Performance of Aptima and Cobas HPV Testing Platforms in Detecting High-Grade Cervical Dysplasia and Cancer

Yimin Ge, MD [1,2]; Paul Christensen, MD[1]; Eric Luna, CT (ASCP)[3]; Donna Armylagos, CT (ASCP)[3]; Mary R. Schwartz, MD[1]; and Dina R. Mody, MD[1,2]

BACKGROUND: Human papillomavirus (HPV) tests and genotyping have been used in clinical risk assessment. The purpose of this study was to analyze the performance of 2 common HPV testing platforms in detecting high-grade cervical lesions (high-grade squamous intraepithelial lesion [HSIL] or worse [≥HSIL]). **METHODS:** Between January 1 and December 31, 2015, 2041 Papanicolaou (Pap) tests with biopsy confirmation were analyzed along with HPV tests performed on Cobas or Aptima platforms. A biopsy diagnosis of grade 2 cervical intraepithelial neoplasia was confirmed with p16/Ki-67 immunohistochemistry. **RESULTS:** In total, 1866 and 175 Pap cases were tested on Cobas and Aptima platforms, respectively. Both platforms were highly sensitive (97% for both) for biopsy-confirmed ≥HSIL. Cobas HPV testing had higher positive rates for the diagnosis of benign lesions (84% vs 51%) and low-grade squamous intraepithelial lesions (89% vs 63%) on biopsy compared with Aptima. Aptima testing had significantly higher specificity for ≥HSIL than Cobas (41% vs 13%; $P < .0001$). Overall, performance of the Aptima platform was superior to that of the Cobas platform in detecting biopsy-confirmed ≥HSIL, resulting from its significantly higher positive predictive value (25% vs 16%; $P < .03$) and overall accuracy (50% vs 26%; $P < .0001$). **CONCLUSIONS:** Although both the Cobas and Aptima platforms offer highly sensitive tests for high-grade cervical lesions, Aptima HPV testing demonstrated significantly higher specificity and positive predictive value than Cobas testing for biopsy-confirmed ≥HSIL. The considerable difference may be related to the significant increase in E6/E7 expression after HPV DNA integration. The significantly higher specificity and overall accuracy of Aptima testing for ≥HSIL, resulting in the identification of high-risk populations that require immediate treatment and close follow-up, may prove useful in clinical risk stratification. ***Cancer Cytopathol* 2017;125:652-7.** *© 2017 American Cancer Society.*

KEY WORDS: Aptima human papillomavirus (HPV) test; cervical cancer; Cobas HPV test; high-grade squamous intraepithelial lesion (HSIL); HPV E6/E7 messenger RNA test; Papanicolaou (Pap) test.

INTRODUCTION

In the past 2 decades, significant advances in the prevention and treatment of cervical cancer have been achieved because of the landmark finding of the causative role of human papillomavirus (HPV) in cervical cancer and its precursor lesions.[1] Virtually all cervical cancers result from a persistent infection of 1 or more HPV genotypes, primarily those classified by the International Agency for Research on Cancer as groups 1 and 2a (conventionally referred to as high-risk HPV [hrHPV]).[2–4] Because of HPV infection, complex genetic and epigenetic changes occur, leading to viral DNA integration and transformation of cervical epithelial cells to precancerous lesions and, eventually, to cancer.[5–8] In recent years, HPV testing has been incorporated into cervical cancer screening and management algorithms to assist in triaging patients with equivocal cytology results or as a cotest with cytology to maximize the detection rate of high-grade cervical lesions (high-grade squamous intraepithelial lesion [HSIL] or

Corresponding author: Yimin Ge, MD, Department of Pathology and Genomic Medicine, Houston Methodist Hospital, Weill Cornell Medical College of Cornell University, 6565 Fannin Street, M227, Houston, 77030; Fax: (713) 793-1603; yge@houstonmethodist.org

[1]Department of Pathology and Genomic Medicine, Houston Methodist Hospital, Houston, Texas; [2]Weill Medical College of Cornell University, New York, New York; [3]BioReference Laboratories, Houston, Texas.

The authors thank Jiaqiong Xu, PhD, for statistical analysis, and Helen Chifotides, PhD, for editing of the manuscript.

Received: February 19, 2017; **Revised:** March 21, 2017; **Accepted:** April 6, 2017

Published online June 2, 2017 in Wiley Online Library (wileyonlinelibrary.com)

DOI: 10.1002/cncy.21875, wileyonlinelibrary.com

worse [≥HSIL]) in women aged ≥30 years.[9] Most recently, the American Society for Colposcopy and Cervical Pathology management guidelines (2012) also recommended the use of HPV genotyping tests as an option in clinical risk management.[10]

There are more than 100 commercially available HPV assays worldwide, and each laboratory may choose its testing platforms based on availability, cost, and other preferences. Among the 4 US Food and Drug Administration-approved HPV tests in the United States, 3 are DNA-based tests: Cobas HPV test (Cobas 4800; Roche Molecular Diagnostics [Roche], Pleasanton, Calif), Hybrid Capture 2 (HC2) (Qiagen, Gaithersburg, Calif), and Cervista (Hologic, Bedford, Mass); whereas 1 test detects E6/E7 messenger RNA (mRNA) (Aptima; Hologic/Gen-Probe, San Diego, Calif). According to a 2014 College of American Pathologists (CAP) survey of laboratories, the most commonly used platforms for HPV genotyping are Roche Cobas (37%) and Hologic Aptima (26%).[11]

In view of the critical role of E6/E7 overexpression after HPV genome integration in the development of cervical malignancy, direct testing of hrHPV E6/E7 mRNA in cervical samples may be more specific than hrHPV DNA testing for the detection of high-grade cervical lesions. Direct comparative data with regard to the performance of the 2 different testing methods in clinical practice are limited. Recent studies suggest that HPV mRNA testing has improved specificity for grade 2 cervical intraepithelial neoplasia (CIN2) or worse (≥CIN2) compared with HPV DNA assays.[12–14] However, other studies concluded that there was no significant difference between HPV DNA and RNA testing methods in clinical performance for the detection of high-grade cervical lesions.[15,16] The purpose of the current study was to analyze the performance of the 2 most common HPV testing platforms (Cobas and Aptima) in detecting high-grade cervical lesions that were confirmed by biopsy.

MATERIALS AND METHODS

Study Design

We retrospectively searched 115,104 Papanicolaou (Pap) tests recorded in our Laboratory Information System database from January 1 to December 31, 2015, and identified 2041 patients who underwent biopsy within 6 months of the Pap test and had hrHPV test performed on either the Cobas (Cobas 4800 system; Roche) or Aptima (Hologic/Gen-Probe) platform. The Aptima HPV test was validated on SurePath (SP) samples (Becton Dickinson, Franklin Lakes, NJ) in our laboratory. The correlation between HPV testing results and biopsy diagnoses was analyzed in an effort to evaluate the performance of the 2 HPV testing platforms in detecting ≥HSIL in biopsy, including HSIL (CIN2/CIN3), squamous cell carcinoma, adenocarcinoma in situ (AIS), and cervical adenocarcinoma.

Pap Tests

All Pap tests were performed with 1 of the 2 liquid-based methods, in accordance with the preference of the referring practitioner: ThinPrep (TP) (Hologic, Madison, Wis) or SP (Becton Dickinson). The study included 1159 TP samples (1049 tested on Cobas platforms and 110 tested on Aptima platforms) and 882 SP samples (817 tested on Cobas platforms and 65 tested on Aptima platforms).

Biopsy Confirmation

The biopsies performed within 6 months of the Pap and HPV tests were included in the study. Most biopsy samples were obtained within 5 weeks after the initial Pap/HPV tests, with an average time of 35 days, including 49 obtained before and 1992 obtained after Pap/HPV tests (range, from −25 to 179 days). The biopsy results were categorized into 3 general groups: benign (including no pathologic alteration and benign or reactive changes), low-grade squamous intraepithelial lesion (LSIL, CIN1), and high-grade cervical lesions (≥HSIL, including CIN2/CIN3, squamous cell carcinoma, AIS, and adenocarcinoma). All CIN2 lesions were confirmed by immunohistochemical staining for p16 and Ki-67. In patients who had more than 1 tissue sample, the highest grade diagnosis was recorded. Endometrial lesions were excluded from the study. Real-time histocytologic correlation was performed at the time of the biopsy sign-out, which is our routine clinical practice and is in accordance with the mandates of the 1988 Clinical Laboratory Improvement Amendments required by the Laboratory Accreditation Program of the CAP. Board-certified cytopathologists or gynecologic pathologists interpreted the Pap tests and biopsies at an academic medical center.

Statistical Analysis and Institutional Review Board Approval

Accuracy and 95% confidence intervals (CIs) of Cobas and Aptima HPV testing for the detection of ≥HSIL cervical lesions were compared using the Fisher exact test. All

analyses were performed with the STATA software package (version 14; StataCorp LP, College Station, Tex). Significance was defined as a 2-tailed P value $< .05$. The study was conducted with approval from the Institutional Review Board at Houston Methodist Hospital.

RESULTS

The results from hrHPV testing and biopsies are summarized in Table 1. Among the 2041 cases, biopsies revealed no pathologic alteration and benign or reactive changes in 741 patients, LSIL in 993 patients, and ≥HSIL in 307 patients. Cobas and Aptima HPV testing was performed in 1866 and 175 patients, respectively. Both HPV testing platforms were highly sensitive (Cobas: 97% [95% CI, 95%-99%]; Aptima: 97% [95% CI, 80%-100%]; $P = .51$) for biopsy-confirmed ≥HSIL lesions. The negative predictive values for ≥HSIL were essentially identical for both platforms (Cobas: 97% [95% CI, 93%-99%]; Aptima: 98% [95% CI, 90%-100%]; $P = 1.0$). However, rare patients with biopsy-confirmed ≥HSIL lesions tested negative for hrHPV on both platforms (3% for each).

TABLE 1. Correlation of Human Papillomavirus Test Results With Papanicolaou Testing and Biopsy Diagnoses

	Biopsy Results: No. (%)			
HPV Test Results	Benign	LSIL	≥HSIL[a]	Total
Cobas				
Positive	577 (84)	802 (89)	271 (97)	1650
Negative	111 (16)	98 (11)	7 (3)	216
Aptima				
Positive	27 (51)	59 (63)	28 (97)	114
Negative	26 (49)	34 (37)	1 (3)	61
Total	741	993	307	2041

Abbreviations: HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; ≥HSIL, HSIL or worse; LSIL, low-grade squamous intraepithelial lesion.
[a] ≥HSIL includes HSIL, adenocarcinoma in situ, adenocarcinoma, and squamous carcinoma.

The analysis of the 2 HPV testing platforms for the entire cohort revealed higher positive rates with Cobas HPV testing compared with Aptima testing in patients who had benign/reactive changes (84% vs 51%) and LSIL (89% vs 63%) on biopsies, respectively (Fig. 1). Consequently, the Aptima test surpassed the Cobas test because it provided significantly higher specificity (Aptima: 41% [95% CI, 33%-50%]; Cobas: 13% [95% CI, 12%-15%]; $P < .0001$) and positive predictive value (Aptima: 25% [95% CI, 17%-34%]; Cobas: 16% [95% CI, 15%-18%]; $P = .03$) for biopsy-confirmed ≥HSIL (Table 2). The results were observed in both SP and TP liquid-based samples. In summary, the HPV test performance of Aptima

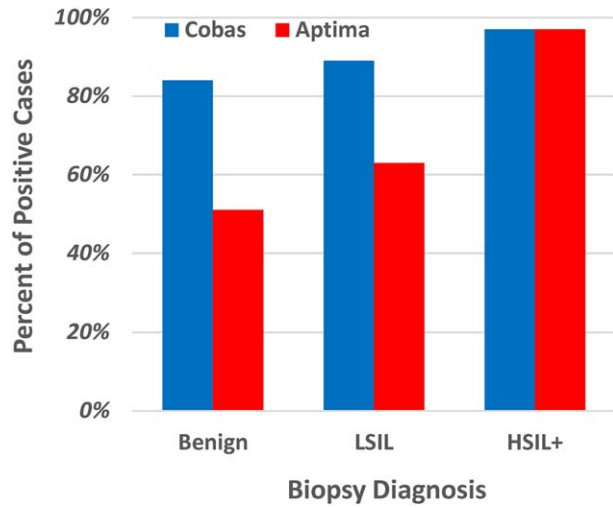

Figure 1. A comparison of results from the Cobas and Aptima human papillomavirus (HPV) testing platforms is illustrated based on HPV-positive rates in cervical biopsy categories. Although both platforms were positive for the majority of high-grade squamous intraepithelial lesions (high-grade cervical lesion or worse [HSIL+]), Cobas HPV testing was positive more often than Aptima in patients who had benign or low-grade squamous intraepithelial lesions (LSIL) on biopsy, resulting in significantly higher specificity and positive predictive accuracy for biopsy-confirmed HSIL+ by Aptima testing compared with Cobas testing.

TABLE 2. Performance of Cobas and Aptima Human Papillomavirus Testing in Detecting Biopsy-Confirmed High-Grade Cervical Lesions

	Cobas		Aptima		
Variable	Percentage (No./Total No.)	95% CI, %	Percentage (No./Total No.)	95% CI, %	P
Sensitivity	97 (271/278)	95-99	97 (28/29)	80-100	.55
Specificity	13 (209/1588)	12-15	41 (60/146)	33-50	< .0001
PPV	16 (271/1650)	15-18	25 (28/114)	17-34	.03
NPV	97 (209/216)	93-99	98 (60/61)	90-100	1.0
Overall accuracy	26 (480/1866)	24-28	50 (88/175)	43-58	< .0001

Abbreviations: CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

was superior to that of Cobas in detecting ≥HSIL in biopsies, because it provided significantly higher overall accuracy (Aptima: 50% [95% CI, 43%-58%]; Cobas: 26% [95% CI, 24%-28%]; $P < .0001$) (Table 2).

DISCUSSION

The Roche Cobas hrHPV test detects viral DNA of 12 hrHPV genotypes in a cocktail manner with concurrent, separate genotyping of HPV-16 and HPV-18. The Hologic Aptima hrHPV assay is a target-amplification test for the detection of viral E6/E7 mRNA from 14 hrHPV genotypes with optional genotyping of HPV-16 and HPV-18/HPV-45. Although both platforms offer highly sensitive tests for ≥HSIL, our study demonstrated that the Aptima HPV assay had significantly higher specificity and positive predictive value compared with Cobas HPV testing for ≥HSIL in biopsies. Our results are consistent with a previous report indicating that the Aptima test was significantly more specific than the Cobas test for ≥CIN2 lesions in follow-up biopsies (63.1% vs 59.3%; $P < .003$) from women who had atypical squamous cells of undetermined significance (ASC-US) on cytology.[13] Similar findings were also observed in a screening population[17,18] and in women who were referred for colposcopy.[12,19] The favorable specificity in detecting ≥HSIL was also reported with several other detection methods based on HPV E6/E7 mRNA testing compared with HPV DNA testing platforms.[20]

hrHPV testing has been recommended to triage women with ASC-US on cytology or follow-up of women after treatment.[10,21] The superior specificity and overall accuracy of HPV mRNA testing for ≥HSIL, without compromising high sensitivity, may prove useful in clinical risk stratification with the identification of high-risk populations that need immediate treatment and close follow-up. HPV mRNA testing reportedly was equally sensitive for but more specific than HPV DNA testing for high-grade dysplasia in triaging both ASCUS and LSIL.[22] Rijkaart et al studied the E6/E7 mRNA status of women who had positive HPV-DNA testing and observed that a positive E6/E7 mRNA test was associated with an increased risk of ≥CIN2 lesions in women who had normal cytology results.[23] Therefore, HPV mRNA testing offers a potentially useful tool for the triage of women who have LSIL or negative cytology but positive HPV-DNA testing, whereas HPV-DNA testing is less useful because it lacks specificity. By identifying a smaller high-risk population, the implementation of HPV mRNA testing is expected to reduce colposcopy referral rates and excessive treatment of women who may have transient HPV infection. In turn, optimal clinical risk stratification with mRNA testing may lead to an overall cost reduction from surveillance and treatment by reducing unnecessary colposcopies, repeated tests, and biopsies.[18]

Emerging data indicate that the HPV mRNA assays are generally more specific than HPV DNA tests in detecting high-grade cervical lesions.[24] The considerable difference between the 2 major HPV testing methods has been attributed to the dynamic changes of tested molecules with progression of HPV infection.[24] HPV infection in basal cells may maintain a stable episomal form as the viral genome is replicated in conjunction with cellular DNA during S-phase (productive infection). This form of infection produces abundant viral DNA and lower grade lesions, which mostly regress within 9 to 12 months.[25] Immune evasion leads to persistence of HPV infection and integration of viral DNA into the host genome, resulting in aborted normal viral life cycle and overexpression of E6 and E7 oncoproteins, a biologic hallmark of high-grade dysplasia and cervical cancer. Ratnam et al reported that the ratio of hrHPV E6/E7 mRNA to hrHPV-DNA positivity increased with the histologic severity of dysplasia.[26] In a 2-year follow-up study using a 5-genotype panel (HPV-16, HPV-18, HPV-31, HPV-33, and HPV-45), Fontecha et al demonstrated that hrHPV E6/E7 mRNA was highly sensitive for high-grade cervical lesions and was correlated with disease progression.[27] The dramatic change in E6/E7 expression with progression of HPV infection may contribute to the high specificity of Aptima testing in the later stage of disease, which usually presents with high-grade cervical lesions.

Although Aptima HPV testing has preferable specificity over DNA-based HPV tests, in general, all of the currently available HPV tests are far from ideal because of their inability to accurately identify women who are at high risk of developing cervical cancer. In view of the relatively low sensitivity of the cytology test alone, in 2014, the US Food and Drug Administration approved the Cobas HPV test as an option for the primary screening of women aged ≥30 years. However, it is expected that this highly sensitive and less specific test will produce considerable numbers of hrHPV-positive cases with negative or equivocal/low-grade findings on cytology or biopsy. A

plausible way to resolve this issue is to apply a combined strategy involving both hrHPV and Pap tests (cotesting), which has demonstrated improved sensitivity and specificity.[28] Alternatively, assays targeting markers rich in the later stage of HPV infection, such as E6/E7, likely provide a reasonable strategy to improve screening performance. In addition to Aptima testing, a few more commercially available or emerging mRNA-based HPV tests achieved similar or even higher specificity for high-grade cervical lesions. Recently, Munkhdelger et al reported 91% sensitivity and 98.6% specificity for ≥CIN2 lesions by using a commercial diagnostic kit that targeted HPV E6/E7 mRNA based on a reverse transcriptase-quantitative polymerase chain reaction assay.[29] Furthermore, monitoring the qualitative changes in E6/E7 over the course of the disease may be more informative with respect to the severity and progression of cervical disease compared with the currently available cross-sectional qualitative test.

Despite the interesting findings, our data should be interpreted with caution. This was a retrospective study that emerged from a review of data from women who were referred for colposcopy biopsy and had cytology-hrHPV cotesting. Therefore, the population was less uniform in terms of prior history of cervical disease, reasons for referral, and selection of cotesting. Although the samples were collected from women in a screening population in the same geographic region and were processed and interpreted in the same laboratory, many other variables were still difficult to control as common limitations in retrospective studies (such as the number of patients, age distribution, previous cervical disease, and treatment history). For example, fewer women in our cohort were tested on the Aptima platform than on the Cobas platform, as noted in our current results. This is generally coincident with recent CAP survey data indicating that Cobas is the leading HPV genotyping test followed by other HPV tests, including Aptima.[11] Clinicians may select HPV tests based on many considerations or preferences. The Aptima HPV test was implemented in our laboratory much later than the well established Cobas system, and many of our clinicians were just getting familiar with the new Aptima test at the time of the data collection. In addition, the lack of general consensus among clinicians regarding the selection between Cobas and Aptima HPV testing could also lead to possible uneven distribution of patients in the 2 testing groups. All of these factors should be considered when interpreting the data, and larger scale prospective studies are needed to validate the findings.

In summary, we demonstrated that both the Aptima and Cobas HPV tests provide similar high sensitivities for ≥HSIL, but the Aptima test had significantly higher specificity and positive predictive value than the Cobas test for biopsy-confirmed ≥HSIL. The considerable difference in detecting ≥HSIL between the 2 common hrHPV testing platforms may be related to the significant increase in E6/E7 expression after HPV DNA integration into the host genome in advanced cervical lesions. Notwithstanding its imperfections, the significantly higher specificity and overall accuracy of Aptima testing for high-grade cervical lesions may prove useful in clinical risk stratification in light of its ability to identify high-risk populations. This advantage may also reduce unnecessary colposcopy referrals and over-treatment, which will eventually drive down the cost of cervical cancer prevention, surveillance, and treatment. Future investigations should focus on validation of the findings in large prospective studies and a search for more effective tests that target the markers of advanced-stage HPV infection associated with high-grade cervical dysplasia and cancer.

FUNDING SUPPORT

No specific funding was disclosed.

CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

AUTHOR CONTRIBUTIONS

Yimin Ge: Conceptualization, methodology, formal analysis, investigation, resources, writing–original draft, writing–review and editing, visualization, supervision, and project administration. **Paul Christensen:** Methodology, software, formal analysis, data curation, and writing–review and editing. **Eric Luna:** Validation, resources, and data curation. **Donna Armylagos:** Validation, resources, and data curation. **Mary R. Schwartz:** Conceptualization and writing–review and editing. **Dina R. Mody:** Conceptualization, methodology, supervision, and project administration.

REFERENCES

1. Crosbie EJ, Einstein MH, Franceschi S, Kitchener HC. Human papillomavirus and cervical cancer. *Lancet.* 2013;382:889-899.
2. Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol.* 1999;189:12-19.
3. Bosch FX, Lorincz A, Munoz N, Meijer CJ, Shah KV. The causal relation between human papillomavirus and cervical cancer. *J Clin Pathol.* 2002;55:244-265.
4. Auger M, Khalbuss W, Nayar R, et al. Accuracy and false-positive rate of the cytologic diagnosis of follicular cervicitis: observations

from the College of American Pathologists Pap Educational Program. *Arch Pathol Lab Med.* 2013;137:907-911.
5. Schiffman M, Castle PE. The promise of global cervical-cancer prevention. *N Engl J Med.* 2005;353:2101-2104.
6. Vinokurova S, Wentzensen N, Kraus I, et al. Type-dependent integration frequency of human papillomavirus genomes in cervical lesions. *Cancer Res.* 2008;68:307-313.
7. Moody CA, Laimins LA. Human papillomavirus oncoproteins: pathways to transformation. *Nat Rev Cancer.* 2010;10:550-560.
8. McLaughlin-Drubin ME, Munger K. Biochemical and functional interactions of human papillomavirus proteins with polycomb group proteins. *Viruses.* 2013;5:1231-1249.
9. Moyer VA. Screening for cervical cancer: US Preventive Services Task Force recommendation statement. *Ann Intern Med.* 2012;156: 880-891, W312.
10. Saslow D, Solomon D, Lawson HW, et al. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. *J Low Genit Tract Dis.* 2012;16:175-204.
11. Zhao C, Crothers BA, Ghofrani M, et al. Human Papillomavirus genotyping testing practice in 2014: results of a College of American Pathologists national survey. *Arch Pathol Lab Med.* 2016;140: 1364-1370.
12. Szarewski A, Mesher D, Cadman L, et al. Comparison of 7 tests for high-grade cervical intraepithelial neoplasia in women with abnormal smears: the Predictors 2 study. *J Clin Microbiol.* 2012; 50:1867-1873.
13. Castle PE, Eaton B, Reid J, Getman D, Dockter J. Comparison of human papillomavirus detection by Aptima HPV and cobas HPV tests in a population of women referred for colposcopy following detection of atypical squamous cells of undetermined significance by Pap cytology. *J Clin Microbiol.* 2015;53:1277-1281.
14. Iftner T, Becker S, Neis KJ, et al. Head-to-head comparison of the RNA-based Aptima human papillomavirus (HPV) assay and the DNA-based Hybrid Capture 2 HPV test in a routine screening population of women aged 30 to 60 years in Germany. *J Clin Microbiol.* 2015;53:2509-2516.
15. Muangto T, Chanthasenanont A, Lertvutivivat S, et al. Experience of combined liquid based cervical cytology and high-risk HPV mRNA for cervical cancer screening in Thammasat University Hospital. *Asian Pac J Cancer Prev.* 2016;17:4409-4413.
16. Virtanen E, Kalliala I, Dyba T, Nieminen P, Auvinen E. Performance of mRNA- and DNA-based high-risk human papillomavirus assays in detection of high-grade cervical lesions. *Acta Obstet Gynecol Scand.* 2016;96:61-68.
17. Cuzick J, Cadman L, Mesher D, et al. Comparing the performance of 6 human papillomavirus tests in a screening population. *Br J Cancer.* 2013;108:908-913.
18. Rebolj M, Bonde J, Preisler S, Ejegod D, Rygaard C, Lynge E. Human papillomavirus assays and cytology in primary cervical screening of women aged 30 years and above [serial online]. *PLoS One.* 2016;11:e0147326.
19. Ovestad IT, Vennestrom U, Andersen L, et al. Comparison of different commercial methods for HPV detection in follow-up cytology after ASCUS/LSIL, prediction of CIN2-3 in follow up biopsies and spontaneous regression of CIN2-3. *Gynecol Oncol.* 2011;123:278-283.
20. Burger EA, Kornor H, Klemp M, Lauvrak V, Kristiansen IS. HPV mRNA tests for the detection of cervical intraepithelial neoplasia: a systematic review. *Gynecol Oncol.* 2010;120:430-438.
21. Massad LS, Einstein MH, Huh WK, et al. 2012 Updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. *Obstet Gynecol.* 2013;121:829-846.
22. Sorbye SW, Fismen S, Gutteberg T, Mortensen ES. Triage of women with minor cervical lesions: data suggesting a "test and treat" approach for HPV E6/E7 mRNA testing [serial online]. *PLoS One.* 2010;5:e12724.
23. Rijkaart DC, Heideman DA, Coupe VM, et al. High-risk human papillomavirus (hrHPV) E6/E7 mRNA testing by PreTect HPV-Proofer for detection of cervical high-grade intraepithelial neoplasia and cancer among hrHPV DNA-positive women with normal cytology. *J Clin Microbiol.* 2012;50:2390-2396.
24. Origoni M, Cristoforoni P, Carminati G, et al. E6/E7 mRNA testing for human papilloma virus-induced high-grade cervical intraepithelial disease (CIN2/CIN3): a promising perspective [serial online]. *Ecancermedicalscience.* 2015;9:533.
25. Kajitani N, Satsuka A, Kawate A, Sakai H. Productive lifecycle of human papillomaviruses that depends upon squamous epithelial differentiation [serial online]. *Front Microbiol.* 2012;3:152.
26. Ratnam S, Coutlee F, Fontaine D, et al. Clinical performance of the PreTect HPV-Proofer E6/E7 mRNA assay in comparison with that of the Hybrid Capture 2 test for identification of women at risk of cervical cancer. *J Clin Microbiol.* 2010;48:2779-2785.
27. Fontecha N, Basaras M, Hernaez S, Andia D, Cisterna R. Assessment of human papillomavirus E6/E7 oncogene expression as cervical disease biomarker [serial online]. *BMC Cancer.* 2016;16:852.
28. Zhou H, Mody RR, Luna E, et al. Clinical performance of the Food and Drug Administration-Approved high-risk HPV test for the detection of high-grade cervicovaginal lesions. *Cancer Cytopathol.* 2016;124:317-323.
29. Munkhdelger J, Kim G, Wang HY, et al. Performance of HPV E6/E7 mRNA RT-qPCR for screening and diagnosis of cervical cancer with ThinPrep Pap test samples. *Exp Mol Pathol.* 2014;97:279-284.