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Loop Mediated Isothermal Amplification: Towards Point-of-Care Diagnostic

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Perspective

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Abbreviations: POCD: Point-of-Care Diagnostics; SDI: Sexually Transmitted Diseases Diagnostics Initiative; ASSURED: Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, Deliverable; LAMP: Loop-Mediated Isothermal Amplification; iRBCs: Infected Red Blood Cells; COC: Cyclic Oleofin Copolymer.

Introduction

In 2002, Daar AS, et al. [1] listed the top ten challenges that biotechnology faced to improve health in developing countries. On the top of that list we could find: "Modified molecular technologies for affordable, simple diagnosis of infectious diseases". This idea has been materialized over the following years into the principle of point-of-care diagnostics (POCD). Several definitions have been published since then. A good example of that is the one given by Schito M, et al. [2], which says: "a diagnostic test that is performed near the patient or treatment facility, has a fast turnaround time, and may lead to a change in patient management". Moreover, the test should not require trained laboratory personnel, clinical laboratory or other infrastructural support. Over ten years ago, this basic concept was divided into specific criteria that must be achieved with any POCD. Those criteria were proposed by the World Health Organization Sexually Transmitted Diseases Diagnostics Initiative (SDI) in the acronym ASSURED (Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, Deliverable) [3]. This term has been revisited recently by Land KJ, et al. [4], updating it to the newest technological innovations. Thus, they established the new acronym REASSURED that now also includes: Real-time connectivity and Ease of specimen collection and Environmental friendliness.

The challenge that Daar AS, et al. [1] recognized often face strong limitations, as classic molecular techniques, such as PCR and its variants, require specialized and expensive equipment and a high degree of expertise to perform them. Thus, their arrival and establishment into low-income developing countries is constantly set back. At present, there is a nucleic acid amplification method named loop-mediated isothermal amplification (LAMP) [5]. Compared to PCR-based techniques, the simplicity of the LAMP method makes it suitable for field testing in developing countries [6,7] and an ideal candidate to be used as a POCD tool. LAMP is a highly specific, sensitive and efficient DNA amplification method based on strand displacement reaction and a stem-loop structure under isothermal condition [5]. It uses the *Bacillus stearothermophilus* DNA polymerase and a set of 4 (or 6) primers that hybridize to 6 (or 8) different regions of the target DNA sequence. The LAMP reaction does not require expensive devices and is completed in a short time. Since its emergence, LAMP technology has been successfully used for the detection of a great number of pathogens, including viruses, bacteria, fungi and parasites [8] and, over the past 10 years the number of publications involving the use of LAMP has showed a multiplied exponentially.

Advantages and Limitations of LAMP Technology

From the point-of-care perspective, LAMP presents several major advantages over other molecular amplification methods. First, the equipment required to perform it, a heating block or a simple water bath, deeply reduces the cost and complexity of the infrastructure needed for the diagnostic. The *rapid and robust* criteria of the ASSURED concept is also fulfilled as LAMP can be performed in just 30 min. Additionally, results can be observed by turbidity (due to the formation of a magnesium pyrophosphate precipitate) and by colorimetric changes (adding different dyes, such as SYBR Green I, Malachite Green or Hydroxy naphthol Blue), making it ideal for an easy monitoring and making it a *user-friendly* assay [9]. Furthermore, LAMP is highly robust under circumstances of impure preparations or minimally processed samples [10].This feature is very useful in field conditions for which conventional nucleic acid extraction prior to diagnostic testing is usually not suited. However, a few limitations still remain unsolved for LAMP technology: (1) it is inapplicable for cloning; (2) the multiplexing approaches of LAMP are considered more difficult and are less developed than for PCR; (3) the primer design is exposed to more constraints thus limiting the sites of amplification in the DNA target and (4) there is high risk of carry-over contamination, which can lead to false-positive results. This problem can be due to the stability of the LAMP product, which does not degraded easily, and thus unintended carry-over contamination can occur [11].

LAMP as POCD: Approaches

In recent years, a number of adaptation procedures of the LAMP technology into a more field-friendly tool have been released. Their approach varies greatly: from lateral-flow to lab-on-chip layouts, microfluidic-based methods orin combination with metallic nanoparticles, even into a red blood cell.

As a first example, Chi J, et al. [12] recently developed a pattern photonic cellulose array inspired in the *Stenocara beetle* bumpy back surface, to develop a droplet separated LAMP display for the detection of *Staphylococcus aureus* DNA. This method allowed reducing reaction volume from 25 μL down to 3μL, without the necessity of complex microfluidic designs and valves. Besides, due to the slow-photon effect of the matrix, they achieved up to a 32-fold improvement of calcein fluorescence dye signal for DNA amplification, reducing reaction time from 60 to 40 min and also improving the detection limit of the LAMP method from 5 to 0.6 copies per μL.

On the other hand, Hashimoto M, et al. [13] developed an *in-situ* LAMP for the identification of *Plasmodium* species DNA confined into red blood cells. The assay consists of forming a monolayer of infected red blood cells (iRBCs) over the entire surface of hydrophilic-treated cyclic oleofin copolymer (COC) plate. The monolayer of iRBCs was quickly dried with a hair drier, fixed with 20% formalin for 20 min and then permeabilized with 0.5% TritonX-100 for 10 min. Subsequently, combining LAMP reaction mix for the amplification of P. *falciparum* DNA with digoxigenin-labelled dUTP, allowed a fluorescence signal within the iRBC to be detected and visualized by fluorescence microscopy.

One of the most important challenges for LAMP diagnosis in endemic regions is the difficulty to maintain the cold-chain to preserve reagents up to the moment there action takes place. Trying to solve this, Hayashida K, et al. [14] developed a Direct Blood Dry LAMP using a two-step dry-up protocol to stabilize pre-mixed LAMP reagents for the diagnosis of African trypanosomiasis. Two major advantages of this method are: (1) LAMP is performed directly in detergent-lysed blood samples and (2) single tube stabilization of reagents in the presence of trehalose, working as a cryoprotectant. Although a stability of at least 7 months at room temperature for the dried master mix was showed, the reaction buffer for LAMP assay is not dried with the rest of the components and must be added subsequently to the drying process, thus increasing the manipulation of the reagents leading up to the reaction. Now, it is known that the drying of the buffer with the rest of reagents of LAMP assay is the major cause of loss in functionality in LAMP stabilized reaction mixes [15].

Dipsticks are also one of the most popular approaches towards POCD. A LAMP test combined with a lateral flow dipstick was recently developed for leptospirosis [16]. This test was able to show three different lines, corresponding to target DNA amplification, an internal LAMP control amplicon and a chromatography control. This possibility of multiplexing LAMP in lateral flow assays, calls for the development of single-strip multiplexed LAMP for several pathogens. In this sense, a multiplexed lateral flow dipstick LAMP for simultaneous detection of *Enterococcus faecalis* and *Staphylococcus aureus* was recently developed in combination gold nanoparticles [17].

Another very interesting approach for LAMP to be used as to POCD is the use of smart phone-based technology. Priye A, et al. [18] developed a multiplexed LAMP for the detection of Dengue, Chikungunya and Zika viruses that was performed in a LAMP box powered by a USB power bank or solar panel. The visual color detection was performed with a phone camera, using a novel algorithm that is able to transform RGB (Red, Green, and Blue) color values into chromaticity values to analyze fluorescence signals, thus improving the discrimination of positive/negative signals by 5-fold when compared to detection with traditional RGB sensors or the naked eye [19].

Conclusion

At present, LAMP technique has the potential to become an effective technique when it comes to POCD. Limitations of the method are well-known; however, the remarkable job of numerous researchers is closing the gap between laboratory and endemic regions. Hopefully, all those combined efforts will bring us closer to overcome the longstanding challenge of diagnostic in developing countries.

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