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Loop-Mediated Isothermal Amplification Promising Molecular Diagnostic Tool for Application in Developing Countries

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Abstract
Loop-Mediated Isothermal Amplification (LAMP), through its uniqueness has provided the world with a great molecular diagnostic tool. This innovative gene amplification technique, amplifies nucleic acid at a very rapid pace, maintaining high sensitivity, specificity and efficiency. The most innovative features of LAMP, is the simplicity of its protocol, and overall low cost of application. The demerits include highly technical and cumbersome primer design protocol, and relative scarcity of some materials needed for the technique. The standout features however, ranges from the strand displacement activity of the Bst and Bsm polymerase enzymes, to its ability to amplify gene in an unprocessed samples. The PCR and other several pre-existing techniques, has contributed immensely in disease diagnosis and molecular biology at large. However, their use in molecular diagnostics is mostly limited to highly resourced establishment, leaving the low resourced settings with little or no hope. The cheapness and user-friendliness of LAMP amongst other advantages made it an ideal diagnostic tool that provides solution to the odds of PCR and its likes. The outstanding qualities displayed by LAMP in various researches, have led to several modifications in the technique. Presently several forms of LAMP kits, such as lyophilized LAMP Kit, electric LAMP device (eLAMP), and combined use of LAMP and Lateral Flow Assay (LFA), have been adopted for diagnosis of many pathogens. This paper provides a brief description of the LAMP assay Vis avis its comparative advantage and its potential application in resource.
Keywords: *Developing Countries, Disease Diagnosis, Loop Mediated Isothermal Amplification (LAMP).* Limited countries and under field conditions.

Introduction

Nucleic acid amplification is one of the greatest achievements of the 21st century. Over the last few decades, nucleic acid based diagnostic techniques significantly alleviated some of the challenges of disease diagnosis. Despite the tremendous success recorded by the introduction of polymerase chain reaction (PCR) based molecular diagnostic methods, there are still limitations in their wide application in the developing world in particular due to problems associated with technical and financial capabilities. Development of a novel gene amplification technique called Loop Mediated Isothermal Amplification (LAMP) is considered to be a promising molecular diagnostic tool useful under field conditions and in countries with limited resources (Abdullahil *et al.,* 2015). In1998, Eiken Chemical Company Ltd developed a novel nucleic acid amplification method termed (LAMP) (Notomi *et al*., 2000).

Loop-mediated isothermal amplification (LAMP) can amplify nucleic acids with high specificity, sensitivity and rapidity under isothermal conditions (Notomi *et al*., 2000). The method was easy to perform (Mori *et al*., 2001; Nagamine *et al*., 2002), which is based on the principle of the reaction performed by a DNA polymerase with strand displacement activity and a set of two specially designed inner primers (FIP and BIP) and two outer primers (F3 and B3). LAMP had high specificity for the target sequence because the target sequence is recognized at six positions by six independent primers (F1c, F2, F3, B1c, B2 and B3) in the initial stage and by four independent primers (F1c, F2, B1c, and B2) in the later stages of the LAMP reaction. The amplification efficiency of the LAMP method is extremely high since there are no time lapse for thermal change as the reaction is carried out under isothermal condition. Therefore, the LAMP assay has comparative advantages in specificity, efficiency of amplification and rapidity over other nucleic acid amplification methods (Mori *et al*., 2001). Moreover, the LAMP reaction can be accelerated by the use of additional forward and backward loop primers (LF and LB) (Nagamine *et al*., 2002). The LAMP method is capable of amplifying a few copies of DNA to a tremendous amount in less than an hour without any additional reagents (Tomita *et al*. 2008).

In case of LAMP, amplification can be interpreted without post amplification processing. Visual turbidity is a good indicator of positive reaction since nucleic acids are amplified in large amount in LAMP reaction. This results in production of large quantities of excess pyrophosphate ions, which will combine with magnesium ions resulting in production of white precipitate of magnesium pyrophosphate responsible for the turbidity seen in case of positive reaction (Mori *et al*., 2001).

Owing to these facts LAMP is thought to address the most apparent limitations of the pre existing molecular techniques such as conventional PCR with respect to cost of operation, specificity and efficiency (Francois *et al*., 2011).

Resources essential for molecular assays, such as bio-safety cabinets, a stable supply of electricity, and well-experienced technicians, are scarce in most of the peripheral laboratories in developing countries

(Yasuyoshi *et al*., 2012). Lack of accessibility to such diagnostic systems has resulted in the deterioration of the situation in most developing countries, especially in sub-Saharan tropical countries. Diagnostics using LAMP have emerged as a promising methodology because of their remarkable high sensitivity, and therefore, they have been applied as diagnostic tools for detecting various kinds of pathogens in clinical settings in developed countries (Rodrigues *et al*., 2010).

Therefore, the objectives of this seminar paper are:

- To briefly review the protocol and technical soundness of LAMP in disease diagnosis
- To show the unique features of LAMP and its potential application in disease diagnosis for resource limited laboratories in developing countries

2. HISTORICAL PERISPECTIVE ON THE DEVELOPMENT OF LOOP MEDIATED ISOTHERMAL AMPLIFICAION

In1998, Eiken Chemical Company Ltd Tokyo Japan developed a novel nucleic acid amplification method termed (LAMP). In 2000, first LAMP paper publication was recorded (Notomi *et al*., 2000).

In 2002 commercial (loopamp^{TM}) reagent was available from Eiken chemical company. Starting from 2000 up to date around 500 publications were registered and greater than 250 gene and species detected by LAMP (Brian, 2009).

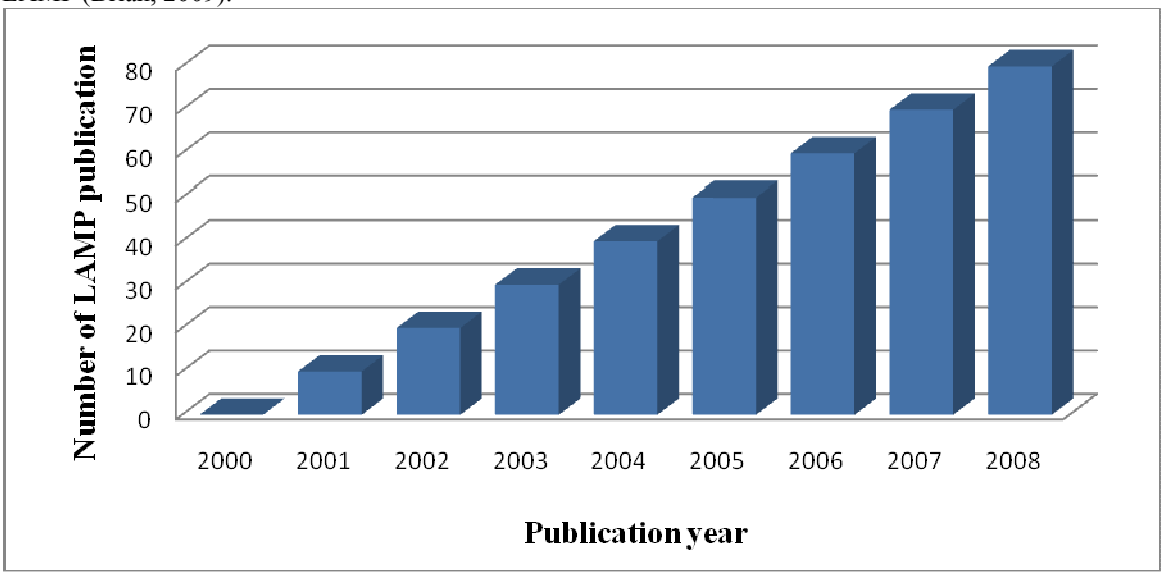

Figure1. LAMP Publications per year. **Source**: **(**Brian, 2009**).**

3. LAMP PROTOCOL

3.1. Essential Components of loop-mediated isothermal amplification

Essential components of LAMP, a simple and cost-effective technique, requires few and inexpensive materials to perform (Nagamine *et al*., 2002).

Primers: The most crucial of all is the primers. LAMP uses four to six primers specially designed to target six to eight regions in a gene of interest. These are Forward Inner Primer (FIP), Forward Outer Primer (F3), Backward Inner Primer (BIP), Backward Outer Primer (B3) and two optional loop primers (LF and LB). The primers are designed manually or by the use of different software's which are available for LAMP primer designing such as Primer Explorer (Eiken Co.) and LAMP Designer (PREMIER Biosoft International). Incorporation of two more primers i.e. loop primers increases the specificity as well as reduce the reaction time (Nagamine *et al*., 2002).

F3 and B3 have their major role during strand displacement. FIP and BIP function in loop formation (Parida *et al*., 2008).The FIP and BIP should be of higher performance Liquid Chromatography (HPLC) purified primer. The primers are designed based on eight target regions present on the target gene i.e. F3c, F2c, F1c and FLP on the 3' end. In 5' side B1, B2, B3 and BLP are present. The inner and loop primer act via different mechanism. The use of loop primers facilitates the LAMP reaction reducing the reaction time to a period of half an hour in comparison to the original LAMP method without loop primers. Loop primer inclusion also yields a large amount of amplified DNA in short time (Nagamine *et al*., 2002b).

In designing LAMP primers the following characteristics need to be considered. The inner primer should not have AT rich sequence at both the end, GC content should be about 50-60%, in case of GC rich sequence the melting temperature should be 60-65^{o}c and for AC rich sequence it should be 50-60^{o}c, and designed primer should not have any secondary loop structure formation (Kuldeep *et al*., 2014).

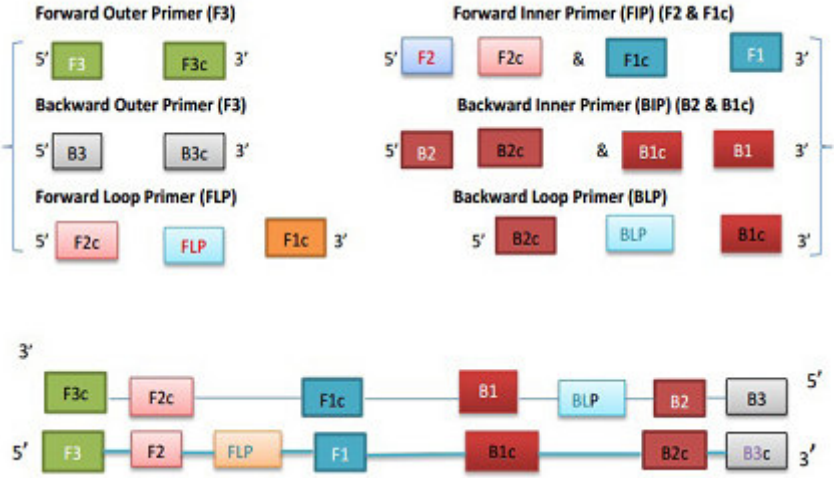

Figure 2. LAMP primer hybridizing to specific site of a target gene. **Source**: (Abdullahil *et al*., 2015).

Polymerase enzyme: The LAMP polymerase enzyme plays a very crucial role, it can be considered as the "brain box" of the technique. They are two polymerase enzymes suitable for LAMP reaction, the most common is the Bst DNA polymerase, and the less commonly used enzyme is the Bsm DNA polymerase. These enzymes are sourced from Bacillus stearothermophilus and Bacillus smithii, respectively. The enzymes possess strand displacement activity and catalyze 5'-3' as well as the 3'-5' DNA polymerization, but lack exonuclease activity (Nagamine *et al*., 2001). The Bst and the Bsm enzyme retain their enzymatic activity at 66ºC and 63ºC, respectively. They however work best at 63°C and 60°C, respectively (Dhama *et al*., 2014).

Reagents: Betaine is used in stabilization of the AT and GC content so as to ensure the stability of the reaction. Other components include Deoxynucleoside Triphosphate (dNTP), which provides the required nucleotides; Magnesium Sulphate (MgSO4) forms the pyrophosphate ions, which accumulates in the reaction mixture to form visible white precipitate (Goto *et al*., 2009). Fluorescent DNA dye can be added to ease the visualization of the turbidity resulting from precipitate of pyrophosphate ions (Hirayama *et al*., 2007). Example of DNA dyes used in LAMP includes SYBR Green, HNB, Picogreen and a Calcein metal ion indicator. Lastly buffer solution containing $(NH4)_2SO_4$, Tris-HCL with pH 8.8, MgSO4and KCl are used as part of the LAMP reaction mixture (Parida *et al*., 2008).

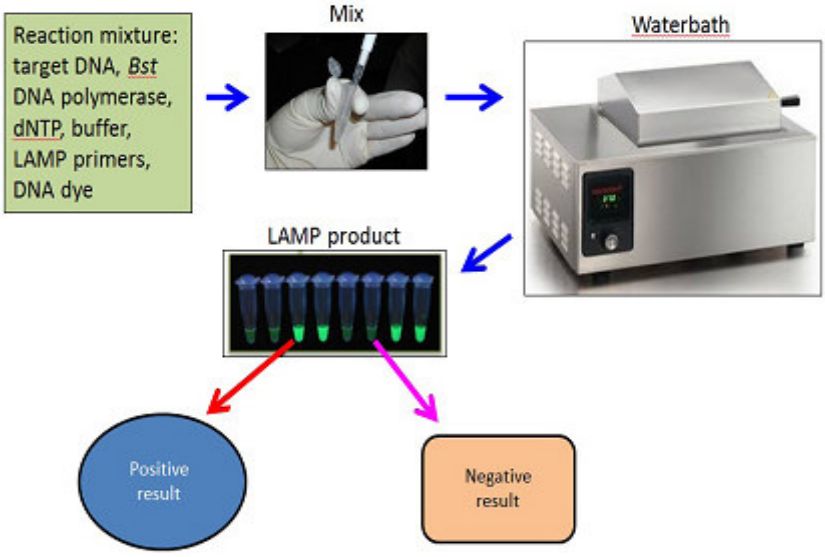

Figure3. Simple and user friendly LAMP assay protocol. **Source**: (Abdullahil *et al*., 2015).

3.2 Principle of LAMP Technique

LAMP as a nucleic acid amplification technique operates under a unique amplification principle which involves two basic steps. These are the non-cyclical and cyclical phase. The non-cyclical precedes the cyclical phase of the amplification where (Parida *et al*., 2008; Ushikubo *et al.,* 2004) all the four primers as well as the Bst DNA polymerase enzyme with a strand displacement activity, play a role in this first stage of LAMP reaction. However, the cyclical phase adds up nucleotides on the product of the noncyclical phase in which case only two

of the outer primers and the BstDNA polymerase enzyme are involved. Loop primers might be involved in the cyclical step when six primers are used (Nagamine *et al*., 2002) to increase the reaction efficiency.

3.2.1. Non-cyclical phase

The non-cyclical phase begins with the annealing of the Forward Inner Primer (FIP) to a complementary sequence at the 5' end of the double stranded DNA, extension from the annealing point occurs following the activity of the Bst DNA polymerase producing a full length of a complimentary strand to the target DNA. The forward outer primer (F3) then anneals to the F3c region outside the FIP initiating displacement of the newly formed DNA strand resulting in a single stranded DNA with a dumb bell shape at the 5' end. The Backward Inner Primer (BIP) now uses the displaced single stranded DNA with a dumb bell shape at the 5' end as a template; it anneals to a complimentary sequence at the B2c region of this new DNA strand and subsequently extended by the Bst DNA polymerase as seen in the first amplification involving the FIP (Parida *et al*., 2008).

Following elongation of the DNA strand with the aid of the enzyme, the backward outer primer (B3) anneals to the B3c outside the BIP to initiate displacement of the DNA, resulting in the formation of a dump bell shaped DNA at the 3' end. The non-cycling step ends with formation of DNA with stem loop structure at both ends ready to go into cyclical step (Parida *et al*., 2008; Ushikubo *et al*., 2004).

3.2.2. Cyclical phase

The cyclical phase builds upon the product of the noncyclical step. This phase basically involves hybridization of the internal primers to the stem loop product from the non-cyclical step to initiate displacement DNA synthesis (Ushikubo *et al*., 2004). This stage of amplification result in the production of stem looped DNA with a stem twice as long as the template stem looped DNA (Notomi *et al*., 200; Ushikubo *et al*., 2004). Ultimately the final products of this phase are stem loop DNA with several repeats of the targets and cauliflower like structure (Notomi *et al*., 2000; Parida *et a*l., 2008; Ushikubo *et al*., 2004).

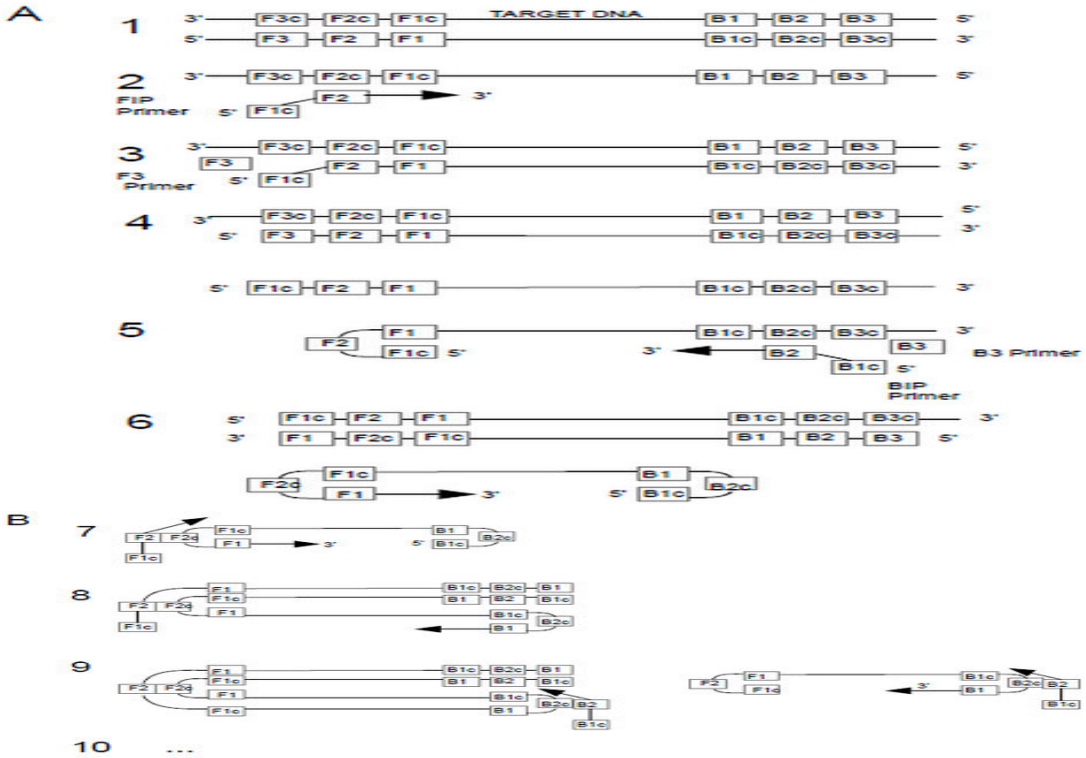

***Figure** 4. Non-cyclic (A) and cyclic (B) step of LAMP **source :**(Parida* et al*., 2008)*

3.3 Mechanism of LAMP reaction

LAMP relies on auto-cycling strand displacement DNA synthesis which is carried out at 60-63°C for 45-60min in the presence of Bst DNA polymerase, dNTPs, specific primers and the target DNA template. The mechanism of the LAMP amplification reaction includes three steps: production of starting material, cycling amplification and elongation, and recycling (Notomi *et a*l., 2000).

To produce the starting material, inner primer FIB hybridizes to F2c in the target DNA and initiates complementary strand synthesis. Outer primer F3 hybridizes to F3c in the target and initiates strand displacement DNA synthesis, releasing a FIP-linked complementary strand, which forms a looped-out structure at one end. This single stranded DNA serves as template for BIP-initiated DNA synthesis and subsequent B3-primed strand displacement DNA synthesis leading to the production of a dumb-bell form DNA which is quickly

converted to a stem loop DNA. This then serves as the starting material for LAMP cycling, the second stage of the LAMP reaction. During cycling amplification, FIP hybridizes to the loop in the stem-loop DNA and primes strand displacement DNA synthesis, generating as an intermediate one gapped stem loop DNA with an additional inverted copy of the target sequence in the stem, and a loop formed at the opposite end via the BIP sequence. Subsequent self-primed strand displacement DNA synthesis yields one complementary structure of the original stem-loop DNA and one gap repaired stem-loop DNA with a stem elongated to twice as long and a loop at the opposite end. Both of these products then serve as templates for BIP-primed strand displacement in the subsequent cycles, the elongation and recycling step. The final product is a mixture of stem loop DNA with various stem length and cauliflower-like structures with multiple loops formed by annealing between alternately inverted repeats of the target sequence in the same strand (Notomi *et al*., 2000).

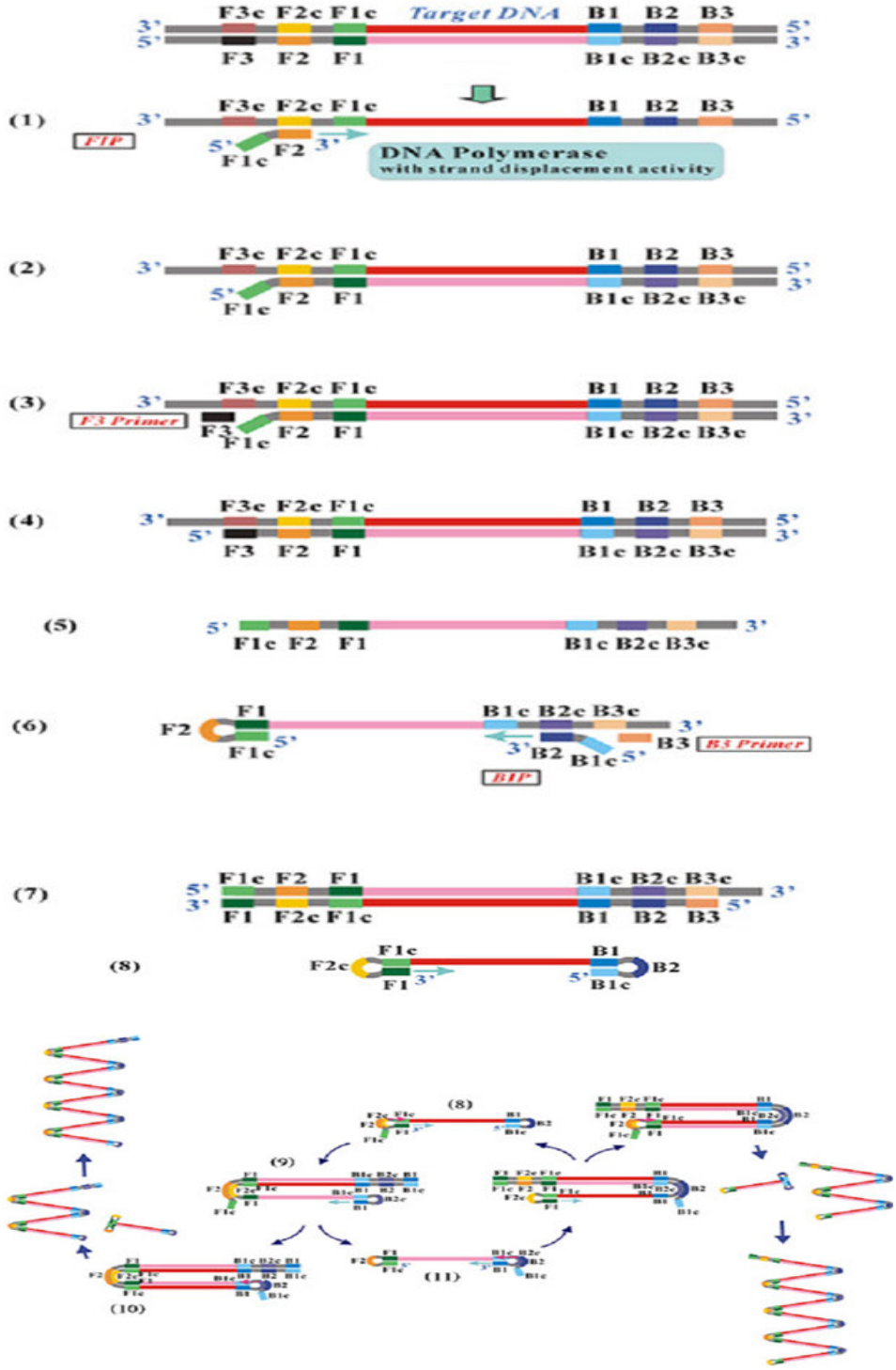

Figure 5. Mechanism of LAMP reaction. **Source**: (Eiken chemical CO.LTD, 2000).

3.4 Detection and Interpretation of Lamp Product

The foremost advantage of LAMP in comparison with PCR is that the result of amplification can be interpreted without post amplification processing. Visual turbidity is a good indicator of positive reaction. Nucleic acids are amplified in large amount in LAMP reaction. This results in production of large quantity of excess pyrophosphate ions, which will combine with magnesium ions resulting in production of white precipitate of magnesium pyrophosphate. This is responsible for turbidity seen in case of positive reaction (Mori *et al*., 2001).

Increase in turbidity is directly proportional to the amount of nucleic acid, which can be measured in real time using real time turbido meter. Turbidity is measured as OD at 400nm in every 6 seconds. It is cheaper than real time PCR machine. For the formation of white precipitate, yield in microgram quantities is required. In LAMP, DNA is amplified to more than 10µg, therefore visual turbidity can be used as an indicator of positive reaction (Parida *et al*., 2008). Polyethylene imine (PEI) can be added to the reaction tube post amplification for the detection of amplification. PEI forms insoluble complex with high molecular weight amplification product, but will not combine with low molecular weight oligonucleotides. Visually detectable clear coloured precipitate is formed on addition of PEI. But PEI cannot be added prior to reaction, as it will inhibit amplification (Mori *et al*., 2006).

Amplified products can also be visualised in presence of fluorescent intercalating dyes such as SYBR Green I, Calcein etc. On addition of SYBR Green I to the reaction tube post amplification, the colour changes from orange to green in case of positive reaction. Fluorescence can be detected visually using handheld UV torch (wavelength 365nm). Calcein is a fluorescent metal ion indicator. It can be added to tubes prior to reaction. Calcein quenches the manganous ions. Before the reaction, the solution appears orange in colour. As the reaction proceeds, manganous ions are released from Calcein and it will combine with pyrophosphate ions, thus increasing fluorescence of calcein. Increased fluorescence can be detected visually as well as by ultraviolet light (Tomita *et al*., 2008).

Colorimetric detection is also possible by addition of 120µM Hydroxynaphthol blue (HNB) to the reaction mix before amplification. HNB is a metal ion indicator. The colour of HNB changes depending on the pH of the solution. Positive reaction is indicated by change in colour from violet to sky blue (Goto *et al*., 2009). Analysis of LAMP reaction products can also be done by agarose gel electrophoresis on 2% agarose gel, followed by staining with ethidium bromide solution and visualization under UV transilluminator as in the standard PCR. In positive cases, it will produce ladder like pattern due to the production of stem-loop structures with different stem lengths. Either restriction enzyme digestion sequencing can be done for the confirmation of specificity of the amplified product in LAMP reaction (Parida *et al*., 2008).

4. DIFFERENT VERSIONS OF LOOP MEDIATED ISOTHERMAL AMPLIFICATION

The outstanding qualities displayed by LAMP in various researches, have led to several modifications in the technique. Presently several version of LAMP, such as lyophilized LAMP, electric LAMP device (eLAMP), Multiplex LAMP (mLAMP), Lateral Flow Assay (LFA) and Micro LAMP have been introduced (Abdullahil *et al.,* 2015).

4.1. Micro LAMP

This form of LAMP utilizes a microfluidic chip for easy detection and quantification of nucleic acid. It works under isothermal condition; it can be used in real-time quantification of amplified genes, by integrating an optic fiber in the microfluidic chip (Fang *et al*., 2010).

4.2. Lyophilized LAMP

This version of LAMP aims to simplify and make the process of LAMP more rapid, through combination of all LAMP reagents into a single mixture called lyophilized LAMP mix. User of this version of LAMP is required to only add the template DNA or sample into the stand by mix to carry out the amplification. Lyophilized LAMP kit is commercially available for Rapid diagnosis of some diseases (Dhama *et al*., 2014).

4.3. Electric LAMP (eLAMP)

Is a device designed to improve the efficiency of testing putative LAMP primers on a target gene sequences. It helps researchers to determine the possibility of using existing primer to amplify a newly discovered sequence variants (Salinas *et al*., 2012).

4.4. Multiplex LAMP (mLAMP)

The 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 (Liang *et al*., 2012) bacteria (kouguchi *et al*., 2010) and viruses (He and Hu, 2011) can be detected simultaneously. Further processing and additional equipment are required for many of previous methods. But *multiplex LAMP* came with Real time detection

(Tanner *et al*., 2012).

4.5. Lateral Flow Assay (LFA)

Lateral flow assay is among the latest advancements in LAMP. It employs an absorbent pad or strip, containing an antibody specific to a target analytic. The absorbent strip binds to the LAMP product in case of a positive reaction. This device is designed to serve as an ideal tool for health care application (Roskos *et al*., 2013).

5. COMPARATIVE ADVANTAGE OF LOOP MEDIATED ISOTHERMAL AMPLIFICATION OVER OTHER DETECTION METHODS

5.1. Longevity

LAMP is more stable compared to PCR and real time PCR (Francois *et al*., 2011). It is secure at a range of temperatures, PH and wide range of elongation time. This stability is helpful in case of incompletely processed or sample that is not processed at all where some components may hinder the reaction. Trace quantities of whole-blood, hemine, blood culture media, N-acetyl cysteine, Nacl and anticoagulant or anti complement compound can inhibit Taq polymerase and hence the PCR cannot detect the DNA of the subject. But LAMP can tolerate these components (kaneko *et al*., 2007; Francois *et al*., 2011). Cold chain is a must while preparing master matrix for PCR which is not a mandate in case of LAMP .The activity of Taq polymerase used in PCR may be hindered in samples like urine or stool (Fredricks and Relman, 1998) while LAMP is not affected.

5.2. Specificity and sensitivity

LAMP is a highly sensitive technique that amplifies few copies of template DNA in a reaction to a detectable level (Notomi *et al*., 2000). As low as a fentogram level of DNA can be detected in a sample with a degraded DNA copies (Dhama *et al*.,2014). Parida demonstrated LAMP to be considerably more sensitive than the conventional PCR, with a 10-100 fold higher sensitivity than PCR and detection limit of 0.01-10 PFU of virus (Parida *et al*., 2005). The high specificity of LAMP reaction is attributed to the nature of primers used (Nagamine *et al*., 2002). The specificity is also demonstrated in its ability to selectively amplify few copies of target DNA, without interference of genomic DNA (Sen *et al*., 2010). This high specificity reflects the strict adherence of the four to six LAMP primers which hybridizes to six or eight distinct regions of a target gene (Parida *et al*., 2008).

5.3. Technicality, Simplicity and Cost Effectiveness

These wonderful properties of LAMP give room for its application both in resourced and resource-limited settings; only a simple water bath is needed for a complete round of test, unlike other conventional methods that require sophisticated instrument, such as PCR thermo cycler (Tomita *et al*., 2008). Although LAMP is a technically sound technique, it is however simple to perform due to its straight forward principles, a semiskilled personnel can effectively perform the assay, and protocols are comprehensive with no sophisticated instruments required. The reaction can be conducted rapidly and efficiently in a single step, hence, can serve as an ideal diagnostic tool (Njiru *et al*., 2012).

5.4. Amplification of Template in a Non-Processed Sample

The ability of LAMP to amplify a target gene in a non-processed sample such as blood has taken the robustness of this technique to another level. The BstDNA polymerase enzyme commonly used in LAMP has been demonstrated to be resistant to presence of anticoagulants, hemin, N-acetylcysteiny, Na Cland other PCR inhibiting substances (Francois *et al*., 2012; Kaneko *et al*., 2005). Application of this property of LAMP will be highly beneficial in health care centers, where accurate and rapid diagnosis is most desired, as time taken for template extraction and purification is eliminated (Abdullahi1 *et al*., 2015).

6. LIMITATION OF LOOP MEDIATED ISOTHERMAL AMPLIFICAION

Despite the robustness of this novel technique there are some few limitations that could impede its smooth application. The most prominent among these limiting factors is the complicated nature of the LAMP primer design. LAMP is yet to be fully recognized as an efficient technique in the field of molecular diagnostics. It is the most crucial step in conducting a successful LAMP assay (Dhama *et al*., 2014; Parida *et al*., 2008).Four to six primers are required to carry out the assay, based on the principle of LAMP, designing of the primers can be a difficult task due to certain conditions that need to be met. For a good LAMP reaction the size of the target DNA should be within 130-200bp (Notomi *et al*., 2000). This condition makes it more difficult to design primers (Parida *et al*., 2008). Therefore the researcher is expected to painstakingly study the sequence of the target gene and apply the primer design guidelines.

Multiplexing in LAMP has been achieved by choosing a target region with a restriction site, and digesting prior to running on a gel, such that each product gives rise to a distinct size of fragment (Iseki *et al*.,

2007) although this approach adds complexity to the experimental design and protocol. The use of a strand-displacing DNA polymerase in LAMP also precludes the use of hydrolysis probes, e.g. Taq Man probes, which rely upon the 5'-3' exonuclease activity of Taq polymerase. An alternative real-time multiplexing approach based on fluorescence quenchers has been reported (Tanner *et al*., 2012).

Free, open-source or commercial software packages are generally used to assist with LAMP primer design, although the primer design constraints mean there is less freedom to choose the target site than with PCR. In a diagnostic application, this must be balanced against the need to choose an appropriate target (e.g., a conserved site in a highly variable viral genome, or a target that is specific for a particular strain of pathogen) (Torres *et al*., 2011).

Carry over contamination has been hunting most researchers who are working on LAMP. Sensitivity level of LAMP contributes to this contamination problem. Moreover, LAMP product is stable that it is not degraded easily which may contribute to the chance of carry over contamination (Bai *et al*., 2011).

7. PROSPECTS OF LOOPMEDIATED ISOTHERMAL AMPLIFICAION AS ADIAGNOSTIC TOOL IN DEVELOPING COUNTRIES

7.1. Identification of human and animal pathogens

Inexpensive and streamlined method can be more readily used in developing countries that do not have access to high tech laboratories. These areas are known for having a multitude of infectious diseases caused by various pathogens such as bacteria and viruses. The LAMP method is very useful for detection of these pathogens (Fu *et al*., 2011) .The LAMP can not only be used for detection of DNA, but can also be used in detection of RNA which is termed as RT-LAMP. Hence it can detect both DNA and RNA viruses (Kalvatchev *et.al*., 2010). More than100 LAMP tests have already been developed for detection of human and animal pathogens (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, it ideal in the diagnosis of such diseases (Morshed *et al*., 2007).

7.3. The Application of LAMP in Ethiopia

The use of LAMP has been documented in Ethiopia. A study to investigate the association of phytoplasmas with papaya dieback and citrus decline syndromes in Ethiopia was carried out between July 2009 and February 2010, with sampling performed in major papaya- and citrus-growing areas of the Rift Valley. A real-time LAMP assay was used for detecting *16SrII* and *XII* phytoplasmas in fruit and weeds of the Ethiopian Rift Valley. Thus, the method can also be adopted for rapid diagnosis of those animal diseases under field conditions for which an already developed and optimized LAMP assay is available (Bekele *et al*., 2011).

8. CONCLUSION AND RECOMMENDATIONS

LAMP is a rapid, cost effective and technically sound test. The pace at which the LAMP has been developed for various agents of medical and veterinary importance, assures the prevention of non-infectious population to unnecessary drug exposure. Efficient primer design is a prerequisite for the use of LAMP in fruit full manner. Advancement in the field of biotechnology and molecular biology has made primer design a bit easier but the chance of false positivity in a LAMP reaction require future investigation. LAMP is much more sensitive and specific when compared to PCR for detection viral disease and has formed an integral parts of the pen-side diagnosis, recommended by both WHO and office des international epizootics (OIE). As a reaction can be performed, a result can be read without opening reaction tubes showing, it shows the greater potential of LAMP in disease diagnosis. In this regard, much is required to develop such a closed reaction system. In developing country, LAMP has a potential for application for clinical diagnosis along with surveillance of infectious disease without requiring sophisticated equipment and skilled personnel. Development of sophisticated lyophilized LAMP kits against chronic infection has already brought success. It must be remembered so 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.

Based on the above conclusion the following recommendations are forwarded:

- Since LAMP is newly developing molecular diagnostic technique, it should be given attention to introduce LAMP protocol as diagnostic technique for animal diseases.
- Educational institutions dealing with teaching and research in veterinary medicine should consider the importance of LAMP method particularly for developing countries and incorporate in their curriculum.
- Government and funding organization should give economic support for the adoption of LAMP in developing countries to ensure its sustainable application.
- Further study should be done to overcome LAMP limitation

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