Detection and Genotyping of Human Papillomavirus DNA by SPF10 and MY09/11 Primers in Cervical Cells Taken From Women Attending a Colposcopy Clinic

Chris Perrons,1* Bernhard Kleter,2 Rosanne Jelley,3 Hamid Jalal,1 Wim Quint,2 and Richard Tedder1
1Department of Virology, Royal Free and University College Medical School, London, United Kingdom
2Delft Diagnostic Laboratory BV, Delft, The Netherlands
3Department of Obstetrics and Gynaecology, University College Hospitals NHS Trust, London, United Kingdom

Human papillomavirus (HPV) is the main etiological agent of cervical cancer. There is a large number of HPV genotypes and therefore a need to distinguish the high risk HPV genotypes associated with invasive cancer from the low risk. Because persistence of high risk HPV infection is necessary for progression of a pre-invasive cervical change one needs to identify the individual genotype to see if it persists. PCR amplification of HPV DNA is described using two consensus primer systems from cervical cells. Amplified HPV DNA was genotyped using a reverse hybridization line probe assay (LiPA). HPV DNA was amplified from 42% of samples by MY09/11 and from 80% by SPF10. In 42 samples HPV DNA was detected by both primer sets and in 38 samples only the SPF10 primers detected HPV DNA. The LiPA detected 21 different HPV genotypes (13 high risk) in this cohort of samples. Forty-three percent contained a single HPV genotype and 24% contained multiple infections (2–5 genotypes). Overall, high risk HPV genotypes were detected in 48% of the cervical samples, the most frequent types were 16, 18, 31, and 51. The proportion of high risk HPV genotypes increased with more severe cytological abnormalities. This study demonstrates that the SPF10 primer set is more sensitive than the MY09/11 primer set and that genotyping by LiPA tells us if the HPV infection is caused by a high risk type and if the infection is mixed. Additionally LiPA provides information about the individual genotype when looking for persistence of infection. HPV DNA detection and genotyping is therefore a useful tool in the colposcopy clinic, used in conjunction with cytology. J. Med. Virol. 67:246–252, 2002.

INTRODUCTION

In countries that have screening programs for cervical cancer using cervical cytology there have been significant reductions in both the incidence of and mortality from cervical cancer. There is still a good deal of ambiguity in the management of women with low grade abnormalities, however, because of the poor sensitivity of cervical smears. Human papillomavirus (HPV) is the main etiological agent of cervical cancer [zur Hausen et al., 1984] and its detection would provide valuable information. HPV DNA has been detected by PCR in over 90% of carcinomas [Resnick et al., 1990; Yoshikawa et al., 1991; van den Brule et al., 1992]. A large number of HPV genotypes have been identified, although not all are associated with the development of cervical carcinoma. HPV genotypes are defined as either high or low risk depending on their association with cervical cancer. Persistent infection with a high risk HPV genotype can increase the risk of cancer.

Many methods have been developed for HPV detection and these vary widely in sensitivity and specificity. Techniques based on southern blotting and hybridization (hybrid capture) have been described [Low et al., 1990; Clavel et al., 1999]. DNA amplification by PCR has shown to be a sensitive method of HPV detection [Morris et al., 1990; Ward et al., 1990; Kuypers et al., 1993]. There are individual PCR assays for HPV genotype determination [Cuzick et al., 1994] and these are highly specific. The problem with these separate amplifications is, because of the large number of HPV types, multiple PCRs have to be performed which is time consuming. Therefore, there is great advantage in a single

*Correspondence to: Chris Perrons, Department of Virology, Windeyer Institute of Medical Sciences, Windeyer Building, 46 Cleveland Street, London W1T 4JP, UK.
E-mail: c.perrons@ucl.ac.uk
Accepted 16 August 2001
DOI 10.1002/jmv.2214
Published online in Wiley Interscience (www.wiley.interscience.com)
assay being able to amplify all the HPV genotypes with universal primers. A number of methods have been described using universal primers to amplify HPV DNA [Manos et al., 1989; Snijders et al., 1990; Kleter et al., 1998]. For genotyping after amplification various methods have been developed either to define the individual genotype or differentiate between high and low risk types. These include restriction fragment length polymorphism [Bernard et al., 1994], detection by a cocktail of probes specific for high risk genotypes [Jacobs et al., 1997] and methods depending on detection by specific probes immobilized onto strips [Gravitt et al., 1998; Kleter et al., 1999].

The amplification of HPV DNA from cervical cells by two PCR systems is described. The MY09/11 [Manos et al., 1989] and the SPF10 [Kleter et al., 1998] primer sets were used separately. Both are consensus primer sets and amplify in the conserved L1 region a fragment of 450 and 65 bp respectively. Any amplified HPV DNA from either PCR was genotyped using a reverse hybridization line probe assay (LiPA). The samples were collected consecutively from women attending a colposcopy clinic. Comparisons were made between the two PCR systems. The LiPA results were correlated with the result of the smear tests taken at the same time and we were able to see the distribution of individual genotypes in the various grades of smears.

**PATIENTS AND METHODS**

**Clinical Samples**

Cervical cells were available from women attending the colposcopy clinic of UCL hospital. The samples were collected consecutively from 100 women to represent a typical cross section of women referred to the colposcopy clinic. The women were attending the clinic for one of three reasons: 1) for the first time, referred from the national screening program because their cervical smear had shown persistent borderline nuclear changes or mild dysplasia when repeated after six months or who presented with one smear showing moderate or severe dysplasia, as according to National Service Cervical Screening Programme (NHSCSP) guidelines [Duncan, 1997]; 2) for treatment of cervical dysplasia; and 3) after treatment for cervical dysplasia. At the time of the colposcopy a smear was taken with a spatula and cervical cells placed onto a slide which was sent for routine cytological analysis. The end of the spatula was then broken off and placed in a universal container with 2 ml of normal saline.

**DNA Isolation**

The cervical cells were removed from the spatula by agitation. The spatula was removed and the suspension spun at 2,000 × g for 5 min. The pelleted cells were resuspended in 2 ml saline and the centrifugation step was repeated. The cells were resuspended in 200 μl saline and the DNA extracted using the QIAmp spin column (Qiagen, Hilden, Germany). The extracted DNA was resuspended in 200 μl DNAse free water (Promega, Southampton, UK) and stored at −70°C. The samples were thawed immediately before testing for the presence of HPV DNA and 10 μl were used in each type of PCR with a 50 μl final reaction volume. All PCRs were performed on the same thermal cycler.

**Amplification of HPV DNA by PCR**

Two different primer sets with separate PCR reactions were used in this study. The first primer set consisted of MY09 and MY11, which are degenerate primers designed to amplify a 450 base pair fragment located in the L1 region of the HPV genome. These primers were designed by Manos et al. [1989]. The primers were synthesized by Oswel (Southampton, UK) and, in order for the amplified PCR product to be typed, it was necessary to have a biotin molecule attached at the 5’ end of the degenerate MY09 primer. This PCR had final concentrations of 1.5 mM MgCl2, 50 mM KCl, 10 mM Tris (pH 8.3), 200 μM dNTP, 1.25 U of AmpliTaq Gold DNA polymerase (Perkin-Elmer, Oak Brook, IL) and 100 ng of each primer. Initial denaturation was at 95°C for 12 min followed by 38 cycles of 95°C for 1 min (denaturation), 55°C for 1 min (annealing), and 72°C for 1 min (extension), with an additional 7 min extension. The products were electrophoresed through a 3% agarose gel, stained with ethidium bromide and visualized under ultraviolet light. Because the PCR products were biotinylated, detection was confirmed using the DNA enzyme immunoassay [Kleter et al., 1998]. The sensitivity of this PCR was accessed with a full length HPV16 sequence cloned in the plasmid pBR 322 (provided by E-M de Villiers, Heidelberg, Germany). Using a dilution series of this plasmid it was shown that this PCR could detect down to 100 HPV copies/ml.

The second primer set SPF10 contained a mixture of 10 sequences designed to amplify a 65 bp fragment [Kleter et al., 1999] that was located within the sequence amplified by the MY09/11 primers. The primer set had been described originally as a 6 primer set [Kleter et al., 1998] but was modified to include another four sequences. The PCR had final concentrations of 2.0 mM MgCl2, 50 mM KCl, 10 mM Tris (pH 9.0), 0.1% Triton X-100, 0.01% gelatin, 200 μM dNTP, 1.5 U of AmpliTaq Gold DNA polymerase (Perkin-Elmer) and 15 pmol of each primer. Initial denaturation was at 95°C for 9 min followed by 40 cycles of 94°C for 30 sec (denaturation), 52°C for 45 sec (annealing), and 72°C for 45 sec (extension), with an additional 5 min extension. The biotinylated PCR products amplified by the SPF10 primer set were detected by the DNA enzyme immunoassay [Kleter et al., 1998].

**DNA Enzyme Immunoassay**

This microtiter based hybridization assay was used to detect biotinylated PCR fragments amplified by both the SPF10 and the MY09/11 primer sets. The method is outlined by Kleter et al. [1998]. Briefly, the PCR
products were captured onto a streptavidin-coated microtiter plate and washed 3 times. Then the non-biotinylated complementary strands were separated by denaturation with alkaline NaOH and removed by washing three times. The next stage involved hybridization with a mixture of nine oligonucleotides labeled with digoxigenin (DIG). After incubation the wells were washed three times and anti-DIG alkaline phosphate (BCIP) and nitroblue tetrazolium (NTB) added. After a further incubation, washing five times, the substrate was added, incubated and the reaction stopped with acid. The optical densities were determined at 450 nm. The presence of HPV DNA is determined by comparing the optical density with those obtained by the positive borderline and negative controls.

**Genotyping by Line Probe Assay**

The biotinylated products amplified from both MY09/11 and SPF10 primers were typed using reverse hybridization with the INNO-line probe assay (LiPA) HPV prototype research genotype assay. This was based on a method described for hepatitis C virus (HCV) [Stuyver et al., 1996] using oligonucleotide probes developed at Delft Diagnostics Laboratory [Kleter et al., 1999]. The probes were immobilized as parallel lines on nitrocellulose membrane strips. They were able to detect 25 different HPV genotypes and differentiate between low risk types (6, 11, 34, 40, 42–44, 53, 54, 70, 74) and high risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68). The method is outlined by Kleter et al. [1998]. Briefly, the biotinylated PCR products were denatured by alkaline NaOH in individual troughs of a tray and then the LiPA strips added. All the following steps were performed automatically in an Auto LiPA (Innogenetics, Belgium). Hybridization buffer was added and incubated at 50°C for 1 hr, then the strips were washed twice in hybridization solution. The strips were incubated in alkaline phosphate-streptavidin conjugate for 30 min at room temperature. After three washes the substrates 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitroblue tetrazolium (NTB) were added, incubated at room temperature for 30 min and the reaction stopped. Hybridization was shown by a purple color on the probe and the strips were interpreted by comparing the hybridization pattern to those given by type specific HPV.

**RESULTS**

**Comparison of PCR Consensus Primer Sets**

The 100 cervical smears were tested for the presence of HPV DNA with both primer sets. HPV DNA was amplified from 42 samples by the MY09/11 primers and 80 samples by the SPF10 primer set (Table I). In 42 samples HPV DNA was detected by both primer sets, whereas in 38 samples HPV DNA was detected only by the SPF10 primers (Fig. 1). Twelve of these 38 samples, of which the MY09/11 primers had not shown any detectable HPV DNA, were shown to contain high risk HPV types by the LiPA (genotypes 16, 18, 31, 39, 45, 51, 52, 56, 68). In the samples HPV DNA was detected by the MY09/11 primers, it was also detected by the SPF10 primers.

**Prevalence of HPV Genotype**

Twenty-one different HPV genotypes were detected by the LiPA in this cohort of 100 samples, 13 of which were classed as high risk types (Table II). Forty-three samples contained a single HPV genotype. Twenty-four of the cervical samples contained multiple infections with between 2–5 HPV genotypes being detected. Twenty-one HPV genotypes were detected in the 24 mixed infections therefore representing a wide range of genotypes in the coinfected patients.

LiPA did not detect a genotype in 33 samples, even though HPV DNA had been detected in 13 of these by the SPF10 PCR primer set. This is because the LiPA does not detect all low risk types, and therefore these 13 could be low risk types or new sequences not yet determined.

Overall, high risk HPV genotypes were detected in 48% (n = 48) of the cervical samples, of which 21 were mixed infections. These 21 mixed infections comprise of two groups: multiple high risk genotypes only (n = 10), and a mixture of high/low risk genotypes (n = 11). The most prominent high risk genotypes detected from this group of patients were type 16 (n = 14), 18 (n = 7), 31 (n = 10) and 51 (n = 10). The LiPA detected low risk HPV genotypes in 19% (n = 19) of the samples, of which three contained mixed infections.

In the cervical samples where HPV DNA had been detected by the SPF10 primers but not by the MY09/11 primers, there was a wide range of genotypes identified

**TABLE I. Detection of HPV DNA by PCR in Cervical Smears Using the MY09/11 and SPF10 Primer Sets, Showing the Genotypes Detected by LiPA**

<table>
<thead>
<tr>
<th>Cytology</th>
<th>Number of samples</th>
<th>HPV DNA detected</th>
<th>High risk HPV genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MY09/11 SPF10</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>54</td>
<td>12 (22%) 39 (72%)</td>
<td>16, 18, 31, 39, 45, 51, 52, 56, 58, 66, 68</td>
</tr>
<tr>
<td>Borderline dysplasia</td>
<td>11</td>
<td>5 (45%) 10 (91%)</td>
<td>16, 33, 35, 58</td>
</tr>
<tr>
<td>Mild dysplasia</td>
<td>18</td>
<td>12 (71%) 17 (94%)</td>
<td>16, 18, 31, 39, 45, 51, 52, 56, 58, 66</td>
</tr>
<tr>
<td>Moderate dysplasia</td>
<td>9</td>
<td>6 (67%) 6 (67%)</td>
<td>16, 31, 33, 51, 58, 66</td>
</tr>
<tr>
<td>Severe dysplasia</td>
<td>8</td>
<td>7 (88%) 8 (100%)</td>
<td>16, 18, 31, 51</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>42 (42%) 80 (80%)</td>
<td></td>
</tr>
</tbody>
</table>
Samples from this group contained 30 single and eight mixed infections. The single infections comprise of eight high risk and 22 low risk genotypes. The mixed infections comprise multiple high risk ($n=1$), multiple low risk only ($n=2$) and a mixture of high/low risk ($n=5$) genotypes. There was no significant correlation between any particular HPV genotype and the inability of MY09/11 to amplify it.

**TABLE II. HPV Genotypes Detected by LiPA in the 100 Cervical Smears**

<table>
<thead>
<tr>
<th>HPV genotype</th>
<th>High or low risk</th>
<th>Amplified by SPF10</th>
<th>Amplified by MY09/11</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Low</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>Low</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>High</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>18</td>
<td>High</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>31</td>
<td>High</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>33</td>
<td>High</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>35</td>
<td>High</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>39</td>
<td>High</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>42</td>
<td>Low</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>44</td>
<td>Low</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>45</td>
<td>High</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>51</td>
<td>High</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>52</td>
<td>High</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>53</td>
<td>Low</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>54</td>
<td>Low</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>56</td>
<td>High</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>58</td>
<td>High</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>66</td>
<td>High</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>68</td>
<td>High</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>70</td>
<td>Low</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>74</td>
<td>Low</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

* 12 of these contain high risk types

Fig. 1. Comparison of MY09/11 and SPF10 primer sets.

(6, 11, 16, 18, 31, 39, 42, 44, 45, 51–54, 56, 68, 70, 74). Samples from this group contained 30 single and eight mixed infections. The single infections comprise of eight high risk and 22 low risk genotypes. The mixed infections comprise multiple high risk ($n=1$), multiple low risk only ($n=2$) and a mixture of high/low risk ($n=5$) genotypes. There was no significant correlation between any particular HPV genotype and the inability of MY09/11 to amplify it.

**HPV Genotype in Different Grades of Cervical Smear**

There was an increase in the proportion of high risk HPV genotypes in the cells from more severe lesions (Fig. 2). In women with normal borderline smears there were high risk HPV genotypes detected in nearly 50% of the samples. This proportion increased to 76% in the

![Bar chart](image.png)

* High risk HPV  ■ Low risk HPV □ HPV type not identified by LiPA

Fig. 2. Relationship between high and low risk HPV DNA detected by SPF10/LiPA and grade of cervical smear.
samples from women classed with mild dysplasia and to 100% in the samples from women with moderate or severe dysplasia. The mix of HPV genotypes detected in cervical smears varied. At the lower clinical grade there was a mixture of low risk and high risk HPV but, at higher clinical grades, the high risk types completely dominated (Fig. 2). There were 13 different HPV genotypes (high and low risk) detected in the samples from women with normal or borderline smears (Table I) but, in the samples graded with severe dysplasia, there were only genotypes 16, 18, 31, and 51 (all high risks) detected.

In the group of women who were attending the clinic for the first time or who were under long time review, 83% had HPV DNA detected in their cervical cells of which 75% were from high risk HPV genotypes. In the group of women being seen following treatment, 75% had HPV DNA detected in their cervical cells of which only 25% were high risk HPV. The length of time since these women had undergone treatment varied considerably (6 months–10 years).

**DISCUSSION**

Clearly, with such a wide range of HPV genotypes it is impossible to design a primer set that is highly complementary to all. The MY09/11 primer set used in this study were the original ones described by Manos et al. [1989]. MY09/11 are degenerate primers and therefore a mixture of 24 unique oligonucleotide sequences. The SPF10 primer set are not degenerate primers but a mixture of 10 primers.

In this study there were differences in the amplification ability of the two PCR systems, SPF10 and MY09/11. The SPF10 primer set detected HPV DNA in 80% of the samples compared to 42% by the MY09/11 primer set. This different amplification ability could be due to a number of reasons, one of which is that specific HPV genotypes are not amplified by one of the PCR systems. There have been reports that MY09/11 primers are less efficient at detecting certain genotypes such as HPV 35 [Qu et al., 1997], although in this study it detected HPV DNA in the single sample where it was present. In this set of samples, MY09/11 primers did not detect the HPV genotypes 42, 68, 70, and 74 as determined by the SPF10/LiPA system. It is not possible to determine if this is to do with sensitivity or specificity of the MY09/11 primers. The HPV genotypes 6, 11, 16, 18, 31, 39, 44, 45, 51–54, 56, and 68 were detected in fewer samples by the MY09/11 primers compared with the SPF10 primer set. Because it is generally believed that consensus primer sets do not have a uniform efficiency of amplification across the HPV genotypes, this discrepancy could be due to a low viral load.

The variation in the sensitivity to individual HPV genotypes by consensus primers is due to primer design and the number and amount of each oligonucleotide sequence present. With degenerate primers the synthesis relies on the random insertion of bases, and therefore an equal proportion of each sequence cannot be guaranteed. It is possible to obtain sequences in optimal proportions by synthesizing them separately and then mixing them, as is done with the SPF10 primer set. Recently, the MY09/11 primers have been modified [Gravitt et al., 1998]. This new primer set, PGMY09/11 is a mixture of defined oligonucleotide sequences targeted at the same region as the MY09/11 primers. By this modification an increased sequence complementarity to a broader range of HPV types was obtained.

Another reason for the different amplification ability of HPV DNA by PCR systems is the difference in the amplified product size. In certain archival samples, such as those in paraffin embedded materials, primers that produce long PCR products may have a lower amplification rate because of the damaged DNA [Baan et al., 1996; Karlsen et al., 1996]. Therefore in archival samples it is possible that the SPF10 PCR could have a higher amplification rate than the MY09/11 PCR because it produces smaller PCR products (65 bp compared to 450 bp). In this study the samples were frozen at −70°C and thawed just before testing, therefore there would be less de-grading of DNA and so this would not account for the low amplification rate by the MY09/11 PCR.

In two of the samples HPV16 was amplified by SPF10 but not by the MY09/11 primers, although it was shown that MY09/11 could detect a single copy HPV DNA in dilution series. This sensitivity result was based on purified HPV16 DNA (plasmid) that does not always reflect the sensitivity in DNA rich cells.

The largest group in this cross-section of samples taken from patients attending a colposcopy clinic were those determined normal by cytology (Table I). These patients would have originally have had a problem with their smear taken for primary screening. In this group the MY09/11 primer set amplified HPV DNA from 22% of the samples that is similar to other studies [Young et al., 1997; Kotloff et al., 1998], in contrast the SPF10 amplified HPV DNA in a much higher percentage (72%) than as been reported before.

The LiPA was capable of detecting 25 different genotypes, 14 high risk, and 11 low risk. In this study a wide range (n = 13) of high risk HPV was detected, the predominant types were HPV16 (14%), HPV31 and HPV51 (10% each) and HPV18 (7%). This wide range of genotypes only occurred in women with low grade lesions, with the range being restricted to four types in those graded with severe dysplasia. Interestingly these were also the four genotypes most frequently detected in the whole group.

The HPV genotypes detected in low grade lesions were a mixture of low and high risk HPV, while with progression of severity of the lesion the high risk HPV appears to dominate. A large proportion (24%) of the samples in this study had mixed infections with some having up to five different genotypes. The LiPA proved to be effective in detecting genotypes in these mixed infections that often comprised a mixture of high and low risk HPV.
The guidelines established by the British Society for Colposcopy and Cervical Pathology advises treatment of women with moderate and severe dysplasia. In this study there is a high correlation between women with these grades of smear and the presence of high risk HPV, confirming this advice. The group for which HPV typing is likely to be most useful is the women with smears reported as Borderline Nuclear Changes (BNC) or mild dysplasia. These are required to be kept under review by colposcopy and many remain in this status for several years, undergoing repeated colposcopic examinations. A single HR HPV test is unlikely to be helpful, but the persistence of high risk HPV combined with BNC would be useful in patient management. It is also important to know if it is the same high risk HPV genotype that is persisting. The absence of high risk HPV in this group would allow discharge from colposcopic follow-up.

The women whose smears become negative would at present be discharged from colposcopy. In this study 36% of women in this group had high risk HPV detected and indicating the need for them to kept under close review, whereas the remainder could safely be discharged to primary screening.

In the post-treatment follow up group the absence of high risk HPV provides reassuring confirmation of cure and, therefore, discharge of the patient. It is not possible to apply any conclusions to the presence of high risk HPV in 30% of the women in this study who had been previously treated because in some there had been a considerable length of time since treatment prior to the sample being taken. The use of HPV DNA detection in the colposcopy clinic provides us with more information that could help in more accurate targeting of treatments and decisions on when to release women back to primary screening.

In conclusion, HPV DNA detection is a useful tool when used in conjunction with cytology in the early diagnosis of pre-cancer of the cervix. Both the SPF10 and MY09/11 PCRs are useful in HPV DNA detection, with the SPF10 being more sensitive. It is important to know if the HPV infection is caused by high risk type and the LiPA can provide this information. Additionally, the LiPA can detect mixed infections and provides information about the individual HPV genotype that is very useful when looking for persistence of infection.

ACKNOWLEDGMENT

We thank Lynn Hyams for help in the preparation of the manuscript.

REFERENCES


学霸图书馆

www.xuebalib.com

本文献由“学霸图书馆-文献云下载”收集自网络，仅供学习交流使用。

学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，提供一站式文献检索和下载服务”的24小时在线不限IP图书馆。图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

图书馆导航：

图书馆首页 文献云下载 图书馆入口 外文数据库大全 疑难文献辅助工具