THE SCIENCE WORLD
A Monthly e Magazine
ISSN:2583-2212
Jan 2024 Vol.4(1), 235-241

Popular Article

Loop-mediated Isothermal Amplification (LAMP) Assay as a Diagnostic Tool in Veterinary Medicine

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https://doi.org/10.5281/zenodo.10512854

Abstract

For making appropriate and most suitable therapeutic strategies for infectious diseases, accurate and timely identification of infectious agents is crucial. Conventional methods like microbial isolation from clinical samples followed by its identification and serological tests have been regarded as gold standard methods of diagnosis for most of infectious diseases. Microbial isolation method is time consuming and having risk to laboratory personnel. Serological techniques are useful only after development of specific antibody in the body and sometimes produce false positive reactions. Molecular techniques like polymerase chain reaction (PCR) and real-time PCR also have been used in detection of infectious agents since last three decades. In these techniques, unique region of pathogen's genome is amplified under different temperature cycles followed by end products, diagnosis rapid without compromising accuracy. However, major obstacles in adoption of PCR and real-time PCR based diagnosis are high-cost laboratory setup, high-cost equipment, skilled laboratory personnel, sample transportation to testing laboratory and efficient sample preparation. These limitations make PCR and real-time PCR unsuitable being used as regular diagnostic methods in low resource laboratory settings. Recently, loop-mediated Isothermal amplification (LAMP) has come up, which amplifies the target region of DNA at a single temperature. The LAMP assay can be performed in a simple water bath with un-purified samples. Features like low-cost equipment, rapid result, high sensitivity, and high specificity make researchers to assess possibilities of LAMP being used as cheap diagnostic tool in low-cost laboratory setting. LAMP assay also satisfying the WHO's ASSURED guidelines' minimal requirements to act like point of care (POC) analysis. Now-a-days, LAMP is used as a preferred diagnostic tool around the world in detection of many infectious agents of veterinary importance.

Keywords: Isothermal Amplification; Loop-mediated isothermal amplification, ASSURED format, Point-of-care diagnostics

Published 14.01.2024

1. Introduction

To implement appropriate treatment and/or preventative measures and hence restrict spread of the diseases, rapid and accurate detection of causative agent is crucial. Over the time, many methods/tools of disease diagnosis have been developed. The isolation and identification of the etiological agent from clinical specimens served as the foundation for diagnosis in the past. As per the World Organization of Animal Health (WOAH), the microbial isolation is considered as reference/gold standard method of identification of infectious agents for most of infectious diseases. But this method involves transmission risk to laboratory personnel; require containment laboratory for high-risk pathogens and sample-to- result report may take 4-5 days. By the time the result report received, and preventive strategies are made, the disease may spread to larger population. These limitations motivated scientists to develop rapid, safe and user-friendly diagnostics methods for infectious diseases. Later, diagnostic time is decreased by the development of serological testing techniques. The serological methods detect specific anti-microbial antibodies developed after infection. The specific antibodies appeared in body approximately 2 weeks after first time infection. Sometimes, serological techniques produce false positive reaction due to cross reactivity among species of micro-organisms. Serological methods are preferred during sero-surveillance and sero-monitoring epidemiological studies. During last 2-3 decades, development of various molecular diagnostic techniques preferably targeted nucleic acid sequence amplification assays, resulted in reduction of sample-to-diagnosis report time to hours. Nucleic acid amplification based diagnostic assays are useful, particularly for conditions where an early diagnosis is crucial to the prognosis. Furthermore, high risk micro-organism causing diseases may make it challenging to isolate etiological agents, which could be a significant obstacle to correctly diagnosing the underlying cause. Nucleic acid sequence amplification-based detection techniques serve as good substitutes in these circumstances.

Nucleic acids amplification methods like PCR, real-time PCR and their variants involves three temperature cycles of activity: Initial denaturation, annealing and elongation (Mullis and Faloona, 1987). A unique region of disease-causing pathogen can be amplified to billions of copies in a tube containing suitable reaction mixture of enzymes/salts and providing appropriate temperature cycles in a thermo-cycler. The amplification of targeted region is initiated using micro-organism specific (or target sequence specific) two primers (each of 15-20 nucleotide sequence). The billions of amplified targets are further subjected to agarose gel electrophoresis for result documentation. Similarly, real-time PCR is the modified version of

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Published 14.01.2024

PCR, where the amplification of target nucleic acid sequence is monitored in real time during the time of amplification process. Real-time PCR doesn't require post-amplification electrophoretic result analysis. Both PCR and Real-time PCR have higher sensitivity in detecting target sequence with high specificity. Many studies have reported that PCR and real-time PCR can detect as little as picogram of target sequence and comparatively, real-time PCR has higher sensitivity than conventional PCR. The limitations associated with both PCR and real-PCR are high equipment cost, need of superior infrastructure/laboratory setting, high-cost reagents, sample preparation and skilled personnel to perform the experiment. The sample-to-result report time varies from few hours to days depending upon sample size, type of sample, methods of processing and end products analysis. These limitations restrict PCR and real-time PCR being used as preferred diagnostic tools in animal health in developing countries where limited laboratory infrastructure is present. Also, PCR and real-time PCR are unsuitable being considered in point-of-care (POC) diagnostic devices development. The World Health Organization (WHO) enlisted features like affordability, sensitivity, specificity, user-friendliness, rapid and robustness, equipment-free and deliverable (collectively called ASSURED format) as prerequisites of POC diagnostic devices (Martzy *et al.*, 2019).

Recently, many new nucleic acid sequence amplification methods have been come up, where amplification of target sequence can be done at single temperature (*i.e* isothermal amplification) unlike PCR and real-time PCR. Loop-mediated isothermal amplification (LAMP) assay is one of among them. This assay has successfully been used for detection of human as well as animal pathogens. This article is designed to give an overview of LAMP assay and its application in detection of animal pathogens.

2. Isothermal Amplification Methods

Isothermal nucleic acid amplification is a promising method for rapid and efficient nucleic acid amplification at a constant temperature and thus does not require a thermo-cycler like in PCR. Most of these methods have high sensitivity in target sequence detection and some of them become commercially successful. Recently developed isothermal-based nucleic acid amplification methods are: Loop-mediated isothermal amplification (LAMP), Strand displacement amplification (SDA), Nucleic acid sequence-based amplification (NASBA), Helicase dependent amplification (HDA), Rolling circle amplification (RCA), Exponential amplification reaction (EXPAR) and whole genome amplification (WGA). Isothermal amplification methods use DNA polymerase enzymes with strand displacement abilities to

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create continuous growing nucleic acid sequence target in a self-displacing cycle. LAMP is widely used isothermal amplification method in human and animal health diagnostics.

3. LAMP (Loop-mediated Isothermal Amplification)

In 1998, a Japanese company called Eiken Chemical Co., Ltd. designed a method known as loop-mediated isothermal amplification (LAMP) of DNA that eliminates some difficulties inherent in PCR (Notomi, T *et al.*, 2000).

Like PCR, the LAMP amplifies target DNA region to billion copies. LAMP assay uses *Bst* DNA Polymerase which has strand displacement property as well as polymerase activity. This property of the enzyme negates the use temperature cycles as in PCR. Hence the amplification process can be performed in a temperature regulated machine provides constant temperature such as heat block and water bath. Another innovative feature of LAMP is its high specificity due to the use of multiple primers (four to six) corresponds to specific sites of the target region on DNA template, as compared to only two in a typical PCR (Parida, M *et al.*, 2008). Amplification process is carried out at 60-65°C for 1 hour. However, amplification process can be accelerated by inclusion of two loop primers, LF (loop forward) and LB (loop backward), and reaction is completed more quickly than conventional PCR. In addition, this method can be used to amplify the target RNA sequence. In that case, one-step amplification, same as DNA amplification, can be done by simultaneously adding reverse transcriptase, since reverse transcriptase also has strand displacement activity.

A major advantage of LAMP is the ability to assess amplified end products using multiple methods. Like PCR, the amplified end products can be detected in agarose gel electrophoresis. The positive LAMP reaction end products show multiple bands on 2-2.5% agarose. However, in-tube positive LAMP reactions can also be identified even with the naked eye. In case of positive amplification reaction, the reaction mixture appears slightly turbid as compared to control reaction mixture. Many metal indicator dyes like hydroxynaphthol blue, calcein, Eriochrome Black T etc. and some DNA intercalating dyes like SYBR green, EvaGreen etc. can also be used. The use of dyes in reaction mixture, negate post-amplification electrophoretic analysis and hence environmental contamination with amplified products. These methods can be combined with real-time analysis to allow simultaneous quantitative evaluation of the amplified product.

4. LAMP as a diagnostic tool in Veterinary Medicine

Shorter sample-to-report time, without compromising sensitivity and specificity and non-requirement of high-cost equipment make LAMP assay suitable for disease diagnosis in

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low-level laboratory settings. Due to its high sensitivity, the technology can also detect low-level of microbial infection cases. Today, the widespread use of LAMP is possible due to the commercial availability of primers tested against various viruses, bacteria, fungi and parasites that cause infections in human and animals.

LAMP has the potential to be used as a simple screening assay in field or at pen-side by veterinarian. LAMP assays have been developed for the detection of 18 viruses deemed notifiable of ruminants, swine, and poultry by the World Organization for Animal Health (Mansour, S.M *et al.*, 2015). Among animal viruses, a one-step RT-LAMP assay has been developed for detection of FMD virus (Dukes, J.P *et al.*, 2006), Viral haemorrhagic septicaemia virus targeting G-protein sequence in less than 1 hour. LAMP assay coupled with lateral flow dipstick for the detection of classical swine fever virus has been developed by Chowdry *et al* (2014). LAMP test targeting the p40 gene of *Mycoplasma agalactiae*, for the diagnosis of classical contagious agalactia also reported. LAMP was also used for the successful detection of a wide range of viruses such as adenovirus, varicella zoster and cytomegalovirus.

Das *et al.* (2012) developed the first LAMP assay to detect capripox viruses (CaPV) by targeting the conserved poly (A) polymerase small subunit (VP39) and found that developed test has similar limit of detection with gold standard real-time qPCR. Mwanandota *et al.* (2018) compared the detection rate (DR) of two LAMP assays versus PCR for the detection of capripox virus (CaPV) genus responsible for Lumpy skin disease (LSD) in apparently health animals and 59 clinically sick animals and found that DR was 13.6% for PCR and 39.0% for LAMP it was 39.0%. Good sensitivity and specificity results of this LAMP assay supports its use in routine diagnosis of LSD. Also, I.A. Bhat *et al* (2019) developed probe-based RT-LAMP assay, which is extremely rapid, cost effective, highly specific, and sensitive and has potential usefulness for rapid *Brucella* surveillance.

5. Conclusion

With the ever-increasing advancements in the field of molecular techniques, more focus is given to detect microorganisms at molecular level for disease diagnosis. There have been many reports already proved that; LAMP is a successful molecular tool for the amplification of target DNA sequence and pathogen detection. LAMP diagnostic kits are currently available for clinical diagnosis of some infectious diseases, which reflects the quality and usefulness of the technique. The features of LAMP assay like higher sensitivity and specificity, robust nature, easy result evaluation and high-cost equipment-free nature makes it fit for development point-of-care disease diagnostics in veterinary medicine. Therefore, more efforts need to be done to

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utilize the full potentials of this wonderful diagnostic tool and promote its application in other aspects of research.

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