THE LAMP AT THE END OF THE TUNNEL
LOOP-MEDIATED ISOTHERMAL AMPLIFICATION ASSAY (LAMP) AS A POTENTIAL ALTERNATIVE TO PCR-BASED DIAGNOSTICS

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Abstract

Diagnostic testing is key in mitigating the spread of infectious diseases. Although current nucleic acid amplification tests such as PCR-based tests are usually highly accurate, they utilise expensive equipment and complicated processes. These qualities make it largely inaccessible, especially to low- and middle-income countries. An emerging technology called loop-mediated isothermal amplification (LAMP) fosters the potential to be an accessible, cost-effective, yet comparably accurate alternative to existing gold standard methods. LAMP is a rapid single-tube technique that amplifies the target gene at a constant temperature, thus eliminating the need for the expensive thermocycler. Results can be determined by simply looking at a colour change in the sample. Furthermore, it only requires assay reagents and a heating source such as a water bath or a heat block, which are suitable for low-resource settings. This method has already been deployed in the field of primary care settings for COVID-19 and other diseases. Although the assay still leaves room for improvement, it has the potential to provide diagnostics more equitably and control outbreaks more efficiently.

Whether it is for COVID-19 or other infectious diseases, diagnostic testing is a ubiquitous public health tool. Diagnostics play a pivotal role in containing the spread of outbreaks and endemic diseases [1]. However, in resource-limited areas, the main challenge is accessibility to these diagnostic tests that consequently result in the delay of initial detection of community transmission. This diagnostic bottleneck has been linked to the accelerated spread of recent outbreaks such as SARS-CoV-2, Ebola, Zika, and yellow fever [1, 2]. The poor level of surveillance delays the time to containment and increases the difficulty of controlling the outbreak [1].

While diagnostic tests can be quickly developed for various diseases, they are not commonly suitable for deployment at the community and point-of-care (POC) level [3]. For example, polymerase chain reaction (PCR) is considered the gold standard in detecting an array of pathogens in humans, animals and plants [4]. Although this method is robust and accurate, performing PCR requires an expensive lab testing facility with skilled technicians. Both of which limit the test's affordability, physical accessibility, and turnaround time of results. Therefore, it is vital to innovate and develop strategies to overcome these challenges, especially for low- and middle-income countries.

An emerging technology called Loop-mediated Isothermal Amplification (LAMP) assay has been studied to be a potentially simpler and more accessible diagnostic tool than current gold standard tools such as PCR [5]. The World Health Organization described that an ideal test for controlling infectious diseases is not necessarily the most accurate one; instead, it has to be evaluated more holistically [6]. The characteristics of an ideal test include affordability by those at risk, sensitivity, specificity, user-friendliness, rapidity, and being equipment-free [6]. All these characteristics ensure that the diagnostic tests are available at POC to those at high risk, such as individuals belonging to disadvantaged backgrounds [6]. In addition, POC testing facilitates rapid diagnostics near the patient to subsequently prompt immediate yet informed health interventions.

There are various other nucleic acid amplification tests (NAAT) that use a wide array of techniques to initiate DNA synthesis and directly detect genetic material [7]. However, most of them require sophisticated instruments or elaborate methods to perform [7]. With more development and optimisation down the road, LAMP stands out to meet the criteria mentioned above.

A Brief Introduction to the LAMP Assay

LAMP is a simple single-tube NAAT that does not require thermocycling equipment to perform [8]. Instead, it only needs a simple heater block or a water bath. Unlike other NAAT methods, results can be determined through a visual colour change readout [9]. Thus, eliminating the need for confirmatory measures, such as agarose gel electrophoresis or fluorescence quantification through machines. The LAMP assay was first described in the year 2000 as a novel NAAT that amplifies small amounts (or copies) of DNA into a million copies within an hour (Figure 1) [8]. The target gene is amplified at an isothermal temperature of 60-65°C using multiple primers and a polymerase with high strand-displacing activity. The high strand-displacing activity of the polymerase aids in denaturing double-stranded DNA at lower temperatures [10].

The process requires a set of four to six primers that correspondingly bind to six to eight different regions of a target gene [8]. The binding of primers to multiple gene regions makes LAMP highly specific in pure samples. Primer sets commonly contain two outer primers (FP3 and RP3), two inner primers (FP2 and RP2), and sometimes two additional loop primers [8]. In addition to the multiple primers, another essential component of LAMP is the strand-displacing polymerase that facilitates the displacement of downstream DNA without the need for fluctuating temperatures [10].

Complementary sequences bond together, resulting in a dumbbell structure product that contains multiple sites to initiate DNA synthesis [11]. Ultimately, this process yields rapidly accumulating amplicons [11].

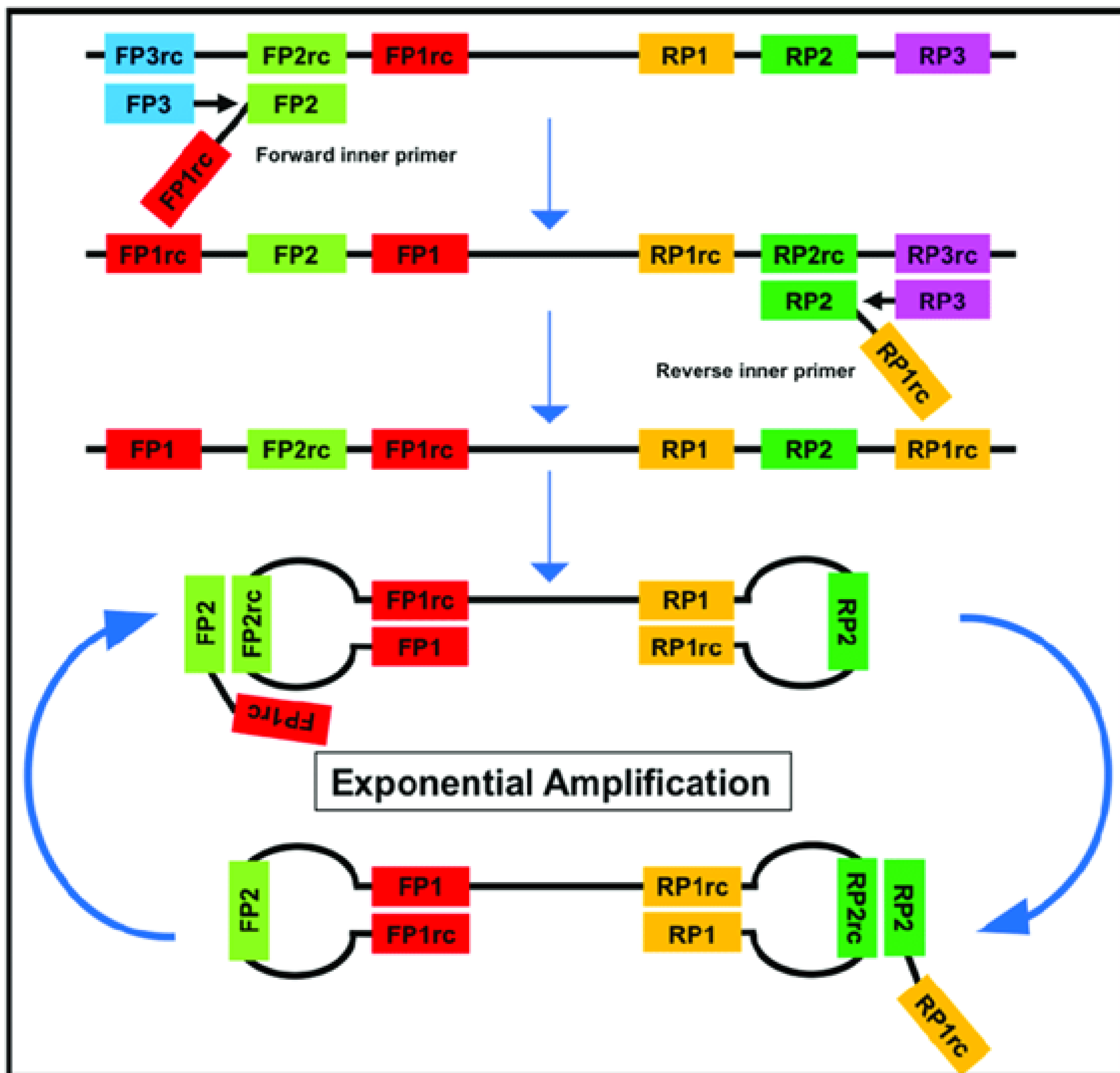

Figure 1: *Illustration of the amplification mechanism of LAMP. Amplification initiates through strand displacement by a forward inner primer. The strand-displacing activity of the polymerase helps in disrupting the double-stranded DNA, after which the reverse inner primer attaches to the newly synthesised strand. Complementary sequences bond together, resulting in a dumbbell structure product that contains multiple sites to initiate DNA synthesis. Ultimately, this process yields rapidly accumulating amplicons [11].*

LAMP is, by no means, a perfect assay. While it has high sensitivity, it also presents workflow challenges such as carryover contamination and mispriming [12, 13]. Nevertheless, researchers have been working their way towards the end of the tunnel. The method has been continuously optimised for practical applications in detecting various pathogens, primarily for providing effective POC diagnostic testing in resource-limited areas [7]. LAMP can even be adapted for a more comprehensive detection (or diagnosis) of an array of diseases in combination with other molecular approaches [14].

LAMP in COVID-19…

At the dawn of the COVID-19 pandemic, countries all over the world scrambled to realise a sudden rise in Real-Time quantitative PCR (RT-qPCR) testing demand [15]. The pandemic itself shed light on the costly laboratory set-ups, limited reagents, and a shortage of healthcare workers that contributed to a bottleneck for testing. Countries such as South Korea and New Zealand were able to keep up with the initial surge, but resource-limited and densely populated countries such as the Philippines and India struggled to keep up [15]. In an attempt to augment testing capacity for COVID-19, studies were done to adapt LAMP for SARS-CoV-2 RNA detection using an additional reverse transcription step. The modified assay called "reverse transcription LAMP" (RT-LAMP) was considered a promising POC and cost-efficient test that does not compromise accuracy [16].

To eliminate the need for skilled workers in obtaining nasopharyngeal swabs, multiple projects have successfully developed saliva testing in tandem with RT-LAMP [9, 17-22]. All these assays produced visual results in less than 30 minutes. In addition, one of these studies reported that their version of a deployable one-step RT-LAMP protocol had a specificity of >96% and sensitivity of >97% compared to the RT-qPCR and nasopharyngeal swab gold standard [23].

Currently, countries such as the USA and the Netherlands already recognise RT-LAMP as a valid alternative to RT-qPCR [24, 25]. In Austria, the Austrian Agency for Health and Food Safety (AGES) has already recommended the use of saliva-based RT-LAMP in hospitals and laboratories that have not established PCR-based diagnostics [26]. RT-LAMP's simple workflow application in COVID-19 is a leap towards achieving POC and even at-home testing. This cost-effective and rapid method may sustain routine mass testing strategies that will aid the complete reopening of the economy.

Even with the availability of vaccines, diagnostic testing remains a relevant tool in detecting "breakthrough" cases in vaccinated individuals and in testing unvaccinated people. Routine testing in a population will also determine emerging variants that may be more pathogenic. Doing so would aid in the immediate containment of these variants.

Aside from NAATs, other methods of rapid POC testing are also available such as antigen testing, but it comes with a trade-off. Although antigen tests are easily deployable and rapid, their relatively low sensitivity raises concerns among experts [15]. The test's propensity to miss infectious cases could give people a false sense of security and elicit outbreaks in countries with fewer restrictions [15]. However, it remains a valuable tool to use while other rapid yet sufficiently accurate tools are being developed [15].

...and beyond

Prior to the COVID-19 pandemic, conventional LAMP had already been utilised and developed for various infectious pathogens, such as the causative agents of pneumonia and tuberculosis [27, 28]. One of the most notable studies using LAMP was an *in vitro* study on *Trichomonas vaginalis*, the most common sexually transmitted infection in women, caused by a parasite [29]. The study found that the sensitivity of LAMP was up to 1000 times higher than the sensitivity of PCR testing. This is possibly due to the LAMP DNA polymerase's tolerance to inhibitors found in urine samples [29]. However, the LAMP test also had false-positive issues due to carryover contamination [29]. These contamination issues can be minimised through aseptic techniques or other preventive measures [13, 29].

RT-LAMP has also been used successfully in detecting other viruses such as Zika, HIV, and Ebola in recent outbreaks [30, 31]. More than plainly detecting the presence of a pathogen, RT-LAMP has the capability to distinguish pathogen subtypes. For example, a study developed a fluorescent RT-LAMP assay that could consistently detect HIV subtypes A, B, C, D, and G [32]. Furthermore, it can also be a useful POC tool to quantify HIV RNA copy numbers in low-resource, primary care, and hospital settings [32].

Surpassing human diseases, LAMP and RT-LAMP have practical applications in plants and animals, which is particularly useful for field testing in the aqua- and agriculture industries. A prime example is its use in the detection of the two most common shrimp pathogens in the Philippines: White Spot Syndrome Virus and *Vibrio* spp. [33]. Early diagnosis of disease outbreaks in shrimp populations can help farmers carry out intervening measures. Outbreaks like these could wipe out entire shrimp populations and incur significant losses on small-scale farms when not detected on time [33]. Surprisingly, the results showed that the more rapid LAMP assay was ten-fold more sensitive than conventional RT-PCR testing in detecting the White Spot Syndrome Virus [33]. On the other hand, LAMP proved to be more time- and labour-efficient in the bacterial identification of *Vibrio* spp. [33].

Similar findings apply to other pathogens such as the Batai virus in cattle and mosquitoes and the Hepatitis E virus in shellfish [34, 35]. All these demonstrate the breadth of LAMP's versatile potential to provide robust, cost-effective, and physically accessible diagnostics for various species.

Conclusion

LAMP is undergoing continuous optimisation and development with regard to disease diagnostics. Its subsequent applications demonstrated that it can be a cost-effective, physically accessible, yet sufficiently accurate alternative to RT-PCR. However, like most assays, LAMP has its drawbacks, and there is a long road ahead for improving and adapting it to detect different diseases. LAMP has always been framed as a diagnostic tool for neglected tropical diseases in low-resource settings [14]. However, the COVID-19 pandemic has exposed the vast scale of diagnostic bottlenecks beyond the developing world, and we are now seeing the first large-scale deployment of LAMP diagnostics [14]. Further optimisation of this testing strategy can build strong foundations in preparation for future outbreaks by containing the spread of pathogens. For COVID-19 and other infectious diseases, this relatively novel technology has the potential to be the LAMP at the end of the tunnel.

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