Analytical Performance of Sensitivity and Specificity for Rapid Multiplex High Risk Human Papillomavirus Detection Kit: HPV ViroCheck

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INTRODUCTION

High risk (HR) human papillomavirus (HPV) is a major cause of cervical cancer [1]. Epidemiologic evidence in cervical cancer was shown 16 HR HPVs, following (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, and 69) among 100 known HPV types [2]. Especially, HPV 16 and 18 are accounted for approximately 70% of all cervical cancers [2,3]. The presence of HR-HPV is complementarily used as a cervical cancer screening with cervical cytology test for preventing invasive cervical cancer [4].

HPV DNA tests, as developed for early cancer screening
using molecular biology techniques, were recommended by the clinical guidelines implemented from American society for colposcopy and cervical pathology. The combination of an HPV DNA test and a Pap test in cervical cancer screening have been focused since 2009 [4,5]. Now, HPV and Pap co-testing were required for routine screening in initial assessment and the management guidelines for over 20s old women [6].

In the Korean Society of Gynecologic Oncology, importance of HPV tests was stand out. It was recommended that specific HPV types in HR HPV-positive/cytology-negative women, and a positive test for HPV 16 or 18 should lead to colposcopy [7]. Especially, women for HPV 16 and/or 18 with ASC-US or LSIL cytology can be diagnosed directly to colposcopy and shown positive results [7,8].

As HPV infection on diagnosis of cervical cancer is increasingly important, the oncogenic process in cervical cancer is studied and the upregulation of HPV E6/E7 oncoproteins is a marker for an increased risk of cervical cancer [9,10]. HPV E6 oncoproteins promote degradation of p53 and disrupts the function of the apoptosis [11,12]. HPV E7 oncoproteins inactivates pRb and dysregulates cell cycle [13,14]. Therefore, detection of E6/E7 oncogene expression could be more specific and a better predictor of cervical cancer risk than the detection of HPV DNA.

In our previous study, HPV E6/E7 oncogenic detection showed better specificity than HPV commercialized DNA kit [15]. Now, we developed E6/E7 based tests and commercialized. In this study, we evaluated the newly developed HPV E6/E7 tests, which detects 16 HR HPVs as well as major types of HPV 16 and HPV 18. HPV plasmid DNA and HPV infected cells were examined by analytical sensitivity, specificity, and reproducibility test.

MATERIALS AND METHODS

1. Control plasmid DNA

Plasmid HPV DNA was prepared for usage as a positive control to determine the analytical sensitivity and specificity of the HPV genotyping kit. The plasmid DNA was prepared by inserting the synthesized E6 and E7 regions of each HPV genotype into pCR2.1 vectors (Invitrogen, Carlsbad, USA), and the concentration was adjusted to $2 \times 10^8$ copies with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and mixed with C33A (HPV negative) genomic DNA. The E6 and E7 regions of the 16 high-risk HPV types were confirmed on the basis of Genbank blast. All plasmid HPV DNA constructs were confirmed by PCR-amplified DNA sequencing.

2. Cell lines

SiHa, HeLa, and C33A cells were purchased from American Type Culture Collection (ATCC, Manassas, USA) and Korean Cell Line Bank (Seoul, Korea). SiHa cells and HeLa were cultured in Dulbecco’s MEM (DMEM) Eagle, with 10% fetal bovine serum (FBS), and standard antibiotics. C33A cells were cultured in Minimal Essential Medium (MEM) Eagle with 10% FBS, and standard antibiotics. Cells were counted using a T20eral Automated cell counter (Bio-Rad, Hercules, USA) according to manufacturer’s instructions. Each cell was measured by $10^6$ cells/mL.

3. DNA extraction

DNA was extracted from 1.0 mL of each counted cell line using the 5% chelax-100 resin solution with boiling 5 min [15-17]. Template DNA was stored at $-20^\circ$C until use.

4. Multiplex HPV E6/E7 real time PCR assay

Detection of HPV E6/E7 in extracted DNA was performed by real time PCR. HPV ViroCheck assay kit (Optipharm M&K, Osong, Korea) was performed using CFX-96 (Bio-Rad, Hercules, USA) real-time PCR systems for thermocycling and fluorescence detection, according to the manufacturers’ instructions. The PCR primers and the corresponding TaqMan® probes were designed for three different sets of HPVs, in each case targeting their common sequence (Group I: HPV genotypes 16, 31, 33, 35, 52, and 58; Group II: HPV genotypes 18, 39, 45, 51, and 68; and Group III: HPV genotypes 53, 56, 59, 66, and 69). Real-time PCR amplification for HPV E6/E7 gene was performed in a total volume of 20 μL containing 10 μL 2 × Thunderbird probe qPCR mix (Toyobo, Osaka, Japan), 5 μL primer and TaqMan probe mixture, 2 μL template cDNA, and 3 μL DW.
for each well.

The multiplex RT-qPCR assay detected HPV E6/E7 genes, simultaneously in three tubes by incorporating alpha 9, alpha 7, and alpha 5,6-specific TaqMan probes (HEX), HPV 16 and HPV 18 specific probes (FAM), and internal controls (CY5) labeled with different fluorophores. Positive controls were included throughout the procedure. No-template controls with sterile DW instead of template DNA were incorporated into each run. Cycling conditions were 95°C for 3 min, followed by 40 cycles of 95°C for 3 s and 55°C for 30 s. The detection of HPV was determined by the cycle threshold (Cq), which is the number of PCR cycles required for the fluorescence to exceed a value significantly higher than the background fluorescence. To avoid false negatives of HPV infection in samples, glycer-

5. Statistical analysis

All statistical analyses were performed using Prism 5 software (GraphPad, La Jolla, CA, USA) and SPSS statistics software version 21.0 (IBM, Armonk, NY, USA). Cq average, 95% confidence interval (CI), and coefficient of variation (CV) for HPV ViroCheck analytical sensitivity of cloned DNA and cell lines.
RESULTS

1. Analytical sensitivity in HPV copies of plasmid HPV DNA

The limit of detection (LoD), which indicate 95% detection rates, were determined according to the methods in the KFDA HPV guideline document. The cloned 16 HR HPV types plasmid DNAs with 1000 copies, 100 copies, 10 copies, and 1 copy were measured in replicates of 10 times, resulting in a total of 20 replicates, over 3 days by RT-qPCR. The representative 16 HR HPV types with 1000 copies, 100 copies, 10 copies, and 1 copy from real time PCR were shown in Figure 1. The 1 copy of LoD in HPV 16 and HPV 31 was shown. 10 copies of LoD were HPV 33, HPV 52, HPV 35, HPV 53, HPV 59, HPV 66, and HPV 69. 100 copies of LoD were HPV 18, HPV 39, HPV 45, HPV 51, HPV 56, HPV 58, and HPV 68. All of cloned 16 HPV types plasmid DNAs were detected below 100 copies/test and CV was below 3% (Table 1).

2. Analytical sensitivity in HPV infected cell lines

To find out how many cells from cervical cancer can be detected, the LoD of cervical cancer cell lines, which were HPV 16 infected SiHa, HPV 18 infected HeLa, and HPV negative C33A, was performed in replicates of 10 times, resulting in a total of 20 replicates, over 3 days by RT-qPCR. The mean of GAPDH in 20 replicates was 38.7 (95% CI 38.6 ~ 38.8) and 39.1 (95% CI 39.1 ~ 39.2) in SiHa

Table 1. Limit of detection for 16 high risk HPVs through 20 repeated tests

<table>
<thead>
<tr>
<th>HPV</th>
<th>Copy number of plasmids per test</th>
<th>1000 copies</th>
<th>100 copies</th>
<th>10 copies</th>
<th>1 copy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cq avg (95%CI)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>HP16</td>
<td>31.7 (31.6 ~ 31.8)</td>
<td>35.1 (35.0 ~ 35.2)</td>
<td>37.8 (37.6 ~ 38.1)</td>
<td>39.2 (39.0 ~ 39.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CV 0.6%</td>
<td>0.7%</td>
<td>1.6%</td>
<td>1.5%</td>
<td></td>
</tr>
<tr>
<td>HP31</td>
<td>32.5 (32.3 ~ 32.6)</td>
<td>35.0 (34.9 ~ 35.1)</td>
<td>37.1 (36.8 ~ 37.3)</td>
<td>39.2 (38.9 ~ 39.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CV 1.1%</td>
<td>0.9%</td>
<td>1.4%</td>
<td>1.5%</td>
<td></td>
</tr>
<tr>
<td>HP33</td>
<td>33.0 (32.9 ~ 33.1)</td>
<td>36.1 (35.8 ~ 36.4)</td>
<td>39.1 (38.8 ~ 39.3)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CV 0.6%</td>
<td>2.0%</td>
<td>1.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP52</td>
<td>32.9 (32.8 ~ 33.0)</td>
<td>36.2 (35.9 ~ 36.4)</td>
<td>39.0 (38.8 ~ 39.3)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CV 0.9%</td>
<td>1.3%</td>
<td>1.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP58</td>
<td>33.5 (33.4 ~ 33.6)</td>
<td>36.8 (36.7 ~ 37.0)</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CV 0.8%</td>
<td>1.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP39</td>
<td>32.8 (32.5 ~ 33.2)</td>
<td>36.6 (36.3 ~ 36.8)</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CV 2.5%</td>
<td></td>
<td>1.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP53</td>
<td>32.7 (32.6 ~ 32.8)</td>
<td>36.0 (35.8 ~ 36.3)</td>
<td>37.7 (36.8 ~ 38.6)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CV 0.9%</td>
<td>1.7%</td>
<td>5.7%</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>HP18</td>
<td>31.4 (30.9 ~ 32.0)</td>
<td>34.8 (34.5 ~ 35.1)</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CV 4.3%</td>
<td>2.2%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP56</td>
<td>31.5 (31.4 ~ 31.6)</td>
<td>35.0 (34.8 ~ 35.3)</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CV 0.7%</td>
<td>1.4%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP35</td>
<td>30.3 (30.2 ~ 30.4)</td>
<td>33.4 (33.3 ~ 33.5)</td>
<td>36.6 (36.3 ~ 36.9)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CV 1.0%</td>
<td>0.8%</td>
<td>1.9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP45</td>
<td>31.8 (31.7 ~ 31.9)</td>
<td>35.5 (35.3 ~ 35.6)</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CV 1.0%</td>
<td>1.1%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP51</td>
<td>33.4 (33.2 ~ 33.5)</td>
<td>36.9 (36.7 ~ 37.2)</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CV 1.1%</td>
<td>1.7%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP59</td>
<td>31.4 (31.3 ~ 31.5)</td>
<td>34.7 (34.5 ~ 35.0)</td>
<td>37.9 (37.6 ~ 38.2)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CV 0.8%</td>
<td>1.5%</td>
<td>2.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP66</td>
<td>31.5 (31.4 ~ 31.6)</td>
<td>34.7 (34.5 ~ 35.0)</td>
<td>38.1 (37.7 ~ 38.5)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CV 0.8%</td>
<td>1.6%</td>
<td>2.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP68</td>
<td>34.7 (34.5 ~ 35.0)</td>
<td>38.6 (38.3 ~ 38.9)</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CV 1.6%</td>
<td>1.8%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP69</td>
<td>33.7 (33.5 ~ 33.9)</td>
<td>37.3 (36.9 ~ 37.6)</td>
<td>39.4 (39.3 ~ 39.6)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CV 1.5%</td>
<td>2.3%</td>
<td>0.8%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Cq avg, average of quantification cycles in 20 repeated tests; 95% CI, 95% confidence interval; N/A, not amplified.
and HeLa cells and detected by 10 cells each. The mean of Group I in SiHa cell was 39.1 (95% CI 39.0 to 39.2) and the mean of Group II in HeLa cell was 38.9 (95% CI 38.9 to 39.0). Both were detected by 10 cells (Table 2).

3. Cross-reactivity for 16 HR HPV types

In order to confirm whether bacteria and virus, which are normal flora or sexually transmitted infectious agents in female genitalia show cross-reactivity, analytical specificity were performed with 23 bacteria and 23 virus including 16 HR HPV types. The $10^5$ PFU/mL for virus and $10^6$ CFU/mL for bacteria at medically significant concentrations with HPV negative C33A cell lines were used. C33A with bacteria or virus and without bacteria or virus alternately were repeated 3 times at one reaction and performed over 5 times, resulting in a total of 15 times. No cross reactions were confirmed for Groups I, II and III (Table 3).

4. Precision of plasmid HR HPV DNA

For precision test, a total number of measurements per sample was 144 (12 days × 2 runs/day × 2 replicates × 3 lots). HPV 16 clone DNA for FAM dye of Group I, HPV 33 clone DNA for HEX dye of Group I, HPV 18 clone DNA for FAM of Group II, HPV 39 clone DNA for HEX of Group II, HPV 53 clone DNA for HEX with 500 copies, 100 copies, and negative were used. The total within-run CV ranged from 0% to 0.2% for HPV 16, 0.2% to 0.3% for HPV 33, 0.1% to 0.4% for HPV 18, 0.1% to 0.3% for HPV 39, 0.4% to 0.5% for HPV 53, and 0.2 to 0.3 for HPV 56. The total between-run CV ranged from 0% to 0.2% for HPV 16, 0% for HPV 33, 0.3% to 0.4% for HPV 18, 0.1% to 0.2% for HPV 39, 0.3% to 0.4% for HPV 53, and 0.1 to 0.2 for HPV 56. The total before-between day CV ranged from 1.3% to 2.8% for HPV 16, 1.9% to 2.1% for HPV 33, 2.0% to 2.5% for HPV 18, 2.1% for HPV 39, 1.9% to 2.9% for HPV 53, and 2.5% to 3.2% for HPV 56. The total lot to lot CV ranged from 0.0% to 0.2% for HPV 16, 0.4% to 0.6% for HPV 33, 0.3% for HPV 18, 0.5% to 1.0% for HPV 39, 0.1% to 0.4% for HPV 53, and 0.5% to 0.6% for HPV 56 (Table 4).

DISCUSSION

HPV ViroCheck kit detecting HPV 16 and HPV 18 genotyping as well as 16 HR HPVs demonstrated a high degree of analytical sensitivity, analytical specificity, within laboratory precision. To assess the performance of the HPV ViroCheck assay, the cloning and sequencing of plasmid HPV DNA, which is confirmed to obtain the reliable results, was used as a reference standard. Also, HeLa and SiHa cell lines were confirmed, respectively. HeLa cell lines contain 10 to 50 copies of HPV 18 DNA/cell and SiHa cell lines contain 1 to 2 copies and HPV 16 DNA/cell from Mincheva et al study [18]. Considering the reference, HPV 18 was detected by 100 copies/test and HPV 16 was detected by 1 copy/test. Each minimum detection sensitivity of HR HPVs ranged from 1 copy/test to 1000 copies/test in both plasmid HR HPV DNA and HPV infection cells was same (Table 1 and Table 2) and shown the DNA concentration dependent HPV detection (Figure

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### Table 2. Limit of detection in cervical cancer cells

<table>
<thead>
<tr>
<th>Cells per test</th>
<th>1000</th>
<th>1000</th>
<th>100</th>
<th>10</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiHa (HPV 16) (N=20) GADPH Cq</td>
<td>28.3 (28.3 to 28.4)</td>
<td>31.6 (31.5 to 31.7)</td>
<td>35.1 (34.9 to 35.2)</td>
<td>38.0 (37.7 to 38.4)</td>
<td>38.7 (38.6 to 38.8)</td>
</tr>
<tr>
<td>Group I Cq</td>
<td>0.5%</td>
<td>0.8%</td>
<td>1.0%</td>
<td>2.1%</td>
<td>0.8%</td>
</tr>
<tr>
<td>SiHa (HPV 18) (N=20) GADPH Cq</td>
<td>29.1 (29.0 to 29.2)</td>
<td>32.5 (32.2 to 32.8)</td>
<td>36.0 (35.9 to 36.2)</td>
<td>38.0 (37.7 to 38.2)</td>
<td>39.1 (39.1 to 39.2)</td>
</tr>
<tr>
<td>Group II Cq</td>
<td>0.5%</td>
<td>2.3%</td>
<td>1.3%</td>
<td>1.7%</td>
<td>0.6%</td>
</tr>
<tr>
<td>HeLa (HPV 18) (N=20) GADPH Cq</td>
<td>29.2 (29.1 to 29.3)</td>
<td>32.6 (32.5 to 32.7)</td>
<td>36.4 (36.1 to 36.8)</td>
<td>38.9 (38.9 to 39.0)</td>
<td>N/A</td>
</tr>
<tr>
<td>Group I Cq</td>
<td>0.8%</td>
<td>0.7%</td>
<td>2.4%</td>
<td>0.4%</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Abbreviations: Cq, quantification cycles; N/A, not amplified.

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Therefore, the analytical detection limit of the assay was determined to provide enough fluorescence intensity to serve as the minimum detection sensitivity. Moreover, the analytical specificity was tested also using HPV negative cervical cancer cell line C33A along with 46 other types of microbial DNA including viruses and bacteria mixed with. There was no cross-reaction, demonstrating that the presence of other cervical related microbial DNA mixed with HPV DNA did not affect the performance of the assay (Table 3).

Currently, there are several commercially available HPV tests. Hybrid Capture2, which is approved by the FDA, is
### Table 4. Performance of inter-assay and intra-assay coefficients of variation

<table>
<thead>
<tr>
<th>Copies N</th>
<th>Total</th>
<th>Within-run</th>
<th>Between-run</th>
<th>Between-day</th>
<th>Lot to lot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cq avg SD CV (%)</td>
<td>Cq avg SD CV (%)</td>
<td>Cq avg SD CV (%)</td>
<td>Cq avg SD CV (%)</td>
<td>Cq avg SD CV (%)</td>
<td></td>
</tr>
</tbody>
</table>

**Group I** (HPV16)  
0 144 N/A - - N/A - - N/A - - N/A - - N/A - -  
100 144 33.9 0.6 1.80% 33.9 0 0.00% 33.9 0.1 0.20% 33.9 0.4 1.30% 33.9 0 0.00%  
500 144 31.3 1 3.00% 31.4 0.1 0.20% 31.3 0 0.00% 31.3 0.9 2.80% 31.3 0 0.20%  

**Group I** (HPV33)  
0 144 N/A - - N/A - - N/A - - N/A - - N/A - -  
100 144 34.9 0.7 2.10% 34.9 0.1 0.30% 34.9 0 0.00% 34.9 0.7 1.90% 34.9 0.1 0.40%  
500 144 32.3 0.8 2.40% 32.3 0.1 0.20% 32.4 0 0.00% 32.4 0.7 2.10% 32.4 0.2 0.60%  

**Group II** (HPV18)  
0 144 N/A - - N/A - - N/A - - N/A - - N/A - -  
100 144 34.9 1 2.80% 34.9 0.1 0.40% 34.9 0.1 0.30% 34.9 0.9 2.50% 34.9 0.2 0.60%  
500 144 31.9 0.7 2.30% 32 0 0.10% 31.9 0.1 0.40% 31.9 0.6 2.00% 31.9 0.1 0.30%  

**Group II** (HPV39)  
0 144 N/A - - N/A - - N/A - - N/A - - N/A - -  
100 144 35.9 0.9 2.50% 35.9 0 0.10% 35.9 0 0.10% 35.9 0.8 2.10% 35.9 0.2 0.50%  
500 144 33.2 0.8 2.30% 33.2 0.1 0.30% 33.2 0.1 0.20% 33.2 0.7 2.10% 33.2 0.3 1.00%  

**Group III** (HPV53)  
0 144 N/A - - N/A - - N/A - - N/A - - N/A - -  
200 144 36.6 0.8 2.20% 36.6 0.2 0.50% 36.6 0.1 0.40% 36.6 0.7 1.90% 36.6 0 0.10%  
1000 144 33.2 1 3.10% 33.4 0.1 0.40% 33.3 0.1 0.30% 33.3 1 2.90% 33.3 0.1 0.40%  

**Group III** (HPV56)  
0 144 N/A - - N/A - - N/A - - N/A - - N/A - -  
200 144 35.3 1.2 3.50% 35.3 0.1 0.20% 35.3 0.1 0.20% 35.3 1 3.20% 35.3 0.2 0.60%  
1000 144 32.4 0.9 2.70% 32.4 0.1 0.30% 32.4 0 0.10% 32.4 0.8 2.50% 32.4 0.2 0.50%  

**Abbreviations:** N, test number of inter- and intra-assay; Cq, Quantification cycles; CV, coefficients of variation; SD, standard deviation; N/A, not amplified.

Detected by 5000 copies of HPV per assay [19]. However, HC2 lacks internal standards to assess sample quality and cannot detect genotypes of HPVs [20–22]. HPV ViroCheck includes internal control in each well to see whether samples contain PCR inhibitors. Also, major HPV 16 and HPV 18 genotypes can be detected simultaneously. Recently, new genotyping kits using a variety of measuring instruments have been commercialized: Cobas™ HPV test (Roche Diagnostics Gmbh), the Cervista™ HPV HR test (Hologic), and the Abbott Real time high risk HPV test (Abbott Laboratories, IL, USA) performed by real-time PCR assay [23,24]. Further, comparative testing is necessary to assess the relative advantages of these systems.

Therapeutic vaccines using HPV E6/E7 have been generated to treat HPV-associated lesions and cancers, and focus on its efficacy in clinical trials. The DNA vaccine pNGVL4a-sig/E7(detox)/HSP70 DNA vaccine was shown to enhance the HPV-16 E7 antigen-specific T cell mediated immune responses in a preclinical model [24]. Another therapeutic vaccine GX-188E, a therapeutic HPV DNA vaccine engineered to express HPV16 and HPV18 proteins E6/E7 fused to the extracellular domain of Flt3L. The clinical trial Phase I with 9 volunteers demonstrated GX-188E is safe and well tolerated by patients and shown statistically significant cellular immune response and three patients showed a weak antibody response against E7 protein [25]. The other HPV vaccine was VGX-3100. VGX-3100 is a combination of two plasmids encoding optimized HPV16 and 18 E6 and E7 antigens [26]. VGX-3100 was administered through intramuscular injection followed by electroporation to 18 female patients who had been previously treated for CIN2/3 lesions [27]. Each patient received three rounds of vaccination, which was well tolerated with no observed dose-limiting toxicities. As therapeutic vaccines using HPV E6/E7 were developed, the complementary diagnosis for follow-up of vaccine efficacy will be needed.

The HPV ViroCheck assay uses the fluorescence to detect specific HPV 16 and HPV 18 with FAM dye and 16 HR HPVs with HEX dye in only three wells. The advantages of the rapid and handy screening method. For HPV genotyping or detection, an appropriate assay should be selected on the basis of the specific purpose and conditions of each laboratory. The HPV ViroCheck genotyping kit offers another valuable option for use in the future.
요 약

인간유두종 바이러스 (HPV)는 자궁경부암의 주요 원인이며, 자궁경부암의 주요 원인 바이러스는 16종의 고위험군 유전자 HPV 16, HPV 18, HPV 33, HPV 35, HPV 39, HPV 51, HPV 53, HPV 56, HPV 58, HPV 59, HPV 66, HPV 68, HPV 69 이다. 특히, HPV 16형과 HPV 18형이 HPV 양성 암환자의 70%에서 발견된다. 따라서, 바이러스의 존재 유무를 확인하는 것은 환자의 스크리닝에 도움을 주며, 최근에 세포학적 검사와 함께 보조적인 검사법으로 사용되고 있다.

본 연구는 16종의 고위험군 바이러스와 HPV 16, HPV 18 유전자형을 검출 할 수 있는 HPV ViroCheck의 발암 유전자의 분석 성능을 확립하기 위한 목적으로 수행되었다. 먼저, 16종의 고위험군 HPV의 발암유전자 E6/E7 유전자형의 검출한계를 확인하기 위하여, 분석적 민감도를 수행하였다. 그리고, 관련된 미생물 및 바이러스에서의 교차반응 및 정확도를 비교하여 평가하였다.

고위험군 HPV 유전자형의 민감도는 Clone DNA를 이용 하였을 때, 최대 1카피에서 100 카피까지 검출이 가능하였고, SiHa 세포와 Hela 세포의 경우 최소 10 세포까지 검출이 가능하였다. 그리고, 관련된 미생물 및 바이러스에서의 교차반응을 비교하여 평가하였다.

고위험군 HPV 발암유전자 이질도는 Clone DNA를 이용 하였을 때, 최대 1카피에서 100 카피까지 검출이 가능하였고, SiHa 세포와 Hela 세포의 경우 최소 10 세포까지 검출이 가능하였다. 그리고, 관련된 미생물 및 바이러스에서의 교차반응을 비교하여 평가하였다.

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