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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 10^9 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; Lauerman, 2004; Black *et al*., 2002;

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Belak, 2007; Deb and Chakraborty, 2012; Dhama *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, immunocapture PCR, PCR-ELISA etc.), detection of nucleic acid polymorphism (Rapid amplification of polymorphic DNA, RAPD). A large number of diagnostic tests including immunochromatographic methods are available for increasing the accuracy of penside diagnostic tests (Black *et al*., 2002; Kataria *et al*., 2005; Schmitt and Henderson, 2005; Dhama *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, arcobacter, mycoplasma, chicken anaemia 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; Dhama *et al*., 2005, 2008b, c, 2009, 2011, 2012a; Jones *et al*., 2008; Kumar *et al*., 2009, 2012a, b; Pawaiya *et al*., 2009; Bhatt *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; Ratcliff *et al*., 2007; Dhama *et al*., 2008b; Balamurugan *et al*., 2010; Siddiqui, 2010; Taddele *et al*., 2011; Dhama *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; Lauerman, 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 interpretated. 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 (3SR) 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) Adoptable 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 10^9 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 "PrimerExplorer" which is commonly used for LAMP primer designing.

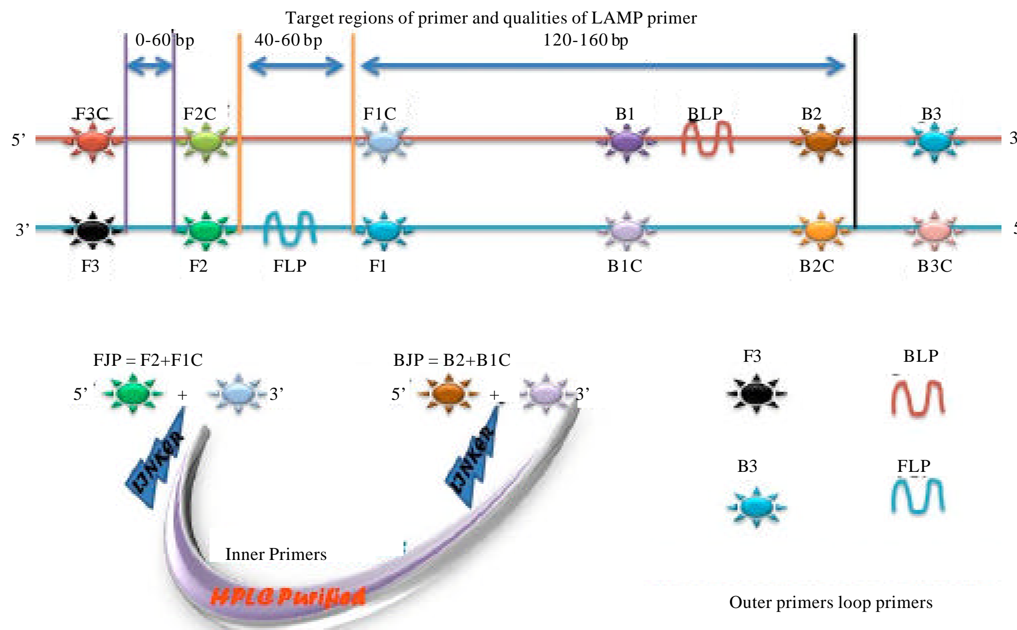

Fig. 1: Target region of primers and qualities of LAMP primer

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 recommended 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, Tris-HCl with a pH 8.8, $(NH_4)_2$ SO_4, $MgSO_4$ 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 (Njiru *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 1: Comparative analysis of PCR and LAMP

Properties	PCR	LAMP
Denaturation	Required for separation of strands, enabling primer binding.	Denaturation step is not a mandate, as the enzyme displaces the strand and takes care of it.
Annealing, extension	Usually employs 3 steps as denaturation, annealing and extension, working at different temperature and timing.	Works under a constant temperature usually between 60-65°C.
Time required	Takes 2-3 h based on the different parameters.	Sixty min usually. Loop primer inclusion can shorten the time taken for completion of the reaction.
Post amplification process	Needs agarose gel electrophoresis for knowing the result.	By incorporating DNA binding dyes like SYBR green or any metal indicator like calcein or other dyes like hydroxy naphthol blue the results can be interpreted visually Tomita *et al*. (2008) and Goto *et al*. (2009).
Sensitivity	Can detect up to nanogram level of DNA.	Can detect up to femtogram level of DNA in the sample. So minute quantity can be amplified and result interpretation becomes easy.
Instruments	Needs sophisticated instrument in order to maintain different temperature within a given time.	No need of expensive instruments. Water bath, a common instrument can serve the purpose.
DNA template preparation	Requires template DNA preparation which should be pure and impurities can hinder the PCR reaction	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).

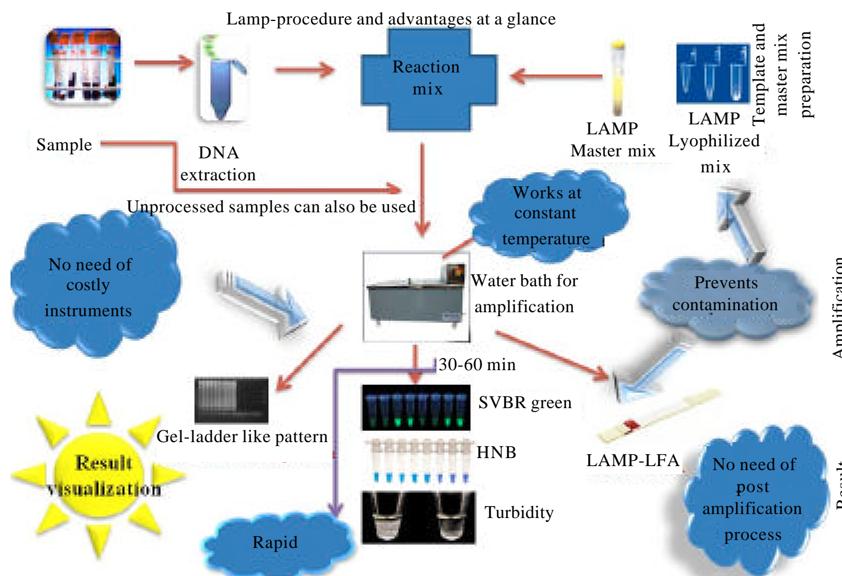

Fig. 2: LAMP Procedure and advantages at a glance

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 visualisation, 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 likes urine or stools (Fredricks and Relman, 1998) have no effect on LAMP.

Table 2: DNA binding dyes and their interpretation

Agent	Positive	Negative
SYBR green	Green	Orange
Calcein	Green	Yellow
HNB	Sky blue	Violet

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 (Kalvatchev *et al*., 2010). More than 100 LAMP tests have already been developed for both human and animal pathogens (Bendall *et al*., 2002; Karanis and Ongerth, 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 (Morshed *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 (Hatano *et al*., 2010), Cholera organism (Ymazaki *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 herpesvirus 6 (Ihira *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 bronchio alveolar lavage samples were used for diagnosis. Parasitic organism like *Taenia* species (*Taenia saginata*,

T. asiatica and *T. solium*) which can cause cysticercosis can be detected by LAMP (Nkouawa *et al*., 2010).The LAMP has also been developed for protozoan parasites like *Trypanosoma* species (*Trypanosoma brucei rhodesiense*) (Thekisoe *et al*., 2007) *Giardia duodenalis* (Plutzer and Karanis, 2009), Brugian filariasis (Poole *et al*., 2012), *Entamoeba histolytica* (Liang *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 cytomegalo virus (Yang *et al*., 2012), various pox viruses like camel pox virus (Venkatesan *et al*., 2012), capripox viruses (Das *et al*., 2012), Porcine circo virus 2 (Zhou *et al*., 2011), RT LAMP developed for ND virus (Kirunda *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* (Chantratita *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* (Huy *et al*., 2012), *Riemerella anatipestifer* infection of ducks (Han *et al*., 2011) parasitic pathogens like *Babesia gibsoni* in dogs (Ikadai *et al*., 2004), *Plasmodium* spp. (Han *et al*., 2007), *Leishmania* spp. (Adams *et al*., 2010), Thelieria infection ovines (Liu *et al*., 2008), *Anaplasma phagocytophilum* infection of dogs (Lee *et al*., 2012). The LAMP has also come into detection of Rickettsial 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 (Thekisoe *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

Microbe category	LAMP test for	Microbe's target	References
Viral	SARS	Human	Notomi *et al.* (2004) and Poon *et al.* (2004)
	Hepatitis B virus	Human	Moslemi *et al.* (2009)
	Human papilloma virus type 6, 11, 16 and 18	Human	Hagiwara *et al.* (2007)
	Dengue	Human	Parida *et al.* (2005)
	Enterovirus	Human	Xia *et al.* (2011)
	Norovirus	Human	Fukuda *et al.* (2006)
	West nile virus	Poultry	Parida *et al.* (2004)
	Coronavirus	Ruminants	Poon *et al.* (2005b)
	Highly pathogenic avian influenza	Poultry	Imai *et al.* (2006), Ito *et al.* (2006) and Dinh *et al.* (2011)
	Infectious bronchitis (IBV)	Poultry	Luo *et al.* (2012)
	Chicken anaemia virus	Poultry	Huang *et al.* (2010)
	FMD	Ruminants	Dukes *et al.* (2006)
	Classical swine fever	Pigs	Chakraborty and Choudhury (2012)
	Porcine cytomegalo virus	Pigs	Yang *et al.* (2012)
	Camel pox	Camel	Venkatesan *et al.* (2012)
	Porcine circo virus 2	Pigs	Zhou *et al.* (2011)
	ND	Poultry	Kirunda *et al.* (2012)
	IBD	Poultry	Xue *et al.* (2009)
	MD	Poultry	Angamuthu *et al.* (2012)
	Capripox	Goats	Das *et al.* (2012)
Bacterial	Leptospirosis	Human	Sonthayanon *et al.* (2011)
	Staphylococcus aureus	Human	Lim *et al.* (2013)
	Listeria monocytogenes	Human	Tang *et al.* (2011)
	Tuberculosis	Animals/human	Enosawa *et al.* (2003), Iwamoto *et al.* (2003), Boehme *et al.* (2007), Pandey *et al.* (2008) and Geojith *et al.* (2011)
	Streptococcus suis	Pigs	Huy *et al.* (2012)
	Shigella and enteroinvasive *Escherichia coli*	Pigs	Song *et al.* (2005) and Wang *et al.* (2012)
	Yersinia enterocolitica	Pigs (pork)	Gao *et al.* (2009)
	Staphylococci strains	Animals	Xu *et al.* (2012)
	Brucella spp.	Animals	Song *et al.* (2012)
	Riemerella anatipestifer	Ducks	Han *et al.* (2011)
	Edwardsiella ictaluri	Cat fish	Savan *et al.* (2004) and Yeh *et al.* (2005)
Rickettsial	*Anaplasma phagocytophilum*	Dogs	Lee *et al.* (2012)
	Anaplasma ovis	Small ruminants	Ma *et al.* (2011)
Mycotic	*Pneumocystis pneumonia*	Human	Uemura *et al.* (2008)
	Candidiosis	Human	Inacio *et al.* (2008)
	Paracoccidioides brasilieusis	Human	Endo *et al.* (2004)
Parasitic	Cysticercosis (*Taenia saginata*, *T. asiatica* and *T. Solium*)	Human	Nkouawa *et al.* (2010)
	Giardia duodenalis	Human	Plutzer and Karanis (2009)
	Brugian filariasis	Human	Poole *et al.* (2012)
	Babesia gibsoni	Dogs	Ikadai *et al.* (2004)
	Plasmodium spp.	Animals	Moody (2002), Han *et al.* (2007) and Poon *et al.* (2006)
	Leishmania spp.	Animals	Adams *et al.* (2010)
	Thelieria infection	Ovine	Liu *et al.* (2008)

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).

SALIENT 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 (10^9-10^{10} 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.

DRAWBACKS 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 calcein 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.

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