agents of LAMP assay is needed for prompt development. Besides, detecting infectious agents in low amounts of tissue is challenging due to the stochastic effect; thus, the enrichment of DNA target before DNA amplification should be further investigated to decrease this limit.

In perspective, LAMP assay would be combined with the latest technology, CRISPR-cas system to accelerate the powerful diagnostic toolkits with ultra-sensitivity, specificity, and high speed (Chen et al., 2018; Li et al., 2018; Mukama et al., 2020). CRISPR-cas has not been employed only for genome editing to modify a specific base or a portion of DNA in certain genes, but has also been recognized as a promising approach for developing powerful and ideal diagnostic toolkits with high specificity, sensitivity, speed, and simplicity to assay (Chen et al., 2018; Li et al., 2018; Mukama et al., 2020). The isothermal nucleic acid amplification including LAMP enables acceleration of CRISPR-cas-based diagnosis to be better until comparable to ideal platform (Craw et al., 2015). We strongly believe that the combination of LAMP and CRISPR-cas is a great alternative platform and powerful tool for accommodating the development of rapid diagnostic toolkits for several infectious diseases in animals. Also, the add-on of CRISPR-cas to LAMP might establish the feasibility for multiplexed assay, meaning that multiple pathogens would be detected in one single reaction. At present, various platforms, such as paper and microfluid-based diagnosis developed to include all steps involved in the nucleic acid detection, from DNA isolation to readout in one drop sample on one station, or so-called lab-on-chip/paper (Craw et al., 2015; Zhang et al., 2019). LAMP can be used for amplifying DNA targets to display the presence or absence of agents on the putative chip/paper.

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