

Development of multiplexing LAMP on lateral flow assay (LAMP-LFA) for rapid detection of respiratory viruses

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Summary:

Respiratory viruses such as influenza viruses and coronaviruses are among the most significant contributors to emerging infectious diseases due to the substantial threat they pose to public health. Diagnostics of respiratory viruses have become increasingly important. Here we report the development of lateral flow assay for rapid detection of respiratory viruses based loop-mediated isothermal amplification (LFA-LAMP). This assay enables rapid, sensitive, and user-friendly detection without the need for the complex laboratory infrastructure. The proposed method has potential applications in point-of-care testing and large-scale screening, particularly in resource-limited settings.

Keywords: Point-of-care (POC), LAMP-LFA, respiratory viruses, Lateral flow assay (LFA), Loop-Mediated isothermal amplification (LAMP).

Background

Respiratory viral infection remains a persistent challenge to global health systems, causing seasonal epidemics and occasional pandemics [1,2]. Influenza viruses and coronaviruses have demonstrated their capacity to spread rapidly across populations [3,4]. Their high mutation rates and zoonotic potentials contribute to the continuous emergence of new variants. Effective surveillance and early detection are critical to limiting transmission and reducing disease impact [7]. However, standard method techniques such as polymerase chain reaction (PCR) are often time-consuming and dependent on centralized laboratory facilities [8]. This creates a gap in timely diagnosis, especially in remote or low-resource settings [9]. As a result, there is increasing in developing rapid and portable diagnostic tools [10]. Isothermal amplification methods, such as loop-mediated isothermal amplification (LAMP), offer a promising alternative due to their speed, simplicity and sensitivity [11]. Coupling LAMP with lateral flow assay enables easy interpretation of results without specialized equipment [12]. These advances highlight the importance of developing innovative diagnostic strategies for improved management of respiratory viral infections.

Principles of LAMP-LFA

The principles of LAMP-LFA include 2 main steps. In the first step, multiplexing LAMP is carried out to produce products labeled with biotin, which serve as detectable targets in the second step using LAMP-LFA strip. To enable this, an additional oligo is attached to the 5' end of loop

primers (Figure 1). Furthermore, a complementary sequence to this added oligo is modified with biotin at 5' end.

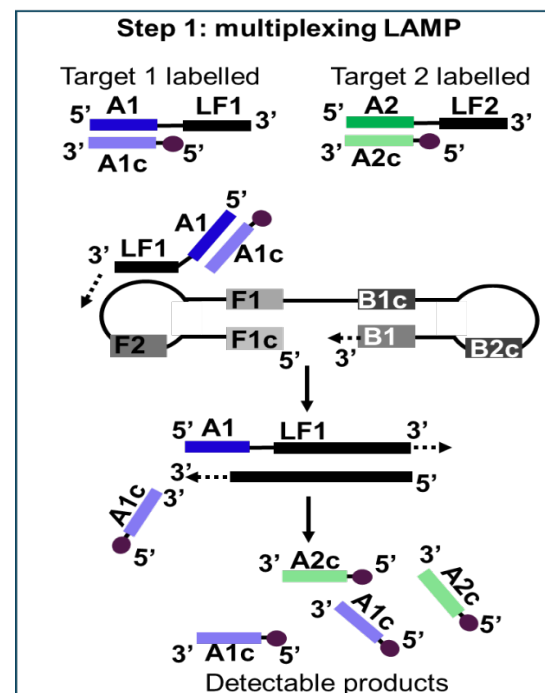


Figure 1. Multiplexing LAMP to produce detectable products.

During LAMP amplification, this biotin-labeled oligo is released. The released complementary oligo then binds to the nitrocellulose paper membrane. Subsequently, a streptavidin-gold nanoparticle complex (SA-GNP) attaches to the biotin on the membrane, generating a detectable signal (Figure 2).

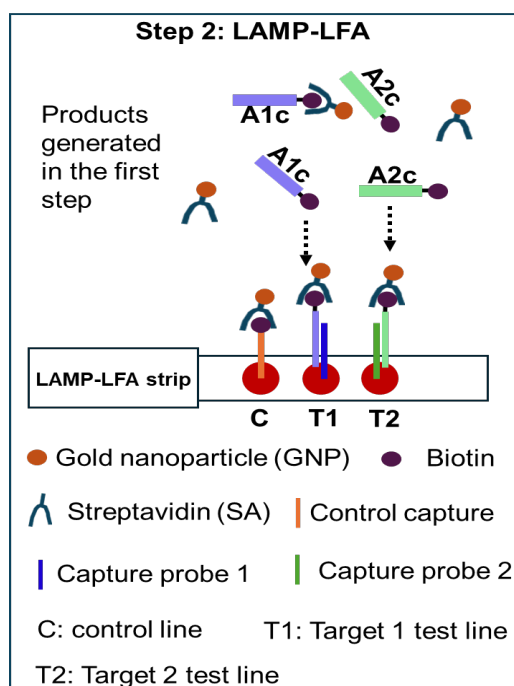


Figure 2. LAMP-LFA principle.

Results

The multiplexing LAMP assay was successfully developed to detect three targets SARS-CoV-2, influenza B, and an internal control (enhance green fluorescent protein, eGFP) within 20 min.

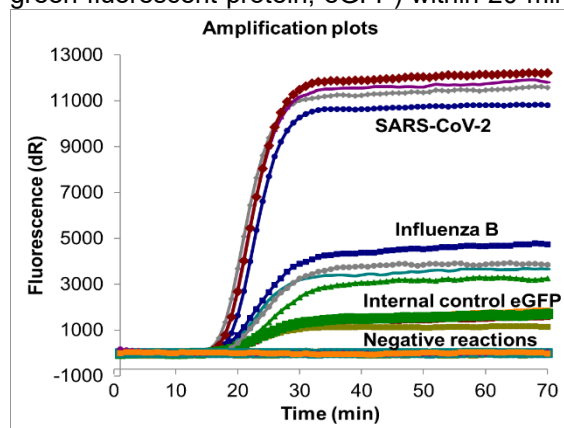


Figure 3. Multiplexing LAMP assay for detection of three targets.

Detection was achieved using assimilating probes, with a fluorescent label attached to the 5' end of the additional oligo on the loop primers and a quencher incorporated into its complementary sequence, enabling efficient signal generation. The results demonstrate the robustness of the amplification assay and its compatibility with downstream detection strategies. Furthermore, successful incorporation of biotin-labeled products indicates that the assay can be efficiently adapted to a LFA format, where labeled products can be captured on the strip and visualized via SA-GNP detection, supporting its potential for rapid POC diagnosis (Figure 4).

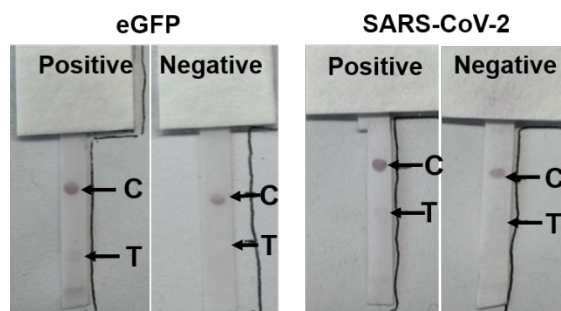


Figure 4. Primary LAMP-LFA results.

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