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## ORIGINAL ARTICLE

# Colorimetric Detection of 23 Human Papillomavirus Genotypes by Loop-Mediated Isothermal Amplification

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## SUMMARY

***Background:*** **Human papillomavirus (HPV) infection is linked to cervical cancer. With the technological development of molecular biology and epidemiology, detection and treatment of HPV has become an important mean to prevent cervical cancer.**
***Methods:*** **A simple, rapid, and sensitive colorimetric loop-mediated isothermal amplification (LAMP) method was established herein to detect 23 HPV genotypes. The sequences of the primers for the LAMP reaction were located in the L1 gene of the HPV genome. As it is a fluorescent dye, calcein was added before the reaction. The reaction was run under isothermal conditions at 65°C for 40 minutes. A positive reaction was indicated by a color change from yellow to fluorescent green. The fluorescence curve diagram represents the monitoring of real time quantitative instrument. 450 cervical swab samples from patients with single infections of 23 different HPV genotypes were examined to evaluate the specificity.**
***Results:*** **The results revealed no cross-reaction with other HPV genotypes. A serial dilution of a cloned plasmid containing 23 HPV L1 gene sequences was employed to evaluate the sensitivity. Different HPV subtypes have different detection capability. The sensitivity of different HPV subtypes tested by LAMP assay was in the range from 1.0 x10 to 4.0 x $10^3$ copies per reaction. The LAMP assay and the RDB (reverse dot blot) were compared for detecting and genotyping HPV among the 450 clinical samples. There were 385 (85.6%) and 375 (83.3%) HPV positive specimens detected by LAMP and RDB, respectively, as well as 306 (68.0%) and 296 (65.8%) for HR-HPV positive specimens. The agreement between the LAMP and RDB assays was 93.3% (κ = 0.75) for HPV positivity and 94.7% (κ = 0.88) for HR-HPV positivity.**
***Conclusions:*** **It was concluded that this colorimetric LAMP assay had potential application for the rapid screening of the HPV infection in resource-limited hospitals or rural clinics.**
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**KEY WORDS**

human papillomavirus (HPV), cervical carcinoma, loop-mediated isothermal amplification (LAMP), visualization

## INTRODUCTION

Human papillomavirus (HPV) infection is linked to cervical cancer [1-3]. Worldwide, carcinoma of the uterine cervix is a common cancer in women, being second on-

ly to breast cancer. Since the pioneer work by Harald zur Hausen in the 1970s that suggested a role for HPV in the development of cervical cancer, there have been a number of molecular, epidemiological, and clinical observational studies implicating HPV as an etiological agent in various anogenital cancers [4].

Genotyping, through comparison of viral sequences and comparison of genetic homology of viral genomes, has shown HPV to be remarkably heterogeneous, with the presence of over 100 genotypes fully sequenced and identified to date [5]. Biologically, HPV can be divided into "high-risk (HR)" and "low-risk (LR)" groups on the basis of their pathogenicity and cervical lesions [6-8]. The HR group (containing probable high risk) includes HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82, and 83, while the low-risk group includes types 6, 11, 42, 43, and 81 [8-10]. With the technological development of molecular biology and epidemiology, HPV DNA testing and treatment became an important method of preventing cervical cancer. As such, HPV genotyping is important in HPV treatment and vaccine research and helps epidemiological prevention and control strategies.

The routine cervical-screening procedures applied in hospital for genotyping of HPV need more time to get test results. These methods were operated in fully equipped laboratories with good infrastructure, reliable electrical supply, and with highly trained staff. It is not convenient for detecting objects quickly. Over the last decade, some molecular methods, particularly amplification technology, were developed in detection of the clinical samples [11]. Han Jian et al. utilized multiplex PCR technology to amplify and identify of 25 human papillomavirus types simultaneously [12]. However, this method might not be suitable in ordinary clinical settings in developing countries or for field use, because of sophisticated instrumentation, complicated assay procedures, and expensive reagents [13]. Therefore, an expanding variety of novel nucleic acid amplification technologies has been developed to specifically meet the challenges of performing diagnostics outside of well-equipped facilities in low resource settings. LAMP assay is an excellent diagnostic tool due to its simplicity, cost-effectiveness, high efficiency, and specificity [14]. Under isothermal conditions, the LAMP reaction can be completed using a water bath or heating block, which are readily available in common clinical laboratories. This method needs a four-primer set, designed to recognize six distinct regions on the target gene, and uses enzyme *Bst* polymerase which has strand displacement activity. In addition, loop primers can be added to the reaction, which is designed based on the four primer set, to enhance efficiency and increase specificity of the assay [15]. The use of calcein dye is carried out in our laboratory, which made it convenient for researchers to observe the result directly with the naked eye. The samples that tested positive by the LAMP assay would make the tube green and negative samples would be yellow [16]. This improvement can reduce the risk of pollution generated in the amplification process and eliminate the need to open the tubes after reactions. In this study, a simple and visual type-specific LAMP assay for detection of 23 commonly encountered HPV genotypes was described, including 16 HR-HPV types (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82, and 83), 2 pHR-HPV types (types 53 and 66), and 5 LR-HPV types (types 6, 11, 42, 43, and 81). In order to achieve the optimal amplification effect, we adjusted the amount of each component in the reaction system. On that basis, the sensitivity and specificity of the experiment were carried out. Moreover, the assay was further evaluated with 450 clinical specimens that had been analyzed by a commercial HPV Genotyping Kit.

## MATERIALS AND METHODS

### Clinical specimens and DNA extraction

Between September 2013 and January 2015 clinical specimens were collected from 450 women (age from 18 to 45 years old) visiting the gynecological outpatient clinic of Wenzhou People's Hospital in China. These specimens were liquid-based with abnormal or normal cytology samples, which were collected as part of routine cervical-screening procedures. All specimens selected consecutively and were anonymized and randomized prior to testing. Total cellular DNA was extracted by a commercial kit (Viral genomic DNA Extraction Kit, Bioteke, China) according to the manufacturer's instructions. After extraction, DNA was stored at -80°C until tested.

### The commercial HPV Genotyping Kit

The commercial HPV Genotyping Kit (PCR-RDB, YanengBio, China) is widely used at clinics in China for HPV diagnosis. There are 23 HPV types that the kit can detect; these are: HPV6, 11, 16, 18, 31, 33, 35, 39, 42, 43, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 81, 82, and 83. The method which is used in the kit is the reverse dot blot hybridization, and the detection limit of the kit is $10^3$ copies and the specificity can reach 98% according to the manufacturer's instructions. All 450 samples were first confirmed by the kit in Wenzhou MeiZhong Medical Laboratory and then evaluated by the LAMP assay.

### HPV sequence alignment and Genotype-specific primer designs

The L1 gene sequences of HPV genotypes 6, 11, 16, 18, 31, 33, 35, 39, 42, 43, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 81, 82, and 83 were obtained from GenBank in the NCBI database. Most established HPV typing assays used in epidemiologic studies were based on consensus PCR to amplify the relatively conserved L1 gene region with hybridization [17,18]. The widely used L1 consensus primer PCR systems include the $GP5^+/6^+$, PGMY09/11, and SPF systems [19-22]. The length of

the target fragment (Figure 1) in different HPV subtypes was different. Although it is different, all fragments were designed by the conserved L1 regions. After aligning by using Clustal X software, we found all fragments were located in the $GP5^+/6^+$ regions, which is the same target fragment on hybridization. According to the target fragment, the pairs of type-specific primers (Supplemental Table A1) were designed by using free software (Primer Premier 5.0). These primers were only used to produce the recombinant plasmids of 23 HPV types, and they were synthesized by Invitrogen Biotechnology Co., Ltd. Each primer was dissolved in deionized water and adjusted to 100 mM as a stock solution and stored at -20°C.

**Standard Plasmids**

To evaluate the analytical sensitivity and type specificity of the HPV assay, all standard plasmids were performed as positive controls of the LAMP assay [23,24]. These primers are listed in Supplemental Table A1 and the clinical samples were used to construct the standard plasmids. The steps of the construction of the standard plasmid are as follows:

First, each pair of genotype-specific primers was diluted to 10 mM and added into the PCR system (Promega, Shanghai, China). The thermal cycle program was as follows: 95°C for 2 minutes, followed by 32 cycles of 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds, and a final extension step of 72°C for 5 minutes. The products were then detected by electrophoresis and gel purified using the MiniElute Gel Extraction Kit (Genebase Bioscience, GuangZhou, China). Finally, every target fragment of each HPV genotype was ligated into the pMD19-T simple vector (TaKaRa) by using DNA Ligation Kit Ver.2.1 (TaKaRa), and then transformed into *Escherichia coli* DH5a using standard procedure. The recombinant plasmids were confirmed by cloning PCR, as well as by sequencing. The positive recombinant plasmids were extracted as the standard plasmids for the test. Extracts were stored at -20°C until further assessment.

**Primer designs for LAMP**

The type-specific primers for LAMP amplification of HPV DNA were based on the HPV sequences information obtained from GenBank [accession numbers: HPV6 (AF092932), HPV11 (M14119), HPV16 (K02718), HPV18 (AY262282), HPV31 (J04353), HPV33 (M12732), HPV35 (M74117), HPV39 (M62849), HPV42 (M73236), HPV43 (AJ620205), HPV45 (X74479), HPV51 (M62877), HPV52 (X74481), HPV53 (X74482), HPV56 (X74483), HPV58 (D90400), HPV59 (X77858), HPV66 (U31749), HPV68 (X67161), HPV73 (X94165), HPV81 (AJ620209), HPV82 (AF293961) and HPV83 (AF151983)]. The primers of the plasmids' construction and the LAMP primers both spanned the $GP5^+/6^+$ regions, which indicated the detection aimed at the same sequence. The Primer Explorer Version 3 was a primer designing software specifically for LAMP (http://primerexplorer.jp/e/), which was produced by Eiken Chemical (Tokyo, Japan). In the design process, the BLAST software program was used to avoid the probability of cross-reactivity with heterologous HPV virus. The LAMP primer set listed in Supplemental Table A2 contained two outer primers (F3 and B3), two inner primers (FIP and BIP), and loop primer (LF or LB). The number of loop primers might be one or two. All oligonucleotide primers were synthesized by Invitrogen Biotechnology Co., Ltd.

**Visualization and optimization of LAMP conditions**

Calcein, a metal indicator that yields strong fluorescence by forming complexes with divalent metallic ions, such as calcium and magnesium, is used for various analyses. Optimization of LAMP conditions was assessed via amplifying $10^8$ copies of the positive plasmid and the amplification reaction was finally carried out in a 25 μL volume. Plasmid clones for each of the HPV genotypes were added to the amplification reagent containing LAMP primers for the corresponding HPV type together with 20 mM Tris-HCl (pH 8.8), 10 mM KCl, 8 mM $MgSO_4$, 10 mM $(NH_4)_2SO_4$, 10% Tween20 (Sigma-Aldrich), 0.4 M Betaine, 0.2 mM Calcein, 0.5 mM $MnCl_2$, 1.4 mM dNTPs (Thermo Scientific, USA), and *Bst* DNA polymerase. On the basis of the same amplification efficiency, we reduced the added amount of enzyme to make the method cheaper. According to the different amount of enzyme, the HPV types could be grouped into four sets. Set A contains 2U of *Bst* DNA polymerase for HPV16; set B contains 4U of *Bst* DNA polymerase for HPV11, HPV31, HPV33, HPV51, HPV52, HPV53, HPV58, HPV59, HPV66, HPV73 and HPV83; set C contains 6U of *Bst* DNA polymerase for HPV39 and HPV56; and set D contains 8U of *Bst* DNA polymerase for HPV6, HPV18, HPV35, HPV42, HPV43, HPV45, HPV68, HPV81 and HPV82. All reactions were conducted at 65°C for 60 minutes and there was an obvious color change in positive tubes (Figure 2). In order to facilitate the collection and collation of data for the sensitivity and specificity, the mixtures were reacted at 65°C in the instrument ($CFX96^{TM}$, BioRad, USA). If the sample is positive, the amplification curve of the LAMP reaction will appear in the instrument and a color change will occur at the same time.

**Sensitivity of type-specific LAMP assays**

In the sensitivity experiment, the template concentration was diluted by 10 times and the copy number was $10^8$ to $10^1$ copies. Every diluted plasmid was used as template to add into the reaction. In the specified time, the fluorescent signal can be obtained, which proves the copy number could be detected. We could get the minimum copy number from the curve, which represented the detection limit [25]. These tests were carried out in triplicate for each type. With the log starting quantity/copy number (from $10^8$ to $10^1$) as the abscissa, the amplification start time (AST) as the ordinate, a standard curve

was established for linear relationship analysis between reaction time and the template concentration.

**Specificity of type-specific LAMP assays**

In the specificity experiment, each genotype specific plasmid will be added into 23 different reaction tubes, and every tube has type-specificity primers for each HPV type. If the fluorescence signal detected was less than the negative control or there was no change in color, cross-reactivity was defined negative. In each specificity experiment, one clone plasmid was used as the amplification target with a set of uniplex primers.

**Evaluation of LAMP with clinical specimens**

We evaluated and compared the clinical performance of the LAMP and RDB assays for HPV genotyping. Data were analyzed by Cohen's kappa coefficient test for concordance for qualitative items and the Cochran Mantel Haenszel chi-square test for the association between two qualitative variables using the statistical package SPSS (version 19.0). P-values of $< 0.05$ were considered statistically significant [26].

## RESULTS

**Optimization of visual LAMP assay**

The amplification efficiency would be enhanced if we optimized the parameters in the reagents, such as the salt concentrations of magnesium ions and manganese ions, the concentration of betaine and calcein, and the ratio among outer primers and inner primers. The same conditions were repeated twice. The reaction was conducted with different concentrations of magnesium ions, ranging from 0 to 12 mM. According to the AST, the final concentration of $Mg^{2+}$ was determined as 8 mM (Figure 3a). Similarly, the concentrations of manganese ions ranged from 100 to 800 μM, the final was 500 μM (Figure 3b). The concentration of betaine ranged from 0 to 1.4 mM, and 0.4 mM was optimum (Figure 3c). Similarly, the concentration of calcein was set from 100 μM to 800 μM. To distinguish clearly by the naked eye, the final calcein concentration was 200 μM (Figure 3d). The inner primer of the LAMP reaction was mainly involved in the synthesis of the latter stem-loop structure, and the outer primers only participated in the reaction in the initial reaction. Therefore, it was better to fix the concentration of the outer primers, and the inner primer concentration was changed gradually. The ratios were set to 1:1, 1:2, 1:4, 1:8 and 1:16. The outer and inner primer ratio was finally identified as 1:8 (Figure 3e).

**Specificity and sensitivity of HPV type-specific LAMP**

Each HPV type-specific LAMP primer set spans a region within the $GP5^+/6^+$ regions. The optimal amount of *Bst* DNA polymerase for HPV18 was shown (Figure 4a). In the specificity experiment, a sample will be added into 23 different reaction tubes, and every tube has type-specificity primers for each HPV type. The primer sets for each HPV type amplified only the template of the corresponding type, and there were no cross-amplification products detected in the reactions carried out with the other 22 HPV types. As a case, the result of HPV18 type for the specificity was shown (Figure 4b). According to the change of color, it was easy to distinguish the positive samples from the negatives ones after the reactions (Figure 4c). The results (Table 1) showed the time (in minutes) until an increase of the fluorescence value caused by LAMP amplification was detected. Serial dilutions of the pMD19-T plasmids to cover the range of $10^8$ to $10^1$ copies were added to the corresponding tube as template to determine the detection limits of HPV type specific LAMP. The result of HPV18 type for the sensitivity was shown (Figure 4d). The amount of time needed to develop a visual fluorescence value unit in these experiments was inversely proportional to the amount of template, demonstrating that the signal is quantitatively proportional to the abundance of template [27]. The results of the standard curves indicated that there were significant correlations between the reaction time and the template concentration of each HPV type (each $R^2 > 0.90$) (Supplemental Table A3). The sensitivity of type-specific LAMP for HPV genotypes were respectively different, and could be divided into several groups: HPV6 and 68 were 4.0 x $10^3$ copies per reaction; HPV11, 31, 39, 45, 52, 58, 81, 82, and 83 were 1.0 x $10^3$ copies per reaction; HPV18, 43, 56, and 66 were 4.0 x $10^2$ copies per reaction; HPV35, 42, 51, and 53 were 1.0 x $10^2$ copies per reaction; HPV16, 33, 59, and 73 were 1.0 x $10^1$ copies per reaction.

**Evaluation of HPV type-specific LAMP with clinical specimens**

The RDB HPV genotyping test included PCR amplification of target DNA followed by hybridization using a reverse blot system for simultaneous detection of 23 HPV genotypes. The test was according to the manufacturer's instructions. A total of 450 cervical scrape samples, including samples positive for HPV6 (n = 3), HPV11 (n = 14), HPV16 (n = 28), HPV18 (n = 25), HPV31 (n = 18), HPV33 (n = 21), HPV35 (n = 9), HPV39 (n = 9), HPV42 (n = 17), HPV43 (n = 25), HPV45 (n = 5), HPV51 (n = 15), HPV52 (n = 60), HPV53 (n = 32), HPV56 (n = 13), HPV58 (n = 21), HPV59 (n = 8), HPV66 (n = 13), HPV68 (n = 9), HPV73 (n = 2), HPV81 (n = 20), HPV82 (n = 5), HPV83 (n = 3), and HPV-negative samples (n = 75), were detected by the RDB assay (Table 2).

Of the 450 specimens, 385 (85.6%) contained HPV genotypes by LAMP and 375 (83.3%) by RDB assays. There were 306 (68.0%) and 296 (65.8%) HR-HPV positive specimens detected by LAMP and RDB assays, respectively. Complete concordance for the presence or absence of HPV and HR-HPV genotypes by the two tests were 93.3% ($\kappa = 0.75$, 95% CI) and 94.7% ($\kappa = 0.88$, 95% CI), respectively (Table 3). In the indi-

**Table 1. Type-specific amplification by the LAMP primer sets in HPV assay.**

| | Plasmid clones of 23 types for HPV | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Primers** | **6** | **11** | **16** | **18** | **31** | **33** | **35** | **39** | **42** | **43** | **45** | **51** | **52** | **53** | **56** | **58** | **59** | **66** | **68** | **73** | **81** | **82** | **83** |
| **HPV6** | **30*** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **HPV11** | - | **30** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **HPV16** | - | - | **26** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **HPV18** | - | - | - | **20** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **HPV31** | - | - | - | - | **35** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **HPV33** | - | - | - | - | - | **35** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **HPV35** | - | - | - | - | - | - | **32** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **HPV39** | - | - | - | - | - | - | - | **30** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **HPV42** | - | - | - | - | - | - | - | - | **35** | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **HPV43** | - | - | - | - | - | - | - | - | - | **40** | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **HPV45** | - | - | - | - | - | - | - | - | - | - | **32** | - | - | - | - | - | - | - | - | - | - | - | - |
| **HPV51** | - | - | - | - | - | - | - | - | - | - | - | **30** | - | - | - | - | - | - | - | - | - | - | - |
| **HPV52** | - | - | - | - | - | - | - | - | - | - | - | - | **40** | - | - | - | - | - | - | - | - | - | - |
| **HPV53** | - | - | - | - | - | - | - | - | - | - | - | - | - | **30** | - | - | - | - | - | - | - | - | - |
| **HPV56** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | **45** | - | - | - | - | - | - | - | - |
| **HPV58** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | **35** | - | - | - | - | - | - | - |
| **HPV59** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | **25** | - | - | - | - | - | - |
| **HPV66** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | **25** | - | - | - | - | - |
| **HPV68** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | **55** | - | - | - | - |
| **HPV73** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | **40** | - | - | - |
| **HPV81** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | **35** | - | - |
| **HPV82** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | **35** | |
| **HPV83** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | **40** |

*** - Time (min) until the results can be observed and determined by Calcein dye-mediated visualization using the naked eye.**

vidual comparison of all 23 genotypes, no statistically significant differences were detected.

## DISCUSSION

In this study, a rapid, efficient, and sensitive colorimetric loop-mediated isothermal amplification with calcein is established to test 23 human papillomavirus genotypes. LAMP was performed at 65°C for 40 minutes. The amplification system was finally carried out in 25 μL volumes, containing every optimal parameter, such as the salt concentrations of magnesium ions and manganese ions, the concentration of betaine and calcein, the ratio among outer primers and inner primers, and the amount of enzyme.

The most attractive feature of the calcein dye-based LAMP assay is the visual observation of the reaction result, which reduces the risk of cross-contamination during amplification by omitting the uncapping steps [16]. Moreover, the LAMP assay just needs constant temperature equipment, such as an ordinary water bath or a metal bath, and does not require expensive instruments. The LAMP assay is more cost-effective than hybridization, because of the equipment and specific probes in RDB assay. We estimate a reagent cost of RDB about $20.00 per assay. However, using the LAMP method, we estimate a reagent cost of about $12.00 per person. The LAMP assay performed with high specificity, and the sensitivity is comparable to that of hybridization (according to the manuscript of the HPV Genotyping Kit). In the meanwhile, there is a linear correlation between the genome quantity and reaction time by the LAMP method, making quantitative HPV type-specific LAMP detection of HPV DNA possible in clinical samples [13,27]. Our research showed that there were significant correlations between the reaction time and the template concentration. Thus, we will do further quantitative research in the future.

We evaluated the clinical performance of the LAMP

**Table 2. Kappa values for individual HPV genotypes detectable by LAMP and RDB.**

| Oncogenic potential | Genotype | Numbers [a] of genotypes found positive by: | | | Kappa value (95% CI) |
|---|---|---|---|---|---|
| | | LAMP | RDB | LAMP and RDB | |
| High risk | HPV16 | 29 | 28 | 27 | 0.94 |
| | HPV18 | 26 | 25 | 25 | 0.98 |
| | HPV31 | 18 | 18 | 17 | 0.94 |
| | HPV33 | 22 | 21 | 21 | 0.97 |
| | HPV35 | 10 | 9 | 9 | 0.94 |
| | HPV39 | 9 | 9 | 9 | 1.00 |
| | HPV45 | 5 | 5 | 5 | 1.00 |
| | HPV51 | 15 | 15 | 14 | 0.93 |
| | HPV52 | 61 | 60 | 59 | 0.97 |
| | HPV56 | 14 | 13 | 12 | 0.88 |
| | HPV58 | 22 | 21 | 21 | 0.97 |
| | HPV59 | 8 | 8 | 7 | 0.87 |
| | HPV68 | 9 | 9 | 8 | 0.89 |
| | HPV73 | 2 | 2 | 2 | 1.00 |
| | HPV82 | 5 | 5 | 5 | 1.00 |
| | HPV83 | 3 | 3 | 3 | 1.00 |
| Probable high risk | HPV53 | 34 | 32 | 32 | 0.96 |
| | HPV66 | 14 | 13 | 13 | 0.92 |
| Low risk | HPV6 | 3 | 3 | 3 | 1.00 |
| | HPV11 | 14 | 14 | 13 | 0.92 |
| | HPV42 | 17 | 17 | 16 | 0.94 |
| | HPV43 | 25 | 25 | 24 | 0.96 |
| | HPV81 | 20 | 20 | 20 | 1.00 |

[a] - The result for 385 positive samples after analysis are shown, and all samples were single genotype.

**Table 3. Concordance between LAMP and RDB tests in specimens for detection of HPV and HR-HPV genotypes.**

| Test result | Numbers of samples (%) with RDB | | Total number of samples (%) | Absolute agreement (%) | Kappa value (95% CI) |
|---|---|---|---|---|---|
| | HPV(+) | HPV(-) | | | |
| LAMP result [a] | | | | | |
| HPV(+) | 365 | 20 | 385 (85.6) | 93.3 | 0.75 |
| HPV(-) | 10 | 55 | 65 (14.4) | | |
| Total | 375 (83.3) | 75 (16.7) | 450 | | |
| | Number of samples (%) with RDB | | | | |
| | HR-HPV(+) | HR-HPV(-) | | | |
| LAMP result [b] | | | | | |
| HR-HPV(+) | 289 | 17 | 306 (68.0) | 94.7 | 0.88 |
| HR-HPV(-) | 7 | 137 | 144 (32.0) | | |
| Total | 296 (65.8) | 154 (34.2) | 450 | | |

CI - confidence interval, HR-HPV - high-risk HPV. [a] - HPV(+) result refers to HPV detection, HPV(-) result reflects the absence of HPV. [b] - HR-HPV(+) result refers to HR HPV genotypes, HR-HPV(-) result reflects the absence of HR HPV genotypes. HR HPV is comprised of HR and probable HR HPV genotypes.

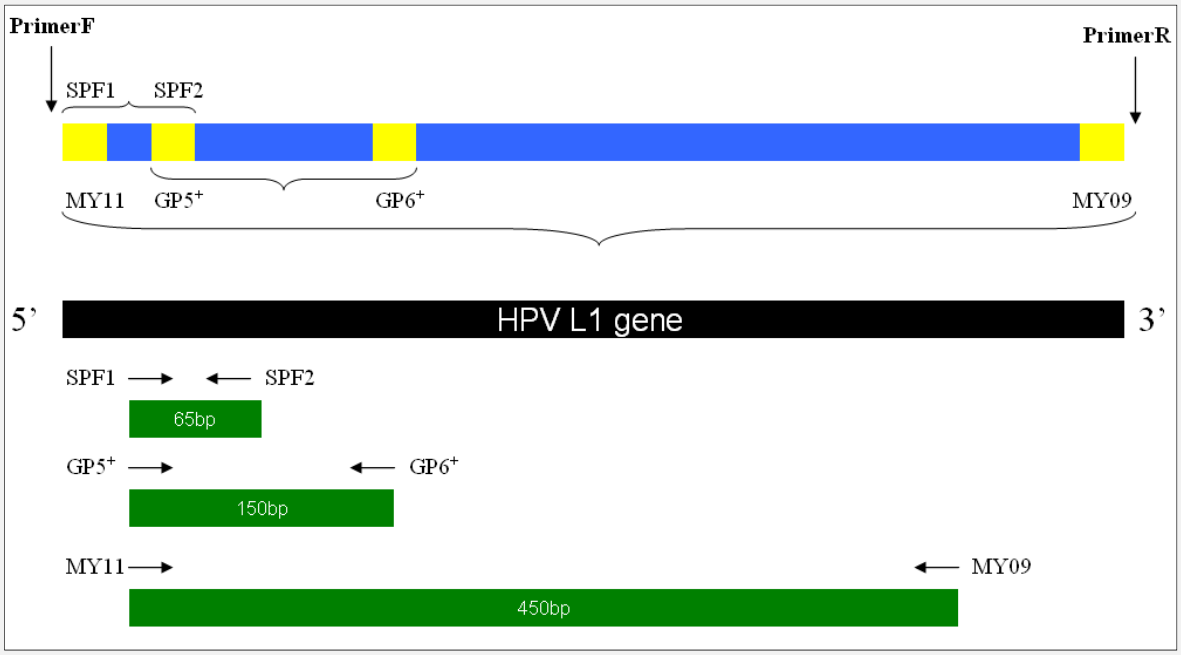


**Figure 1. The particular GP5$^+$/6$^+$ regions, containing several heterogenous sites, were selected as the target sequences for designing the primers.**

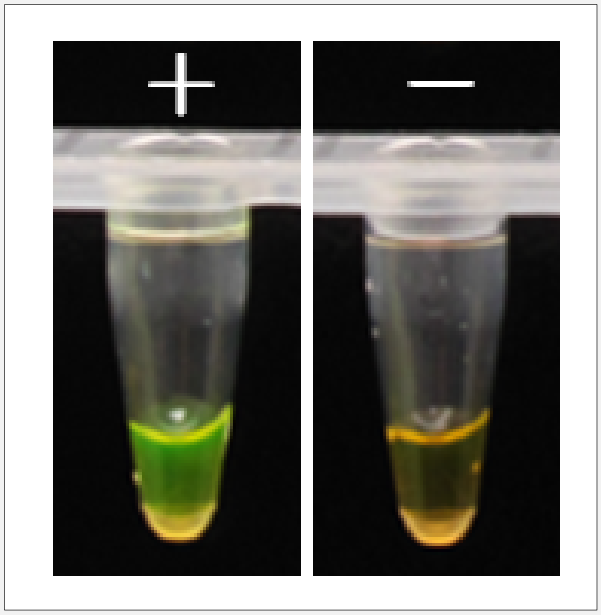


**Figure 2. Detection of the LAMP reaction using fluorescent metal indicator under daylight. Plus sign denotes positive reaction (with target DNA), minus sign denotes negative reaction (without target DNA).**

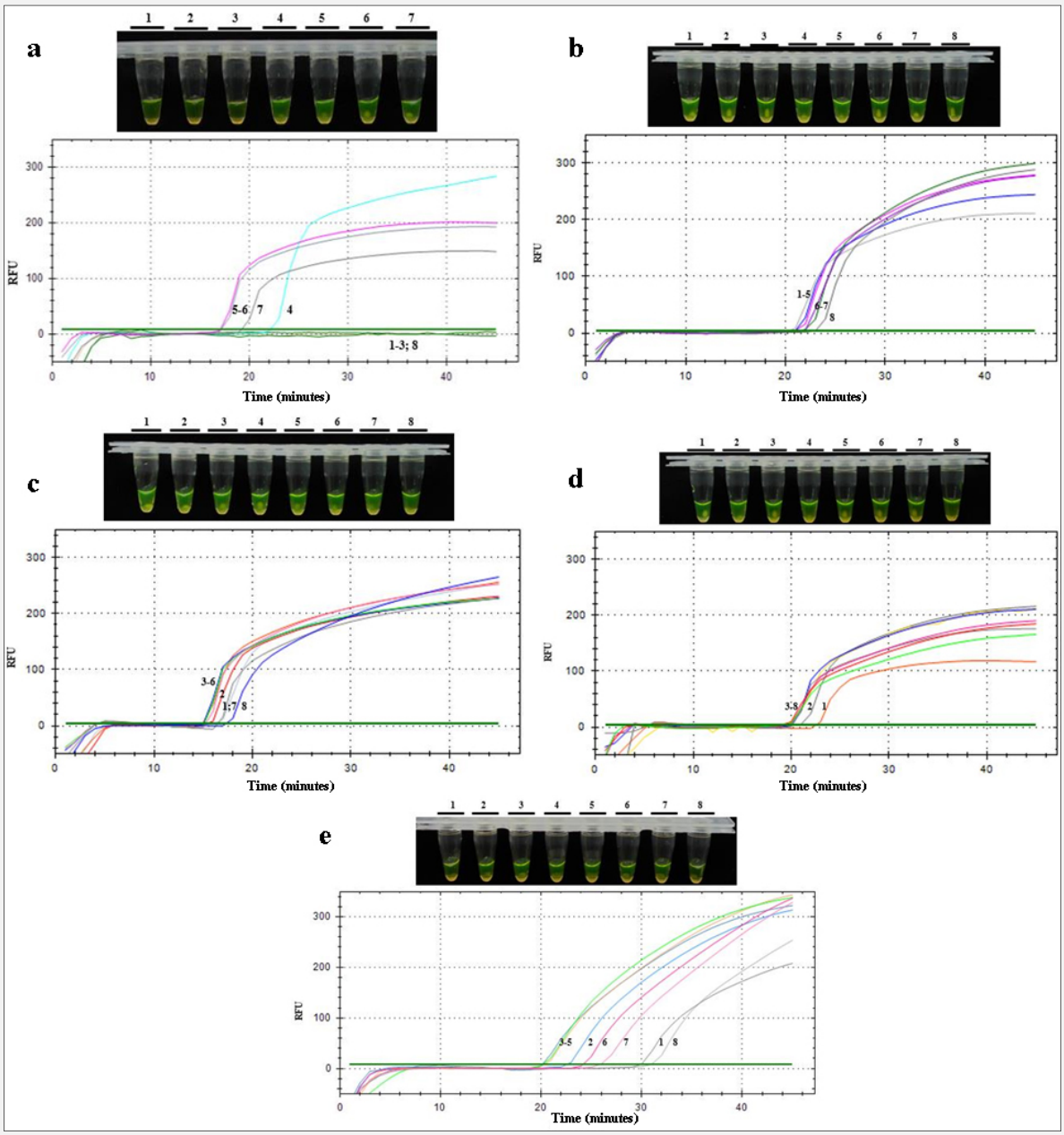


**Figure 3. Optimization of visual LAMP assay. (a) Optimization of Mg2+ concentration.**

**1: 0 mM; 2: 2 mM; 3: 4 mM; 4: 6 mM: 5: 8 mM; 6: 10 mM; 7: 12 mM. (b) Optimization of Mn2+ concentration. 1: 100 µM; 2: 200 µM; 3: 300 µM; 4: 400 µM; 5: 500 µM; 6: 600 µM; 7: 700 µM; 8: 800 µM. (c) Optimization of betaine concentration. 1: 0 M; 2: 0.2 M; 3: 0.4 M; 4: 0.6 M: 5: 0.8 M; 6: 1 M; 7: 1.2 M; 8: 1.4 M. (d) Optimization of calcein concentration. 1: 100 µM; 2: 200 µM; 3: 300 µM; 4: 400 µM; 5: 500 µM; 6: 600 µM; 7: 700 µM; 8: 800 µM. (e) Optimization of primer concentration ratio. The ratios of inner primer concentration and outer primer concentration. 1-8: 1:1, 1:2, 1:4, 1:6, 1:8, 1:10, 1:12, 1:16.**

HPV genotyping assay against that of the RDB test for the detection and genotyping of HPV using cytology specimens, thereby providing insight into their clinical utility. The RDB test is a PCR-based HPV genotyping test detecting HPV L1 regions from 23 different HPV types using DNA hybridization. Under isothermal conditions, the LAMP assay is able to detect amounts of oligonucleotides by using *Bst* polymerase directly,

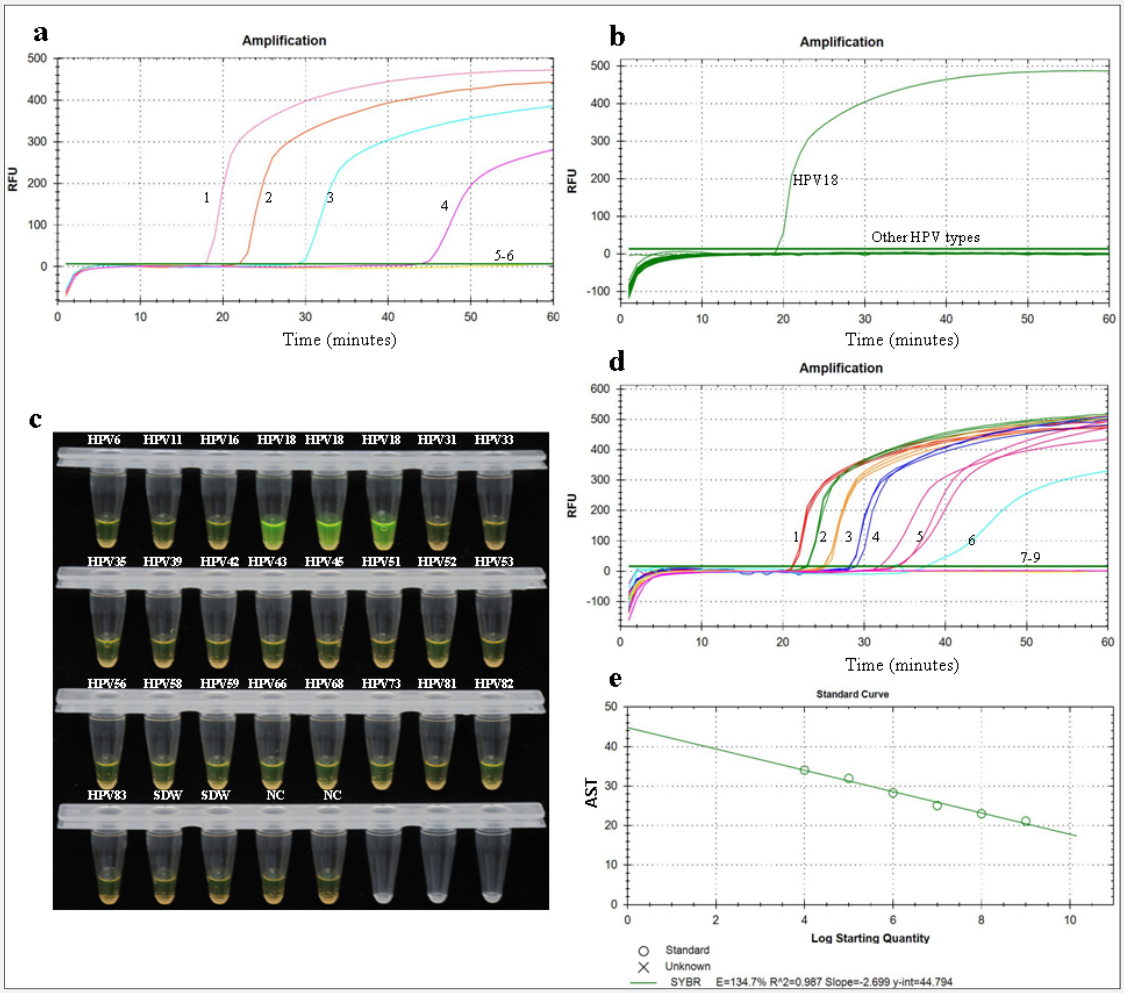


**Figure 4. The result of HPV 18 type for the LAMP assay.**

**(a) Different volume of *Bst* DNA polymerase added in LAMP assays. 1: 8U; 2: 6U; 3: 4U; 4: 2U; 5: 1U; 6: Negative control. (b) Specificity analysis of LAMP assays by the amplification curve. (c) Specificity analysis by the method of fluorescent dye. (d) Sensitivity analysis. 1: $10^8$ copies/μL; 2: $10^7$ copies/μL; 3: $10^6$ copies/μL; 4: 105 copies/μL; 5: 104 copies/μL; 6: 103 copies/μL; 7: 102 copies/μL; 8: 101 copies/μL; 9: Negative control. Type 18 was shown as the representative of the experimental results to display.**

which has strand displacement activity. Overall, the agreement between the two tests was considered moderate, with a Kappa value of 0.75. While 426 specimens (94.7%, κ = 0.88) generated concordant results for the presence or absence of high-risk HPV (HR-HPV) by the two assays. The agreement between the two assays for HR genotypes is good.

The LAMP assay positively identified 10 HPV samples which were not detected by RDB assay (Table 3). In the detection step, the LAMP assay depends upon the amount of the amplification products. The RDB method uses hybridization, which may be limited by the different binding affinities of the HPV genotype products to the probes [26].

## CONCLUSION

The present study demonstrated that HR-HPV genotyping results obtained by the LAMP assay are highly comparable to those obtained by the RDB assay in examining clinical specimens.

The present study is a new test that can potentially be applied to HPV genotypes. The subsequent experiment-

ation of the LAMP test needs significantly more clinical data to verify the feasibility to promote the technique. If the method works as expected, it might be a diagnostic tool for women's health. Accordingly, the LAMP assay may be suitable for the rapid screening of 23 HPV types in resource-limited hospitals or rural clinics in China.

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**Author Contributions:**
Mingzhou Zhang and Jiehong Fang conceived and designed the experiments;
Junxiao Lin, Biao Ma, and Wei Lin performed the LAMP experiments;
Ye Wang, Haizhen He, and Wei Su performed the RDB experiments;
Junxiao Lin, Biao Ma, and Jiehong Fang analyzed the data;
Junxiao Lin, Biao Ma, Jiehong Fang, and Mingzhou Zhang wrote the paper;
Mingzhou Zhang supervised the work.
All authors have approved the present article.

**Ethical Approval:**
The ethical committee of Wenzhou People's Hospital approved the study.

**Declaration of Interest:**
All authors declare that they have no conflicting or dual interests.

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**Supplemental material: Appendix A. Supplementary data.**

**Table A1. HPV genotype-specific primers of standard plasmids in this study.**

| Type | Primer names | Length of target fragment | Sequence (5' to 3') | L1 gene position |
|---|---|---|---|---|
| HPV6 | Forward | 594bp | GAACCTGTGCCTGATACTCT | 792 - 812 |
| | Reverse | | CTTGCGTCCCAAAGGATA | 1368 - 1386 |
| HPV11 | Forward | 419bp | TGTGCCTGATGACCTGTT | 800 - 818 |
| | Reverse | | TTGGTGGAGGCGATAAAC | 1201 - 1219 |
| HPV16 | Forward | 436bp | TCTGGGTCTACTGCAAAT | 915 - 933 |
| | Reverse | | ATTGCCTGGGTTACAAAC | 1334 - 1352 |
| HPV18 | Forward | 747bp | AGGCACTGCTTGTAAATC | 695 - 713 |
| | Reverse | | CGATATGTATCCACCAAAC | 1423 - 1442 |
| HPV31 | Forward | 493bp | ATCAGGCACGGTTGGTGA | 791 - 809 |
| | Reverse | | GTAATGGCCTGTGAGGTG | 1266 - 1284 |
| HPV33 | Forward | 422bp | TGTTCCCGATGACCTGTA | 809 - 827 |
| | Reverse | | CAGATGGAGGAGGTGTTA | 1216 - 1234 |
| HPV35 | Forward | 443bp | TAGGGCTGGAACTGTAGG | 794 - 812 |
| | Reverse | | CAGAAGGCGGTGGTGTAA | 1219 - 1237 |
| HPV39 | Forward | 518bp | GGGGACAGTATGTTCTTC | 723 - 741 |
| | Reverse | | AAACTGGCAGATGGTGGA | 1223 - 1241 |
| HPV42 | Forward | 648bp | CCTCCAAAGCTGAGGTAC | 716 - 734 |
| | Reverse | | GACAGCGAATAGCTTCTG | 1346 - 1364 |
| HPV43 | Forward | 541bp | CAGTGGGTCTTTGGTTAC | 962 - 980 |
| | Reverse | | CAAACCCGCCTGCATAAC | 1485 - 1503 |
| HPV45 | Forward | 651bp | GGAATAGGGCAGGTGTTA | 862 - 880 |
| | Reverse | | GTAACCCAGCCTGAACTA | 1495 - 1513 |
| HPV51 | Forward | 493bp | TTACGCAGGGAGCAAATC | 744 - 762 |
| | Reverse | | TAGCAGACGGAGGTAATG | 1219 - 1237 |
| HPV52 | Forward | 414bp | CCCTGTGCCAGGTGATTT | 893 - 911 |
| | Reverse | | AGGCCAAATTGCCAGTCC | 1289 - 1307 |
| HPV53 | Forward | 432bp | GGCGTTATTGGTGAGGAA | 786 - 804 |
| | Reverse | | AGGAGGCGACAAACCTAT | 1200 - 1218 |
| HPV56 | Forward | 488bp | GCAGATGCCTATGGTGAT | 816 - 834 |
| | Reverse | | CAGTCCTCCAGTAGGTTA | 1289 - 1307 |
| HPV58 | Forward | 428bp | GGCTGTCCCAGATGACCTTTAT | 884 - 906 |
| | Reverse | | TGGCAGACGGAGGAGGTGTTAA | 1290 - 1312 |
| HPV59 | Forward | 484bp | GGCGAGAACAGGTTTTTGCC | 751 - 771 |
| | Reverse | | GTAGGAGGTGGTGTAACACC | 1215 - 1235 |
| HPV66 | Forward | 594bp | CCTCCTCCCAGTTCTGTA | 850 - 868 |
| | Reverse | | TACACTAGCCTTGGGTCT | 1426 - 1444 |
| HPV68 | Forward | 531bp | TATGGTGCTATGGACTTT | 612 - 630 |
| | Reverse | | AATTGCTGCTGATTGTAG | 1263 - 1281 |
| HPV73 | Forward | 609bp | TAGGCATGGCTGCTGATC | 697 - 715 |
| | Reverse | | CTTTAGGAGGTTGAGGAC | 1288 - 1306 |
| HPV81 | Forward | 497bp | TTACAAATGGCTGCTGAG | 702 - 720 |
| | Reverse | | AACAGTGCCTTGTTCATA | 1181 - 1199 |
| HPV82 | Forward | 440bp | GCTATACCCACCACTTTG | 804 - 822 |
| | Reverse | | AAACTAGCAGTGGGAGGT | 1226 - 1244 |
| HPV83 | Forward | 696bp | ATGGCGACATGGTGGAAA | 589 - 607 |
| | Reverse | | AGGTAATAGCACGGGACT | 1267 - 1285 |

**Table A2. Genotype-specific LAMP primer sets for HPV test.**

| Type | Primer name | Sequence (5' to 3') | L1 gene position |
|---|---|---|---|
| HPV6 | F3 | CTCTTTGGTGTCCTCTGAG | 878 - 897 |
| | B3 | ACATGACGCATGTACTCTT | 1066 - 1085 |
| | FIP | AGTTGATTACCCCAACAAATACCAT-GCACAATTGTTTAATAAGCCATA | 949 - 974/897 - 920 |
| | BIP | GTTTGTTACTGTGGTAGATACCACA-TGGTGTATGTGGAAGATGT | 974 - 999/1035 - 1054 |
| | LF | GTCCCTGGGCTTTTTGTAGC | 923 - 943 |
| | LB | ACATGACATTATGTGCATCCGT | 1009 - 1031 |
| HPV11 | F3 | AACAGATCATCTGTAGCTAGT | 834 - 855 |
| | B3 | GCAGATTTAGACACAGATGCA | 1025 - 1046 |
| | FIP | GCCTTTTGAAGCCAATATGGTTTAT-AGTATTTATGTACATACACCTAGTG | 913 - 938/855 - 880 |
| | BIP | TCAGGGACATAACAATGGTATTTGC-TGTCATATTTGTACTGCGTGT | 938 - 963/999 - 1020 |
| | LF | CCTCTGAAGACACCAATGAGC | 880 - 901 |
| | LB | CCACTTGTTTGTTACTGTGGTAGA | 971 - 995 |
| HPV16 | F3 | GCCATATCTACTTCAGAAACTACA | 1116 - 1140 |
| | B3 | TTGCCTGGGTTACAAACC | 1333 - 1351 |
| | FIP | TGCAGTTAAGGTTATTTTGCACAGT-TACTAACTTTAAGGAGTACCTACG | 1208 - 1233/ 1148 - 1172 |
| | BIP | TTCCACTATTTTGGAGGACTGGA-AGTATCTTCTAGTGTGCCTC | 1262 - 1285/ 1309 - 1329 |
| | LF | CTGTAAATCATATTCCTCCCCATGT | 1172 - 1197 |
| | LB | TTGGTCTACAACCTCCCCC | 1288 - 1307 |
| HPV18 | F3 | CACTGTGCCTCAATCCTT | 989 - 1007 |
| | B3 | ACTGGGAGTGGTATCTACC | 1181 - 1200 |
| | FIP | GGTAACAATAGAGCCACTTGGAGAG-ACAGGTATGCCTGCTTCA | 1061 - 1086/ 1020 - 1038 |
| | BIP | TGACTCCCAGTTGTTTAATAAACCA-AATAATTGATTATGCCAGCAAAC | 1088 - 1113/ 1149 - 1172 |
| | LB | GGTTACATAAGGCACAGGGTC | 1117 - 1138 |
| HPV31 | F3 | ATCGGTCCCTACTGACTT | 809 - 827 |
| | B3 | AAATAACTGATTGCCCCAAC | 973 - 993 |
| | FIP | CGCTAGGTGTAGGAAAGTATGTACT-ATATATTAAAGGCTCCGGTTCA | 867 - 892/827 - 849 |
| | BIP | GCTCCATGGTTACTTCAGATGC-AAATACCATTATTGTGTCCCTG | 892 - 914/951 - 973 |
| | LB | CCATATTGGATGCAACGTGCT | 930 - 951 |
| HPV33 | F3 | GAACTACTGCCTCTATTCAAAG | 841 - 863 |
| | B3 | CACTAGTTACTTGTGTGCATAA | 1030 - 1052 |
| | FIP | ACGTTGTAGCCAATATGGCTTATTA-GTGCTTTTTTTCCCACTCC | 920 - 945/865 - 884 |
| | BIP | ATGGTATTTGTTGGGGCAATCA-AGTCATATTAGTACTGCGAGT | 962 - 984/1009 - 1030 |
| | LF | ACTGAGATTCGGAAGTAACCATTG | 892 - 916 |
| HPV35 | F3 | GGTACCACTGGCACATTG | 843 - 861 |
| | B3 | TGTACTGTCACTAGAAGACAC | 1044 - 1065 |
| | FIP | CACGTTGCAACCAATATGGTTTATT-AGTACTAGTTATTTTCCTACTCCTA | 924 - 949/864 - 889 |
| | BIP | TTGTTGGAGTAACCAATTGTTTGTT-AGCAGAACACACAGACAT | 971 - 996/1026 - 1044 |
| | LF | ATCGGAGGTTACCATAGAGCC | 891 - 912 |
| | LB | ACTGTAGTTGATACAACCCGTAGT | 996-1020 |

| | | | |
|---|---|---|---|
| HPV39 | F3 | CAAACCCCGGTAGTTCTG | 847 - 865 |
| | B3 | GAGGTAGATAATGTAAAGTTGGTA | 1013 - 1037 |
| | FIP | GGCCTTATGTAGCCAATAAGGCTTA-TATACTGCCCCTCTCCCA | 920 - 945/865 - 883 |
| | BIP | CAGGGCCACAACAATGGTATATG-CTACGGGTAGTGTCCACA | 945 - 968/995 - 1013 |
| | LF | ACTGGGAATCAGAGGTTACCA | 892 - 913 |
| HPV42 | F3 | GTATTTATTATCCTACCCCTAGTG | 947 - 971 |
| | B3 | AGCAGCTGTATATGTATCACC | 1132 - 1153 |
| | FIP | TCCTTGTGCTTGTTGTAACCAATAT-GTTCTATGGTAACATCTGATGC | 1011 - 1036/971 - 993 |
| | BIP | AATGGTATATGTTGGGGAAATCAGC-CAGTGGCACACAAAGTCA | 1042 - 1067/1106 - 1124 |
| | LB | ACTGTGGTTGATACTACCCGT | 1075 - 1096 |
| HPV43 | F3 | ATACCACTCGTAGTACAAACT | 1081 - 1102 |
| | B3 | AGTCCTCTAATAATGTGGGATC | 1263 - 1285 |
| | FIP | CGCAGGTATTCCTTAAACTTTGCA-CGTTATGTGCCTCTACTGAC | 1151 - 1175/1105 - 1125 |
| | BIP | CATGTGGAAGAATATGATCTGCAGT-TATGAATATATGTCATAACCTCTGG | 1176 - 1201/1233 - 1258 |
| | LF | TCATATGTACTGGGCACAGTAGG | 1125 - 1148 |
| HPV45 | F3 | ACAATGGTATTTGTTGGCATAA | 1045 - 1067 |
| | B3 | GGATATATGACATAACCTCTGCA | 1241 - 1264 |
| | FIP | AGGATTTTGTGTAGAGGCACATAAT-AGTTGTTTGTTACTGTAGTGG | 1115 - 1140/1069 - 1090 |
| | BIP | GCCAAGTACATATGACCCTACTAAG-GCACAACTGAAAAATAAACTGT | 1142 - 1167/1205 - 1227 |
| | LF | GTTAAATTAGTACTGCGGGTAGTGT | 1090 - 1115 |
| | LB | GCAGTATAGTAGACATGTGGAGGA | 1172 - 1196 |
| HPV51 | F3 | GTCTATGATAACATCTGATTCTCAA | 890 - 915 |
| | B3 | TACTCTTCCCCATGCCTAA | 1090 - 1109 |
| | FIP | TGATTGTTCCAGCAAATGCCATT-TAATAAGCCTTATTGGCTCCA | 960 - 983/920 - 941 |
| | BIP | ACCTGTGTTGATACTACCAGAAGTA-GGAGTAAATGTTGGGGAAAC | 993 - 1018/1050 - 1070 |
| | LF | ATTGTGACCCTGCGCACG | 942 - 960 |
| | LB | ATTTAACTATTAGCACTGCCACTGC | 1021 - 1046 |
| HPV52 | F3 | AGCAGTGCTTTTTTTCCTAC | 954 - 974 |
| | B3 | CTCAGCACATAAAGTCATGTT | 1113 - 1134 |
| | FIP | ACGTTGTAACCAGTACGGTTTATT-TCCTAGTGGTTCTATGGTAAC | 1014 - 1038/974 - 995 |
| | BIP | GGCCACAATAATGGCATATGTTG-GTGCTACGAGTGGTATCC | 1044 - 1067/1094 - 1112 |
| | LB | GGCAATCAGTTGTTTGTCACAG | 1068 - 1090 |
| HPV53 | F3 | GGTAGTAATGGCAGGGAC | 828 - 846 |
| | B3 | TTGCGGAAAGAGTCATGT | 1015 - 1033 |
| | FIP | GCAGCCAATATGGCTTATTAAACAA-TGTATATGTTGCTACACCTAGT | 909-934/860-882 |
| | BIP | AACGTGCCCAGGGACATAATA-TTGTATTCCTGGTGGTATCC | 934 - 955/995 - 1015 |
| | LF | AGCCTCTGAAGTTATCATAGACCC | 882 - 906 |
| | LB | GGCATCTGTTGGAACAATCAGTT | 957 - 980 |
| HPV56 | F3 | GAACAATTATTTGCCAGACATT | 858 - 880 |
| | B3 | GCCATTATTATGGCCTTGG | 1049 - 1068 |
| | FIP | GGTTCTCTACCATTGCTACCCTTTA-ATTTTAATAGGGCTGGTAAAGTTG | 931 - 956/880 - 904 |
| | BIP | AGTTCTGTATATGTTGCTACGCC-CGTTGCAACCAATAAGGTT | 963 - 986/1027 - 1046 |

| | | | |
|---|---|---|---|
| | LF | TAACTCTGCAGGTATTGTTTCCC | 904 - 927 |
| | LB | CTATGATTACGTCTGAGGCACAG | 994 - 1017 |
| HPV58 | F3 | CGGTAATACTGCAGTTATCCAA | 917 - 939 |
| | B3 | TTTACTTCAGTGCATAATGTCA | 1102 - 1124 |
| | FIP | CGCTGTAGCCAATAAGGCTTATTAA-AGTAGTGCATTTTTTCCAACT | 997 - 1022/939 - 960 |
| | BIP | TGCACAAGGTCATAACAATGGC-TATTAGTGCTACGAGTGGTATC | 1022 - 1044/ 1080 - 1102 |
| | LF | CTGAGGTAACTATAGAGCCACTAGG | 960 - 985 |
| | LB | GGGCAATCAGTTATTTGTTACCGT | 1052 - 1076 |
| HPV59 | F3 | GAGAACAGGTTTTTGCCAG | 754 - 773 |
| | B3 | TTGTTTAAACCCTGAGCCT | 943 - 962 |
| | FIP | GGCACGTATGTCAGTACCTTTAATA-ACATTTTTGGAATAGATCTGGT | 827 - 852/773 - 795 |
| | BIP | GGCAGTTATTTATATTCCCCTTCCC-TGTGCAGCCAATATGGTT | 858 - 883/925 - 943 |
| | LF | GATTCAGGAAGTTGATCACCCAT | 798 - 821 |
| | LB | TGGGTCTGTGGTTACTTCTGA | 887 - 908 |
| HPV66 | F3 | CCTCCCAGTTCTGTATATGTTG | 853 - 875 |
| | B3 | ATGTGCTTTTAGCTGCATTA | 1029 - 1049 |
| | FIP | CACGTTGCAACCAATAAGGTTTATT-CTACTCCTAGTGGGTCCA | 919 - 944/875 - 893 |
| | BIP | CACAGGGCCATAATAATGGCATA-AGTCATGTTGGTGCTTCT | 944 - 967/1009 - 1027 |
| | LF | AATAATTGGGCCTCAGAGGTAATC | 894 - 918 |
| | LB | GGGGTAATCAGGTATTTGTTACTGT | 971 - 996 |
| HPV68 | F3 | GCCAGGCATTTTTGGAAT | 768 - 786 |
| | B3 | ATTGTTGTGTCCCTGTGC | 945 - 963 |
| | FIP | GTTTCACGAATGTCAGTGCCC-GCATGGTAGGGGACACTA | 833 - 854/793 - 811 |
| | BIP | CCTAGTAGTTATGTGTATGCCCCC-CAGCCAATAGGGCTTGTT | 855 - 879/921 - 939 |
| | LB | CTATGGTGTCCTCTGACTCCC | 892 - 913 |
| HPV73 | F3 | CGACACTTATTTAACAGGGC | 765 - 785 |
| | B3 | GCTTCTAGTAGTATCTACAACAGTT | 986 - 1011 |
| | FIP | TGGATGGTGTTGCAGTATTGC-GGTGATAAAATCCCAGATGAC | 835 - 856/795 - 816 |
| | BIP | CATGGTTTCTTCAGATGCACAGT-ACAAATACCATTATTTTGTCCCT | 887 - 910/943 - 966 |
| | LB | CCTTATTGGTTGCAAAAGGCAC | 921 - 943 |
| HPV81 | F3 | CCCGGGAGTTATATTTATGCC | 861 - 882 |
| | B3 | AGCAGATGTAGCTGTGCA | 1038 - 1056 |
| | FIP | CCGTTGTAGCCAATAAGGCTTATTA-CCTACACCTAGTGGGTCT | 926 - 951/882 - 900 |
| | BIP | CACAGGGCCATAATAATGGCA-AATTGGTGCTTCTGGTAGT | 952 - 973/1011 - 1030 |
| | LF | AGCTGGGAATCAGAGGATACC | 902 - 923 |
| HPV82 | F3 | TTCTATTTATTCTGCTACACCTAG | 863 - 887 |
| | B3 | ATGGTTTAAATGGAGTGGATG | 1054 - 1075 |
| | FIP | TGCACGTTGTAACCAGTATGG-TGGCTCTATGGTTACTTCG | 927 - 948/887 - 906 |
| | BIP | TGGGGCAATCAATTGTTTGTTACC-CAGATGCTGCAGATAATGTACT | 972 - 996/1032 - 1054 |
| | LB | TGTGTTGATACCACCCGCA | 996 - 1015 |
| HPV83 | F3 | GGAACACTCTTACCAGCT | 853 - 871 |
| | B3 | GTATTCATTAGCCTGTGTAGC | 1047 - 1068 |
| | FIP | TGCAGCCAGTAGGGCTTATTAAATA-ACATCTATGCTCCTACTCCT | 922 - 947/871 - 891 |
| | BIP | TGCCCAGGGACATAATAATGGC-ATATTGGTACTGCGGGTAGT | 950 - 972/1011 - 1031 |
| | LF | ACGATACCAGGGAGCCACT | 891-910 |

**Note: Inner primers recognize two different regions of the target DNA. The forward inner primer (FIP) consists of the F2 region (3' end) that is complementary to the F2c region and has the same sequence as the F1c region at the 5' end. Similarly, the backward inner primer (BIP) consists of the B2 region (3' end), which is complementary to the B2c region and has the same sequence as the B1c region at the 5' end. Dashes in the FIP and BIP primer sequences indicate the two regions linked by a TTTT linker in the FIP and BIP primers.**

**Table A3. The standard curves of 23 HPV types specific LAMP.**

| Type | Standard curve | Correlation coefficient |
|---|---|---|
| HPV6 | y = -8.745x + 103.334 | $R^2$ = 0.982 |
| HPV11 | y = -1.824x + 32.242 | $R^2$ = 0.980 |
| HPV16 | y = -1.544x + 31.814 | $R^2$ = 0.980 |
| HPV18 | y = -2.699x + 44.794 | $R^2$ = 0.987 |
| HPV31 | y = -2.475x + 49.455 | $R^2$ = 0.972 |
| HPV33 | y = -2.124x + 43.698 | $R^2$ = 0.971 |
| HPV35 | y = -2.916x + 52.320 | $R^2$ = 0.930 |
| HPV39 | y = -2.603x + 44.539 | $R^2$ = 0.976 |
| HPV42 | y = -4.389x + 70.232 | $R^2$ = 0.977 |
| HPV43 | y = -5.080x + 87.064 | $R^2$ = 0.976 |
| HPV45 | y = -3.457x + 59.772 | $R^2$ = 0.951 |
| HPV51 | y = -1.738x + 31.473 | $R^2$ = 0.958 |
| HPV52 | y = -7.440x + 97.798 | $R^2$ = 0.963 |
| HPV53 | y = -2.096x + 35.154 | $R^2$ = 0.948 |
| HPV56 | y = -5.903x + 94.250 | $R^2$ = 0.954 |
| HPV58 | y = -1.761x + 34.686 | $R^2$ = 0.983 |
| HPV59 | y = -2.890x + 46.488 | $R^2$ = 0.974 |
| HPV66 | y = -1.619x + 29.207 | $R^2$ = 0.983 |
| HPV68 | y = -11.469x + 149.864 | $R^2$ = 0.917 |
| HPV73 | y = -3.100x + 50.339 | $R^2$ = 0.970 |
| HPV81 | y = -4.344x + 69.091 | $R^2$ = 0.970 |
| HPV82 | y = -2.954x + 48.721 | $R^2$ = 0.951 |
| HPV83 | y = -7.333x + 103.901 | $R^2$ = 0.967 |

**Note: These curves show that there is a link between reaction time and the template concentration. In these equations, AST (y) is equivalent to the reaction time, and the number (x) is the log starting quantity/copy number (from $10^8$ to $10^1$).**