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Loop-mediated Isothermal Amplification of DNA (LAMP): A New Diagnostic Tool Lights the World of Diagnosis of Animal and Human Pathogens: A Review

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Abstract: Diagnosis is an important part in case of animal husbandry as treatment of a disease depends on it. Advancement in molecular biology has generated various sophisticated tools like Polymerase Chain Reaction (PCR), its versions along with pen-side diagnostic techniques. Every diagnostic test however has both advantages and disadvantages; PCR is not an exception to this statement. To ease the odds faced by PCR several non-PCR techniques which can amplify DNA at a constant temperature has become the need of hour, thus generating a variety of isothermal amplification techniques including Nucleic Acid Sequence-Based Amplification (NASBA) along with Self-Sustained Sequence Replication (3SR) and Strand Displacement Amplification (SDA) and Loop mediated isothermal amplification (LAMP) test. LAMP stands out to be a good and effective diagnostic test for empowering in developing countries as it does not require sophisticated equipments and skilled personnel and proves to be cost-effective. Performance of LAMP mainly relies on crafting of six primers (including 2 loop primers) ultimately accelerating the reaction. LAMP amplifies DNA in the process pyrophosphates are formed causing turbidity that facilitates visualisation in a more effective way than PCR. The Bst and Bsm polymerase are the required enzymes for LAMP that does not possess 5'-3' exonuclease activity. Results can be visualized by adding DNA binding dye, SYBR green. LAMP is more stable than PCR and real-time PCR. Non-involvement of template DNA preparation and ability to generate 10^6 copies of DNA are added benefits that make it more effective than NASBA or 3SR and SDA. Thus, it fetches researcher’s interest in developing various versions of LAMP viz., its combination with lateral flow assay or micro LAMP and more recently lyophilized and electric (e) LAMP. Availability of ready to use LAMP kits has helped diagnosis of almost all pathogens. LAMP associated technologies however needs to be developed as a part of LAMP platform rather than developing them as separate entities. This review deals with all these salient features of this newly developed tool that has enlightened the world of diagnosis.

Key words: Bst polymerase, isothermal amplification, lateral flow, lyophilized LAMP, micro LAMP, pyrophosphate, SYBR green, template, animal, human, pathogen, diagnosis

INTRODUCTION

Treatment of a disease can begin based on the diagnosis of the disease; hence diagnosis is the important part in case of animal husbandry. Although new diagnostic techniques have come into market, yet isolation and identification of pathogen remains gold standard. But it is not an easy job because of the hazard involved in case of some harmful zoonotic pathogens and also requires will and skill, apart from being a time consuming process. Polymerase Chain Reaction (PCR) has come to rescue the world of diagnosis by detecting pathogens accurately and easily (Saiki et al., 1985; Mullis et al., 1986; Laueran, 2004; Black et al., 2002;

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Belak, 2007; Deb and Chakraborty, 2012; Dharma et al., 2012a, b; Singh et al., 2012). The advent in the field of molecular biology has led to the development of various molecular detection techniques, which include: various versions of PCR (e.g., Real time PCR, quantitative PCR, multiplex PCR, immunoassay PCR, PCR-ELISA etc.), detection of nucleic acid polymorphism (RAPD, RAPD). A large number of diagnostic tests including immunochromatographic methods are available for increasing the accuracy of bedside diagnostic tests (Black et al., 2002; Kataria et al., 2005; Schmitt and Henderson, 2005; Dharma et al., 2008a, 2012a, 2013; Nath et al., 2010; Deb and Chakraborty, 2012; Singh et al., 2012).

Moreover, in the changing world scenario, high population density, global warming, threat of economically important animal diseases along with presently emerging, re-emerging, variant and newer animal pathogens/diseases (foot and mouth disease, rotavirus, parvovirus, campylobacter, arbovirus, mycoplasma, chicken anemia virus, Marek’s disease, infectious bronchitis virus, etc.) and zoonotic and pandemic threats (salmonellosis, brucellosis, rabies, tuberculosis, leptospirosis, West Nile virus, etc.; avian/bird flu, swine flu, plague and others) are haunting the animal health and production systems (Daszak et al., 2000; Dharma et al., 2005, 2008b, c, 2009, 2011, 2012a; Jones et al., 2008; Kumar et al., 2009, 2012a, b; Pawana et al., 2009; Bhut et al., 2011; Patyal et al., 2011; Verma et al., 2011, 2012a, b; Jain et al., 2012; Mahima et al., 2012; Singh et al., 2012, 2013; Sumi et al., 2012). Therefore, it is the need of the hour that special attention need to be given for their effective diagnosis and control; especially supporting and strengthening the surveillance and monitoring systems with tools and techniques unravelled by the novel advances in molecular biology, biotechnology and nanotechnology (Black et al., 2002; Belak, 2007; Bollo, 2007; Deb and Chakraborty, 2012; Ratchiff et al., 2007; Dharma et al., 2008b; Balamurugan et al., 2010; Siddiqui, 2010; Taddele et al., 2011; Dharma et al., 2008b; Deb et al., 2013).

Every diagnostic test however has both advantages and disadvantages; PCR is not an exception to this statement (Mullis and Faloona, 1987; Lauermer, 2004; Kataria et al., 2005; Jones et al., 2008). Drawbacks of PCR are (1) It needs sophisticated instrument in order to maintain different temperature with different time (2) Needs post amplification protocols like electrophoresis in order to know the result (3) Takes 3-4 h to know the result and (4) DNA should be extracted from the samples before carrying out PCR. Though it is an enthralling test for diagnosis of microbes, these negative points became a hurdle which needs some alterations so that the end user is benefited. Real time PCR, yet another gift for diagnosis can quantitatively assess the DNA (Mackay et al., 2002). However, these tests can be used only in well equipped laboratories because of the need of expensive instruments and skills required for results to be interpreted. To ease the odds faced by PCR, several non PCR techniques which can amplify DNA at a constant temperature was the requirement of the hour. Several isothermal amplification tests have been developed which include Nucleic Acid Sequence-Based Amplification (NASBA) (Mugasa et al., 2009), Transcription Mediated Amplification (TMA) or Self-Sustained Sequence Replication (SSR) and Strand Displacement Amplification (SDA) (Walker et al., 1992) along with Rolling Circle Amplification (RCA) (Lizardi et al., 1998), Signal Mediated Amplification of RNA Technology (SMART), loop-mediated isothermal amplification of DNA (LAMP), Isothermal Multiple Displacement Amplification (IMDA), helicase-dependent amplification (HDA), Single Primer Isothermal Amplification (SPIA) and circular Helicase Dependent Amplification (cHDA) (Gill and Ghaemi, 2008). Notomi et al. (2000) came up with a technique called as Loop mediated isothermal amplification test (LAMP). Among all the isothermal amplification techniques LAMP was unique and easy so it fetched researcher’s interest. LAMP has stormed into diagnosis of almost all pathogens and there are already some ready to use LAMP kits are available in the market.

AN IDEAL DIAGNOSTIC TECHNIQUE

Prevention of disease is becoming harder day by day due to emergence of new and re-emergence of all ready existing diseases/pathogens. Prevention of a disease can be achieved only by proper diagnosis of the pathogen which has been a chaotic job in case of developing countries. The criterion for ideal diagnostic tests (According to World Health Organization are (1) Sensitivity, (2) Specificity (3) Considerably of low cost (4) Simplicity (5) Rapidity (6) Adaptable to any sort of climatic variation and (7) Easy availability of instruments (Njiru, 2012). The PCR based DNA detection methods seem to have the qualities of sensitivity and specificity (Saiki et al., 1988) but lacks other qualities like swiftness and requires costly instruments, etc. Isothermal amplification techniques satisfy other qualities stated by WHO. Among the isothermal amplification techniques LAMP stands out to be a good and effective diagnostic test. 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 (Hotez et al., 2007).
LAMP: Loop mediated isothermal amplification as the name implies the reaction takes place at an isothermal temperature which is the greatest credit to the test. LAMP uses a DNA polymerase which has unique property of strand displacement along with the usual polymerization property. LAMP is a simple screening assay and when compared to PCR is more sensitive and specific, so that it can amplify a negligible amount of DNA to more than \(10^7\) copies which happens within an hour’s time (Notomi et al., 2000; Sen and Ashbolt, 2010). LAMP amplifies DNA at greater concentration when compared to PCR based amplification and allows easier visualisation due to release of pyrophosphate that causes turbidity due to precipitation. The amplification technique can also be quantitative (Mori et al., 2004) and depends on DNA synthesis by autocycling strand displacement, performed by a DNA polymerase with high strand displacement activity (Whiting and Champoux, 1998; Ayyadevara et al., 2000; Shijun et al., 2011).

Requirements of LAMP

Primer: Performance of LAMP mainly relies on crafting of Primers which should be very specific. Unlike PCR, LAMP requires minimum 4 primers which are named as F3 (Forward outer), B3 (Backward outer), FIP (Forward inner) and BIP (Backward inner) primers. Two more primers namely LF (Loop forward) and LB (Loop backward) can also be incorporated which accelerates the reaction hence completes the reaction still faster (Nagamine et al., 2002a). The F3 and B3 have their major role during strand displacement and called as strand displacing primers. FIP and BIP have their function in loop formation (Parida et al., 2008). The FIP and BIP should be of High Performance Liquid Chromatography (HPLC) purified primers. The primers are designed based on the eight target regions present on the gene: F3c, F2c, F1c and FLP which are in 3' side and B1, B2, B3 and BLP in the 5' side (Fig. 1). The inner and loop primers act via different mechanisms. Moreover, the loop primers facilitate the LAMP reaction specifically within a period of half an hour in comparison to when original LAMP method is used. Loop primer inclusion yields a large amount of DNA in short time (Nagamine et al., 2002b). There are certain characters which are to be considered while designing a primer for LAMP which are:

- Inner primers should not have AT rich sequence at both the ends
- GC content should be about 50-60%
- In case of GC rich sequence the melting temperature should be 60-65% and for AT rich sequence it should be within 55-60%
- Designed primers should not have any secondary loop structure formation
- Distance between 5' end of F2 and B2 should be 120-180 bp and that of F2 and F3 is 0-20 bp. The same can be considered for B2 and B3 also

These primers can be designed online through “PrimerXplorer” which is commonly used for LAMP primer designing.

Fig. 1: Target region of primers and qualities of LAMP primer
**Enzyme:** Enzyme can be called as the heart of the LAMP. An enzyme which has DNA polymerizing capacity along with the crucial ability to displace the strand is selected for LAMP. The enzymes with recomended ability are Bst polymerase isolated from *Bacillus stearothermophilus* and *Bsm* polymerase isolated from *Bacillus smithii*. Both enzymes have unique property of strand displacement and can catalyse 5’-3’ DNA polymerization but they don’t have 5’-3’ exonuclease activity (Nagamine et al., 2001; Wozniakowski et al., 2012). The Bst polymerase has its enzyme activity till 66°C and Bsm polymerase has its activity till 63°C and best at 60°C. Other components which are required for LAMP are dNTPs for providing required nucleotides, Magnesium sulphate which forms magnesium pyrophosphate during the course of the reaction which enables to visualize the result based on the turbidity formed (Tomita et al., 2008). Betaine is a chemical used to stabilize the AT and GC content and finally buffer which contains tween, Tri-HCl with a pH 8.8, (NH₄)₂ SO₄, MgSO₄ and KCl are commonly used.

**Template preparation: a need?** In case of LAMP there is no need for preparation of template DNA for amplification. It can work well with moderately prepared template or the sample as such can be used (Kaneko et al., 2007). This exclusion of DNA extraction step not only shortens the reaction time and result interpretation but also eliminates the chance of contamination. The ultimate sample of choice for LAMP has not been clearly found but reports are there for the straight away use of CSF, heat treated blood (Njimu et al., 2008), serum. The procedure of LAMP assay is depicted in a pictorial presentation in Fig. 2. The comparison of PCR and LAMP is given in Table 1.

<table>
<thead>
<tr>
<th>Properties</th>
<th>PCR</th>
<th>LAMP</th>
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<tbody>
<tr>
<td>Denaturation</td>
<td>Required for separation of strands, enabling primer binding</td>
<td>Denaturation step is not a mandate, as the enzyme displaces the strand and takes care of it.</td>
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<tr>
<td>Annealing, extension</td>
<td>Usually employs 3 steps as denaturation, annealing and extension, working at different temperature and timing.</td>
<td>Works under a constant temperature usually between 60-65°C.</td>
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<tr>
<td>Time required</td>
<td>Takes 2-3 h based on the different parameters.</td>
<td>Sixty min usually. Loop primer inclusion can shorten the time taken for completion of the reaction.</td>
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<td>Post amplification process</td>
<td>Needs agarose gel electrophoresis for knowing the result.</td>
<td>By incorporating DNA binding dyes like SYBR green or any metal indicator like calcine or other dyes like hydroxy napthol blue the results can be interpreted visually Tomita et al. (2008) and Goto et al. (2009).</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Can detect up to nanogram level of DNA.</td>
<td>Can detect up to femtogram level of DNA in the sample. So minute quantity can be amplified and result interpretation becomes easy.</td>
</tr>
<tr>
<td>Instruments</td>
<td>Needs sophisticated instrument in order to maintain different temperature within a given time.</td>
<td>No need of expensive instruments. Water bath, a common instrument can serve the purpose.</td>
</tr>
<tr>
<td>DNA template preparation</td>
<td>Requires template DNA preparation which should be pure and impurities can hinder the PCR reaction</td>
<td>Robust technique no need for processing of DNA. Samples as such can be integrated to the test. Impurities won’t hinder the reaction Kaneko et al. (2007) and Francois et al. (2011).</td>
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**Fig. 2:** LAMP Procedure and advantages at a glance
Methods for detection of amplified LAMP products and results interpretation: Lamp results can be interpreted with naked eye which is the phenomenal thing about LAMP. Because of this feature it can be effortlessly applied in the field as a diagnostic technique and a semiskilled person can interpret the results. Magnesium pyrophosphate is produced as a by-product of amplification turbidity due to which indicates the formation and quantity of targeted genomic region. Gene amplification products can be detected by agarose gel electrophoresis as well as by real-time monitoring in a relatively inexpensive turbidimeter which in addition to visualization, aid in quantification of the gene copy number. Varying sizes of bands (i.e., ladder pattern) are observed from the amplified LAMP products in agarose gel electrophoresis. New LAMP protocols must be verified by restriction enzyme analysis alone or followed by nucleotide sequencing when tested for the first time (Notomi et al., 2000). Results can be visualized by adding up SYBR green (Table 2) which is a DNA binding dye (Monis et al., 2005; Parida et al., 2005, 2008). The DNA binding dyes like ethidium bromide, PicoGreen (Dukes et al., 2006; Curtis et al., 2008), or propidium iodide (Hill et al., 2008) can also be used. Metal ion indicator like calcein can be used to visualize the result (Tomita et al., 2008). Hydroxy naphthol blue a colouring dye can also be used (Goto et al., 2009). The benefit about calcein and Hydroxyl Naphthol Blue (HNB) is that both can be added during the start of the reaction unlike SYBR green which has to be added once the reaction is completed. Calcein and HNB are added in the start of the reaction; hence chance of carry over contamination is very less. Turbidity can also be seen once the amplified product is spun for a short burst of time, white precipitate settles down in the bottom of the tube (Mori et al., 2001).

Longevity of LAMP: LAMP is more stable compared to PCR and real time PCR (Francois et al., 2011). It is secure at a range of temperature, pH and a wide range of elongation time. This stability is helpful in case of incompletely processed or non processed samples where some components try to hinder the reaction. Trace quantities of whole-blood, hemin, blood culture media, N-acetyl cystein, NaCl and anticoagulant or anti-complement compounds can inhibit Taq polymerase and hence the PCR cannot detect the DNA of subject. But LAMP can tolerate these components (Kaneko et al., 2007; Francois et al., 2011). Cold chain is a must while preparing master mix for PCR which is not a mandate in case of LAMP. The Taq polymerase hinderers in samples like urine or stools (Fredricks and Relman, 1998) have no effect on LAMP.

LAMP IN THE FIELD OF DIAGNOSIS

About a decade ago, LAMP had been invented and at present, it has given a new impetus towards development of new diagnostic tests. It is considered as a technology precisely suitable for well-developed laboratories. The LAMP can not only be used for detection of DNA, but also can be used for detection of RNA which is termed as RT-LAMP. Hence it can detect both DNA as well as RNA viruses (Kalvatech et al., 2010). More than 100 LAMP tests have already been developed for both human and animal pathogens (Bendall et al., 2002; Karanis and Orgerter, 2009; Deb and Chakraborty, 2012). In case of Neglected Tropical Diseases (NTD), access to reliable diagnosis is severely limited leading to misdiagnosis. As LAMP is user-friendly and cost effective and at the same time having higher sensitivity and specificity (as discussed earlier), it is ideal to limit such diseases i.e., NTDs (Mosher et al., 2007).

Human pathogens: LAMP has been developed for diagnosis of a list of human pathogen which include tuberculosis (Pandey et al., 2008, Geojith et al., 2011), leptospirosis (Sonthayanon et al., 2011), Staphylococcus aureus detection (Lim et al., 2013), Listeria monocytogenes (Tang et al., 2011), anthrax (Hatanaka et al., 2010), Cholera organism (Yamazaki et al., 2008), Clostridium difficile toxin (Boyanton et al., 2012), for detection of Coronavirus accountable for the development of Severe Acute Respiratory Syndrome, SARS (Notomi et al., 2004, Poon et al., 2004), human influenza A virus (Poon et al., 2005a), H1N1 Swine flu (Kubo et al., 2010), Hepatitis B virus (Moslemi et al., 2009), human herpes virus 6 (Ilhara et al., 2007), herpes simplex and Vericella zoster (Okamoto et al., 2004; Kaneko et al., 2005), Human papilloma virus type 6, 11, 16 and 18 (Hagiwara et al., 2007), Dengue (Parida et al., 2005), Japanese Encephalitis virus (Parida et al., 2006), hepatitis B (Cai et al., 2008), Entero virus detection (Xia et al., 2011). The LAMP is also employed for detection of fungal infections like Pneumocystis pneumonia which is an opportunistic infection in case of patients infected with the deadly disease HIV (Uemura et al., 2008), Candidiasis (Inacio et al., 2008), Paracoccidioides brasiliensis (Endo et al., 2004). Here sputum samples and bronchoalveolar lavage samples were used for diagnosis. Parasitic organism like Taenia species (Taenia saginata, Taenia solium, and Taenia asiatica) were also diagnosed with the help of LAMP.
T. asiatica and T. solium) which can cause cysticercosis can be detected by LAMP (Nkouwa et al., 2010). The LAMP has also been developed for protozoan parasites like Trypanosoma species (Trypanosoma brucei rhodesiense) (Thekiso et al., 2007) Giardia duodenalis (Plutzer and Karamis, 2009), Brugian filariasis (Poole et al., 2012), Entamoeba histolytica (Li et al., 2009) and malarial parasite (Plasmodium falciparum) (Poon et al., 2006; Rodger et al., 2008).

Animal pathogens: LAMP has attracted a lot of attention as a potentially rapid, accurate and cost-effective novel nucleic acid amplification method for detection of micro-organisms. LAMP assay has been successfully used to detect viral diseases/pathogens like West Nile virus (Parida et al., 2004), Coronavirus (Poon et al., 2005b), Norovirus (Fukuda et al., 2006), highly pathogenic avian influenza (Imai et al., 2006; Ito et al., 2006; Dinh et al., 2011), FMD (Dukes et al., 2006), Classical swine fever (Chakraborty and Choudhury, 2012), Porcine cytomegalovirus (Yang et al., 2012), various poxviruses like camel pox virus (Verkatesan et al., 2012), capripox viruses (Das et al., 2012), Porcine circovirus 2 (Zhou et al., 2011), RT LAMP developed for ND virus (Kuruma et al., 2012), IBD (Xue et al., 2009), MD, Infectious bronchitis (IB) virus, Chicken anaemia virus (Huang et al., 2010; Angamuthu et al., 2012; Luo et al., 2012), bacterial diseases/pathogens such as tuberculosis (Enosawa et al., 2003; Iwamoto et al., 2003; Boehme et al., 2007), Burkholderia pseudomallei (Chattratitla et al., 2008), Shigella and enteroinvasive Escherichia coli, (Song et al., 2005), Brucella spp., (Song et al., 2012), Yersinia enterocolitica in pork meat (Gao et al., 2009), various types of Staphylococci strains (Xu et al., 2012), Streptococcus suis (Hu et al., 2012), Riemerella anatipestifer infection of ducks (Han et al., 2011) parasitic pathogens like Babesia gibsoni in dogs (Ikada et al., 2004), Plasmodium spp. (Han et al., 2007), Leishmania spp. (Adams et al., 2010), Therieleria infection ovines (Liu et al., 2008), Anaplasma phagocytophilum infection of dogs (Lee et al., 2012). The LAMP has also come into detection of Rickettsia pathogens like Anaplasma ovis which commonly affects small ruminants (Ma et al., 2011). The LAMP has also been developed for detection of Edwardsiella ictaluri which infects cat fish (Savan et al., 2004; Yeh et al., 2005). Diagnostic application of LAMP for human and animal pathogens is summarized in Table 3.

Furthermore, LAMP assay also has been used for rapid sexing of bovine pre-implantation embryos (Hirayama et al., 2004, 2007); birds (Chana et al., 2012); salmon (Hsu et al., 2011), papaya (Hsu et al., 2012) and also for diagnosis of plant pathogens.

VERSIONS OF LAMP

Micro LAMP: The LAMP carried out on a microfluidic chip for easy read out of results just by visualizing the precipitation or can be measured by optic sensor. This set up seems to be easy, fast, simple and handy for detection of pathogen (Fang et al., 2010).

LAMP combined with lateral flow assay (LFA): Both LAMP and lateral flow assays are newer techniques, easy, faster and cost efficient techniques. Conjoined LAMP and LFA has been effective to find out pathogen of interest. The amplified product whose primers were tagged already, moves through the LFA pad where binding with tag specific antibodies occur which can be visualized and hence results can be interpreted (Soliman and El-Matbouli, 2010; Surasilp et al., 2011).

Lyophilized LAMP: Lyophilized LAMP mixture makes the reaction much easier as it requires only sample addition. The reaction mixture is lyophilized so that only template has to be added and kept for incubation at the desired temperature. This lyophilisation has prepared LAMP as a technique for field conditions. It is not feasible to develop a general LAMP kit requiring addition of primers specific to a pathogen and under such circumstances lyophilized LAMP kits draw the attention. Such kit is already available for diagnosis of tuberculosis which is marketed by Eiken chemical Co. Ltd. Another experimental kit currently under evaluation, developed by the International Atomic Energy Agency (IAEA) is showing promising results. Use of high containment, multi-chamber tube enabling a non-instrumented and portable LAMP kit is however under development (Thekiso et al., 2009; LaBarre et al., 2009, 2011).

Electric LAMP (eLAMP): The eLAMP allows users to efficiently test putative primers on a target sequence set by the use of a PERL script with Tk graphical interface that electronically simulates LAMP. The eLAMP can match primers to templates using either exact via built-in PERL regular expressions; or approximate matching via the electronic tools are however limited to primer design alone and in this regard application of Lamp Assay Versatile Analysis (LAVA) is quiet noteworthy (Anonymous, 2009; Torres et al., 2011; Salinas and Little, 2012).
Table 3: Detail application of LAMP in field of diagnosis of human and animal pathogen

<table>
<thead>
<tr>
<th>Microbe category</th>
<th>LAMP test for</th>
<th>Microbe’s target</th>
<th>References</th>
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<tbody>
<tr>
<td>Viral</td>
<td>SARS</td>
<td>Human</td>
<td>Notomi et al. (2004) and Poon et al. (2004)</td>
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<td></td>
<td>Hepatitis B virus</td>
<td>Human</td>
<td>Mosteki et al. (2009)</td>
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<td></td>
<td>Human papilloma virus type 6, 11, 16 and 18</td>
<td>Human</td>
<td>Faghi et al. (2007)</td>
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<td></td>
<td>Dengue</td>
<td>Human</td>
<td>Parida et al. (2005)</td>
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<td>Enterovirus</td>
<td>Human</td>
<td>Xia et al. (2011)</td>
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<td>Norovirus</td>
<td>Poultry</td>
<td>Fukuda et al. (2006)</td>
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<td></td>
<td>West Nile virus</td>
<td>Poultry</td>
<td>Parida et al. (2004)</td>
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<td>Coronavirus</td>
<td>Ruminants</td>
<td>Poon et al. (2005)</td>
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<td>Highly pathogenic avian influenza</td>
<td>Poultry</td>
<td>Imai et al. (2006), Ito et al. (2006) and Dinh et al. (2011)</td>
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<td></td>
<td>Infectious bronchitis (IBV)</td>
<td>Poultry</td>
<td>Lu et al. (2012)</td>
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<td>Chicken anemia virus</td>
<td>Poultry</td>
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<td>FMD</td>
<td>Ruminants</td>
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<td>Classical swine fever</td>
<td>Pigs</td>
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<td>Porcine cytomegalovirus</td>
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<td>Tuberculosis</td>
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<td>Pigs</td>
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<td>Riemerella anatipestifer</td>
<td>Ducks</td>
<td>Han et al. (2011)</td>
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<td></td>
<td>Edwardsiella ictaluri</td>
<td>Cat fish</td>
<td>Sivan et al. (2004) and Yeh et al. (2005)</td>
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<td>Rickettsial</td>
<td>Anaplasma phagocytophilum</td>
<td>Dogs</td>
<td>Lee et al. (2012)</td>
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<td></td>
<td>Anaplasma ovis</td>
<td>Small ruminants</td>
<td>Ma et al. (2011)</td>
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<td>Myxotic</td>
<td>Pasteurella pneumonia</td>
<td>Human</td>
<td>Uemura et al. (2008)</td>
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<td></td>
<td>Candidiasis</td>
<td>Human</td>
<td>Inacio et al. (2008)</td>
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<td></td>
<td>Pneumocystis brasiliensis</td>
<td>Human</td>
<td>Endo et al. (2004)</td>
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<td>Parasitic</td>
<td>Cysticercosis (Taenia saginata, T. asiatica and T. Solium)</td>
<td>Human</td>
<td>Nkoua et al. (2010)</td>
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<td></td>
<td>Giardia duodenalis</td>
<td>Human</td>
<td>Plutzer and Karais (2009)</td>
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<td>Brucia flanarinis</td>
<td>Human</td>
<td>Poole et al. (2012)</td>
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<td>Babesia gibsoni</td>
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<td>Bidal et al. (2004)</td>
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<td>Leishmanias spp.</td>
<td>Animals</td>
<td>Adams et al. (2010)</td>
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<td>Theileria infection</td>
<td>Oxine</td>
<td>Liu et al. (2008)</td>
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**Multiplex LAMP (mLAMP):** Majority of the current methods for detection of amplified LAMP cannot be applied to multiple targets as they measure total DNA.

Majority of the current methods for detection of amplified LAMP products cannot be applied to multiple targets as they measure total DNA. Multiple targets for parasites (Aonuma et al., 2010, Liang et al., 2012), Bacteria (Kouguchi et al., 2010, Tanner et al., 2012) and viruses (He and Xu, 2011; Liang et al., 2012) can be detected simultaneously. Further processing and additional equipments are required for many of these methods including protocols of mLAMP helping to achieve Real-time detection (Kouguchi et al., 2010; Zerilli et al., 2010; Tanner et al., 2012).

**SALENT ADVANTAGES OF LAMP**

- LAMP can be called as an equipment free technique because it don’t require costly equipments and only water bath which is commonly available can be used. Hence, under field condition, the technique is suitable Notomi et al. (2000)
- LAMP is both sensitive as well as specific when compared with other DNA detection methods like PCR. Large quantities of a targeted sequence (10^1-10^9 copies) are produced in less than an hour increasing sensitivity and the specificity is attributed by the use of 6 separate genomic regions in the initial stage and four in the later stage for a positive reaction (Noren et al., 2011, Khan et al., 2012)
• Diagnosis by LAMP is rapid; gets completed within an hour or 30 min (when loop primers are used) (Nagamine et al., 2002a)
• Unprocessed or partly processed samples can be used as template and hence it a robust technique
• LAMP works at a constant temperature
• No need of post amplification processing. Results can be seen directly by adding SYBR green, HNB or Calcein. So electrophoresis is not needed which also reduces the time (Njiru, 2012)

Advantages/benefits of LAMP over other nucleic acid detection methods: The use of different sets of primers (four in the initial and two in the subsequent steps) ensures high specificity for target amplification. The detection limit is much high compared to NASBA, 3SR and SDA, all of which having a limit of detection less than 10 copies. Moreover, most of the nucleic acid detection methods have lower specificity requiring either a precision method or an elaborate method for amplification compared to LAMP (Abramowitz, 1996; Vrana, 1996). When combined with reverse transcription, amplification of RNA sequences can be done by it with high efficiency (Nagamine et al., 2002b). Advantages are also depicted in a pictorial representation in Fig. 2.

**DRAWDACKS OF LAMP**

Carry over contamination has been hunting most researchers who are working on LAMP. Sensitivity level of LAMP contributes to this contamination problem. The LAMP product is so firm that it is not degraded easily and chance of carry over contamination exist (Bai et al., 2011). To counter act this problem scientist have come out with some solutions. The LAMP master mix can be pre incubated with UNG which shows promising results to prevent the (He and Xu, 2011). But the easier way to prevent carry over contamination will be to stop doing gel electrophoresis for result viewing and can go for DNA binding dyes or metal ion indicator. Use of indicators like HNB and calcine can be suggested as best while performing LAMP because it can be incorporated when the reaction mix is made i.e., at the start of the reaction and hence there is no need for opening the tube after the reaction completes and this becomes a closed tube technology (Chen et al., 2012). Use of impure DNA or partially purified DNA as template does not facilitate amplification (Deguo et al., 2008). LAMP is affected by the amplification time. Francois et al. (2011) found that the least time taken for the amplification of LAMP varies from 60 min-120 min and negative control shows amplification at 180 min (Francois et al., 2011). But this science has no hypothesis. Reaction carried out on ice to check contamination and time taken for master mix preparation should be less than 30 min to get better and satisfying results (Francois et al., 2011).

**CONCLUSION AND FUTURE PERSPECTIVES**

Asymptomatic infection often makes it critical to diagnose and detect particularly most of the diseases for which disease transmission process must be broken down by using sensitive diagnostic tests. Loop mediated isothermal amplification (LAMP) is a rapid and cost-effective, technically sound test. The pace at which the LAMP has been developed for various infectious agents of medical and veterinary importance, assures proper guidance in treatment and so also exposure of the non-infected population to unnecessary drug exposure can be prevented. Efficient primer design is a pre-requisite for the use of LAMP in fruitful manner. Advancement in the field of biotechnology and molecular biology has made primer designing a bit easier but the chances of false positivity in a LAMP reaction requires further investigations. LAMP is much more sensitive and specific when compared to PCR or detection of viral diseases and has formed an integral part of the pen-side diagnosis, recommended by both WHO and Office des International Epizootics (OIE). As reactions can be performed and results can be read without opening reaction tubes, it shows the great potential of LAMP in disease diagnosis. In this regard, much is required to develop such a closed reaction system. In developing countries, LAMP has potential application for clinical diagnosis along with surveillance of infectious diseases without requiring sophisticated equipments and skilled personnel. Development of sophisticated lyophilised LAMP kits against chronic infections has already brought success. It must be remembered though that the technologies associated with LAMP should be taken into consideration and their development needs as a part of LAMP platform rather than developing them as separate entities. By this way, it will be possible to undertake a same day testing strategy.

**REFERENCES**


