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ORIGINAL RESEARCH

Integration of Portable PCR and a Lateral Flow Assay for the Rapid Detection of HPV Type 16

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Background: Human papillomavirus (HPV) is a leading cause of cervical cancer worldwide, with HPV type 16 accounting for roughly half of cases. Although polymerase chain reaction (PCR) is considered the gold standard for HPV detection, its reliance on gel electrophoresis can be costly and require specialized facilities.

Objective: We developed a diagnostic approach integrating PCR with a lateral flow assay to detect HPV type 16 using gold nanoparticles as visible labels.

Methods: Primers targeting the L1 gene of HPV 16 were labeled with 6FAM (forward) and biotin (reverse). Amplified products were applied to lateral flow strips preloaded with streptavidin–gold nanoparticle conjugates. Visible red bands on the test and control lines indicated successful detection.

Results: The optimized assay produced a clear band at ~333 bp by gel electrophoresis and yielded distinct red lines on the lateral flow strip for positive samples. Compared to electrophoresis, this format has a faster turnaround and can reduce costs by eliminating bulky equipment.

Conclusion: This simplified and cost-effective method provides a user-friendly alternative to traditional electrophoresis, making it suitable for resource-limited settings. Further large-scale clinical validation is warranted, including cost analyses and multiplexing for additional genotypes.

Keywords: HPV type 16, PCR, lateral flow assay, cervical cancer diagnostics, gold nanoparticles

Introduction

Cervical cancer remains one of the leading causes of cancer-related deaths among women worldwide. Persistent infection with high-risk human papillomavirus (HPV), particularly types 16 and 18, is the primary etiological factor for cervical cancer. Globally, HPV type 16 alone accounts for approximately 50% of cervical cancer cases, while types 16 and 18 together contribute to over 70% of the disease burden. Despite the availability of effective vaccines and screening programs, the disease remains a significant public health challenge, especially in resource-limited settings where access to diagnostics is limited.[1]

HPV is a double-stranded DNA virus that infects epithelial tissues and is primarily transmitted through sexual contact.[2] To date, over 400 HPV types have been identified and classified into five major genera: Alpha, Beta, Gamma, Mu, and Nu. Among these, the Alpha genus is responsible for mucosal infections and is strongly associated with anogenital cancers, including cervical cancer.[3] High-risk HPV (hrHPV) types, particularly HPV 16, are the most carcinogenic and are associated with the majority of HPV-related malignancies.[2]

The L1 gene, encoding the major capsid protein, is an ideal target for molecular diagnostics due to its high conservation across HPV types and its established role in the viral life cycle.[4] While the gene is highly conserved, it also contains regions of variability that allow for the differentiation of individual HPV types, including high-risk and

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low-risk variants. These properties make the L1 gene a robust and reliable target for PCR-based detection and genotyping assays.[5,6] Additionally, its universal presence across all HPV types ensures consistent amplification during PCR, while its conserved nature enables the development of primers with broad-spectrum compatibility. The L1 gene's relevance to viral assembly and infection further underscores its utility in diagnostics, particularly for identifying persistent HPV infections that may progress to cervical cancer.[7]

Polymerase chain reaction (PCR) is widely regarded as the gold standard for HPV detection due to its high sensitivity and specificity.[8] However, the reliance on gel electrophoresis for amplicon visualization presents a barrier in low-resource settings where access to specialized equipment and trained personnel is limited.[9,10] To address these challenges, we developed a diagnostic system that integrates PCR amplification with a lateral flow assay (LFA) to visualize HPV type 16 amplicons. The primers targeting the L1 gene were labeled with 6FAM and biotin to enable compatibility with the LFA.[11,12] The LFA system incorporates an optimized streptavidin-gold nanoparticle (AuNP) conjugate as the detection label and utilizes anti-6FAM antibodies and biotinylated L-protein immobilized on the test and control lines, respectively. By eliminating the need for electrophoresis, this system provides a rapid, user-friendly, and cost-effective diagnostic alternative.

Here, we describe the design and development of this integrated approach, using primers labeled with 6FAM and biotin for compatibility with an in-house LFA strip loaded with streptavidin–gold nanoparticle conjugates. We also discuss its potential scalability as a cost-effective diagnostic tool for cervical cancer screening.

Materials and Methods

Primer Design

Primers were designed to target the highly conserved L1 gene region of HPV type 16. The sequences were adapted from the study by Safitri et al[13] demonstrating their specificity and utility for detecting HPV types 16 and 18. Primer design was conducted using the NCBI (National Center for Biotechnology Information) Primer-BLAST tool and validated for specificity using BLAST to ensure no off-target amplification. The selection criteria included a melting temperature (Tm) of 58–60°C, GC content between 40–60%, and minimal self-complementarity to avoid primer-dimer formation. The forward primer was labeled with 6FAM (GenScript, Singapore) at the 5′ end, and the reverse primer was labeled with biotin (GenScript, Singapore) at the 5′ end to enable compatibility with the lateral flow assay (Table 1). These primers were optimized during subsequent PCR validation.

DNA Template

The synthetic gene containing the target L1 gene sequence was purchased in plasmid form (GenScript, Singapore). This plasmid DNA was used as the template for PCR amplification. The concentration and purity of the plasmid were verified using a UV-Vis micro-spectrophotometer (Nano-400A, Allsheng, China).

PCR Amplification

PCR amplification was performed using the designed primers to amplify the target L1 gene region of HPV type 16. The reaction was prepared in a total volume of 25 µL, consisting of 12.5 µL of GoTaq® Green Master Mix (Promega, USA), forward and reverse primers with a final concentration of 0.32 µM, 20 and 40 ng of plasmid DNA template, and nuclease-free water (Servicebio, China) to complete the volume. Thermal cycling conditions included an initial denaturation at 95°C for 60 seconds, followed by 30 cycles of denaturation at 95°C for 15 seconds, annealing at 59°C for 15 seconds, and extension at 72°C for 15 seconds, with a final extension at 72°C for 60 seconds. Reactions were performed using a miniPCR thermal cycler (miniPCR, USA).

Table 1 Primers Used in This Study for the HPV 16 L1 Gene Amplification

Primer Name	Sequence (5′ → 3′)	Label	Length (bp)	GC Content (%)	Tm (°C)
Forward Primer	TTTGGGCCTGTGTAGGTGTT	6FAM	20	50	59.5
Reverse Primer	AGTCCATAGCACCAAAGCCA	Biotin	20	50	59.3

Gel Electrophoresis

The PCR products were analyzed using 1.7% agarose gel electrophoresis prepared in a TAE buffer. The gels were run at 80 V for 45 minutes, and the DNA bands were visualized under UV light after staining with a safe DNA dye. A 100-bp DNA ladder was used to confirm the size of the amplified product (~333 bp). Positive and negative controls were included in each run to ensure the reliability of the results.

Conjugate Synthesis and Optimization

The streptavidin-gold nanoparticle (AuNP) conjugate was synthesized and optimized for use in the LFA. A 1 mL suspension of AuNPs (Nanoflow, Belgium) was mixed with 100 µL of streptavidin solution prepared in 10 mM Trizma buffer pH 9 (Promega, USA) to get a final concentration of 60 ug/mL. The mixture was incubated with vortexing at 600 rpm for 30 minutes. Bovine serum albumin (BSA) (Sigma-Aldrich, USA) was then added to the mixture at a final concentration of 0.02% to stabilize the conjugate, and the solution was incubated again under the same conditions. The conjugate was centrifuged using a High-Speed Refrigerated Microcentrifuge (DLAB, China) at 10,000 rpm for 20 minutes to remove unbound components, and the resulting pellet was resuspended in the running buffer for use in LFA testing. The optimized conjugate was tested on half-strip prototypes, producing clear red bands on both the test (T) and control (C) lines, confirming successful synthesis.

LFA Assembly

The LFA strips were assembled using standard conditions. Nitrocellulose membranes were selected for optimal wicking properties, while conjugate and wicking pads were chosen for compatibility with the nitrocellulose membrane. The test (T) line was coated with anti-6FAM antibodies (Medix Biochemica, Finland), and the control (C) line was coated with biotinylated L-protein (GenScript, Singapore). To ensure uniform and precise application, both lines were applied to the membrane using a spray machine XYZ Dispenser HPY001 (Werfen, China). The components, including the nitrocellulose membrane, conjugate pad preloaded with AuNP-streptavidin conjugate, and wicking pad, were layered onto a backing card with slight overlaps to maintain smooth flow. The assembled sheets were cut into 4-mm strips using a precision cutter and stored in a desiccated environment until use. A schematic of the LFA system is shown in Figure 1.

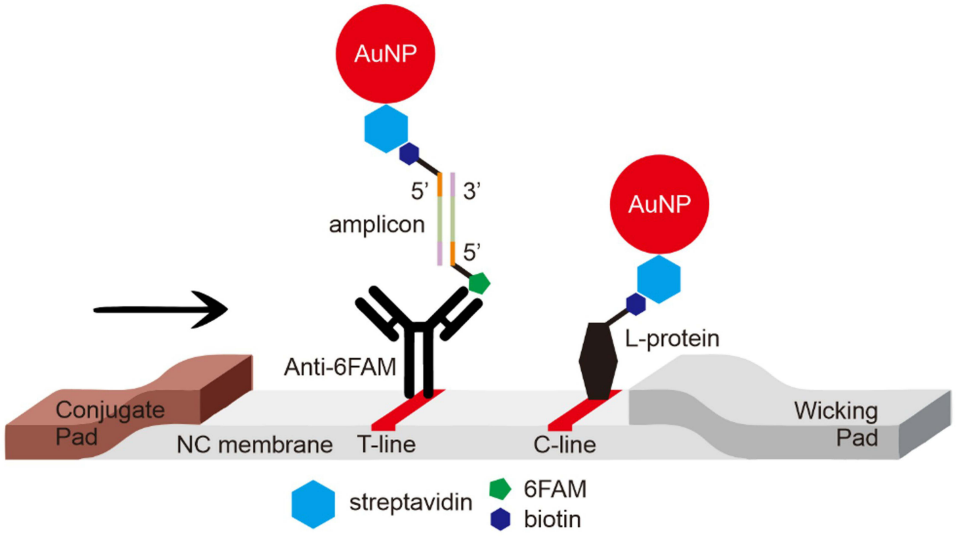

Figure 1 Schematic representation of the lateral flow assay (LFA) for visual detection of PCR amplicons. The conjugate pad contains streptavidin-AuNP conjugate, which binds to the biotin-labeled amplicon. The test line (T-line) is immobilized with anti-6FAM antibodies to detect 6FAM-labeled amplicons, and the control line (C-line) is immobilized with biotinylated L-protein to confirm proper flow and assay functionality.

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Amplicon Detection Using LFA

To detect PCR amplicons, the amplified products were mixed with a running buffer containing phosphate buffer and Tween-20 and applied to the conjugate pad of the LFA strip. The strips were allowed to run, and the results were visually interpreted. A positive result was indicated by the appearance of two red bands (T-line and C-line), while a single red band on the C-line indicated a negative result. The clarity and intensity of the bands confirmed the functionality and effectiveness of the LFA.

Results and Discussion

PCR Amplification and Gel Electrophoresis Analysis

PCR amplification was performed to evaluate the specificity and efficiency of the designed primers. Optimized PCR conditions included a final reaction volume of 25 µL with a commercial PCR master mix, labeled primers (6FAM and biotin), and synthetic plasmid DNA at two concentrations (20 ng/µL and 40 ng/µL). The thermocycling protocol included an annealing temperature of 59°C, as predicted from primer design calculations, and a final extension step to ensure complete amplification.

The gel electrophoresis results confirmed successful amplification of the target L1 gene region, with a product size of 333 bp (Figure 2). Bands corresponding to the amplicons were observed in lanes with 20 ng/µL and 40 ng/µL DNA templates. In Lane 2 (20 ng/µL) and Lane 3 (40 ng/µL), clear and specific DNA bands corresponding to the expected product size of 333 bp were observed. The band intensity in Lane 3 (40 ng/µL DNA input) was notably stronger than that in Lane 2 (20 ng/µL DNA input), indicating that higher DNA input improves amplification efficiency. Lane 4, containing

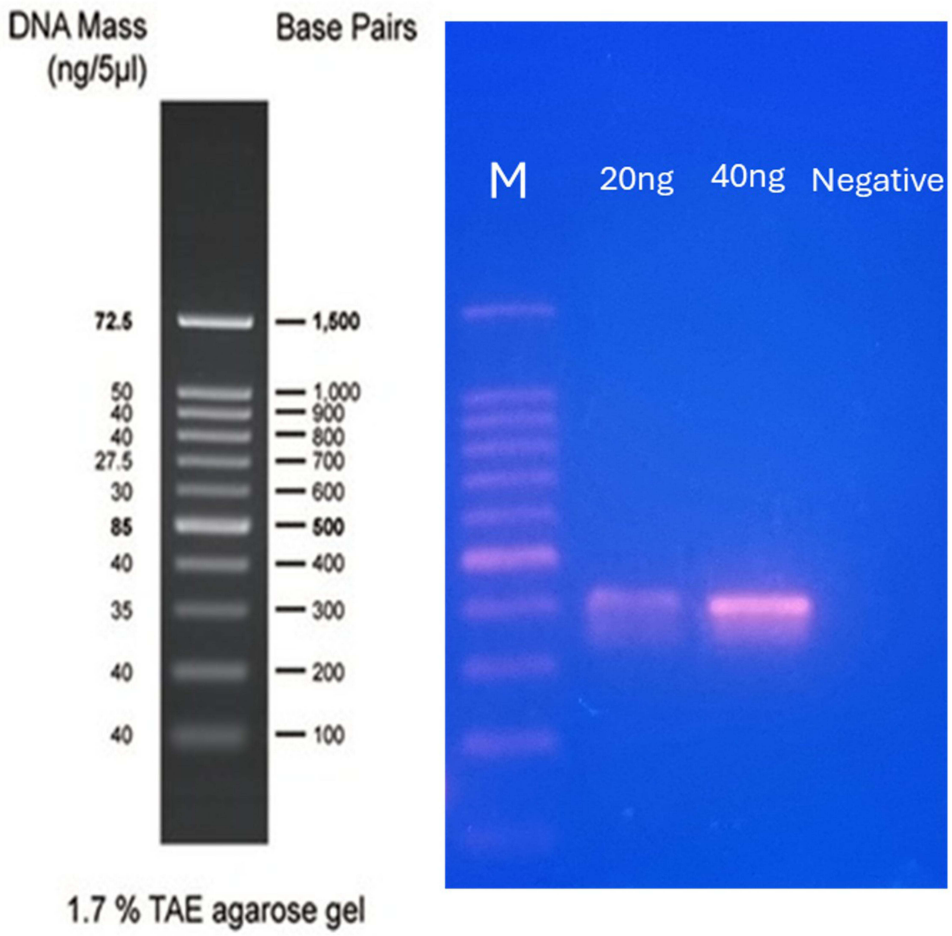

Figure 2 Gel electrophoresis results of PCR amplification of the L1 gene from HPV type 16. Lane M: 100-bp DNA marker; Lane 2: 20 ng/µL DNA template; Lane 3: 40 ng/µL DNA template; Lane 4: negative control. Amplified products are visible at 333 bp, with higher band intensity for 40 ng/µL input DNA.

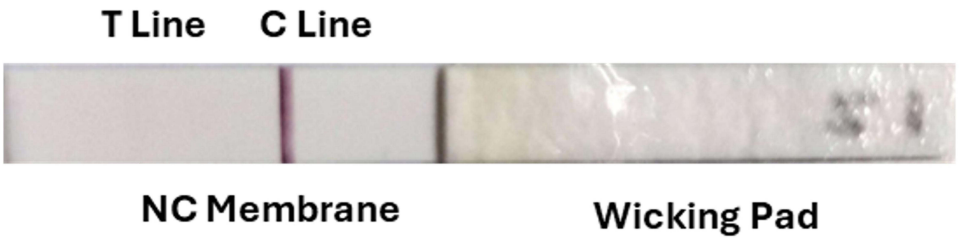

Figure 3 Half-Strip evaluation of streptavidin-AuNP conjugate synthesis and optimization.

the negative control, showed no visible bands, confirming the absence of contamination or nonspecific amplification. This result validated both the specificity of the designed primers and the optimized PCR conditions.

Conjugate Synthesis and Optimization

To enable the detection of PCR amplicons, streptavidin-gold nanoparticle (AuNP) conjugates were synthesized and optimized. The quality and functionality of the conjugates were evaluated using LFA half-strips. Clear and distinct red bands were observed at the C-line containing biotinylated L-protein, confirming successful streptavidin-AuNP conjugate synthesis (Figure 3).

Amplicon Detection Using the LFA System

To simplify PCR result visualization and eliminate the need for gel electrophoresis, an in-house LFA system was developed and tested. The LFA utilized the optimized AuNP-streptavidin conjugate as a detection label. PCR amplicons were mixed with running buffer, and the mixture was applied to the LFA strip. For positive samples, two red bands appeared at the T-line and C-line, indicating the presence of the target amplicon. Negative samples showed only the C-line, confirming the absence of nonspecific binding and validating the specificity of the assay (Figure 4).

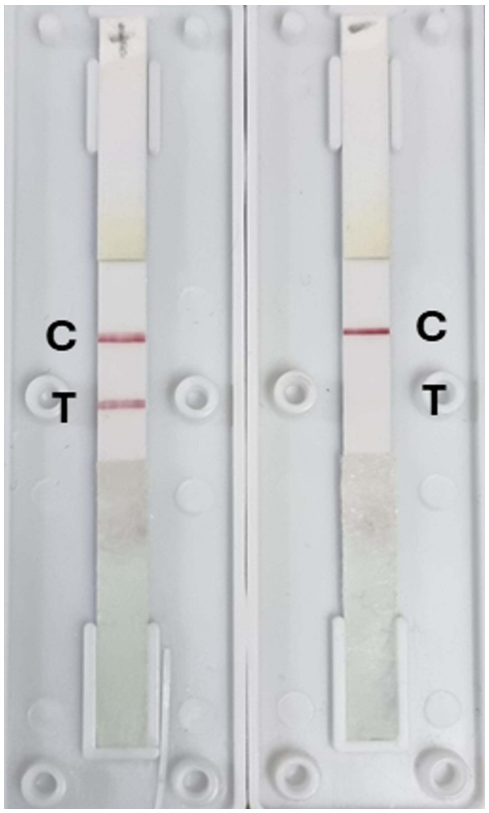

Figure 4 Results of the in-house LFA system for detecting PCR amplicons. Positive samples show two red bands at the test (T) and control (C) lines, while negative samples show only the control line. Red bands are produced by the AuNP label.

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Table 2 Comparison of Traditional Gel Electrophoresis vs PCR–LFA Visualization

No.	Feature	Gel Electrophoresis	PCR–LFA Readout
1.	Equipment Requirement	Gel tank, power supply, UV imager	Minimal (portable PCR + strips)
2.	Processing Time	≥1 hour	~15–20 min
3.	Interpretation	Requires imaging system, trained personnel	Direct visual read (bands on strip)
4.	Cost per Test	Moderate to high (equipment + reagents)	Lower ongoing costs; simple materials
5.	Suitability in Low-Resource Settings	Limited	Highly suitable

Note: This table summarizes key technical and logistical differences between conventional gel electrophoresis and lateral flow–based PCR readouts. Data compiled from previously published studies describing electrophoresis setup, processing time, equipment needs, and suitability for low-resource applications, not directly adapted or reproduced from any previously published figure or table. Data from these studies.[9,10,14–16]

The LFA results were consistent with gel electrophoresis findings, demonstrating reliable detection of the L1 gene amplicons. The red bands produced by the AuNP label were highly visible, making the results easy to interpret visually. Compared to gel electrophoresis, the LFA system provided significant advantages, including shorter processing times, simplicity, and accessibility, as reviewed in Table 2. The lateral flow strip offers faster processing and interpretable results without bulky instrumentation in resource-limited settings. The approximate reagent-based cost per test is also lower once strips are produced in bulk, making it attractive for low-throughput or field diagnostics.

This study demonstrates the feasibility of integrating polymerase chain reaction (PCR) and a lateral flow assay (LFA) for the rapid and accessible detection of HPV type 16. The optimized system eliminates the reliance on gel electrophoresis for PCR result visualization, replacing it with a simple, user-friendly LFA. This approach provides significant advantages in resource-limited settings,[14] where access to advanced diagnostic tools and trained personnel is often restricted.

A key feature of this study is the use of a portable and affordable thermal cycler, which offers several benefits over traditional laboratory-grade PCR machines. The compact design of the thermal cycler makes it highly suitable for decentralized diagnostic workflows, allowing it to be used in smaller laboratories or field settings. Despite its simplicity, the thermal cycler performed reliably, providing consistent amplification results that were successfully validated through gel electrophoresis and the in-house LFA system.

The integration of this portable thermal cycler with the in-house LFA further streamlines the diagnostic workflow. The LFA provides a rapid and visually interpretable method for detecting PCR amplicons labeled with 6FAM and biotin. By combining the accessibility of a portable thermal cycler with the simplicity of the LFA, this system addresses key barriers in molecular diagnostics, particularly for HPV screening. This approach is highly scalable and adaptable for other molecular targets, offering a pathway for accessible diagnostics in both clinical and field settings.[15]

Recent evidence underscores the importance of prophylactic HPV vaccination in reducing the incidence of cervical lesions,[17] yet access to diagnostic tools remains crucial for populations already infected or outside vaccine coverage. Adequate management of HPV-related cervical dysplasia relies on timely screening, diagnosis, and follow-up.[18] Thus, simple and rapid tests such as our PCR–LFA system may complement existing prevention strategies, particularly where conventional laboratory infrastructure is lacking.

Future studies should focus on the clinical validation of this system in diverse populations, as well as exploring its multiplexing capabilities to detect multiple HPV genotypes simultaneously. The combined PCR-LFA platform has the potential to transform molecular diagnostics in underserved regions,[16] enabling early detection and improved management of cervical cancer and other diseases.

Conclusion

This study successfully developed and optimized an integrated diagnostic system combining polymerase chain reaction (PCR) and a lateral flow assay (LFA) to detect HPV type 16. The primers targeting the L1 gene were validated through specific amplification of the 333 bp target region, as confirmed by gel electrophoresis and LFA. Optimization of the streptavidin-gold nanoparticle (AuNP) conjugate ensured high signal intensity and clear test results. The LFA system

demonstrated excellent compatibility with PCR amplicons, providing accurate, rapid, and user-friendly detection compared to traditional gel electrophoresis.

While we acknowledge that PCR and LFA have been explored in other contexts, our adaptation for HPV detection provides a unique approach with significant potential for improving cervical cancer diagnostics. Nonetheless, further clinical validation in larger, more diverse populations is essential before widespread implementation. Ongoing work will explore multiplex detection and more detailed cost analyses to solidify its utility as a practical screening tool for cervical cancer prevention.

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Disclosure

The authors report no conflicts of interest in this work.

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