Comparison of GeneFinder human papillomavirus (HPV) Liquid Beads Microarray PCR Kit and Hybrid Capture 2 Assay for Detection of HPV Infection

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Background: Along with advances in methodological technologies, various assays for detecting high-risk human papillomavirus (HR HPV) have been introduced. The GeneFinder HPV liquid beads microarray PCR kit is one of the recently developed. Our aim was to compare the performance of GeneFinder to Hybrid Capture 2 for detection of HR HPV. **Methods:** A total of 900 cervical swab specimens were obtained. All specimens were submitted for HR HPV detection with Hybrid Capture 2 (HC2) and GeneFinder and then additionally analyzed the discordant or both positive results using restriction fragment mass polymorphism (RFMP) genotyping analysis. **Results:** Hybrid Capture 2 detected 12.8% cases and GeneFinder detected 15.8% cases with 13 HR HPV types. Also, GeneFinder detected 27.4% cases for 32 detectable HPV types. The overall agreement rate was 93.2%

with 0.724 kappa coefficient. Discordant results between these two assays were observed in 56 cases. HC2 showed sensitivity of 83.5% and specificity of 95.9%, while GeneFinder showed sensitivity of 85.4% and specificity of 91.9%. For HPV 16 or HPV 18 detection, GeneFinder showed 95.0% or 66.7% of sensitivity and 99.2% or 100%, respectively. Overall coinfection rate was 15.4% (38/247) in GeneFinder analysis. **Conclusions:** Considering the high agreement rate with HC2, high sensitivity and the ability to differentiate 32 HPV genotypes including HPV 16/18, GeneFinder could be used as a laboratory testing method for the screening of HPV infections. The use of GeneFinder may also contribute to future research associated with the significance of various HPV types and multiple coinfections. J. Clin. Lab. Anal. 31:e22025, 2017. © 2016 Wiley Periodicals, Inc.

Key words: GeneFinder assay; genotyping; human papillomavirus; hybrid capture assay

INTRODUCTION

The human papillomavirus (HPV) is a small double-stranded DNA virus that infects the epithelium (1). It is well known that persistent infection of high-risk (HR) genotypes is necessary for the development of cervical cancer, the second most common malignancy in women worldwide (2–4). Among the HR types, HPV 16 and HPV 18 are the two most common oncogenic types, causing approximately 70% of all cervical cancers (5, 6). The clinical impact has been evaluated in several studies, and HPV 16/18 strains are associated with poor prognosis in cervical cancer patients, poor overall survival, and cancer relapse (7–9).

While the Papanicolaou (Pap) smear cytology test has some limitations including interoperator variability

and low sensitivity, HR HPV testing improved patient management by providing a more accurate risk assessment for cervical cancer or its precursors (10, 11). The Hybrid Capture 2 (HC2; Qiagen, Gaithersburg, MD) was the first HR HPV screening test approved by the Food and Drug Administration (FDA), which is a sandwich capture hybridization assay with chemiluminescence using unlabeled single-stranded RNA probes

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(10). Along with advances in methodological technologies, various assays for detecting HR HPV have been introduced later on (12–14). Moreover, HC2 is recommended for use as a reference test and as a benchmark for the performance assessment of newly developed HPV detection assays (15).

The GeneFinder HPV liquid beads microarray PCR kit (GeneFinder; Infopia Inc., Anyang, Korea) is one of the recently developed assays, which is based on Luminex technology for HPV detection. This assay is designed to detect 19 HR and 13 low-risk (LR) HPV types and to simultaneously distinguish the 32 unique HPV types.

The primary objective of this study was to compare the performance of GeneFinder to HC2 for the screening of HR HPV infection. Additional analysis of the discordant or both positive results using restriction fragment mass polymorphism (RFMP) genotyping analysis based on MALDI-TOF mass spectrometry was performed to evaluate the genotype detection.

MATERIALS AND METHODS

Subjects and Sample Collection

This study was conducted in a routine diagnostic laboratory setting and used 840 cervical swab specimens obtained from Korean patients seeking routine health checkups between January and April 2015. The medical records of each participant were reviewed using an electronic medical records system to collect information regarding age and cytology results. The specimens were obtained using a DNA PAP Cervical Sampler and specimen transport medium (Qiagen). Each specimen was divided into two aliquots and then promptly submitted for HPV detection with HC2 and GeneFinder. RFMP analysis was additionally performed for discordant or both positive results using the residual specimens, which were stored at -70°C . Additional cervical swab specimens of 60 subjects who accompanied high-grade squamous intraepithelial lesion (HSIL) cytologic findings were collected from the Green Cross Reference Laboratory, which were placed in ThinPrep PreservCyt solution (Hologic Inc., Marlborough, MA) and kept in room temperature ($15\text{--}30^{\circ}\text{C}$) as instructed by the manufacturer. Among the 13 HR HPV genotypes detectable by HC2, a total of 146 specimens were referred for additional RFMP genotyping analysis to investigate the discordant or both positive results of the two assays. This study was approved by the institutional review board of Kangbuk Samsung Hospital (2014-11-033).

Assays for HPV detection

Hybrid Capture 2 assay

All specimens were tested with HC2 according to the manufacturer's protocol. HC2 is a sandwich capture molecular hybridization assay that uses chemiluminescent detection to provide a semi-quantitative result. Briefly, HPV DNA was denatured, and then, the single-stranded HPV DNA was hybridized with a mixture of single-stranded, full-genomic-length RNA probes specific for 13 HR HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). The RNA–DNA hybrids were then captured on the surface of an antibody-coated microtiter plate. Immobilized hybrids were detected by adding an alkaline phosphatase-conjugated antibody to the RNA–DNA hybrids, followed by the addition of a chemiluminescent substrate that is cleaved by the action of alkaline phosphatase to produce light. A luminometer semi-quantitatively measured the emission of light as relative light units (RLU). Measurements below the RLU cutoff of 1.0 were scored as negative. Positive and negative controls provided by the manufacturer were included in each run.

GeneFinder HPV liquid beads microarray PCR kit

The GeneFinder assay utilizes $5.6\mu\text{m}$ polystyrene microspheres (as called beads), which are internally dyed with specific intensities of red and infrared fluorophores. Each microsphere comprises oligonucleotide specific to respective 32 kinds of HPV genotypes. Using different amounts of two dyes for batches of microspheres, microsphere sets with up to 100 different addresses can be formed. After the microspheres react with HPV DNA, which had been amplified during PCR, they were detected in a fluid stream using the Luminex 200 system (Luminex company, Austin, TX), and HPV genotypes were defined by a unique signature of each microsphere. The GeneFinder can detect 19 HR HPV (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 70 and 73) and 13 low-risk (LR) HPV (6, 11, 32, 34, 40, 42, 43, 44, 54, 55, 62, 81, and 83) genotypes. Results are reported as each specified genotypes.

RFMP genotyping assay

The details of the RFMP assay protocol were described previously (16, 17). Briefly, $4\mu\text{l}$ of DNA was amplified with PGMY09/11 primers, comprising of two nondegenerate pools of L1 consensus primers. Second-round primer pairs comprised a sense primer RFMP specific to bases 6584 to 6603

(5'-GCMCAGGGHCAAYAAGGATGAATGG-3') and an antisense primer RFMP specific to bases 6657 to 6626 (5'-GTACTDCKDGTRGTATCHACMACGGATGTAACAAA-3'). The 5-nucleotide sequence (GGATG) embedded in the primers introduced a *FokI* site (a neoschizomer of *BtsCI*) in the amplicon. Restriction enzyme digestion of PCR products was performed by mixing the PCR mixtures with 10 µl of buffer containing 50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 1 mM dithiothreitol, and 1 unit of *FokI* and *BtsCI*. The reaction mixtures were incubated at 37°C for 1 hr. The resulting digest was purified by vacuum filtration through 96-well Oasis® µElution Plates (Waters, Tokyo, Japan). The desalted reaction mixtures were resuspended with matrix solution containing 15 mg/ml 3-hydroxypicolinic acid, 0.023 M ammonium citrate, and 12% acetonitrile and spotted in 3 µl volume on a polished MTP AnchorChip™ plate (BrukerDaltonics, Bremen, Germany). Mass spectra were acquired with the aid of installed software (flexcontrol 3.0) on a Microflex linear MALDI-TOF mass spectrometer (BrukerDaltonics). This assay detects 82 HPV genotypes (1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 81, 82, 83, 84, 86, 87, 89, 90, 91, and 102) including 20 HR HPV genotypes (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 70, 73, and 82). Results are reported as each specified genotypes. However, among the uncertain-risk (UR) HPVs, which are HPV types that are classified as neither high risk nor low risk by the International Agency for Research on Cancer working group, 36 genotypes (1, 2, 4, 5, 7, 8, 9, 10, 12, 13, 14, 15, 17, 19, 20, 21, 22, 23, 24, 25, 28, 29, 36, 37, 38, 41, 47, 48, 49, 50, 57, 60, 63, 65, 90, and 91) are reported as "other."

Statistics

The IBM SPSS program version 18.0 (IBM, Armonk, NY) was used for statistical analysis. Chi-squared test was used to compare the proportions of positive samples according to genotypes and cytology results. Concordance rates and kappa coefficients with 95% confidence intervals (CIs) were calculated to analyze the concordance between HC2 and GeneFinder. *P* value <0.05 was considered to be significant.

RESULTS

This study included 900 cervical swab specimens from patients whose mean age was 41.9 years (range

22–83 years). Upon cytological examination, most cases were diagnosed as normal: 821 (91.2%) normal, 14 (1.6%) atypical squamous cells of undetermined significance (ASCUS), five (0.6%) low-grade squamous intraepithelial lesions (LSIL), and 60 (6.7%) high-grade squamous intraepithelial lesions (HSIL).

The numbers and positive rates of cervical HPV DNA tests by the HC2 and GeneFinder are summarized in Table 1. In all of the 900 specimens, HC2 detected 12.8% cases with HR HPV. GeneFinder detected 27.4% cases for the 32 detectable types represented by GeneFinder; among them, 15.8% cases were detected when applied HC2 detectable 13 HR HPV types. The concordance rate of the results between HC2 and GeneFinder in the detection of the 13 HR HPV types is shown in Table 2. The results of GeneFinder agreed with those of HC2 in 93.2% of all samples with 0.724 kappa coefficient.

Additional RFMP results of the 56 samples that were discordant between HC2 and GeneFinder and both positive 90 samples are summarized in Table 3. Among the 15 cases that were positive with HC2 and negative with GeneFinder, 14 specimens were identified a single HPV genotype: six were positive for non-16/18 HR HPV, two were positive for LR HPV, one was positive for uncertain-risk (UR) HPV, and five showed no amplification, and one specimen was identified with multiple HPV genotypes of HPV 51 and 56. Within the 41 cases that were negative with HC2 and positive with GeneFinder, 38 specimens were identified a single HPV genotype: three were positive for HPV 16 or 18, five were positive for non-16/18 HR HPV, two were positive for LR HPV, and 28 showed no amplification. Multiple genotypes were identified in the other three specimens: two were positive for non-16/18 HR HPVs, and one was positive for non-16/18 HR plus LR HPVs.

Table 4 shows the coinfection rate of GeneFinder. GeneFinder showed overall coinfection rate of 15.4%, with coinfection rates ranging from 7.6% to 33.9% depending on the different cytologic results. When comparing HSIL and non-HSIL results, HSIL showed significantly higher coinfection rate compared to non-HSIL (HSIL 33.9% vs. non-HSIL 9.6%, *P* < 0.05).

DISCUSSION

GeneFinder and HC2 showed good agreement rate of 93.2%, which was higher than the value described in a previous study (12). Among the 15 discordant results: positive for HC2 and negative for GeneFinder, when compared to RFMP results, HC2 showed 10 false-positive cases [HPV-53 (*n* = 2), HPV-34 (1), HPV-43 (1), HPV-86 (1), and no amplification (5)] and

TABLE 1. HC2 and GeneFinder Comparison According to Cytological Findings (*n* = 900)

Cytology	HC2		GeneFinder		Total (<i>n</i>)
	Positive	Negative	Positive	Negative	
HSIL	58 (96.7%)	2 (3.3%)	59 (98.3%)	1 (1.7%)	60
LSIL	4 (80.0%)	1 (20.0%)	5 (100%)	0 (0%)	5
ASCUS	8 (57.1%)	6 (42.9%)	11 (78.6%)	3 (21.4%)	14
Normal	44 (5.4%)	777 (94.6%)	171 (20.8%)	650 (79.2%)	821
Total	115 (12.8%)	785 (87.2%)	247 (27.4%)	653 (72.6%)	900

HC2, Hybrid Capture 2; ASCUS, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion.

TABLE 2. Concordance Rate between HC2 and GeneFinder in the Detection of the 13 HR HPV Genotypes (*n* = 827)

		HC2 result		Concordance rate (%)	Kappa coefficient	95% CI
		Positive	Negative			
GeneFinder result	Positive	90	41	93.2	0.724	0.655–0.793
	Negative	15	681			

HC2, Hybrid Capture 2; CI, confidence interval.

GeneFinder showed nine false-negative cases [HPV-34 (1), HPV-39 (1), HPV-43 (1), HPV-52 (2), HPV-53 (2), HPV-59 (1), and HPV-51, 56 (1)]. For the 41 discordant results: negative for HC2 and positive for GeneFinder, when compared to RFMP results, HC2 showed nine false-negative cases [HPV-16 (1), HPV-18 (2), HPV-35 (1), HPV-51 (2), HPV-58 (1), HPV-35, 58 (1), and HPV-68, 73 (1)] and GeneFinder showed 28 false-positive cases [no amplification (28)].

When comparing the false-negative results, it is noticeable that HC2 included three cases: HPV-16 (1) and HPV-18 (2), while GeneFinder did not include any false-negative cases for HPV 16/18. According to the “The 2012 Updated Consensus Guidelines for the Management of Abnormal Cervical Cancer Screening Tests and Cancer Precursors,” women 30 years or older who are HPV 16 or HPV 18 positive should be directly recommended for colposcopy (18). Considering the importance of the detection of HPV 16/18 for cervical cancer screening, false-negative results for these two HPV types may lead to critical outcomes of patients. On the other hand, GeneFinder showed more false-positive cases when compared to HC2. Among the 28 false-positive cases found by GeneFinder, all cases showed no amplification by RFMP. This result may be due to the difference in the analytical sensitivities between assays. Considering the result, GeneFinder may have higher analytical sensitivity compared to both HC2 and RFMP, which means GeneFinder is less likely to fail at detecting HPV genotypes with low copies. However, the viral loads of the samples could not be determined. In some studies, significant

differences in viral load were observed according to higher grades of cervical intraepithelial neoplasia (CIN) and sample volume for HPV DNA quantity or cellularity (19–21). As a prospective and nonselective study with clinical specimens from health checkup examines was performed, the cytology findings of nearly all enrolled cases were normal (91.2%), implying that the enrolled participants might have a lower HPV DNA viral load. Additionally, some aliquots of the original specimens might have an insufficient volume of cells needed to acquire adequate results.

The overall coinfection rate of GeneFinder (15.4%) was higher compared to other assays described in a previous study (9.4% and 12.0%) (13). GeneFinder can detect and report various HPV genotypes compared to other assays, and this should be the reason for the higher coinfection rate. While the significance of multiple HPV infections for cervical cancer is unclear, some studies have suggested that multiple infections may increase the odds of CIN development (14, 22, 23). Multiple infections have also been found to be associated with poor response to treatment and reduced survival in cervical cancer patients (24). On the other hand, some studies could not prove significant association between multiple infections and risk of CIN development (25, 26).

More than 120 HPV genotypes have been characterized based on sequence information, and there is evidence that even more exist (27). The 51 HPV types recognized by the International Agency for Research on Cancer working group are further divided into three groups based on their epidemiological

TABLE 3. The Results of RFMP in the Discordant between HC2 and GeneFinder and Both Positive Results (*n* = 827)

Agreement between assays	<i>N</i>	RFMP results
Concordant		
Both negative	681	ND
Both positive	90	
Single type	80	16 or 18 (23 ^{a1}), Non-16/18 HR (44 ^{a2}), LR (5 ^{a3}), NA (8)
Multiple types	10	16/18 plus other HR (1 ^{b1}), Non-16/18 HR (7 ^{b2}), Non-16/18 HR plus LR (1 ^{b3}), Non-16/18 HR plus UR (1 ^{b4})
Discordant		
HC2 (+), GeneFinder (-)	15	
Single type	14	Non-16/18 HR (6 ^{c1}), LR (2 ^{c2}), UR (1 ^{c3}), NA (5)
Multiple types	1	Non-16/18 HR (1 ^{d1})
HC2 (-), GeneFinder (+)	41	
Single type	38	16 or 18 (3 ^{e1}), Non-16/18 HR (5 ^{e2}), LR (2 ^{e3}), NA (28)
Multiple types	3	Non-16/18 HR (2 ^{f1}), Non-16/18 HR plus LR (1 ^{f2})

HC2, Hybrid Capture 2; RFMP, restriction fragment mass polymorphism; HR, high risk; LR, low risk; UR, uncertain risk; ND, not done; NA, no amplification.

^{a1}HPV-16 (19), HPV-18 (4), ^{a2} HPV-31 (1), HPV-33 (7), HPV-35 (3), HPV-51 (6), HPV-52 (9), HPV-56 (1), HPV-58 (15), HPV-66 (1), HPV-70 (1), ^{a3} HPV-55 (1), HPV-62 (4).

^{b1}HPV-18, 39 (1), ^{b2} HPV-35, 58 (2), HPV-51, 53 (1), HPV-56, 58 (1), HPV-68, 73 (3), ^{b3} HPV-6, 58 (1), ^{b4} HPV-39, 74 (1).

^{c1}HPV-39 (1), HPV-52 (2), HPV-53 (2), HPV-59 (1), ^{c2} HPV-34 (1), HPV-43 (1), ^{c3} HPV-86 (1).

^{d1}HPV-51, 56 (1).

^{e1}HPV-16 (1), HPV-18 (2), ^{e2} HPV-35 (1), HPV-51 (2), HPV-53 (1), HPV-58 (1), ^{e3} HPV-62 (1), HPV-81 (1).

^{f1}HPV-35, 58 (1), HPV-68, 73 (1), ^{f2}HPV-62, 66 (1).

TABLE 4. HPV Coinfection Rates Using by GeneFinder

Cytology (<i>n</i>)	Positive for HPV DNA (<i>n</i>)	Coinfection (<i>n</i>)	Coinfection rates (%)
HSIL (60)	59	20	33.9
LSIL (5)	5	1	20.0
ASCUS (14)	11	4	36.4
Normal (821)	171	13	7.6
Total (900)	247	38	15.4

ASCUS, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion.

association with cervical cancer: group 1 carcinogens (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59), group 2A probable carcinogens (68), and group 2B possible carcinogens (26, 53, 66, 67, 70, 73, and 82) and 31 LR HPV types (28, 29). Despite continuous updates, clinically significant HR types including HPV 16 and HPV 18 have been stable. On the other hand,

other genotypes (such as HPV-26, 53, 61, 62, 69, 70, 73, and 82) have been alternately classified as either HR or LR groups (28, 30). Also, currently, it is not clear whether the detection of HPV genotypes with low or uncertain risk is helpful for the screening of HPV infections, because the genotypes are detected in only a minority of HPV infections (12). However, such minor detection of LR or UR HPV may be due to limitation in detection methods, as those capable of differentiating a wide range of HPV genotypes have not been commonly used. We should not regard that current LR or UR HPV does not have clinical significance, but instead make efforts to keep on investigating. HPV genotyping assays that can cover a broad range of HPV genotypes, which can simultaneously differentiate not only HPV 16/18, but also other HR and LR HPV types, should contribute to future studies in better understanding the significance of various HPV types.

There are a few limitations in this study. First, the present study was limited to using clinical specimens, most of which were cytologically normal, for the detection of HR HPV infection. Further evaluation of the clinical performance of GeneFinder with cytologically abnormal CIN2+ specimens from a clinical setting will be necessary. Second, additional genotyping methods or sequencing analysis could not be performed due to circumstantial limitation, including lack of specimen amount.

In conclusion, evaluation of the recently developed GeneFinder HPV liquid beads microarray PCR kit for the detection of HR HPV genotypes was performed in comparison with HC2. Considering the high agreement rate with HC2 and the ability to differentiate 32 HPV genotypes including HPV 16/18, GeneFinder could be used as a laboratory testing method for the screening of HPV infections. The use of GeneFinder may also contribute to future research associated with the significance of various HPV types and multiple coinfections.

REFERENCES

1. Clifford GM, Smith JS, Plummer M, Munoz N, Franceschi S. Human papillomavirus types in invasive cervical cancer worldwide: A meta-analysis. *Br J Cancer* 2003;88:63–73.
2. Cuschieri KS, Cubie HA, Whitley MW, et al. Persistent high risk HPV infection associated with development of cervical neoplasia in a prospective population study. *J Clin Pathol* 2005;58:946–950.
3. Dalstein V, Riethmuller D, Pretet JL, et al. Persistence and load of high-risk HPV are predictors for development of high-grade cervical lesions: A longitudinal French cohort study. *Int J Cancer* 2003;106:396–403.
4. Schlecht NF, Kulaga S, Robitaille J, et al. Persistent human papillomavirus infection as a predictor of cervical intraepithelial neoplasia. *JAMA* 2001;286:3106–3114.

5. Bosch FX, Burchell AN, Schiffman M, et al. Epidemiology and natural history of human papillomavirus infections and type-specific implications in cervical neoplasia. *Vaccine* 2008;26(Suppl 10):K1–K16.
6. Li N, Franceschi S, Howell-Jones R, Snijders PJ, Clifford GM. Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: Variation by geographical region, histological type and year of publication. *Int J Cancer* 2011;128:927–935.
7. Baleriola C, Millar D, Melki J, et al. Comparison of a novel HPV test with the Hybrid Capture II (hcII) and a reference PCR method shows high specificity and positive predictive value for 13 high-risk human papillomavirus infections. *J Clin Virol* 2008;42:22–26.
8. Lai CH, Chang CJ, Huang HJ, et al. Role of human papillomavirus genotype in prognosis of early-stage cervical cancer undergoing primary surgery. *J Clin Oncol* 2007;25:3628–3634.
9. Schwartz SM, Daling JR, Shera KA, et al. Human papillomavirus and prognosis of invasive cervical cancer: A population-based study. *J Clin Oncol* 2001;19:1906–1915.
10. Cuzick J, Clavel C, Petry KU, et al. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. *Int J Cancer* 2006;119:1095–1101.
11. Ratnam S, Franco EL, Ferenczy A. Human papillomavirus testing for primary screening of cervical cancer precursors. *Cancer Epidemiol Biomarkers Prev* 2000;9:945–951.
12. Choi J, Park Y, Lee EH, Kim S, Kim JH, Kim HS. Detection and genotyping of human papillomavirus by five assays according to cytologic results. *J Virol Methods* 2013;187:79–84.
13. Park Y, Lee E, Choi J, Jeong S, Kim HS. Comparison of the Abbott RealTime High-Risk Human Papillomavirus (HPV), Roche Cobas HPV, and Hybrid Capture 2 assays to direct sequencing and genotyping of HPV DNA. *J Clin Microbiol* 2012;50:2359–2365.
14. Wong AA, Fuller J, Pabbaraju K, Wong S, Zahariadis G. Comparison of the hybrid capture 2 and cobas 4800 tests for detection of high-risk human papillomavirus in specimens collected in PreservCyt medium. *J Clin Microbiol* 2012;50:25–29.
15. Meijer CJ, Berkhof J, Castle PE, et al. Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older. *Int J Cancer* 2009;124:516–520.
16. Hong SP, Shin SK, Lee EH, et al. High-resolution human papillomavirus genotyping by MALDI-TOF mass spectrometry. *Nat Protoc* 2008;3:1476–1484.
17. Lee EH, Um TH, Chi HS, Hong YJ, Cha YJ. Prevalence and distribution of human papillomavirus infection in Korean women as determined by restriction fragment mass polymorphism assay. *J Korean Med Sci* 2012;27:1091–1097.
18. Massad LS, Einstein MH, Huh WK, et al. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. *J Low Genit Tract Dis* 2013;17:S1–S27.
19. Schmitz M, Scheungraber C, Herrmann J, et al. Quantitative multiplex PCR assay for the detection of the seven clinically most relevant high-risk HPV types. *J Clin Virol* 2009;44:302–307.
20. Carcopino X, Henry M, Mancini J, et al. Significance of HPV 16 and 18 viral load quantitation in women referred for colposcopy. *J Med Virol* 2012;84:306–313.
21. Wanram S, Limpiboon T, Leelayuwat C, et al. The use of viral load as a surrogate marker in predicting disease progression for patients with early invasive cervical cancer with integrated human papillomavirus type 16. *Am J Obstet Gynecol* 2009;201:79. e1–7.
22. Fife KH, Cramer HM, Schroeder JM, Brown DR. Detection of multiple human papillomavirus types in the lower genital tract correlates with cervical dysplasia. *J Med Virol* 2001;64:550–559.
23. Lee SA, Kang D, Seo SS, et al. Multiple HPV infection in cervical cancer screened by HPV DNChip. *Cancer Lett* 2003;198:187–192.
24. Bachtiry B, Obermair A, Dreier B, et al. Impact of multiple HPV infection on response to treatment and survival in patients receiving radical radiotherapy for cervical cancer. *Int J Cancer* 2002;102:237–243.
25. Cuschieri KS, Cubie HA, Whitley MW, et al. Multiple high risk HPV infections are common in cervical neoplasia and young women in a cervical screening population. *J Clin Pathol* 2004;57:68–72.
26. Rolon PA, Smith JS, Munoz N, et al. Human papillomavirus infection and invasive cervical cancer in Paraguay. *Int J Cancer* 2000;85:486–491.
27. Antonsson A, Forslund O, Ekberg H, Sterner G, Hansson BG. The ubiquity and impressive genomic diversity of human skin papillomaviruses suggest a commensal nature of these viruses. *J Virol* 2000;74:11636–11641.
28. Bouvard V, Baan R, Straif K, et al. A review of human carcinogens—Part B: Biological agents. *Lancet Oncol* 2009;10:321–322.
29. Schiffman M, Clifford G, Buonaguro FM. Classification of weakly carcinogenic human papillomavirus types: Addressing the limits of epidemiology at the borderline. *Infect Agent Cancer* 2009;4:8.
30. Arney A, Bennett KM. Molecular diagnostics of human papillomavirus. *Lab Med* 2010;41:523–530.