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Loop-Mediated Isothermal Amplification (LAMP): Comparative Advances over Conventional PCR and Other Molecular Techniques

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Gene amplification technology is essential in the fields of diagnostic medicine. The polymerase chain reaction (PCR) is central in the molecular studies and provides ways for diagnostic advancement in the areas. However, the requirement for thermal cycler in a dedicated facility for amplification of target genes in the PCR technique has been a bottleneck to many researchers. The limitations associated with PCR include cost implication, strict expertise necessity and relatively higher turn-around time. The emergence of loop-mediated isothermal amplification (LAMP) in the last two decades assists in bridging the undesirable gaps. This review aims to highlight the natural advantages of the LAMP technique over the existing conventional PCR and other isothermal molecular techniques. Available published articles on LAMP techniques reviewed, listed many outstanding advances of the method in comparison to traditional PCR technique. The mentioned advantages include simplicity, affordability, naked-eye result detection and many more. That made LAMP becomes a rapidly accepted method in the field of molecular diagnosis. Other essential features of LAMP in comparison with other emerging nucleic acid amplification techniques were

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adequately explained and presented in tabular form for research and quick reference purposes. Though LAMP has some few limitations, its advantages outweigh its flaws by filling the gap in the field of molecular biology diagnostics.

Keywords: Advantages; gaps LAMP; nucleic acid; PCR.

1. INTRODUCTION

Accuracy in molecular diagnostic studies on diseases is necessary for effective treatment outcome and control [1]. In recent years, nucleic acid-based techniques have increased the speed, sensitivity and specificity of the diagnosis of infections [2]. The requirement for thermal cycler machine and a dedicated facility for amplification of target genes using PCR technique has been a bottleneck to many researchers. The limitations involved the cost burden, strict expertise and relatively higher turn-around time (TAT) [3]. Despite many excellent improvements associated with these techniques, mostly attributed to PCR, there exist some limitations not adequately addressed, as reported by [4]. The emergence of LAMP technique introduced first in the early 2000s has helped to fill those gaps. It is an excellent isothermal rapid diagnostic technique used for detection and identification of infectious agents [5–7] food safety assessment [8,9], a study on genetic disorders and genetic traits, single nucleotide polymorphism (SNP) study [10], and many other research applications [11].

The technique is simple and it facilitates the detection through rapid amplification of a few target DNA into billions of copies at a constant temperature. The temperature is within the range of 60–65ºC in the presence of 4–6 primers specially designed to target 6–8 specific regions on the target DNA. It works on the auto cycling displacement activity of *Bacillus stearothermophilus* (*Bst*) large fragment on double-stranded target DNA. The amplification cycles occur within one hour and is a cheap alternative approach for detection of diseases [12]. It has the advantage of having many modes of amplicons detection methods after the LAMP reaction. The first method used for detection was naked eye monitoring using DNA intercalating agents such as SYBR green I or ethidium bromide (EtBr) [13]. Recently, many methods of detection to improve sensitivity have emerged. These include gel electrophoresis, real-time turbidimeter, real-time fluorescence and electrochemical biosensors [12]. Its relevance in the molecular field lies on its non-requirement of thermal cycler machine used in PCR for amplification. It requires only a heat block or a water bath to achieve amplification. Its simplicity, rapidity and non-machine dependence are the significant attributes that make it potentially applied in point-of-care-testing (POCT). It helps in early diagnosis of diseases, offer therapeutic measures and preventative actions when an outbreak of disease is anticipated, particularly in low-resource settings. The review aimed to elucidate the advantages of LAMP over PCR and other molecular techniques.

2. SUPERIOR ATTRIBUTES OF THE LAMP ASSAY

There are many advantages of LAMP assay over other nucleic acid-based tests. These are mostly associated with standout qualities like simplicity, low TAT, precision, high yield of amplicon and less costly as explained as separate benefits below. According to the World Health Organization (WHO) definition, POCT must conform with a set criterion, put together in an acronym "ASSURED". These are affordable, sensitive, specific, user-friendly, rapid/robust, equipment-free or minimal and deliverable to the concerned who are in dare need [14].

2.1 Visual Detection of Results

LAMP assay can be detected using visual detection with the naked eye as a result of the accumulation of turbid white magnesium pyrophosphate as a by-product of DNA amplification. LAMP products visualised by direct fluorescence using fluorescence dyes such as ethidium bromide (EtBr), SYBR green, Quant-iT picoGreen, GeneFinder, polyethylenimine, or Evergreen [12,15]. The dyes are applicable for qualitative and quantitative measurement. The hydroxy naphthol blue (HNB) [16] and calcein [12,17] are indirect colourimetric indicators used for one-step reaction by adding them during LAMP mixture preparation. Calcein dye also applicable in real-time monitoring of LAMP reaction [17]. In limited-resource settings, naked eye monitoring of LAMP result will be paramount in speeding clinical judgment and reducing mortality rate to common and treatable infections.

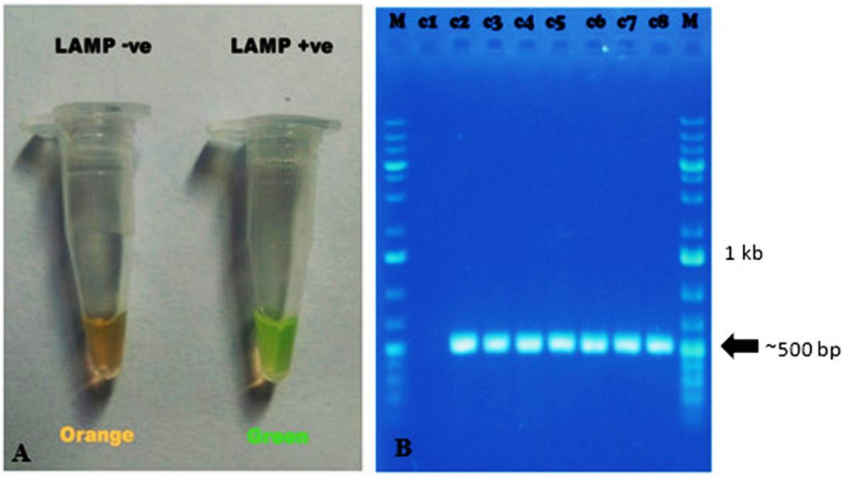

Fig. 1. A: LAMP assay visual detection results using calcein dye indicator; orange indicates negative, while green indicates positive to the target gene. B: Gel electrophoresis for detection of PCR results

Since quantitative detection is complicated and needs further approach, the naked eye could easily indicate whether a LAMP reaction is positive or negative. Based on the developed turbidity [18] or change of colour of the dye to the final positive colour after the reaction [16] as shown in Fig. 1. Here, the colour changed from orange to green using calcein dye. In contrast to traditional PCR that relies only on gel electrophoresis for DNA analysis. The UV transilluminator necessary need to view and identify the target DNA.

2.2 The High Sensitivity of LAMP Assay

The sensitivity of the LAMP is mainly dependent on the primer set and its low ability to amplify non-template DNA. Primers design to produce an amplicon size of approximately <300 bp allows for excellent sensitivity and increases the reaction speed [19]. Tian and coworkers [20] also reported the use of high concentrations of inner primers (FIP and BIP, 1.6 µM each) accelerates LAMP reaction. It thus results in high yield of amplicons [20]. The sensitivity is measured based on either the detection of low copy number or a high dilution of genomic DNA of the target template or detection limit from the spiked sample in comparison to the standard gold method. LAMP assay found to be very sensitive in detecting the target template [21]. Typical of LAMP assay is the dynamic range of amplicon concentration within 10^4–10^8 copies. It can detect as low as 1 – 1000 copies of the template, and that shows its robustness in terms of sensitivity as reported by Wang et al. [15], with the limit of detection of 10 copies/µL of target DNA. Notomi et al. [11] also reported a detection limit of 6 copies/µL of hepatitis B virus (HBV) DNA template. The findings supported other results, including Chen et al. [22] that reported high sensitivity as low as 3 copies/µL using multiplex microfluidic LAMP assay.

The detection limit, when compared to PCR sensitivity, as published by Adao and Rivera [5], was 0.1 cells/ml which is much lower than 100 cells/mL in PCR for detection of *Trichomonas vaginalis*. It further lends more credence to LAMP over PCR technique in terms of sensitivity. Concordant findings of Seki et al. [23] reported a low detection limit of 10 cells/reaction of pneumococci from purified DNA. Spiked CSF sample within 30 min with 100-fold sensitivity over conventional PCR detected 10^4 cells/reaction. Some sensitivity findings were reported in the colony-forming unit per millilitre (CFU/mL) as reported by Park et al. [24] that indicated 2 CFU/200µL. Wang et al. [25] also reported a detection limit of 4.2×10^2 CFU/mL of *V. parahaemolyticus* DNA from the spiked oyster homogenate. The above findings show higher sensitivity benefits of LAMP over conventional PCR.

2.3 The High Specificity of the LAMP Assay

The specificity testing of LAMP assay usually tested using genomic DNA of closely related organisms. Its preference over conventional PCR is due to the specificity of the primers targeting

template, as shown in Fig. 2 [26]. According to the findings by Kasahara et al. [27], the specificity of primers sets for conventional LAMP assays tested with 10 ng of the purified DNA of the 20 strains of target species and 33 related species from 10 genera. The primer sets detected all the 20 strains of interest successfully without amplification of non-target species, within 30 min of LAMP reaction. Despite the higher number of primers for LAMP assay, LAMP multiplex assay is practically applicable with robust specificity. Mandappa and Joglekar [28] reported high specificity of LAMP their primer sets on the extracted DNA of four different genes of *Bacillus cereus* (hemolysin-A *(hblA),* Enterotoxin-T *(bceT),* Enterotoxin-FM *(entFM/cwpFM) and* Cytotoxin-K *(CytK*) and the primers selectively targeted diarrhoeal genes. No cross-reactivity noticed with other organisms. The relatively higher specificity is one of the significant advantageous attributes of LAMP when compared to other nucleic acid amplification-based techniques. Table 1 below indicates findings from different authors that affirm LAMP as a method that has high yield ability in comparison to PCR technique.

2.4 Target Template Amplification at a Constant Temperature

Loop-mediated isothermal amplification technique has the capability of revolutionising the molecular-based diagnostic approach to infections. It is through reducing the dependence of sophisticated, expensive, and facility-domiciled instruments resulting in low running cost and short TAT. Moreover, LAMP anticipated being a low cost molecular diagnostic tool, particularly in limited-resource environments [29]. The LAMP test materials easily transported because of small size with minimal facilities requirement, maintenance and do not require highly skilled staff to run it [30]. The LAMP assay has superiority based on its ability to operate at a constant temperature.

Contrary to other molecular-based amplification techniques such as PCR that requires thermal cycler, set at alternating temperature cycles and necessary steps. The primers are designed and synthesised with an optimum temperature range between 60–65°C, which is also optimum for *Bst* enzyme displacement and amplification activities [13,24]. The main advantage of LAMP techniques in POCT lies on its reagents' ability to remain stable at room temperature during storage. According to Thekisoe et al. [31] and Notomi et al. [32], newly developed LAMP reagents can be stored efficiently both at 25°C and 37°C. Thus, supported the use of LAMP in the field as well as in resource-limited settings where there is no stable power supply for refrigeration of reagents.

PCR amplification steps
Denaturation 92°C–95°C → Annealing 52°C–60°C → Extension 70°C–75°C

LAMP amplification
Constant temperature
60°C–65°C

2.5 Various Methods of Detection

Loop-mediated isothermal amplification, unlike other nucleic acid-based techniques, could monitor the amplification reaction of template in diverse approaches. It solely depends on the availability of resources in the laboratory and the required standards of sensitivity and specificity used. The search for an efficient method of detection lies in need of improving sensitivity, specificity, the stability of the amplified LAMP amplicons, simplicity and POCT [12]. For example, as shown in Fig. 2, in a resource-limited setting, naked-eye monitoring is enough for detection of template of interest. When using hydroxy naphthol blue (HNB), the colour changes from violet to sky blue if the reaction turns positive. When the result turns out negative, the colour remains violet. The LAMP assay progression monitored using calcein dye to give a change of tone from orange (negative) to green (positive). Thus, it shows its simplicity and cost advantage compared to PCR, which uses only gel electrophoresis for target identification. Also, the additional cost of gel electrophoresis machine and staining reagents is another disadvantage. Of note, LAMP genetic analysis depends not only on the efficacy of template amplification but based on the practical method of reaction monitoring since there are many methods of choice [12,33].

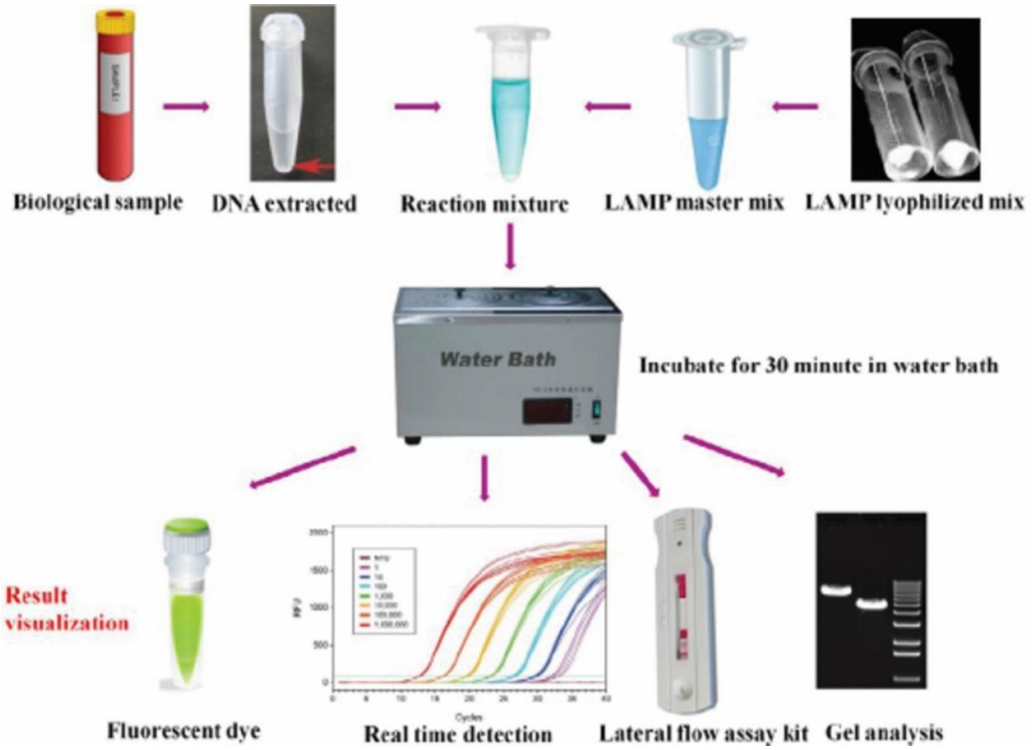

Fig. 2. Examples of various methods of detection and advantages at a glance of the LAMP technique. Image adapted from Ranjan [34]

2.6 Low Susceptibility to Reaction Inhibitors

The LAMP excellent property of makes it less sensitive to reaction inhibitors such as Ca^{2+} [35], fat, casein [36], proteinases [37] and urine helps it adopted by many researchers in the field of molecular diagnostic. The substances are known to affect PCR based reactions adversely [24]. The technique uses a powder in the reaction components, which removes all inhibitors of the reaction and makes DNA free in the solution [38]. Reports indicated that the sensitivity of LAMP is not affected by the presence of non-target DNA in the reaction tube [39,40]. Supported by Kiddle et al. [41] that LAMP is tolerant of inhibitory materials known to inhibit PCR reactions. These include blood, serum, and food ingredients, suggesting omission of template purification in LAMP protocol and therefore, lowers time to result in analysis.

2.7 Direct Sample Preparation without DNA Extraction

The traditional methods of detecting infectious agents are culture, microscopy and histopathology and these methods have their disadvantages in terms of sensitivity and specificity. Moreover, they are known for being laborious and time-consuming [42]. Hence, it is necessary to emphasise the development of more sensitive molecular techniques. These are based on nucleic acid pathogenic agents and not based on its growth on microbiological culture media. Such methods include PCR and hybridisation, but they are still laborious and expensive [43]. These limitations led to the search for novel approaches, and one of them is the LAMP technique. This technique can directly process sample in the field without extraction of the target nucleic acid template, and that increases its rapidity and subsequently reduces TAT. It is relatively more superior in terms of sensitivity and specificity with comparably low susceptibility to reaction inhibitors that generally inhibit PCR and its cohorts as reported by Niessen et al. [44]. The ability of LAMP technique to exhibit less sensitivity to reaction inhibitors such as a urine, serum, plasma, and culture medium present in biological samples is critical, particularly in the field of POCT. Therefore, it indicates it robustness, reduces cost and time-to-result, unlike PCR that

can efficiently be inhibited by these substances [23].

2.8 High Yield of *Bst* DNA Polymerase

The success of sound amplification of target template and reaction sensitivity lies in the type of DNA polymerase used in the reaction. The *Bst* DNA polymerase enzyme, used in LAMP assay is a large fragment engineered polymerase derived from *Bacillus stearothermophilus* with improved properties of rapid amplification, 5'–3' exonuclease activity and template strand displacement. *The Bst* enzyme was also known to have the tolerance to reaction inhibitors, thermostability. It incorporates deoxyuridine triphosphate (dUTP) for DNA sequencing and cloning applications. Furthermore, these properties occur both in *Bst* 2.0 and *Bst* 3.0 [45]. The *Bst* also shows significant reverse transcriptase activity up to 72°C used for RT-LAMP reaction as occurred only in *Bst* 3.0 [46,47]. The significant difference between *Bst* DNA polymerase and the *Taq* polymerase used in PCR reactions is the intense strand displacement activity of the former. At the same time, the latter has weaker or no displacement activity and therefore not fit for LAMP assay as no significant amplicon would be produced [48]. Niessen et al. [44] relate the high molecular weight of DNA built through the excellent activity of *Bst*. Besides, Sahoo et al. [34] reported high amplification efficiency of *Bst*, amplifying DNA 10^9–10^{10} times within one hour.

2.9 Formation of Loop Amplicons after LAMP Reaction

LAMP amplification reaction exists in two steps, namely: non-cyclic and cyclic phase. The end-product of the non-cyclic period serves as material for the cyclic phase. When loop primers (forward and backwards) are available, they hybridise to the non-cyclic phase end-product and accelerate the rate of the reaction and enhance sensitivity [23]. The large size of LAMP reaction amplicon with loop shape as an end product makes the result visible to the naked eye [49]. Unlike PCR that has only two primers per reaction, with no formation of loop-shaped end-product. The likelihood of seeing it using naked eye is low because of the smaller size amplicon as well low number of the amplified product when compared to LAMP assay [4].

2.10 LAMP Point-Of-Care Testing (POCT) Ability

POCT refers to medical diagnostic testing that done near a patient at the time the patient receives medical care [50]. Accuracy, precision and timely diagnosis of human infections and genetic disorders are paramount for effective management of patients and reducing the financial burden, slowing the emergence of drug resistance and lowering morbidity and mortality rates [51,52]. The nucleic acid-based technique (NAT) is the gold standard for some gene-based identification of microorganisms. However, such methods like PCR involve multi-analytical steps comprising nucleic acid (DNA/RNA) extraction from the sample, target gene amplification and detection. Such processes require centralised laboratories to carry them out and present hindrance, particularly in resource-limited settings [24]. However, with the advent of the LAMP technique, direct treatment of the sample becomes possible with excellent precision and reproducibility in the field or near the patient in hospital settings [22,51].

3. COMPARATIVE ADVANTAGES OF LAMP TECHNIQUE OVER OTHER NUCLEIC ACID-BASED DIAGNOSTIC TESTS

Nucleic acid amplification is an essential tool for virtually all life science research. Many approaches developed, each with specific innovation to re-initiate nucleic acid amplification. Such techniques include cross-priming amplification assay (CPA), rolling circle amplification (RCA), helicase dependent amplification (HDA), strand displacement amplification (SDA), self-sustained sequence replication (3SR), nucleic acid sequence-based amplification (NASBA) [46,57,58], PCR [59] and LAMP [60]. They mainly differ in reaction time, type of nucleic acid amplified (RNA or DNA), the requirement for a precision thermal cycler, dNTP modification, enzyme involvement, reaction temperature, method of amplification and many more [11]. Table 2 depicts the summary of differences and similarities of the significant techniques based on their mechanisms of action and other properties. LAMP POCT approach to diagnosis has many advantages as listed in Table 2.

Table 1. Comparative accuracy between LAMP and PCR assays for the detection of microbial pathogens

LAMP technique	Target	LAMP specificity	LAMP LoD	PCR LoD	Reference
Conventional LAMP	*Streptococcus pneumoniae/lytA*	83.9% (73/88)	10^1 copies/reaction	10^4 copies/reaction	[23]
LAMP-LFD	*Vibrio parahaemolyticus/tlh*	100%	3 cfu/reaction	30 cfu/reaction	[53]
Conventional LAMP (EtBr)	*Toxoplasma gondii/529 fragment*	17/200 (8.5%)	1 pg/µL	10 pg/µL	[54]
Conventional LAMP (gel)	*Trichomonas vaginalis/*NDMR 100 18S RNA	53/121 (46.06%)	0.036 ng/µL	0.36 ng/µL	[5]
Conventional LAMP (SYBR green)	*Coxsackievirus B3/ CVB3*	100%	10 pg/reaction	100 fg/reaction	[7]
Conventional LAMP (SYBR green)	Enterohemorrhagic *E. coli*/*rfbe*; shiga toxins *stx1*and*2*	100%	10 pg/µL	10 ng/µL	[55]
Conventional LAMP (SYBR green)	*Cryptococcus neoformans/C. gatti/ura5*	5/142(3.5%) (2/107(1.85%) CSF; 3/35 (8.5%) serum)	5 copies/reaction	50 copies/reaction	[56]
AuNP – LAMP	*Vibrio parahaemolyticus* (VP_{AHPND}) $Pir^{vp}A$	100%	10^2 cfu/ml	10^4 cfu/ml	[6]

[a]LoD: Limit of detection; [b]LFD: lateral flow dipstick; [d]AuNP: nanogold probe; LAMP: loop-mediated isothermal amplification

Table 2. Comparative analysis of LAMP and other molecular-based diagnostic tests

No.	Properties	LAMP	PCR	NASBA	3SR	SDA
1.	Naked-eye detection	Amenable to visual (naked) detection based on turbidity	Not responsive to visual detection	Not sensitive to visual detection	Not responsive to visual detection	Not amenable to visual detection
2.	Primers used	Four-six primers	Only two primers	Only two primers	Only two primers	Only one primer
3.	Rapidity	It is rapid, 30 – 60 min	High TAT, > 1 h to amplify	Rapid, 30 min reaction time	1 – 2 h	2 h
4.	Type DNA polymerase used	*Bst* polymerase produced by *Bacillus stearothermophilus*	*Taq* polymerase provided by *Thermus aquaticus*	T7 RNA polymerase derived from T7 bacteriophage	AMV-RT; RNase H and T7 RNA polymerase	*Bst* polymerase produced by *Bacillus stearothermophilus*
5.	Role(s) of DNA polymerase enzyme	Strand displacement and amplification of the target gene	Amplification of target gene	AMV-RT: reverse transcription RNase H: RNA sense strand digestion	AMV-RT: reverse transcription RNase H: RNA sense strand digestion	Two enzymes cut the RE sites for sequence flanking
6.	Temperature Used	Requires isothermal temperature 60-65ºC	It involves the use of alternating temperatures	Requires isothermal temperature 40ºC	Requires isothermal temperature 40ºC	Requires isothermal *T*° 37 – 40ºC; still requires 95°C for denaturation
7.	Inhibitors tolerance	Tolerant to sample matrix inhibitors, e.g. serum, anticoagulant	Sensitive to sample matrix inhibitors	Sensitive to sample matrix inhibitors	Sensitive to sample matrix inhibitors	Reactions inhibited in the presence of high human DNA, glycerol, formamide
8.	Nature of amplicons	Reaction amplicon: a mixture of stem-loop DNAs with various sizes of the stem and cauliflower-like structures	No stem-loop and cauliflower-like structures formed, thus increases TAT	No stem-loop and cauliflower-like structures built thus increases TAT	No stem-loop and cauliflower-like structures formed thus increases TAT	No stem-loop and cauliflower-like structures formed thus increases TAT
9.	Nucleic-acid extraction/Direct sample preparation	It may/may not require DNA extraction and sample preparation.	Necessary	Necessary	Necessary	Necessary
10.	Method of amplification	Strand displacement	Heat denaturation	Reverse transcription	Reverse transcription	Restriction digestion and strand displacement
11.	Nucleic acid detected	DNA/RNA to cDNA	DNA/RNA	RNA	RNA	DNA/RNA

4. LIMITATIONS OF LAMP TECHNIQUE

Though there are many advantages that the LAMP technique has, it also comes with some limitations. One of the striking disadvantages is the possibility of contamination, either by healthy human flora (*Staphylococcus aureus, Staphylococcus sapropyticus*) or cosmopolitan fungi (e.g. *Aspergillus* species). It is the reason for LAMP not to be recommended for the detection of such organisms. Also, the final products of LAMP reaction are a composite of multiple concatemer amplicons with different band sizes observed in a characteristic "ladder-like" pattern on a gel. In contrast, the PCR technique has only a single band per template for easy identification of size and analysis [34]. The multiple size amplicons render it to be unsuitable for cloning work, unlike PCR [61]. Nevertheless, these disadvantages do not discount it from not being used as a detection and identification tool for pathogens for molecular diagnostic purposes.

5. CONCLUSION

In conclusion, our findings from previous studies showed excellent performance of LAMP over PCR technique. The significant advantages of interest to many researchers are the non-requirement for thermal cycler machine and simple expertise requirement. The simplicity, rapidity, high specificity, sensitivity and POCT integration, suggest pieces of evidence of acceptance, especially in resource-limited settings. Understanding the technics shows that LAMP is superior over other molecular-based techniques, including conventional PCR. The LAMP is a perfect molecular technique that satisfied the WHO set criteria on an ideal diagnostic tool. Though it has some few limitations, its advantages outweigh its flaws.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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