Original Article

Obstet Gynecol Sci 2018;61(2):235-241
https://doi.org/10.5468/ogs.2018.61.2.235
pISSN 2287-8572 · eISSN 2287-8580

Obstetrics & Gynecology Science

Sequencing analysis of HPV-other type on an HPV DNA chip

Min-Jeong Kim, Jin Ju Kim, Sunmie Kim

Department of Obstetrics and Gynecology, Healthcare Research Institute, Gangnam Healthcare Center, Seoul National University Hospital, Seoul, Korea

Objective
To identify the specific human papillomavirus (HPV) genotypes from HPV-other type on an HPV DNA chip test by sequencing.

Methods
Among 13,600 women undergoing a routine gynecology examination including Pap smear and/or HPV test by DNA chip test in the healthcare system at Gangnam Center from July 2012 to February 2013, we prospectively collected and performed sequencing for a total of 351 consecutive cervicovaginal samples consisting of 180 samples that tested positive for HPV-other type and 171 samples that tested positive for either high-risk HPV or low-risk HPV.

Results
Of a total of 351 samples, individual HPV genotypes were successfully sequenced in 215 cases: 119 HPV-other type, 82 HPV-high-risk, and 14 HPV-low-risk. Based on the sequencing for 119 HPV-other type samples, 91.6% were detected as HPV types that were not included on the DNA chip; however, 7.6% (9/119) were proven to be high-risk HPV types: HPV 18 (n=4), HPV 33 (n=3), HPV 35 (n=1), and HPV 59 (n=1). For correlation analysis of all high-risk and HPV 16/18, the correlation rate was 76.2% and 86.6% with kappa-value of 0.38 and 0.69, respectively.

Conclusion
HPV-other type on DNA chip test may still have possibility of high-risk HPV, i.e., HPV 18 and thus the significance of HPV-other type in detecting cervical disease remains to be investigated.

Keywords: Papillomaviridae; Genotype; Oligonucleotide array sequence analysis; DNA sequence analysis

Introduction

Numerous human papillomavirus (HPV) genotyping test have been introduced to the market, based on the evidence of high-risk HPV genotypes as the primary risk for the development and progression from mild/moderate precancerous lesion to malignancy [1,2], ranging from between 10% and 15% for HPV 16/18 to less than 3% for all other high-risk HPV types combined [3,4]. The HPV DNA chip test (MyHPV chip; Biomedlab Co., Seoul, Korea), commercial HPV DNA genotyping tool has been used in Korea and other Asian countries [5-7] after approval of the Korean Food and Drug Administration and been shown to perform well in a prevalence and comparison study of HPV DNA [7-9].

However, a number of previous comparison studies reported inferior sensitivity of HPV DNA chip test, compared to other HPV tests [10-12]. In additions, our previous study on type-specific HPV prevalence using HPV DNA chip test (MyHPV chip; Biomedlab Co.) revealed that the proportion of women testing positive for HPV-other type, that is, a positive band on gel electrophoresis but negative hybridized signal for 19 types of HPV, is somewhat high, ranging from 20% to 30% among those that tested positive for all HPVs [13]. Even in abnormal

Received: 2017.08.02. Revised: 2017.11.13. Accepted: 2017.11.23.
Corresponding author: Sunmie Kim
Department of Obstetrics and Gynecology, Healthcare Research Institute, Gangnam Healthcare Center, Seoul National University Hospital, 152 Teheran-ro, Gangnam-gu, Seoul 06236, Korea
E-mail: sunmie@snuh.org
https://orcid.org/0000-0001-5122-2218

cervical cytology, i.e., atypical squamous cells of undetermined significance (ASCUS), the proportion of HPV-other type on DNA chip remains higher than 25% [13]. HPV DNA chip may often have limitation in addressing further surveillance in almost one fourth of women who attend screening programs although in 2012, American society for Colposcopy and Cervical Pathology (ASCCP) updated guideline for the management of abnormal cervical cancer screening tests and cancer precursors [14] based on the study of Katki et al. [15] suggesting 'similar management strategies for similar levels of risk.'

Therefore, we conducted this prospective study to identify specific HPV genotypes from HPV-other type samples on an HPV DNA chip using direct sequencing and hopefully to elucidate the clinical meaning of HPV-other type.

Materials and methods

1. Study samples

This prospective study was conducted during routine health check-ups at Seoul National Hospital Healthcare System, Gangnam Center to determine clinical significance of HPV-other type on HPV DNA chip by identifying specific HPV genotypes of HPV-other type with direct sequencing. Of 13,600 women who underwent cervical cytology and HPV testing using an HPV DNA chip as part of a routine gynecological examination from July 2012 to February 2013, we prospectively collected a total of 351 cervicovaginal samples, for which HPV DNA sequencing was performed; 180 consecutive samples with results of HPV-other type to identify their specific HPV types, and 171 random samples (29 of HPV-low-risk and 142 of HPV-high-risk) to further validate the genotyping obtained from the HPV DNA chip, irrespective of the results of their cervical cytology (Fig. 1). The clinical samples used for this study were alcohol-preserved, liquid-based, cervical cytology specimens (LBC, BD Surepath; Becton, Dickinson and Company, Franklin Lakes, NJ, USA), which were submitted by 3 gynecologists in our center as part of routine gynecologic examinations.

After material was taken from each sample for routine cervical cytology and HPV genotype testing on the DNA chip, approximately 1 mL of the cell suspensions was placed in a 1.5 mL Eppendorf tube, blind-coded with a case number, and transferred to the laboratory at MacroGene Co. Ltd. (Seoul, Korea) for HPV polymerase chain reaction (PCR)/DNA sequencing. Written informed consent was obtained from all participants. This cross-sectional prospective study was approved by the Institutional Review Board of Seoul National University Hospital (IRB-No. H-1205-070-410).

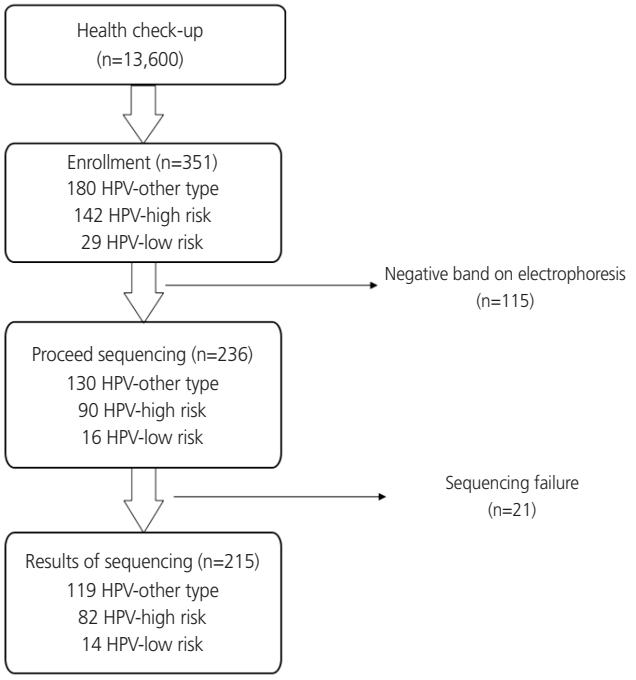

Fig. 1. Eligible study samples.

2. HPV DNA chip

For the HPV genotyping, we used the MyHPV chip kit (AGBIO Diagnostics Co., Seoul, Korea), a commercially available HPV DNA chip, PCR-based microarray system containing 19 type-specific probes for 11 types of high-risk HPV (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, and 58) and 8 types of low-risk HPV (types 6, 11, 34, 40, 42, 43, 44, and 54) according to the manufacturer's instructions [5]. Briefly, the target HPV DNA was amplified using the PCR and the primers (for HPV and β-globin) and conditions provided by the AGBIO Diagnostics Co. The amplified DNA was then labeled with a single dye, indocardocyanine-dUTP (MEM Life Science Products, Inc., Boston, MA, USA). The PCR product size of the HPV DNA was 150 base pairs (bp) on the gel electrophoresis. Next, the PCR product was hybridized onto the chip. Hybridization was performed at 43℃ for 90 minutes; the product was then washed with 3×saline-sodium phosphate-ethylenediamine tetraacetic acid (SSPE) for 5 minutes and with 1× SSPE for 5 minutes before drying at room temperature. The

hybridized signals were visualized using a DNA chip scanner (Scanarray lite; GSI Lumonics, Ottawa, Canada). Samples that showed a positive band of 150 bp on the gel electrophoresis of the HPV-PCR but a negative hybridized signal on the DNA chip scanner were designated (meaning "negative for 11 high-risk HPV and 8 low-risk HPV types but positive for other HPV types") HPV-other type samples [16]. A lack of visualized spots and samples that were negative for the 150 bp HPV-specific band using gel electrophoresis were considered "HPV-negative."

3. HPV sequencing

Genotyping results were confirmed by direct sequencing, as previously described [17]. Direct sequencing was performed by an outside laboratory (MacroGene Co. Ltd.) using a universal HPV primer set (MY 09/11, GP 05/06, KM 38/29) that detects a wide range of HPV types. The primed PCR product of each sample was added to the sequencing reaction mixture, and sequencing was performed bi-directionally using the BigDye3 terminator cycle sequencing kit (PE Applied Biosystems, Foster City, CA, USA) and an ABI PRISM 310 Genomic Analyzer (PE Applied Biosystems) at a dispensing pressure of 600 mbar with 8-ms open times and 65-s cycle times. The sequencing procedure was carried out by stepwise elongation of the primer strand upon cyclic dispensation of the various deoxynucleotide triphosphates (Amersham Pharmacia Biotech, Piscataway, NJ, USA). A charge coupled device camera detected the light output caused by nucleotide incorporation, and the data was obtained in a graphic format. In samples with multiple infections that were identified by the 2 HPV genotyping assays, type-specific primers were used to obtain specific PCR products, which were analyzed again by direct sequencing.

4. Statistical analysis

For descriptive statistics, HPV types were categorized into 3 groups according to their carcinogenic potential; 12 high-risk types (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59), probably high-risk type (HPV types 68)/possibly high-risk types (HPV types 26, 53, 66, 67, 70, 73, and 82), and low-risk types (non-oncogenic; HPV types 6, 11, and other beta/gamma types, such as HPV types 40, 42, 54, 55, 61, 62, 64, 69, 71, 72, 81, 82 subtype IS39, 83, 84, and 89 [formerly CP6108]) [18]. To analyze the MyHPV chip results, however, 'high-risk' was confined to the 11 high-risk HPV types (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, and 58) which can be detected by HPV DNA chip, and HPV patients with single or multiple infection that tested positive for 11 high-risk HPV genotypes were categorized as HPV-high-risk. In cases of multiple infections, subjects were dominantly classified based on the HPV types associated with a higher risk of developing invasive cancer.

Table 1. Sequencing results of human papillomavirus (HPV)-other type on DNA chip

HPV (n=119)	Type	No. of subjects (%)
High-risk	18	4 (3.4)
	33	3 (2.5)
	35	1 (0.8)
	59	1 (0.8)
	Subtotal	9
Probable/possibly high-risk	53	8 (6.7)
	66	11 (9.2)
	70	11 (9.2)
	Subtotal	30
Low-risk	6	2 (1.7)
	61	8 (6.7)
	62	16 (13.4)
	71	3 (2.5)
	72	5 (4.2)
	81	23 (19.3)
	83	8 (6.7)
	84	9 (7.6)
	89	3 (2.5)
	90	1 (0.8)
	102	2 (1.7)
	Subtotal	80

We describe specific types of HPV-other types on HPV DNA chip according to 3 those HPV categories (Table 1). Then, the genotypes obtained using the HPV DNA chip were compared with those obtained using HPV DNA sequencing. In cases of multiple infection, when the type obtained using HPV DNA sequencing was one of the types obtained using the HPV DNA chip test, we considered the type obtained using the DNA chip test 'concordant' with the sequencing type. The concordance rates of the MyHPV chip and the sequencing results were evaluated using the kappa coefficient with 95% confidence intervals and their uneven distribution evaluated

with McNemar's test. Kappa-values are interpreted as follows: 0–0.20 were considered slight, 0.21–0.40 as fair, 0.41–0.60 as moderate, 0.61–0.80 as substantial, and 0.81–1.00 as almost perfect agreement.

Statistical analyses were performed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA).

Result

Of the 351 cervical swab samples obtained from 351 individuals (mean age 49.0; range 23.3–76.3), 89.7% (315/351) had negative for intraepithelial cells or malignancy (NILM) in cervical cytology, followed by 17 of ASCUS, 15 of low-grade squamous intraepithelial lesion, and 4 atypical squamous cells cannot exclude high grade lesion/high-grade squamous intraepithelial lesion. Only 20 cases had available histology by colposcopy-guided biopsy of uterine cervix for their abnormal cervical cytology (data not shown).

Eligible study samples are shown in Fig. 1. DNA sequencing detected specific HPV types in a total of 236 samples, excluding 115 samples that showed no detectable bands on electrophoresis. Additionally excluding 21 cases of sequencing failure, a total of 215 samples were directly sequenced: 14 HPV-low-risk samples, 82 HPV-high-risk samples, and 119 HPV-other type samples.

Sequencing results of 119 HPV-other type samples were shown in Table 1. HPV 81 (19.3%), HPV 62 (13.4%), HPV 66 (9.2%), and HPV 70 (9.2%) were frequently detected. One hundred nine samples (91.6%) out of 119 were positive for HPV genotypes that were not included in the HPV DNA chip test. Nine samples (7.6%) were proven to be high-risk HPV types comprising HPV 18 (n=4), HPV 33 (n=3), HPV 35 (n=1), and HPV 59 (n=1). Restricted to HPV types that are included with 19-probe DNA chip, 8 out of 119 samples turned out to be high-risk HPV undetected by DNA chip. Including types 26, 53, 66, and 68 among the probable or possible high-risk HPV types, almost one fourth (23.5%, 28/119) of the HPV-other type samples were considered as potential high-risk HPV types and the most commonly identified HPV type was HPV 66 (9.2%), followed by HPV 53 (6.7%) and HPV 18 (3.4%).

Among 171 samples positive for either high-risk or low-risk HPV types, 82 of HPV-high-risk and 14 of HPV-low-risk were finally proceeded with sequencing (Fig. 1). There were 64 samples with a single HPV genotype and 32 with multiple HPV genotypes. Of all those 96 samples, 76.0% was concordant between HPV DNA chip and sequencing with 23 samples showing discordant results (Table 2). Among 82 samples positive for high-risk HPV on HPV DNA chip, 21 samples (25.6%) were positive for low-risk HPV on sequencing with the discordance rate of 19.2% and 52.0% for single and multiple high-risk HPV infection, respectively. Eight (23.5%) out of 34 samples positive for HPV 16/18 on HPV DNA chip were positive for HPV types other than HPV 16 or 18 by sequencing with the discordance rate of 12.0% and 55.5% for single and multiple HPV 16/18 infection, respectively (data not shown). The concordance rate for the detection of HPV 16/18 between the two tests was 86.5% with a kappa-value of 0.69, which was higher than the corresponding value for the detection of all high-risk HPV of 76.0% (Table 2).

Discussion

With the exception of the HPV types included in 24 probes but not included in 19 probes of the HPV DNA chip (i.e., HPV 53, 66, and HPV 68, which mainly belong to probable/possible high-risk HPV types), the majority of the HPV types sequenced from HPV-other type samples in present study were identical to those detected in two studies of Choi et al. [9]; mainly HPV 81, 61, 62, and 84. It is still of concern that 7.6% of HPV-other type samples were positive for high-risk HPV, and HPV 18 was most frequently detected high-risk genotype. Our previous study on type-specific prevalence of

Table 2. Concordance rate for human papillomavirus detection between MyHPV chip and DNA sequencing

HPV	Test	Positive	Negative	Overall agreement (%, accuracy)	Kappa coefficient
HPV 16, 18	MyHPV Chip	34	62	86.5 (78.0–92.6)	0.69 (0.53–0.84)
	Sequencing	25	71		
High-risk HPV	MyHPV Chip	82	14	76.0 (66.3–84.2)	0.38 (0.17–0.60)
	Sequencing	63	33		

HPV using HPV DNA chip reported that overall positive rate of HPV-other type on DNA chip was as high as 28% of all HPV-positive samples which accounted for 25% of women with HPV-positive ASCUS [13].

Many comparison studies have reported that HPV DNA chip test is less sensitive than the other HPV DNA tests in detecting cervical intraepithelial neoplasia (CIN) 2+ or CIN3+ [10-12]. A number of previous studies that compared the ability of different primer sets to detect HPV DNA in clinical materials [19,20] demonstrated each primer set had different PCR efficiency to each HPV genotype and different types of HPV could be preferentially amplified depending on the primer used within the chip [21]. Therefore, nested PCR method using consensus primer set that HPV DNA chip utilize in detecting HPV genotypes might cause difference of sensitivity for specific HPV genotype, thereby implicating that HPV DNA chip may be incapable of detecting specific HPV genotypes [11]. The poor clinical and analytic performance of HPV DNA chip may be attributable to less sensitive detection of high-risk HPV genotypes and need to be improved since HPV genotyping test should be able to detect high-risk HPV, in particular, HPV 16 or 18, given that HPV 16/18 accounts for more than 70% of cervical cancer and the ASCCP guideline recommend HPV 16/18 genotyping triage for colposcopy referral in women 30 years or older with normal cytology and positive high-risk HPV results in co-testing [14].

The HPV DNA chip from other studies exhibits wide range of concordance rates from 61.5% and 91.1%, compared with DNA sequencing [9,11]. In our study, the concordance for overall high-risk HPV was slight, 76.0% (73/96) with kappa-value of 0.38, whereas 86.5% was concordant for HPV 16/18 with a kappa-value of 0.69. The similarities in DNA sequence between the type-specific probes attached within DNA chip may lead to 'cross-reactivity,' contributing to discordance. There was a clear tendency toward higher discordance for the detection of multiple HPV types, similar to other studies [22,23]. A global proficiency study of HPV genotyping tests by Eklund et al. [23] also reported that HPV tests have a tendency toward lower sensitivity in multiple HPV infection than in single HPV infection and the detection of high-risk HPV types other than HPV 16/18 by many HPV genotyping tests showed less sensitivity, compared to that of HPV 16/18.

Another possibility is that the sensitivity of HPV detection method is dependent on the threshold value of viral load/viral concentration present in the sample [24] and 'negative' result on the test does not guarantee the absence of HPV DNA in the sample, but may imply that the level is below a defined threshold value based on the clinical characteristics of the test [25]. As 90% of our study samples are negative intraepithelial lesion or malignancy (NILM) in cervical cytology, high drop-out rate of HPV-other types samples for sequencing may inevitably result from negative band on electrophoresis and proceeding failure of sequencing.

There are several limitations in our study. We had no comparison of sequencing results of HPV-other type samples according to the severity of cervical cytology and histologic grade. In additions, the MyHPV chip probe used in our study tests only for 19 HPV types including only 11 high-risk HPV types, unlike the 24-probe DNA chip; therefore, some high-risk or probable high-risk HPV types (i.e., HPV 59 and 68) were inevitably undetected. Another limitation is too small study sample size to achieve the statistical power of 80% and type I error of 0.05, for which considering 10% of drop-out rate, samples size should be at least 510 cases to be recruited. Final sequencing results were obtained from 215 eligible samples and consequently, the study power reached only 66% for comparing HPV high-risk samples between both tests. Nonetheless, the main purpose of this study is but to identify the specific HPV type from HPV-other type samples and to verify the reliability of HPV DNA chip by analyzing the correlation between DNA chip and sequencing, not to compare the clinical performance of HPV DNA chip with sequencing.

As far as we know, among the available published data of sequencing for HPV-other type, this study is the largest study attempting to identify the specific HPV genotypes from the HPV-other type samples. Based on our results, HPV-other type on DNA chip test may be postulated to have certain possibility of high-risk HPV, i.e., HPV 18, and therefore, the clinical significance of HPV-other type remains to be investigated in the context of its association to cervical disease.

Acknowledgements

This study was supported by a grant (0420120840) from Seoul National University Hospital, and sequencing was performed with the support of AGBIO Diagnostics Co.

Conflict of Interest

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

References

1. Clifford GM, Rana RK, Franceschi S, Smith JS, Gough G, Pimenta JM. Human papillomavirus genotype distribution in low-grade cervical lesions: comparison by geographic region and with cervical cancer. Cancer Epidemiol Biomarkers Prev 2005;14:1157-64.
2. Naucler P, Ryd W, Törnberg S, Strand A, Wadell G, Hansson BG, et al. HPV type-specific risks of high-grade CIN during 4 years of follow-up: a population-based prospective study. Br J Cancer 2007;97:129-32.
3. Khan MJ, Castle PE, Lorincz AT, Wacholder S, Sherman M, Scott DR, et al. The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. J Natl Cancer Inst 2005;97:1072-9.
4. Bosch FX, de Sanjosé S. Chapter 1: human papillomavirus and cervical cancer--burden and assessment of causality. J Natl Cancer Inst Monogr 2003:3-13.
5. An HJ, Cho NH, Lee SY, Kim IH, Lee C, Kim SJ, et al. Correlation of cervical carcinoma and precancerous lesions with human papillomavirus (HPV) genotypes detected with the HPV DNA chip microarray method. Cancer 2003;97:1672-80.
6. Oh TJ, Kim CJ, Woo SK, Kim TS, Jeong DJ, Kim MS, et al. Development and clinical evaluation of a highly sensitive DNA microarray for detection and genotyping of human papillomaviruses. J Clin Microbiol 2004;42:3272-80.
7. Lee JK, Kim MK, Song SH, Hong JH, Min KJ, Kim JH, et al. Comparison of human papillomavirus detection and typing by hybrid capture 2, linear array, DNA chip, and cycle sequencing in cervical swab samples. Int J Gynecol Cancer 2009;19:266-72.
8. Hwang TS, Jeong JK, Park M, Han HS, Choi HK, Park TS. Detection and typing of HPV genotypes in various cervical lesions by HPV oligonucleotide microarray. Gynecol Oncol 2003;90:51-6.
9. Choi YD, Jung WW, Nam JH, Choi HS, Park CS. Detection of HPV genotypes in cervical lesions by the HPV DNA Chip and sequencing. Gynecol Oncol 2005;98:369-75.
10. Um TH, Lee EH, Chi HS, Kim JW, Hong YJ, Cha YJ. Comparison of HPV genotyping assays and Hybrid Capture 2 for detection of high-risk HPV in cervical specimens. Ann Clin Lab Sci 2011;41:48-55.
11. Park S, Kang Y, Kim DG, Kim EC, Park SS, Seong MW. Comparison of the analytical and clinical performances of Abbott RealTime High Risk HPV, Hybrid Capture 2, and DNA Chip assays in gynecology patients. Diagn Microbiol Infect Dis 2013;76:432-6.
12. Klug SJ, Molijn A, Schopp B, Holz B, Iftner A, Quint W, et al. Comparison of the performance of different HPV genotyping methods for detecting genital HPV types. J Med Virol 2008;80:1264-74.
13. Kim MJ, Kim JJ, Kim S. Type-specific prevalence of high-risk human papillomavirus by cervical cytology and age: Data from the health check-ups of 7,014 Korean women. Obstet Gynecol Sci 2013;56:110-20.
14. Massad LS, Einstein MH, Huh WK, Katki HA, Kinney WK, Schiffman M, et al. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. Obstet Gynecol 2013;121:829-46.
15. Katki HA, Schiffman M, Castle PE, Fetterman B, Poitras NE, Lorey T, et al. Benchmarking CIN 3+ risk as the basis for incorporating HPV and Pap cotesting into cervical screening and management guidelines. J Low Genit Tract Dis 2013;17:S28-35.
16. Choi YD, Han CW, Chung WJ, Jung WW, Lee JS, Nam JH, et al. Analysis of HPV-other samples by performing HPV DNA sequencing. Korean J Pathol 2009;43:250-3.
17. Gharizadeh B, Oggionni M, Zheng B, Akom E, Pourmand N, Ahmadian A, et al. Type-specific multiple sequencing primers: a novel strategy for reliable and rapid genotyping of human papillomaviruses by pyrosequencing technology. J Mol Diagn 2005;7:198-205.
18. Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, et al. A review of human carcinogens--Part B: biological agents. Lancet Oncol 2009;10:321-2.
19. Schiffman MH, Bauer HM, Lorincz AT, Manos MM, Byrne JC, Glass AG, et al. Comparison of Southern blot hybridization and polymerase chain reaction methods for the detection of human papillomavirus DNA. J Clin Microbiol 1991;29:573-7.

20. Baay MF, Quint WG, Koudstaal J, Hollema H, Duk JM, Burger MP, et al. Comprehensive study of several general and type-specific primer pairs for detection of human papillomavirus DNA by PCR in paraffin-embedded cervical carcinomas. J Clin Microbiol 1996;34:745-7.
21. Kado S, Kawamata Y, Shino Y, Kasai T, Kubota K, Iwasaki H, et al. Detection of human papillomaviruses in cervical neoplasias using multiple sets of generic polymerase chain reaction primers. Gynecol Oncol 2001;81:47-52.
22. Cho EJ, Do JH, Kim YS, Bae S, Ahn WS. Evaluation of a liquid bead array system for high-risk human papillomavirus detection and genotyping in comparison with Hybrid Capture II, DNA chip and sequencing methods. J Med Microbiol 2011;60:162-71.
23. Eklund C, Zhou T, Dillner J; WHO Human Papillomavirus Laboratory Network. Global proficiency study of human papillomavirus genotyping. J Clin Microbiol 2010;48:4147-55.
24. Carvalho Nde O, del Castillo DM, Perone C, Januário JN, Melo VH, Brasileiro Filho G. Comparison of HPV genotyping by type-specific PCR and sequencing. Mem Inst Oswaldo Cruz 2010;105:73-8.
25. Rao A, Young S, Erlich H, Boyle S, Krevolin M, Sun R, et al. Development and characterization of the cobas human papillomavirus test. J Clin Microbiol 2013;51:1478-84.