Bio Vet Innovator Magazine

Volume 2 (Issue 2) FEBRUARY 2025

OPEN ACCESS

Popular Article

LAMP: A Lamp for Diagnosis

J B Rajesh[1], Binipi Debbarma[2] and Payel Kar[3]
[1]Assistant Professor (SG), Department of Veterinary Medicine
[2]MVSc scholar, Department of Veterinary Public Health & Epidemiology
[3]MVSc scholar, Department of Veterinary Medicine
College of Veterinary Sciences and Animal Husbandry, Central Agricultural University (I), Selesih PO, Aizawl, Mizoram: 796015.
***Corresponding Author:** leovet@gmail.com
DOI: https://doi.org/10.5281/zenodo.14969369

Received: February 27, 2025
Published: February 28, 2025

Abstract:

Loop-mediated isothermal amplification (LAMP) is an isothermal nucleic acid amplification method known for its ease, sensitivity and rapidity. LAMP is a molecular detection method in which the multiplication is carried out in isothermal condition and does not need thermocycler. LAMP is a technique for the diagnosis of many infections in the field level where sophisticated equipments and expertise are nominal.

Key Words: LAMP, DNA, amplification, primer, PCR

Introduction:

Loop Mediated Isothermal Amplification (LAMP) is a novel technique developed by Notomi et al in 2000. It is primarily used for the amplification of DNA. It is used for the detection of many agents including disease causing microbes. In comparison with other conventional techniques, LAMP are having the advantage of low cost, quickness, easiness in its operation and doesn't require any sophisticated equipments for its operation. In addition, handling of toxic chemicals such as ethidium bromide can be avoided in LAMP. Well experienced laboratory workers are also not required in working LAMP technique. Within a single tube the whole reaction can be completed. Interesting fact about LAMP is its process takes place at a constant temperature. In other words, in comparison with its counterpart Polymerase Chain Reaction (PCR) which needs a thermocycler for its working, LAMP doesn't need it. If we compare both it can be seen that LAMP is a simpler technique which can be handled by ordinary labs or even in field conditions where qualified and experienced staffs are less in number.

With certain modifications in procedure, LAMP can be used for the detection of RNA too. Primers are short fragments of single stranded DNA that help in amplification of target region. In PCR we need to use two primers, ie., forward and backward. But in LAMP, there are 4-6 primers are used viz. Forward Inner Primer (FIP) (Forward 1 Complementary (F1C), F2) and Backward Inner Primer (BIP) (Backward 1 Complementary (B1C), B2)], external primers (F3, B3) and loop-specific primers, Forward Loop Primer (FLP) and Backward Loop Primer (BLP). Most of the developed LAMP procedure adopt only 4 primers and exclude forward loop primer and backward loop primer.

FIP in DNA pattern will attach to F2-complementing region in the pattern strand and initiate the synthesis of the complementing strand. F3 and B3 primers with the help of DNA polymerase enzyme the strand length will be extended lead to production of a dumbbell-shaped DNA formation. This will act as the LAMP initiator. FIP will attach to stem loop structure of DNA and begin the synthesis of next strand which will create a stem-loop intermitted DNA structure having an opposite copy of target sequence in its stem-loop region formed in the other end of the gen by BIP primer. The free strand forms a structure with external loop which acts as the mold for BIP polymer. The dumbbell-shape DNA is formed which will act as the step material of LAMP cycle. By plan of oligonucleotide probes precise for these structures, they can be applied in hybridization and there is no need of heat denaturation after the multiplication. In short, all the steps from multiplication to detection can be done in the same temperature.

Isothermal Amplification:

Amplification is at a set temperature through a repetition of 2 types of elongation reactions occurring at the loop regions: self-elongation of templates from the stem loop structure formed at the 3′-terminal and the binding and elongation of new primers to the loop region. The LAMP assay can amplify a few copies of DNA to 10^9 in less than one hour under isothermal conditions that makes observable detection of specimens with positive results possible.

LAMP Primer Design:

Primers are designed using software and one such software is given: http://primerexplorer.jp/lampv5e/index.html. Designing of LAMP primers is more complex and less predictable than that designing for PCR. Clear target selection and specific guidelines are needed for designing a highly sensitive and specific LAMP assay. The selected primers are used at a constant temperature of 60-65°C for amplifying the target sequence along with a polymerase which is having strand displacement and replication activity. Six distinct regions on the target gene are identified using 4 different primers and the loop primers further speed up the reaction. The amount of DNA produced in LAMP will be higher due to the specificity of the used primers.

Advantages:

Another added advantage of LAMP is easiness in the detection method of amplified products. The yields in LAMP are often tenfold than that in PCR detection. When PCR needs again machinery for the detection of amplified products, LAMP products can be visualized by naked eye. Dyes like SYBR green is used to create a color change that can be detected with eye.

Applications:

The usefulness of LAMP in different sectors of science is innumerable. Being a simple but sensitive technique, it can be a promising tool for the diagnosis of many infections in the field level. The result is quick and easily readable with naked eye. The technique can be operated with minimum expense. Some of the disease conditions where LAMP can be used for diagnosis are: in human medicine for detecting Tuberculosis and malaria. In Veterinary science LAMP is used for the detection of swine fever, porcine circovirus2 infection, porcine reproductive and respiratory syndrome virus and Pseudorabies virus. In fisheries science it is used for the detection of nocardiosis, enteric red mouth disease and edwardsiellosis. Along with this microbial detection, LAMP is helpful in the detection of parasites like Trypanosomes. Detection of Zika virus and Dengue virus in urine samples and Zika virus in mosquitoes can be done with LAMP. Apart from the above uses, LAMP can be employed for the detection of herbal medicinal plants with

accuracy. It can be used for the species-specific detection of phyto-pathogenic fungi. LAMP is used for the rapid detection of pathogens like Salmonella and Staphylococcus in food and feed samples and by this way food poisoning can be minimized. The pollution of water with microbes like enterococcus which is a major public health threat can be detected using LAMP.

Limitations:

Even though LAMP is having many advantages, just like other technologies, it also has some limitations. Primer designing of LAMP is very complex and utmost care is required. It is inappropriate for large test menu and for detection of un-sequenced targets. LAMP products cannot be used for other applications such as cloning or sequencing. Since LAMP is using large number of primers in its reaction, there may be a chance of primer-to-primer interaction. Another disadvantage of LAMP is its dependence on indirect detection methods like turbidity and non-specific dyes, which may lead to the detection of false positive results.

Conclusion:

Now efforts are being made by scientists to make a cheap, portable, battery operated, simple in assembling, easily usable, open-hardware portable LAMP machines which can be used in field as well as in remote areas where laboratory facilities are minimal.

References:

Keikha, M. (2018). LAMP Method as One of the Best Candidates for Replacing with PCR Method. The Malaysian Journal of Medical Sciences, 25(1), 121-123.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., and Hase, T. (2000). Loop-mediated isothermal amplification of DNA. Nucleic acids research, 28(12), e63-e63.

Velders, A. H., Schoen, C., and Saggiomo, V. (2018). Loop-Mediated Isothermal Amplification (LAMP) Shield for Arduino DNA Detection. BMC Research Notes, 11, 1-5.

Wang, Y., Zhang, B., Sun, Y., Liu, Y., and Gu, Y. (2018). Loop-Mediated Isothermal Amplification on Crude DNA as a Point-of-Care Test for the Diagnosis of Mycoplasma Related Vaginitis During Early Pregnancy. Laboratory Medicine, 49(1), 59-67.