

# Cervical Cancer Screening after Perimenopause: How Is Human Papillomavirus Test Performed?

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Cervical cancer is the third most prevalent cancer in women around the world. Recently in Korea, the incidence of cervical cancer has decreased, but in all stages of cervical intraepithelial neoplasia (CIN), CIN has shown a 91% increase from 1999 to 2008. Persistent human papillomavirus (HPV) infection has been found to be the main cause of cervical cancer. HPV types 16 and 18 have been found in 70% of cervical cancer patients around the world. Cervical cancer screening such as cytology has limitations in terms of sensitivity and specificity. A discussion about the need for the HPV test is becoming active in order to compensate for the limitation of cytology. After the role of HPV in cervical cancer was identified, the importance of HPV detection test as a screening was emphasized. Several tests have been developed and each test has its own advantages and disadvantages, and new test method to overcome the disadvantages is still being developed. Today's guidelines and tests are those you would choose from among the large number of cervical cancer screening guidelines and tests, based on the consideration that the selected guidelines and the test are effective. (**J Menopausal Med 2016;22:65-70**)

**Key Words:** Early detection of cancer · Human papilloma virus · Perimenopause · Uterine cervical neoplasms

Cervical cancer is the third most prevalent cancer in women around the world, and the fourth most common cause of cancer-related deaths in women.<sup>1</sup> Although reduction in mortality has been achieved in developed countries due to effective screening which has reached up to 25 million women worldwide every year, mortality rates in the developing world, including Asia, amount to 80% in the patients. An incidence more than three times compared to that in the Western world indicates a high incidence.<sup>2</sup> Recently in Korea, the incidence of cervical cancer has decreased, but in all stages of cervical intraepithelial neoplasia (CIN), CIN has shown a 91% increase from 1999 to 2008 according to the National Cancer Registry statistics.<sup>3</sup> The advent of cytology has had a tremendous impact on cervical cancer rates—reduction in these rates. Still, 989

Korean women die of cervical cancer each year,<sup>3</sup> although it is a theoretically preventable disease. But, cytology is relatively limited. More than 150 different types of human papillomavirus (HPV) have been identified, and among these, 60 types have genotypes which can cause infection of the human genital organs.<sup>4,5</sup> Persistent HPV infection has been found to be the main cause of cervical cancer.<sup>4</sup> Thus, the need for HPV genotyping methods as a screening test to aid the early detection and intervention is increasing.<sup>6</sup> Recently, the U. S. Food and Drug Administration (FDA) approved the first HPV DNA test for women aged 25 years or older that can be used alone as a screening test for uterine cervical cancer.<sup>7</sup> Many countries have used cytology as a screening for uterine cervical cancer to support the national fund. However, the screening guidelines are different, and there

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is a difference in the timing or number of times. In recent years, the screening test has been used to compensate for cytology, including the HPV test or the HPV test alone. We would like to discuss this point.

## HPV and Infection

HPV is a 55 nm in diameter, non-enveloped double-stranded DNA virus that infect epithelial cells such as those in the skin and mucous membranes.<sup>8</sup> HPV, multiplies in the nucleus as an episome, and is primarily found in the cytoplasm in that form.<sup>9</sup> HPV genome is a circular DNA double-stranded chain of about 8 kb, an open reading frame (ORF) is applied to the transformation of its DNA replication and E1, E2, E4, E5, E6, E7 genes.<sup>9</sup> More than 150 different types of HPV have been confirmed till today and among these 60 types have genotypes which can cause infection of the human genital organs. About one in four women will get infected with the types of HPV that are related to cervical cancer in their lifetime, although most of the HPV infected people do not have any signs.<sup>10</sup> HPV is classified into three groups according to its carcinogenic potential; high-risk (HR), probable HR, low-risk (LR).<sup>11</sup> Genotypes that belong to the HR group are 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 types, and genotypes that belong to the probable HR group are 26, 53, and 66 types, and genotypes that belong to the LR group are 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, and 81 types.<sup>10</sup> Nearly all cases of cervical cancer are caused by infection with oncogenic or HR type of HPV. HPV types 16 and 18 have been found in 70% of cervical cancer patients around the world. LR HPV types are commonly detected in genital warts.<sup>10</sup> These sexually transmitted HPV viruses also cause most of the anal cancers; many vaginal, vulvar, and penile cancers; and some oropharyngeal cancers.<sup>10</sup> Although HPV infection is very common, most infections will be suppressed by the immune system within 1 to 2 years without causing cancer.<sup>11</sup> If a cervical infection with a HR HPV type persists, the cellular changes can eventually develop into more severe precancerous lesions.<sup>12</sup> If precancerous lesions are not treated, they can progress to cancer.<sup>13</sup> It can take 10 to 20 years or more for a persistent infection with a HR HPV type

to develop into cancer.<sup>14</sup> Persistent HPV infection has been found to be the main cause of cervical cancer.<sup>4</sup>

## Limitation of the Cytology (the Papanicolaou Test [Pap Test])

Precancerous lesion of cervical cancer can be cured in 98% of patients.<sup>15</sup> Once cancer develops or transition occur the five-year survival rate is 20%. Cervical cancer is more important than the lesions detected early through regular screening. Currently in Korea, the primary screening test for cervical cancer is the Pap test for observing the cells scraped off the uterine cervix, but traditional Pap smear has limitations in terms of sensitivity and specificity. A discussion about the need for the HPV test is becoming active in order to compensate for the limitation of cytology. Screening guidelines for HPV test in Korea stated that women younger than 30 years should not be screened because of the high false positive rate and infection clearance rate of HPV test (Table 1). In women more than 30 years of age, HPV test cannot replace cytology, but co-testing (cytology and HPV test) is recommended because it can yield a false negative cervical cytology. In women with HR HPV types 16 and 18 in whom a normal Pap smear was obtained, the probability of developing precancerous cervical lesions is 35 times higher.<sup>16</sup>

## HPV Detection Method

Many methods have been developed for molecular detection of HPV. Among HPV tests, only 5 HPV detection methods were approved by the FDA in United States until the year 2015.<sup>17</sup> These five HPV tests include 3 polymerase chain reaction (PCR) tests, HPV E6/E7 RNA test, and HPV DNA chip test.

### 1. Direct probe methods or non-amplified hybridization assay

Here, the in situ hybridization (ISH) method, detection of a specific DNA by using a radioisotope or fluorescent substance-covered DNA probe, is involved.<sup>18</sup> The presence

**Table 1.** Human papillomavirus test: current screening guidelines (2012)

Population	Korean guideline	ACS/ASCCP/ASCP
Younger than 21 years		Women should not be screened regardless of the age of sexual initiation or other risk factors.
21–29 years	Women should not be screened because of HPV test with high false positive rate and infection clearance rate	Screening with cytology alone every 3 years is recommended.
30–65 years	Screening with cytology alone every 1 year is recommended. HPV test could not take the place of cytology. However, co-testing is recommended because it can give a false negative cervical cytology.	Screening with cytology and HPV testing every 5 years or cytology alone every 3 years is recommended.

ACS: American Cancer Society, ASCCP: American Society for Colposcopy and Cervical Pathology, ASCP: American Society for Clinical Pathology, HPV: human papillomavirus

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of the virus within the cell or chromosome and the site of infection can be confirmed by this method. But, it is rarely used due to the difficult technique required and low sensitivity, despite high specificity.<sup>19</sup> Other methods including Southern transfer hybridization (STH), dot blot (DB) and filter hybridization are also not used due to their low recall ratio.<sup>20</sup>

## 2. Signal amplification

Digene<sup>®</sup> Hybrid Capture 2 (HC2) HR HPV DNA Test and Cervista<sup>™</sup> HPV 16/18, Cervista<sup>™</sup> HPV HR, approved by the FDA in 2003 and 2009 respectively, are included here. HC2 is based on the liquid hybridization method using HR or LR specific RNA probe.<sup>19</sup> The patient's sample is mixed with HR RNA probe mixtures (probe cocktails) to form specific HPV DNA–RNA hybrids, which are placed in the microtiter plate covered by a specific antibody and then captured. These immobilized hybrids are bound to alkaline phosphatase–attached antibody, which is measured by detection of fluorescent material. Cervista<sup>™</sup> HPV 16/18, Cervista<sup>™</sup> HPV HR uses the invader method to measure the amplified signal produced by hybridization with nucleic acid (NA) probe.

## 3. Target amplification

The target HPV DNA is selectively amplified using PCR technique and detected by gel electrophoresis, DB, line–strip

hybridization, matrix assisted laser desorption/ionization time–of–flight (MALDI–TOF), or real–time PCR. The sensitivity and specificity of PCR may vary depending on primer sets, size of the amplified PCR product and DNA polymerase.<sup>21</sup> PCR has been recognized as the most sensitive method to detect HPV, which is also possible with small amount of sample. It can also detect type–specific HPV infection; thus, multiple infections can be confirmed.<sup>19,20</sup> This method is used in the restriction fragment mass polymorphism (RFMP) assay, Aptima<sup>®</sup> HPV 16 18/45 genotype assay, Aptima<sup>®</sup> HPV assay, Cobas<sup>®</sup> HPV Test, and Roche Linear Array. Among these, Aptima<sup>®</sup> HPV 16 18/45 Genotype Assay, Aptima<sup>®</sup> HPV Assay and Cobas HPV Test were approved by the FDA in 2012. Although L1 consensus primers are used in most tests, Aptima<sup>®</sup> uses amplified E6/E7 viral mRNA for the detection.

## Value of HPV Testing in Preventing Cervical Cancer after Perimenopausal Women

The Korean HPV screening guidelines, stated that HPV test cannot replace cytology. However, co–testing (cytology and HPV test) is recommended because it can yield a false negative cervical cytology when used alone

(Table 1). In our country, all women more than 30 years of age undergo cytology without any cost every two years for national insurance. Although physicians are aware of the importance of the HPV DNA test, the public is still not aware of its importance; hence, primary screening with the HPV test is restricted and limited. The application of the test for detecting HR HPV types has allowed clinicians to identify women at highest risk of cervical cancer more accurately and earlier during the progression of the disease.<sup>17</sup> Cytology, while valuable, merely detects abnormal cellular changes which may be the consequences of HPV infection or possibly inflammation.<sup>22</sup> HPV DNA testing is less subjective and more sensitive in detecting precancerous lesion than cytology alone, particularly for the detection of adenocarcinoma precursors. In a systematic evidence review of randomized studies conducted for the U.S. Preventive Services Task Force, HPV DNA testing was found to demonstrate an average of 35.7% greater sensitivity than cytology in the detection of CIN2 or CIN3.<sup>17</sup> Based on these data, it should come as no surprise that the potential benefit of HPV DNA testing have been recognized in the U.S. guidelines, to help clinicians identify and triage women at the highest immediate risk of high-grade cervical disease.<sup>15</sup> Lesions more than CIN2 in post-menopausal women are not recognized by a single Pap smear, but a large fraction of pre-invasive cervical cancer cases in post-menopausal women result from infections by HPV types.<sup>23</sup> There is no difference in the screening tests according to the menopausal condition. The HR HPV test was three times as sensitive as the Pap test in detecting grade 2 and 3 CIN lesions in women aged 50 to 65 years.<sup>24</sup> Postmenopausal women are infected with persistent oncogenic HPV at a substantial rate, supporting the need for continued screening in postmenopausal women to detect pre-neoplastic genital lesions.<sup>25</sup> In postmenopausal women, cervical cancer primary screening with HPV test show various results, but cervical cancer screening can help to determine future directions of the HR treatment. The major cause of cervical cancer is HPV, and early diagnosis and early treatment are possible, and it is a possibly curable disease. Cervical cancer screening guidelines differ from country to country, but cervical cancer prevention and early detection is done through the national support programs. Current guidelines

recommend that all women 21 years or older with atypical squamous cells of undetermined significance (ASCUS) cytology should be tested for HR HPV, and that only those women with ASCUS who are positive for HR HPV should be referred for colposcopy.<sup>15</sup> Also, in women aged 30 to 65 years, co-testing using the combination of Pap cytology plus HPV DNA testing is the preferred cervical cancer screening method.<sup>15</sup> In Korea, women aged more than 20 years, Pap test is recommended every year.<sup>26</sup> After the role of HPV in cervical cancer was identified, the importance of HPV detection test as a screening test was emphasized. Several tests have been developed and each test has its own advantages and disadvantages, and a new test method to overcome the disadvantages is still being developed. As the main aim of cervical cancer screening is to prevent morbidity and mortality from cervical cancer, the ideal screening test should be able to identify cervical cancer precursors likely to progress to invasive cancer, while minimizing unnecessary treatment for LR types. Today's guidelines aim to do that, while balancing varying levels of risk for women of different ages and encouraging clinicians to make recommendations based on individual patient histories. Today's guidelines and tests are those you would choose from among the large number of cervical cancer screening guidelines and tests, based on consideration that the selected guidelines and tests are effective. According to the menopausal condition, there is no difference in the screening tests for cervical cancer, and HPV test can help to determine future directions of the HR treatment. But, how it will recommend the HPV test as cervical cancer screening? The choice of tests will depend upon you.

In conclusion, HPV test can be used in cervical cancer screening. Also, depending on the HPV DNA test results may be further performed cytology and colposcopy.

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## Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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