

# Comparison of human papillomavirus (HPV) detection in urine and cervical swab samples using the HPV GenoArray Diagnostic assay

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Human papillomavirus (HPV) is the leading cause of cervical cancer. Urine-based HPV testing would be a simple and non-invasive method. It may have the advantage of increasing acceptance of screening. A total of 164 pairs of cervical swab and urine samples from Thai women who underwent cancer screening were used for HPV testing with HPV GenoArray Diagnostic Kits. The overall concordance percentage for HPV detection in the cervical swab and urine samples was 65.2%. The HPV genotypes most commonly detected were HPV16 and HPV18. An analysis of the urine samples and a second analysis of the cervical swab samples showed that the differences in the overall HPV detection rate between women with normal and abnormal cytology were not significant ( $p > 0.05$ ). Urine samples processed with the HPV GenoArray assay may be useful for the clinical management of HPV infection and the technique could be an accurate, noninvasive method for monitoring HPV infections in women.

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# 21 ABSTRACT

22 Human papillomavirus (HPV) is the leading cause of cervical cancer. Urine-based HPV testing  
 23 would be a simple and non-invasive method. It may have the advantage of increasing acceptance  
 24 of screening. A total of 164 pairs of cervical swab and urine samples from Thai women who  
 25 underwent cancer screening were used for HPV testing with HPV GenoArray Diagnostic Kits.  
 26 The overall concordance percentage for HPV detection in the cervical swab and urine samples  
 27 was 65.2%. The HPV genotypes most commonly detected were HPV16 and HPV18. An analysis  
 28 of the urine samples and a second analysis of the cervical swab samples showed that the  
 29 differences in the overall HPV detection rate between women with normal and abnormal  
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# 41 INTRODUCTION

42 Human papillomavirus (HPV) causes cervical cancer (*Koutsky, 1997*). Approximately 170  
43 genotypes have been identified (*De Villiers et al., 2004*) and at least 40 genotypes infect the  
44 human anogenital tract (*De Villiers, 2013*). The genital HPVs are classified into high-risk and  
45 low-risk genotypes depending on their association with uterine cervical cancer (*Muñoz et al.,*  
46 *2003*). The high-risk genotypes most commonly detected in uterine cervical cancer are HPV16,  
47 18, 31, 33, 35, 45, 52, 58, 39, 51, 56, and 59 (*Bouvard et al., 2009*).

48 The Papanicolaou (Pap) test is a cost-effective way to screen for cervical cancer. The test  
49 results help physicians to detect precancerous lesions and determine the course of treatment. Pap  
50 test has been shown to reduce the incidence of mortality from cervical cancer (*Mählck et al.,*  
51 *1994*). However, it is primarily used for detecting invasive cervical cancer and cannot identify  
52 asymptomatic HPV infection (*Safaeian et al., 2007; Leyden et al., 2005*). Nevertheless,  
53 supplementary HPV DNA assays are often used in combination with the traditional Pap smear  
54 test (*Cox et al., 1995*).

55 HPV DNA detection in urine samples may be a feasible alternative to HPV DNA detection in  
56 cervical specimens. Urine collection could provide an especially simple, non-invasive method  
57 for screening (*Prusty et al., 2005*). The benefits of using urine for HPV DNA detection have  
58 been evaluated in disease surveillance and screening for cervical cancers involving specific  
59 genotypes. HPV DNA urine testing can be used to identify abnormal cells in adolescent girls and  
60 young women who do not wish to have a vaginal examination (*Vorstors et al., 2014; Enerly et*  
61 *al., 2013*). Some studies have reported a high HPV detection sensitivity for urine-based assays  
62 (*Forslund et al., 1993; Hagihara et al., 2016; Bernal et al., 2014*), while other studies have

reported a low HPV detection sensitivity from urine-based assays (*D'Hauwers et al., 2007*;  
*Nilyanimit et al., 2013*).

Molecular methods for HPV testing have been explored, such as PCR/sequencing (*De Roda  
Husman et al., 1995*), the INNO-LiPA HPV Assay (*Van Hamont et al., 2006*), and the Hybrid  
Capture 2 test (HCII) assay (*Kubota et al., 1998*). In addition, the HPV GenoArray Diagnostic  
Kit (Hybribio Ltd., Sheung Wan, Hong Kong) is a recently developed PCR-based HPV  
genotyping assay, which uses L1 consensus primers to amplify 21 HPV genotypes. It is then  
followed by flow-through hybridization with immobilized genotype-specific probes. This test is  
currently used in several hospitals in China (*Liu et al., 2010*). A previous study showed that the  
sensitivity of the HPV GenoArray assay was 97.8% and the specificity was 100%. (*Juan et al.,  
2013*).

The aim of this study was to evaluate the use of a urine-based assay as a non-invasive method  
for HPV detection and to genotype the samples using the HPV GenoArray assay.

# **MATERIALS & METHODS**

The research protocol was approved by the Ethics Committee of the National Cancer Institute, Bangkok, Thailand (number EC COA 037/2012), and the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (number 389/2555). The objective of the study was explained to the patients, and written consent was obtained from all participants. Each specimen was sent to be tested in an anonymized way, with a participant-specific numerical code and the participant's age.

## **Clinical specimens**

The 164 pairs of specimens (a Pap smear sample from the cervix and a first-void urine sample) were divided into three groups: 95 samples indicating normal cytology, 50 samples indicating low-grade squamous intraepithelial lesions (LSIL), and 19 samples indicating high-grade squamous intraepithelial lesions (HSIL). The ages of the patients enrolled in this study were between 19 and 69 years.

## **Sample preparation**

Each Pap smear sample (which are the standard samples for HPV genotyping) was evaluated by a specialized cytotechnologist and the results were confirmed by a pathologist. All the Pap smear samples were kept in liquid-based cytology (LBC) buffer (ThinPrep, Hologic, Marlborough, MA, USA).

The participants were asked to collect first-void urine (FVU) samples of 30–50 mL in a sterile Cell PrepPlus (Biodyne, Gyeonggi-do, Korea) urine bottle. Samples were stored at 4 °C and processed within 3 days. Approximately 15 mL of each FVU was centrifuged at 3000 rpm for 5

min, and the supernatant was removed. Subsequently, 800  $\mu$ L of the sample was added to a 1.5-mL tube to wash the pellet, the sample was centrifuged at 8000 rpm for 5 min, and the supernatant was removed. The DNA was extracted from the cervical swabs and urine pellets using a DNA Prep Kit (Chaozhou Hybribio Biochemistry Ltd., Guangdong, China) and stored at -20°C until testing.

### **HPV GenoArray Diagnostic Assay**

The extracted DNA from the cervical swab and urine samples was subjected to an HPV genotyping assay using HPV GenoArray Diagnostic Kits (Hybribio Ltd., Sheung Wan, Hong Kong). These kits utilized both DNA amplification and a flow-through hybridization technique to identify 21 HPV genotypes including 13 high-risk types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68), two probable high-risk types (HPV 53 and 66), and six low-risk or unknown risk types (HPV 6, 11, 42, 43, 44, and CP8304 [HPV-81]). This assay uses an L1 consensus primer-based PCR and is different from the Linear Array HPV Genotyping Test, which uses MY09 and MY11 primers (*Liu et al., 2010*). Subsequently, a flow-through hybridization on a nylon membrane covered in immobilized HPV genotype-specific oligonucleotides probes was performed. The presence of a positive result for the internal control and the biotin dots within the membrane indicates DNA quality, good enzyme conjugate, and successful hybridization process. Results were manually interpreted using the manufacturer's guidelines. The normal detection limit is ~500 copies/ $\mu$ L of target HPV DNA. Cross-reactivity from the amplification/detection of the 21 HPV genotypes was not reported.

### **Statistical methods**

122 A statistical analysis was performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).  
 123 Pearson's chi-square test for matched pairs was used to compare the performance of the two  
 124 types of samples regarding the detection of HPV genotypes. Statistical significance was defined  
 125 as  $p < 0.05$ .

# RESULTS

The mean age of the 164 participants was 45.8 years. Among the women with normal cytology, the mean age was 50.5 years, while among those with abnormal cytology (LSIL or HSIL), the mean age was 41.1 years. The pairs of cervical swab and urine samples were suitable for analysis for this study because the biotin and internal controls were positive, which means that PCR amplification would be effective.

According to the cervical swab samples, 65 of the 164 samples (39.6%) were HPV DNA positive (Table 1). In total, 18 (11.0%) contained multiple HPV genotypes. The most common HPV genotypes detected were HPV16 (12 samples) and HPV18 (8 samples). Thus, 20 of the 164 samples (12.2%) contained either HPV16 or HPV18. In the normal cytology group, 11 of the 95 samples (11.6%) were HPV DNA positive. In contrast, the LSIL and HSIL groups had 35 (10.0%) and 19 (100.0%) HPV DNA-positive samples, respectively.

Regarding the urine samples, 53 of the 164 samples (32.3%) were HPV DNA positive (Table 1). Of all the urine samples, 13 (7.9%) had multiple HPV genotypes. The most commonly detected HPV genotypes were HPV18 (17 samples) and HPV16 (four samples). In total, 20 of the 164 samples contained HPV16 or HPV18 (12.2%). In the normal cytology group, 10 of the 95 samples (10.5%) were HPV DNA positive. In contrast, the LSIL and HSIL groups had 35 (10.0%) and 8 (42.1%) HPV DNA-positive samples, respectively.

Pap smear samples have been used as standard samples for detecting HPV. The aim of this study was to evaluate the efficacy of detection using urine compared with detection using the standard sample. Comparing the cervical swab and urine specimen results, the overall concordance was 65.2% (107/164). There was a concordance of 71.6% (68/95) in the normal cytology group and a concordance of 56.5% (39/69) in the abnormal cytology group (Table 1).

The sensitivity and specificity of the urine-based assay (using HPV GenoArray Detection Kits), using the Pap smear results as the reference, were 56.5% and 70.6%, respectively. The sensitivity and specificity from this study was lower than other studies (Table 2). The positive and negative predictive values were 53.8% (95% CI = 41.9 to 65.4) and 72.7% (95% CI = 63.2 to 80.5), respectively.

Regarding multiple HPV infections, the cervical swab-based assays were able to detect more HPV genotypes in each sample. However, in the normal cytology group, for each pair of biospecimens, the most common number of genotypes per sample was one. Similarly, in the abnormal cytology group, 36 of the 69 cervical swab samples (52.2%) and 31 of the 69 urine samples (44.9%) had a single genotype (Table 3).

An analysis of the urine samples and a second analysis of the cervical swab samples showed that the differences in the overall HPV detection rate between the normal and abnormal cytology groups were not significant ( $p > 0.05$ ).

# DISCUSSION

As a result of the use of the Pap test, screening attendance remains low (*Gakidou et al., 2008*), while the estimated incidence of invasive cervical cancer remains high (*Leyden et al., 2005*). In Thailand, 25–38% of women aged 30–65 years have had only one Pap test (*Sriamporn et al., 2006*). When cervical testing for HPV is required, these results suggest that urine sample collection could be an alternative non-invasive sampling method for monitoring HPV infection in women. In a previous study, the overall percentage agreement between HPV detection in urine and cervical samples was 88% (*Bernal et al., 2014*) and, in this study, the percentage was 65.2%. However, the results must be interpreted with caution owing to variation between the studies in terms of the participant characteristics, surrogate nature of using cervical HPV detection to screen for cervical disease, and lack of standardized urine testing methods.

Urine sample assays cannot be used to detect all of the genital HPV infections, but these assays provide an alternative for use in epidemiological surveys in which invasive sampling is difficult to perform; in these cases, testing urine for HPV DNA could be considered (*Prusty et al., 2005*). Previous studies have compared HPV detection rates between cervical and urine samples in order to evaluate the ability of urine-based assays to detect the prevalence of HPV independently of cervical cytology assays (*Daponte et al., 2006; Munoz et al., 2013*). Research indicates that the sensitivity of urine testing for HPV 16 and 18 was higher for participants with cervical cancer (88.8%) than for those with high- and low-grade lesions (*Daponte et al., 2006*). These data showed that HPV DNA detection in urine samples from most groups of patients (HSIL, Normal) was lower than detection in cervical swabs. The reasons for this could be low-efficiency amplification (due to the presence of inhibitory substances in the urine), HPV DNA

loss during urine processing, or the urine samples being truly HPV DNA negative (*Brinkman et al., 2004*).

The HPV DNA analysis of urine samples needs to be developed further before a urine-based assay can replace the Pap smear test. It is possible that a greater amount of urethral cells in the urine samples could increase the sensitivity of the test. An analysis of the urine samples and a second analysis of the cervical swab samples showed that the differences in the overall HPV detection rate between women with normal and abnormal cytology were not significant ( $p > 0.05$ ). This result suggests that urine could be used as a substitute for cervical swabs. However, the urine samples should be optimized by preventing DNA degradation during extraction and storage, recovering cell-free HPV DNA in addition to cell-associated DNA, processing a sufficient volume of urine, and collecting the first portion of the urine stream in the morning (*Vorsters et al., 2014*).

Using traditional cytological analysis, it is difficult to determine accurate screening results for HPV-associated anogenital tumors. Therefore, HPV genotyping is an alternative screening method to be used in combination with traditional cytology for identifying patients at high risk of developing squamous cell carcinoma (*Saslow et al., 2012; WHO, 2013*). Nowadays, there are many HPV genotyping techniques for detecting HPV DNA, such as PCR, real-time PCR, restriction fragment length polymorphism (RFLP), Hybrid Capture, and Linear Array (*Bernard et al., 1994; Cox et al., 1995; Castle et al., 2008*). However, PCR and real-time PCR need specific expensive equipment (such as a thermal cycler), and these methods have not yet become common procedures in hospital laboratories (*Hagiwara et al., 2007*). This study used the HPV GenoArray Diagnostic Kit for HPV genotyping, which is a commercial kit that has recently been started to be used, especially in China (*Liu et al., 2010*). The results from the HPV GenoArray

assay used in this study were a percentage-point (39.6%) higher compared to the results from a previous survey of Thai women (7.6%) (*Chansaenroj et al., 2010*) and one of Japanese women (22.5%) (*Onuki et al., 2009*). The higher percentage may be due to the small number of participants in our study sample.

The Linear Array HPV Genotyping Test has been widely used as a standard reference method for evaluating new methods. However, the HPV GenoArray Diagnostic Kit is an alternative technique for studies conducted in resource-limited laboratories because the cost of the HPV GenoArray Diagnostic Kit is lower than that of the Linear Array HPV Genotyping Test and the hybridization time is also lower (*Li et al., 2012; Liu et al., 2010*). Moreover, the HPV GenoArray assay can distinguish and identify HPV 52, which is one of the most common high-risk HPV genotypes in women in eastern and southeastern Asia (*Sukvirach et al., 2003; Takehara et al., 2011*).

In conclusion, the HPV GenoArray assay was shown to possess reliable clinical performance for HPV genotyping both cervical swab and urine samples. Urine assays could be a useful noninvasive method for monitoring HPV infection in women and the use of this method may increase the number of women who undergo cervical cancer screening, as some women avoid this critical preventive screening because of the embarrassment and discomfort associated with the traditional Pap smear method. Additional research based on a larger sample from a general screening population is required.

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## **Conflict of Interest**

The authors have no conflicts of interest to declare.

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## **TABLES**

**Table 1.** Detection of HPV genotypes and concordance between cervical swab and urine

samples.

**Table 2.** Studies of human papillomavirus DNA detected in paired urine and cervical samples

from females of all ages.

**Table 3.** Number of HPV genotypes detected using the HPV GenoArray assay.

**Table 1.** Detection of HPV genotypes and concordance between cervical swab and urine samples.

Cytology	Specimen (% positive)				Concordance (percentage)
	Any HPV positive		HPV16&18		
	Cervical swab	Urine	Cervical swab	Urine	
Normal (N=95)	11 (11.6)	10 (10.5)	1 (1.1)	4 (4.2)	68 (71.6)
LSIL (N=50)	35 (10.0)	35 (10.0)	11 (22.0)	13 (26.0)	31 (62.0)
HSIL (N=19)	19 (100.0)	8 (42.1)	8 (42.1)	3 (15.8)	8 (70.5)
Total (N=164)	65 (39.6)	53 (32.3)	20 (12.2)	20 (12.2)	107 (65.2)

405 **Table 2.** Studies of human papillomavirus DNA detected in paired urine and cervical samples from females of all ages.

Author	Country	HPV detection assay	Age, years range	Total sample size	Lesion/HPV types	Sensitivity (%)	Specificity (%)	Concordance (%)
Struss et al. (1999)	UK	PCR with MY and GP primers	16–57	144	All/any type	76.4	73.3	75.7
Daponte et al. (2006)	Greece	In house type-specific primers and commercial	N/A	77	All/HPV16/18	70.3	100.0	85.7
Gupta et al. (2006)	India	In house L1 consensus primers	N/A	30	All/any type	100.0	100.0	100.0
Cuschieri et al. (2011)	UK	HPV INNO–LiPA	16–25	90	All/any type	90.5	67.6	59.8
Nilyanimit et al. (2013)	Thailand	Electrochemical DNA chip	27–61	116	All/HR-HPV	64.3	100.0	75
Bernal et al. (2014)	Spain	Cobas 4800HPV test	21–65	125	All/any type	90.5	85	88
Hagihara et al. (2016)	Japan	Anyplex II HPV28	19–58	240	All/any type	68.4	99.9	98.4
This study	Thailand	HybriBio GenoArray	19–69	164	All/any type	56.5	70.6	65.2

406  
407 N/A, not applicable.  
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411 **Table 3.** Number of HPV genotypes detected using the HPV GenoArray assay.

No. of HPV genotypes detected	Normal (N=95)		Abnormal (N=69)	
	Cervical swab	Urine	Cervical swab	Urine
0 <sup>a</sup>	84 (88.4)	85 (89.5)	15 (21.7)	26 (37.7)
1	11 (11.5)	9 (9.4)	36 (52.2)	31 (44.9)
2	-	1 (1.1)	14 (20.3)	7 (10.1)
≥3	-	-	4 (5.8)	5 (7.3)

412 <sup>a</sup> samples were HPV DNA-negative

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