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Review Article

Loop-Mediated Isothermal Amplification (LAMP) for Detection of Various Organisms: A Review

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Abstract

Disease diagnosis is important for implementation of proper therapeutic and prophylactic measures. Traditionally, disease diagnosis was depends upon isolation and identification of the causative organisms. This was followed by serology and after that molecular method. Molecular tests are valuable when early diagnosis is important. For this purpose, nucleic acid amplification (PCR, nucleic acid sequence-based amplification, self-sustained sequence replication, strand displacement amplification) is one of the most valuable tools not only for the diagnosis of infectious diseases but also used in advanced level research. The Loop-Mediated Isothermal Amplification (LAMP) is a unique nucleic acid amplification technique for diagnosis of various pathogens introduced at 2000 by Notomi and his colleagues which is simple, easy, rapid and cost effective when compared to PCR due to its high specificity, sensitivity, and rapidity. It uses a set of six primers and a DNA polymerase with stranddisplacement activity. Major advantage of LAMP method is its cost-effectiveness as it can be done simply by using waterbath or heating block.

Key words: Loop-Mediated Isothermal Amplification assay (LAMP), Real-time PCR, *Bst* DNA polymerase, Sensitive, Rapid, Simple.

Introduction

Diagnosis of disease is crucial for implementation of proper therapeutic and prophylactic measures. Over time there has been great revolution in the field of disease diagnosis. Isolation and identification of the etiological agent from clinical specimens was the basis for diagnosis earlier. Later, serological tests developed, which reduced the time for diagnosis. With the evolution of molecular techniques, the time needed for final diagnosis was reducedto hours from days.[1]

Many types of PCR have been developed for the diagnostic purpose. Despite the advantages of PCR based assays like higher sensitivity, the need for costly instruments and post amplification processing makes its use as limited for routine use in low resource setting laboratories. Rather PCR facilities for accurate diagnosis get restricted to specialized diagnostic laboratories. Several isothermal amplification techniques came up over time: Transcription Mediated Amplification[2], Strand Displacement amplification[3], Rolling Circle Amplification[4], Helicase Dependent Amplification[5] etc. One of the most widely used isothermal nucleic acid amplification method is Loop mediated isothermal amplification (LAMP).[6] LAMP tests have been developed for the detection of a vast variety of disease causing agentsfrom its beginning in 2000. It makes an effective alternative diagnostic tool for a number of human, animal as well as plant pathogens.[6]

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Why there is need of LAMP?

The PCR based DNA detection methods seem to have the qualities of sensitivity and specificity but lacks other qualities like swiftness and requires costly instruments, etc. Isothermal amplification techniques assure other qualities stated by WHO. LAMP stands out to be a good and effective diagnostic testamong the isothermal amplification techniques. It is a preferred diagnostic tool because of the ease at which it can be performed even though the principle and reaction mechanism is a bit complicated.[7]

To overcome all above limitations in viraldiagnosis, a loop-mediated isothermal amplification(LAMP) method was developed as an alternativemethod for viral detection. LAMP is a novel nucleic acid amplificationmethod that amplifies a few copies of target DNA with high specificity, efficiency, andrapidity under isothermal conditions,

using a set of four specially designed primers and a DNA polymerase with strand displacementactivity.[7] When detecting the RNA genome of a pathogen such as an RNA virus, LAMP has been merged with reverse transcription (RT) into RT-LAMP to allow nucleic acidamplification.[8] This techniquerequires only simple and cost-effectiveequipment amenable to use in clinical laboratories.[9]

Compared to PCR, reaction simplicity and detectionsensitivity are the advantages of LAMP. LAMP assay is capable to detect as low as 1 pg/µl template DNA. It has been claimed that the LAMP method can detect as few as 100 copies of DNA template in blood samples, equal roughlyto 5 parasites/µl.[3] Thissensitivity is notably higher than any knownimmune chromatography-based RDTs, whichare recommended by WHO as part of the globalmalaria control strategy.[10] LAMP has potential applications for clinical diagnosisas well as surveillance of infectious diseases indeveloping countries without requiring sophisticatedequipment or skilled personnel.[11]

Advantages of LAMP Method over Conventional Methods

- As LAMP don't require costly equipments, so itcan be called as an equipment free technique. It needs only water bath or heating block which is commonly available. Hence, under field condition, the technique is suitable.[8]
- LAMP is not only sensitive but also specific when compared with other DNA detection methods like PCR. Large quantities of a desired sequence (10^9-10^{10} copies) are produced in less than an hour[7]
- Diagnosis by LAMP is rapid; gets completed within an hour or 30 min (when loop primers are used)[12]
- It a robust technique as unprocessed or partly processed samples can be used as template.
- LAMP works at a constant temperature
- No need of post amplification processing. Results can be observed directly by adding SYBR green, HNB or Calcein. So electrophoresis is not needed which also reduces the timeamplifies a few copies of target DNA.[13]
- Easy to handle.
- Cost-effective as it can be carried out withsimplest equipment, i.e., dry bath/water bath.[7]

LAMP method:

The whole procedure of this assay is very simpleand rapid by incubating mixture of gene sample andsix proper designed specific primers in a single tubewith reverse transcriptase and Bst DNA polymerase at 63°C. The LAMP amplification includes sixprimers comprising two outer primers, such as forward outer primer (F3) and backward outer primer (B3), having strand displacementactivity during the non-cyclic step only andalso two internal primers, such as FIP and BIP, havingboth sense and antisense sequence which helps in loopformation. Further, two loop primers, i.e., forwardloop primer and backward loop primer are designedto amplify the additional sites that are not accessedby internal primers. For detection agarose gel electrophoresis and also real timemonitoringby visual turbidity or others can be used. Asa byproduct of amplification,the turbidity can be seen due to magnesium pyrophosphateprecipitate. So, it's possible to detect theamplification product by the naked eye. The detectionprocedure can be done in real time either by measuringthe turbidity or by fluorescence using intercalatingdyes such as SYBR Green. A visible color change canbe seen with naked eyes using dyes such as SYBRgreen, EVA green or hydroxynapthol blue. The dye molecules intercalate the DNA, which can be correlated with the number ofcopies making the LAMP be quantitative. LAMP assay is found to be 10-100 folds more sensitivethan PCR with a detection limit of 0.01-10 pfu ofvirus.[7,8,14]

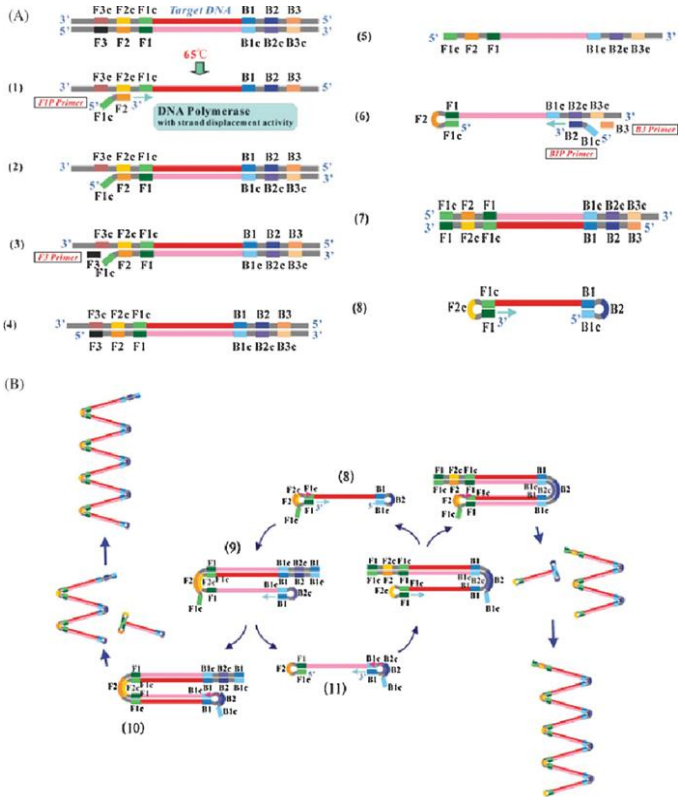

Figure I: Loop Mediated Isothermal Amplification (LAMP) assay[6]

LAMP assay for detection of Human Viruses

LAMP method has been commonly used in detection of DNA and RNA virus.[14] LAMP method has been developed for detection of human viruses including chickenpox virus[15], mumps virus[16], respiratory syncytial virus[17], flavivirus causing West Nile fever[18], enterovirus 71[19], humanimmunodeficiency virus (HIV) causing acquiredimmune deficiency syndrome (AIDS)[20], pandemic (H1N1) 2009 virus[21], cytomegalovirus causing cytomegalovirus inclusion disease[22], chikungunya virus[23], Japanese encephalitis virus[24], herpessimplex virus type 2 causing genital herpes[25], human T-cell lymphotropic-1 and human T-cell lymphotropic-2retroviruses[26], Epstein-Barr virus (EBV)[27], influenza A and B viruses causing influenza[28], Ebola virus[29], human papilloma viruses type 6, 11, 16 and 18[30], and HIV-1 virus.[31]

Their results demonstrated that the LAMP assay could be well-applied to laboratories, as a portable device and valuable tool for differential diagnosis of various viruses in the countryside. LAMP has also been applied successfully for RNA virus detection by directly adding the reverse transcriptase to the reaction mixture, which is termed the reverse transcription LAMP (RT-LAMP).[14]

Hepatitis B virus (HBV) is one of the most important factors for hepatocellular carcinoma and a liverdisease that seems 350-400 million persons are infected with all over the world. Loop mediatedisothermal amplification (LAMP) is a novel nucleic acid amplification technique with high specificity andsensitivity which has been done under isothermal condition.[15] Various authors detected hepatitis B virus by LAMP method and observed that PCR sensitivity and also detected HBV genotypes and it could be used in clinical point-of-care settings, mainly in endemic and resource-limited environments for HBV diagnostics, donor screening, epidemiological studies, and therapeutic monitoring of patients undergoing antiviral treatment.[15,16,17] These results reveal that HBV-LAMP assay is rapid, sensitive and specific, and capableof detecting the HBV. This assay could be used in clinical point-of-care settings, mainly in resource-limited environments for HBV diagnostics, donor screening and also for monitoring of patients undergoing treatment.[17]

As viremic individuals are the main source of infectious virus, so early detection of dengue virus (DENV) infection during the febrile period is crucial for proper patient management and prevention of disease spread. The RT-LAMP assay developed is sensitive, specific and simple to perform. The assay enhanced the detection of dengue when used in combination with serological methods. If RTLAMP assay can be implemented for routine dengue diagnosis, it would greatly improve the diagnostic coverage of suspected dengue cases without the need for costly equipment and reagents.[18]

Japanese encephalitis virus (JEV) is the most common cause of childhood viral encephalitis in the world, causing an estimated 50,000 infections and 10,000 deaths annually. Laboratory diagnosis of JEV relies on virus isolation and characterization, the detection of virus-specific antibodies, and the detection of genomic sequences by nucleic acid amplification techniques. Thus, the RT-LAMP assay reported in this study allows rapid, real-time detection as well as quantification of JEV in acute phase CSF samples without requiring sophisticated equipment and has potential usefulness for clinical diagnosis and surveillance of JEV in developing countries.[19]

Currently, 1 to 10% of renal transplant recipients are diagnosed with BK virus induced renal allograft nephropathy, which can lead to graft loss in 20 to 80% of patients. Detection of BKV in urine and blood can alert a clinician to the possibility of BKVAN in a patient presenting an asymptomatic rise in serum creatinine. However, physicians currently depend on PCR results for BKV detection, which might take 2 to 3 days or longer to arrive in many clinic settings and also it's not available in most of the country. Thus, the LAMP assay for detection of BKV can guide therapeutic decisions in outpatient clinics itself, especially if a urine sample becomes positive after a short, 30 min incubation and is accompanied by a plasma sample that shows positivity, suggesting that the patient is more likely to have a clinically significant viral load.[20]

Having high vaccine coverage, Mumps patients are prone to suffering from secondary vaccine failure (SVF). Due to this critical problem, rapid detection of mumps virus by a fast diagnostic technique was necessary. Development of a fast LAMP assay for detection of mumps virus genome with a detection of 0.1 PFU along with the same sensitivity as RT-nested PCR within 60 min only.[21]

Routine diagnostic testing is essential for the early detection and treatment of HIV infection.As individuals cantransmit the virus during early or acute infection, accurate and timely diagnosis may reduce the transmission of HIV when the individual is mostinfectious. The diagnosis of acutely infected individuals who might be missed by currentrapid antibody tests, HIV-1 RT-LAMP assay can help in this case. If this assay can be implemented atthe POC, where NAAT testing is

currently not feasibledue to cost and time limitations, will be great help.[22]

Influenza virus causes annual epidemics in temperate climates generally lasting 3−8 weeks in the winter months and is a major cause of morbidity and mortality. LAMP showed an excellent sensitivity and specificity for detection of influenza A and B in NP specimens and result is produced in 30 min only.[23]

MERS-CoV is mainly diagnosed byreal-time RT-PCR assay, with at least two different genomic targets required for a positive diagnosis according to the case definition of The World Health Organization (WHO) as of 3 July 2013. So RT-LAMP for MERSCoV was developed, which was capable of detecting as few as 3/4 copies of MERS-CoV RNA.[24] Therefore, it is urgently necessary to develop as many specific genetic diagnostic methods as possible to allow stable diagnosis of MERS-CoV infections.

Herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) and varicella-zoster virus (VZV) are alphaherpesviruses that infect, establish lifelong latency in, and subsequently reactivate from human sensory neuronal ganglia. LAMP assay was used for the detection of HSV-1, HSV-2, and VZV infections in very short time.[25]

Detection of plant viruses:
Viruses are very tiny compared to other groups of plant pathogens like fungi and bacteria which can be visualized through microscopes but plant viruses are too small to observe using light microscopes. They are made of a coat protein and a type of nucleic acid, DNA or RNA based on the nucleic acid which can be seen only using a transmission electron microscope.[26]

LAMP had been developed for many plant viruses such as *Wheat streak mosaic virus* (WSMV)[27], *Wheat yellow mosaic virus* (WYMV), *Japanese soil-borne mosaic virus* (JSBWMV) and *Chinese wheat mosaic virus* (CWMV)[28], *Yellow head virus* (YHV)[29], *bovine viral diarrhea viral* (BVDV)[30], *Potato virus* Y (PVY)[31], *tomato torrado virus* (ToTV)[32], *Sugarcane mosaic virus* (SCMV)[33], *tomato spotted wilt virus, necrotic spots virus,* and *chrysanthemum stunt virus, tobacco mosaic virus, banana streak virus, cauliflower mosaic virus, yellow mosaic virus, potato virus Y, potato spindle tuber viroid, peach latent mosaic viroid, wheat yellow mosaic virus*.[34]

Detection of Animal Viruses:
The LAMP assay has been recently applied for the rapid detection of several viruses in animal, such as Foot-and-mouth disease virus[35], Bovine herpesvirus -4 (BHV-4)[36], viral conjunctivitis[37], porcine circovirus type 2, duck virus enteritis[38], monkey pox virus[39], infectious hematopoietic necrosis virus (IHNV)[40], Newcastle disease virus[41], canine parvovirus and pestiviruses cause diseases in animals such as classical swine fever (CSF) and bovine viral diarrhea/mucosal disease (BVD/MD). Equine arteritis virus (EAV), porcine reproductive and respiratory syndrome virus (PRRSV), lactate dehydrogenase elevating virus (LDV) of mice, and simian hemorrhagic fever virus (SHFV) all belong to the Arteriviridae family of viruses.[34]

In addition, it has been used to determine sex of asparagus, genetically modified organisms (GMOs), and Phytoplasmas.[42]

Detection of pathogenic Bacteria:
Isolation and characterization of pathogens from clinical samples is a tiresome job.[43,44] Lamp assay has been used to detect various pathogenic organism in recent years. It was first used to detect $stxA_2$ in *Escherichia coli* O157:H7 cells.[45]

According to WHO report, TB is the second leading cause of death among infectious disease worldwide after HIV. *Mycobacterium tuberculosis* is a slow growing bacterium that needs to 1 to 2 months for growing in a culture.[45] However, this technique is both labour and timeconsuming. The TB LAMP assay is designed to require, as much as possible, a similar number of steps and biosafety requirements as sputum smear microscopy.[44] MTB-LAMP assay helps direct identification of M. tuberculosis in processed sputum samples.[46]

Salmonella is a common gram-negative bacterium belonging to family Enterobacteriaceae and is an important cause of food-borne bacterial infections in both developed and developing countries.[47] At present, bacteria isolation and biochemical identification are the standard methods for Salmonella detection and identification. Culture-based methods are reliable but laboratory-intensive and time-consuming, while crossbiochemical reaction can occur between different species under Enterobacteriaceae. Salmonella LAMP assay can potentially serve as new on-site diagnostics in the food and agricultural industries.[48]

Toxigenic Vibrio cholerae, the etiological agent of cholera causes severe diarrheal disease affecting thousands of people each year in developing countries. The LAMP assay needs 12-18 min for amplification with a single colony and within 60 min with human feces and seafood samples. This assay is a potent tool for quick, easy, and sensitive detection of CT-producing *V. cholerae* which

may help the investigation of *V. cholerae* contamination in seafood, as well as the early diagnosis of cholera in humans.[49]

LAMP has also been successfully used for specific detection of others organisms likes *Streptococcus pneumoniae*[50], *Staphylococcus aureus*[51], *Bacillus anthracis*[52], and others.

Detection of Fungi:
The LAMP has the advantage of simplicity in detection of fungus compared to the classic diagnostic methods like histopathological test. It detects successfully *C. albicans*[53], *Pneumocystis jirovecii*[54], *Histoplasma capsulatum*[55] and others.

Detection of parasites:
Protozoal parasites are seriously harmful for human and also animals.One of the most neglected tropical diseasesis Visceral leishmaniasis (VL). The annual incidence worldwide is approximately 0.2 to 0.4 million cases with mortality rates of 1.5% (93 deaths/6224 VL cases from 2004-2008) in Bangladesh.[56] LAMP assay has also been established to detect Leishmania donovani DNA from blood samples of VL patients and the results were comparable with that of conventional PCR where 68 case was positive out of 75 confirmed VL cases, and revealed its diagnostic sensitivity of 90.7% (95.84-81.14, 95% CI).[57]

Malaria is alife-threatening disease diseasewhich is caused by protozoan parasites. Clinical diagnosis and parasitological confirmation bymicroscopy using Giemsa-stained blood films ('Giemsamicroscopy') or rapid diagnostic test (RDT) are the malariadiagnostic approaches currently employed throughout world. Compared to nested PCR, the sensitivity andspecificity of the primary NINA-LAMP assay were 96.8%. This assay is highly sensitive for the diagnosis of malaria and detection of Plasmodiumparasite infection at both the genus and species level when compared to PCR. It may be a vital diagnostic modality in efforts to eradicate malaria from areas of low endemicity.[58]

Advantages of LAMP
As it can amplify nucleic acid under isothermal conditions in the range of 60-65°C, it only needs simple and low cost equipment but needs to be effective. LAMP is a good and effective diagnostic test in developing countries as it does not require sophisticated equipment and skilled personnel and proves to be cost effective. Its specificity isextremely high. Its amplification efficiency is very high because there is no time loss of thermal change. Nucleic acid is amplified by the LAMP method and the turbidity derived from the precipitate is produced according to the progress of the reaction and thus making it ideal for easy monitoring through naked eye. It can be detected through fluorescence by utilizing calcein, hydroxy naphthol blue, SYBR Green I etc in naked eye. Both amplification and detection of the gene done here in a single step, by incubating the mixture of gene sample, primers, DNA polymerase with strand displacement activity and substrates at a constant temperature. As ithas high amplification efficiency, DNA can be amplified 10^9-10^{10} times in 15-60 min. Denaturation step is not necessary.[1,59,14]

Disadvantages of LAMP
Although LAMP has some advantages, it hassome disadvantages too. Proper designing of primer is a major constraintin this assay.[60] Multiplexing approaches forLAMP are less developed than PCR. As primers are large in number per target in LAMP, so chance of primer-primer interactions are more.[43] Another problem is the chance of carry over contamination. Contamination chances can be narrowed to some extentby avoiding post amplification opening of the tube. Sample preparation, amplification and post amplification processing (if needed) must be done in separate rooms to avoid chances of contamination at all stages. Another disadvantage is that LAMP cannot be used for amplification of sequences of size more than 300bp.[1]

Conclusion
LAMP has the advantages of reaction simplicityand detection sensitivitycompared with PCR.LAMP is an innovative, new generation, geneamplification technique that can amplify the targetsequence with a high degree of sensitivity and specificityunder isothermal condition.[34]

As detection procedure is easy,i.e., on real-time nucleic acid amplification, this assaycan be used as point of care diagnostic toolfor infectious human or animal disease. Thus, it will provide agreat platform for quick and accurate identification ofdifferent pathogens in medical as well as veterinaryfield.[43]

Furthermore the importance of nucleic acid amplification in various fields of science, such as forensic investigation, food science technology, genetics and clinical diagnosis, underscores the need to develop a robust technique that will link the gaps in molecular diagnostics. Hence, more needs to be done to utilize the full potentials of this wonderful diagnostic tool and also promotes its application in other aspects of biotechnology.

References:

1. Rekha V, Rana R, Arun TR, et al. Loop Mediated Isothermal Amplification (LAMP) Test-a Novel Nucleic Acid Based Assay for Disease Diagnosis. Advances in Animal and Veterinary Sciences 2014 Jul 15;2:344-50.
2. Guatelli JC, Whitfield KM, Kwoh DY, Barringer KJ, Richman DD, Gingeras TR. Isothermal, in vitro amplification of nucleic acids by a multienzyme reaction modeled after retroviral replication. Proc. Natl. Acad. Sci. USA 1990; 87: 1874-1878.
3. Walker GT, Little MC, Nadeau JG, Shank DD. Isothermal in vitro amplification of DNA by a restriction enzyme/DNA polymerase system. Proc. Natl. Acad. Sci. USA 1992; 89: 392-396.
4. Fire A, Xu SQ. Rolling replication of short DNA circles. Proc. Natl. Acad. Sci. USA 1995; 92: 4641-4645.
5. Vincent M, Xu Y, Kong H. Helicase dependent isothermal DNA amplification. EMBO Rep 2004; 5: 795-800.
6. Notomi TH, Okayama H, Masubuchi, et al. Loop-mediated isothermal amplification of DNA. Nucleic Acids Research 2000; 28 (12): e63
7. Dhama K, Karthik K, Chakraborty S, et al. Loop-mediated Isothermal Amplification of DNA (LAMP): A New Diagnostic Tool Lights the World of Diagnosis of Animal and Human Pathogens: A Review. Pakistan Journal of Biological Sciences 2014; 17(2):151-166.
8. Soliman H and Mansour EM. An inexpensive and rapid diagnostic method of Koi Herpesvirus (KHV) infection by loop-mediated isothermal amplification. Virology Journal 2005; 2(1):1.
9. Ihira M, Yoshikawa T, Enomoto Y, et al. Rapid diagnosis of human herpesvirus 6 infection by a novel DNA amplification method, loop-mediated isothermal amplification. Journal of clinical microbiology 2004 Jan 1; 42(1):140-5.
10. Long C, Qi H, Huang SH. Mathematical modeling of cytotoxic lymphocyte-mediated immune response to hepatitis B virus infection. BioMed Research International 2008 Feb 13; 2008.
11. Mori Y and Notomi T. Loop-mediated isothermal amplification (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases. Jounal of Infection and Chemotherapy 2009; 15: 62-69.
12. Nagamine K, Watanabe K, Ohtsuka K, et al. Loop-mediated Isothermal Amplification Reaction Using a Nondenatured Template. Clinical Chemistry 2001; 47(9):1742-1743.
13. Njiru ZK. Loop-Mediated Isothermal Amplification Technology: Towards Point of Care Diagnostics. PLo S Neglected Tropical Disease 2012; 6(6): e1572.
14. Fakruddin MD. Loop Mediated Isothermal Amplification (LAMP)- an alternative to polymerase chain reaction (PCR) Bangladesh research publications journals 2011; 5(4): 425-439.
15. Akram A, Islam SR, Munshi SU, Tabassum S. Detection of Hepatitis B Virus DNA among Chronic and potential Occult HBV patients in resource-limited settings by Loop-Mediated Isothermal Amplification assay. Journal of viral hepatitis 2018 May 16.
16. Moslemi E, Shahhosseiny MH, Javadi G, et al. Loop mediated isothermal amplification (LAMP) forrapid detection of HBV in Iran. African Journal of Microbiology Research 2009; 3(8): 439-445.
17. Nyan DC, Ulitzky LE, Cehan N, et al. Rapid Detection of Hepatitis B Virus in Blood Plasma by a Specific and Sensitive Loop Mediated Isothermal Amplification Assay. Clinical Infectious Diseases 2014; 59(1):16-23.
18. Hu SF, Li M, Zhong LL, et al. Development of reverse-transcription loop-mediated isothermal amplification assay for rapid detection and differentiation of dengue virus serotypes 1-4. BMC microbiology 2015 Dec; 15(1):265.
19. Parida MM, Santhosh SR, Dash PK, et al. Development and evaluation of reverse transcription- loop-mediated isothermal amplification assay for rapid and real-time detection of Japanese encephalitis virus. Journal of clinical microbiology 2006 Nov 1; 44(11):4172-8.
20. Bista BR, Ishwad C, Wadowsky RM, et al. Development of a loop-mediated isothermal amplification assay for rapid detection of BK virus. Journal of clinical microbiology 2007 May 1; 45(5):1581-7.
21. Okafuji T, Yoshida N, Fujino M, et al. Rapid diagnostic method for detection of mumps virus genome by loop-mediated isothermal amplification. Journal of clinical microbiology 2005 Apr 1; 43(4):1625-31.
22. Curtis K, Rudolph D, Owen MS. Sequence-specific detection method for reverse transcription, loop-mediated isothermal amplification of HIV-1. J Med Virol 2009; 81:966-972

23. Ito M, Watanabe M, Nakagawa N, et al. Rapid detection and typing of influenza A and B by loop-mediated isothermal amplification: comparison with immunochromatography and virus isolation. J Virol Methods 2006; 135:272-275

24. Shirato K, Yano T, Senba S, et al. Detection of Middle East respiratory syndrome coronavirus using reverse transcription loop-mediated isothermal amplification (RT-LAMP). Virology journal 2014 Dec; 11(1):139.

25. Reddy AK, Balne PK, Reddy RK, et al. Loop-mediated isothermal amplifi cation assay for the diagnosis of retinitis caused by herpes simplex virus-1. Clin Microbiol Infect 2010; 17:210-213.

26. Jeongl JJ, Ju HJ, Noh J. A Review of detection methods for the plant viruses. Plant Dis 2014; 20(3): 173-181.

27. Lee S, Kim JH, Choi JY, et al. Loop-mediated isothermal amplification assay to rapidly detect wheat streak mosaic virus in quarantined plants. The plant pathology journal 2015 Dec; 31(4):438.

28. Fukuta S, Tamura M, Maejima H, et al. Differential detection of Wheat yellow mosaic virus, Japanese soil-borne wheat mosaic virus and Chinese wheat mosaic virus by reverse transcription loop-mediated isothermal amplification reaction. Journal of virological methods 2013 May 1; 189(2): 348S-54.

29. Khunthong S, Jaroenram W, Arunrut N, Suebsing R, Mungsantisuk I, Kiatpathomchai W. Rapid and sensitive detection of shrimp yellow head virus by loop-mediated isothermal amplification combined with a lateral flow dipstick. Journal of virological methods 2013 Mar 1;188(1-2):51-6.

30. Fan Q, Xie Z, Xie L, et al. A reverse transcription loop- mediated isothermal amplification method for rapid detection of bovine viral diarrhea virus. Journal of virological methods 2012 Dec 1; 186(1-2): 43-8.

31. Przewodowska A, Zacharzewska B, Chołuj J, Treder K. A One-Step, Real-Time Reverse Transcription Loopmediated Isothermal Amplification Assay to Detect Potato Virus Y. American journal of potato research 2015 Jun 1; 92(3): 303-11.

32. Soliman H, El-Matbouli M. Reverse transcription loop-mediated isothermal amplification (RT-LAMP) for rapid detection of viral hemorrhagic septicaemia virus (VHS). Veterinary microbiology 2006 May 31; 114(3-4): 205-13.

33. Keizerweerd AT, Chandra A, Grisham MP. Development of a reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay for the detection of Sugarcane mosaic virus and Sorghum mosaic virus in sugarcane. Journal of virological methods 2015 Feb 1;212:23-9.

34. Saharan P, Khatri P, Dingolia S, Duhan JS, Gahlawat SK. Rapid detection of viruses using loop-mediated isothermal amplification (LAMP): A Review. In Biotechnology: Prospects and Applications 2013; (pp. 287-306). Springer, New Delhi.

35. Dukes JP, King DP, Alexandersen S. Novel reverse transcription loop-mediated isothermal amplifi cation for rapid detection of foot-and-mouth disease virus. Arch Virol 2006; 151: 1093-1106.

36. Ali, Haytham & Keefe, Greg & Cepica, Arnost. Bovine Herpesvirus-4, A Potential Cause of Mastitis in Canadian Dairy Cows. British Journal of dairy science 2011; 2. 31-34.

37. Wakabayashi T, Yamashita R, Kakita T, Kakita M, Oshika T. Rapid and sensitive diagnosis of adenoviral keratoconjunctivitis by loop-mediated isothermal amplification (LAMP) method. Curr Eye Res 2004; 29(4-5): 219-224.

38. Ji J, Du LQ, Xie QM, et al. Rapid diagnosis of duck plagues virus infection by loop-mediated isothermal amplification. Res Vet Sci 2009; 87(1):53-58.

39. Iizuka I, Saijo M, Shiota T, et al. Loop- mediated isothermal amplification- based diagnostic assay for monkeypox virus infections. J Med Virol 2009; 81:1102-1108.

40. Gunimaladevi I, Kono T, Lapatra SE, et al. A loop mediated isothermal amplification (LAMP) method for detection of infectious hematopoietic necrosis virus in rainbow trout 2005; 150: 899-909.

41. Pham HM, Nakajima C, Oaci K, et al. Loop mediated isothermal amplifi cation for rapid detection of Newcastle disease virus. J Clin Microbiol 2005; 43(5): 1646-1650

42. Sankaran S, Mishra A, Ehsani R, Davis C. A review of advanced techniques for detecting plant diseases. Computers and Electronics in Agriculture 2010 Jun 1; 72(1):1-3.

43. Sahoo PR, Sethy K, Mohapatra S, Panda D. Loop mediated isothermal amplification: An innovative gene amplification technique for animal diseases. Veterinary world 2016 May; 9(5):465.

44. Maruyama F, Kenzaka T, Yamaguchi N, Tani K, Nasu M. Detection of bacteria carrying the stx2 gene by in situ loop-mediated isothermal amplification. Applied and environmental microbiology 2003 Aug 1; 69(8):5023-8.

45. WHO T. Global tuberculosis report 2012 World Health Organization. Geneva: http://appswhont/iris/bittream /10665/91355/1/9789241564656_eng. pdf 2012.

46. Pandey BD, Poudel A, Yoda T, et al. Development of an in-house loop-mediated isothermal amplification (LAMP) assay for detection of Mycobacterium tuberculosis and evaluation in sputum samples of Nepalese patients. Journal of Medical Microbiology 2008 Apr 1; 57(4):439-43.

47. European Food Safety Authority (EFSA) (2007) The community summary report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Union in 2006. EFSA J 130, 1-352.

48. Zhuang L, Gong J, Li Q, et al. Detection of Salmonella spp. by a loop-mediated isothermal amplification (LAMP) method targeting bcfD gene. Letters in applied microbiology 2014 Dec; 59(6):658-64.

49. Yamazaki W. Sensitive and rapid detection of cholera toxin-producing Vibrio cholerae using loop-mediated isothermal amplification. InMicrobial Toxins 2011 (pp. 13-22). Humana Press, Totowa, NJ.

50. Seki M, Yamashita Y, Torigoe H, Tsuda H, Sato S, Maeno M. Loop-mediated isothermal amplification method targeting the lytA gene for detection of Streptococcus pneumoniae. Journal of clinical microbiology 2005 Apr 1; 43(4):1581-6.SS

51. Lim KT, Teh CS, Thong KL. Loop-mediated isothermal amplification assay for the rapid detection of *Staphylococcus aureus*. BioMed research international 2013;2013.

52. Kurosaki Y, Sakuma T, Fukuma A, et al. A simple and sensitive method for detection of Bacillus anthracis by loop-mediated isothermal amplification. Journal of applied microbiology 2009 Dec; 107(6):1947-56.

53. Inбcio J, Flores O, Spencer-Martins I. Efficient identification of clinically relevant Candida yeast species by use of an assay combining panfungal loop-mediated isothermal DNA amplification with hybridization to species-specific oligonucleotide probes. Journal of clinical microbiology 2008 Feb 1; 46(2):713-20.

54. Singh P, Singh S, Mirdha BR, Guleria R, Agarwal SK, Mohan A. Evaluation of loop-mediated isothermal amplification assay for the detection of Pneumocystis jirovecii in immunocompromised patients. Molecular biology international 2015; 2015.

55. Scheel CM, Zhou Y, Theodoro RC, Abrams B, Balajee SA, Litvintseva AP. Development of a loop-mediated isothermal amplification method for detection of Histoplasma capsulatum DNA in clinical samples. Journal of clinical microbiology 2014 Feb 1; 52(2):483-8.

56. Alvar J, Velez ID, Bern C, et al. WHO Leishmaniasis Control Team. Leishmaniasis worldwide and global estimates of its incidence. PloS one 2012 May 31;7(5):e35671.

57. Khan MG, Bhaskar KR, Salam MA, Akther T, Pluschke G, Mondal D. Diagnostic accuracy of loop-mediated isothermal amplification (LAMP) for detection of Leishmania DNA in buffy coat from visceral leishmaniasis patients. Parasites & vectors 2012 Dec;5(1):280.

58. Sema M, Alemu A, Bayih AG, et al. Evaluation of non-instrumented nucleic acid amplification by loop-mediated isothermal amplification (NINA-LAMP) for the diagnosis of malaria in Northwest Ethiopia. Malaria journal 2015 Dec; 14(1):44.

59. Parida M, Sannarangaiah S, Dash PK, Rao PVL and Morita K. Loop mediated isothermal amplification (LAMP): a new generation of innovative gene amplification technique; perspectives in clinical diagnosis of infectious diseases. Reviews in Medical Virology 2008; 18: 407-421.

60. Torres C, Vitalis EA, Baker BR , Gardner SN, Torres MW and Dzenitis JM. LAVA: An open-source approach to designing LAMP (loop-mediated isothermal amplification) DNA signatures. BMC Biochem 2011; 12: 240