Research Article

Diagnostic Performance of Urine-based HPV-DNA Test (CerviScan, Bio Farma) as Cervical Cancer Screening Tool in Adult Women

Performa Tes Diagnostik DNA-HPV berbasis Urine (CerviScan, Bio Farma) sebagai Alat Skrining Kanker Serviks pada Perempuan Dewasa

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Abstract

Objective: Detection of high-risk human papillomavirus (hr-HPV) in urine specimens has been introduced recently and a new local PCR kit has been developed in Indonesia (CerviScan, Bio Farma). The objective of this study was to obtain the accuracy of hr-HPV DNA testing using the new kit (CerviScan, Bio Farma) on urine specimens against the gold standard on cervical swabs.

Method: Adult women (aged 20–50 years) underwent routine general check-up or Pap test were enrolled between July and September 2022. Pairs of urine and cervical swab specimens were obtained from all subjects. HPV-DNA tests were performed using the new local PCR kit (CerviScan, Bio Farma) and the standard procedure (COBAS® 6800 HPV, Roche Molecular System). Direct sequencing was done whenever there were dispute results between the two methods. Agreement between both methods was tested using Kappa statistics. Diagnostic performance test was done on CerviScan.

Results: A total of 876 women completed the examination. Agreement between CerviScan and COBAS® 6800 was substantial ($\kappa=0.662$; $p<0.001$) and was almost perfect against COBAS® 6800 plus sequencing ($\kappa=0.828$; $p<0.001$). The accuracy of CerviScan on urine samples was 95.8% against COBAS® 6800 and increased to 97.8% after additional sequencing. The sensitivity and specificity of CerviScan on urine samples compared to cervical swabs are 73.1% and 97.3%, respectively.

Conclusion: Urine-based HPV-DNA testing with CerviScan is a reliable tool to detect high-risk HPV subtypes. It could become an alternative method for HPV-DNA testing to improve the coverage of cervical cancer screening program.

Key words: cervical cancer, HPV-DNA test, HPV molecular test screening, human papilloma virus, urine test.

Abstrak

Tujuan: Deteksi high-risk human papillomavirus (hr-HPV) pada spesimen urin telah diperkenalkan baru-baru ini dan kit PCR lokal baru telah dikembangkan di Indonesia (CervisScan, Bio Farma). Tujuan dari penelitian ini adalah untuk mengetahui keakuratan pengujian DNA hr-HPV menggunakan kit baru (ServiScan, Bio Farma) pada spesimen urin terhadap baku emas pada apusan serviks.

Metode: Perempuan dewasa (usia 20-50 tahun) direkrut untuk menjalani pemeriksaan umum rutin atau Pap tes antara Juli dan September 2022. Spesimen urin dan apusan serviks diperoleh dari seluruh subjek. Tes HPV-DNA dilakukan menggunakan kit PCR lokal baru (CerviScan, Bio Farma) dan baku emas (COBAS® 6800 HPV, Roche Molecular System). Sequencing langsung ditambahkan setiap kali terdapat perbedaan hasil antara kedua metode. Kesepakatan (agreement) antara kedua metode diuji menggunakan statistik Kappa. Uji perfoma diagnostik CerviScan dilakukan terhadap COBAS® 6800 HPV.

Hasil: Sebanyak 876 perempuan dewasa mengikuti pemeriksaan. Ditemukan kesepakatan substansial antara CerviScan dan COBAS® 6800 ($\kappa=0.662$; $p<0.001$) dan hampir sempurna terhadap COBAS® 6800 ditambah sequencing ($\kappa=0.828$; $p<0.001$). Keakuratan CerviScan pada sampel urin adalah 95,8% terhadap COBAS® 6800 dan meningkat menjadi 97,8% setelah ditambah sequencing. Sensitivitas dan spesifisitas CerviScan pada sampel urin dibandingkan dengan apusan serviks masing-masing adalah 73,1% dan 97,3%.

Kesimpulan: Pengujian HPV-DNA berbasis urin dengan CerviScan memiliki keandalan untuk mendeteksi subtipe HPV risiko tinggi. Pemeriksaan ini dapat menjadi metode alternatif untuk pengujian DNA-HPV dalam memperluas program skrining kanker serviks.

Kata kunci: human papilloma virus, kanker serviks, skrining, tes urin, uji HPV-DNA, uji molekuler HPV.

INTRODUCTION

Cervical cancer is the second most common female cancer found in Indonesia. In 2020, the Global Burden of Cancer Study (GLOBOCAN) estimated a total of 36,633 cervical cancer cases or 9.2% of all cancers.[1] The incidence rate of cervical cancer in Indonesia was 27 cases per 100,000 women with mortality rate at 3.95 per 100,000 women at all ages.[2]

Almost all cervical cancer cases are caused by persistent infection with high-risk human papillomavirus (hr-HPV) through the action of its oncoproteins.[3] Screening for hr-HPV is now the gold standard to prevent cervical cancer and other HPV-related diseases.[4] In high-resource countries, screening strategies currently used cytological evaluation (Pap smear), nucleic acid HPV-testing or both. Visual inspection with acetic acid (VIA) is another method commonly done in developing countries that aims to detect pre-cancer and early cancer lesions in apparently normal and asymptomatic women.[5] Previous randomized controlled trials found that hr-HPV screening is more sensitive in detecting precancerous lesions than the cytological screening methods.[6]

Although screening is beneficial in cervical cancer prevention, Pap smear is considered physically uncomfortable for women.[7] Two most common barriers to get cervical screening were pain or discomfort (67.2%) and embarrassment (57.9%).[8] Therefore to encourage screening, other strategy should be thought to increase women willingness to attend screening.

Self-collected urine sampling has been introduced for more than a decade to increase screening uptake rates.[9,10] Meta-analyses showed that urinary HPV test had a pooled sensitivity of 77% and specificity of 88% compared with clinician-collected cervical HPV test (cervical HPV test).[11] In Indonesia, a new PCR-based diagnostic kit (CerviScan) has been developed recently to detect 14 high-risk HPV subtypes based on qualitative polymerase-chain reaction (PCR) assay. The kit is developed by Bio Farma in collaboration with Nusantics, a biotechnology company in Indonesia. Bio Farma is a state-owned pharmaceutical company, while Nusantics is a private company focusing on genetic research. However, before providing it for wide clinical application, a proper diagnostic study should be performed. Therefore, the objective of this study was to obtain the accuracy of hr-HPV DNA testing using the new kit (CerviScan, Bio Farma) against the standard PCR-based HPV-DNA testing and to test diagnostic performance of CerviScan on urine specimens against standard PCR-based HPV-DNA testing on cervical swabs.

METHODS

The study design was a diagnostic study comparing the new HPV-DNA diagnostic kit (CerviScan, Bio Farma) with the standard diagnostic kit (COBAS® 6800 HPV, Roche Molecular system). The study was held between July and September 2022. Sample processing and PCR were done in the laboratory of Clinical Pathology Department, Cipto Mangunkusumo National Central General Hospital, Jakarta. DNA sequencing was done to confirm different results in a private laboratory (Nusantics), Jakarta. Ethics approval was granted from the Health Research Ethic Committee of the Faculty of Medicine Universitas Indonesia Dr. Cipto Mangunkusumo Hospital (No. KET- 674 / UN2.F1/XTIK/PPM.00.0212022).

Study subjects were sexually active women aged 20–50 years who came for routine general check-up or cervical cancer screening using PAP test or IVA. Written consent was obtained from all participants. Subjects were recruited from several clinics of pharmaceutical holding companies where specimens were taken. All subjects provided self-collected urine sample with a minimum volume of 30 mL in a sterile container, and cervical swabs were taken by trained health workers using a cytobrush and promptly preserved in liquid transport medium (ThinPrep PreservCyt Solution® - Hologic, Inc. Malborough, MA, USA). The number of subjects required for this study was estimated using the sample size calculation for sensitivity and specificity,[12,13] the normal distribution value (Z) was set to 1.96 at 95% confidence interval and the maximum acceptable width of the 95% confidence interval (W) was set to 10%. Based on the prevalence (P) of HPV infection among Indonesian women of 5.2%[14] the minimum sample size obtained was 666 women. Sexually active women aged 20–50 years who visit company clinics for PAP smear or IVA test were enrolled. Subjects were excluded if they were pregnant, HIV-infected, having menstruation, or have received completed doses of HPV vaccination.

Diagnostic Kit

Diagnostic kit prototype (CerviScan) was made by Nusantics, which was designed to simultaneously detect 14 high-risk HPV types. It consisted of three components, i.e. qPCR ReadyMix, Nuclease-Free Water (NFW), and HPV Positive Control. The qPCR ReadyMix combined enzymes, probes, and buffered need for qPCR reaction. Cerviscan is a qPCR-based molecular diagnostic kit to detect 14 high-risk HPV types (hr-HPV) associated with cervical cancer, namely HPV types 16,18, 31, 33, 35, 39, 45,51,52,56, 58,59,66 and 68. Furthermore, the kit can be specifically genotyping the HPV type 16, 18, 52. The kit can be applied to detect HPV-DNA from both urine and cervical swab specimens.

HPV-DNA Testing on Urine Samples

Urine samples were self-collected using a sterile urine container allowing a collection of 30 mL first-void urine. Samples were labeled and transferred to the laboratory. Upon arrival in the laboratory, samples were stored at 4oC and processed within 2 days or at -80o C when further process will be delayed more than 3 days.

Briefly, the urine samples were homogenized, and urine sediment were obtained from the 5 ml samples by centrifugation at 800 rcf for 10 min and then the supernatant was discarded. After resuspension, the DNA was extracted from 200 µl of the sample using a standard method of spinned column. The eluent were ready for PCR.

The PCR was done by mixing 5 uL DNA eluent and 15 uL CerviScanReadyMix. Amplification on BioRad CFX-96 thermocycler for 45 cycles. Signal of HPV 16 (HEX), HPV18 (Texas Red), HPV52 (Cy5), Other HR type (FAM), and internal control were considered as detected when Ct value ≤ 40 The result were considered invalid when internal control not detected

HPV-DNA Testing on Cervical Swabs

Each cervical swab specimen was divided into two samples for HPV-DNA testing using CerviScan and COBAS® 6800 system. For CerviScan the sample processing procedure followed the same procedure as the urine sample, and for the COBAS® 6800 system followed the procedure from the manufacturer.

Direct Sequencing

Direct sequencing was performed whenever there was a discrepancy of test results between CerviScan and COBAS® 6800 system using next generation sequencing (NGS) technique.

Statistical Analyses

Agreement between the two methods was tested using Kappa statistics; results were defined as poor ($\kappa = 0$), slight ($0.01 < \kappa < 0.20$), fair ($0.21 < \kappa < 0.40$), moderate ($0.41 < \kappa < 0.60$), substantial ($0.61 < \kappa < 0.80$), almost perfect ($0.81 < \kappa < 1$) or perfect ($\kappa = 1$).

Diagnostic performance test result was expressed as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive and negative likelihood ratios (LRs). Statistical analysis was done using IBP SPSS version 26.

RESULTS

The study population consists of 876 women who completed examination. Invalid results of CerviScan was 3.0% on urine samples and 1.8% on cervical swab samples, whereas COBAS® 6800 system was failed in 23.5% urine specimens and 2.7% on cervical swab samples. The prevalence of HPV infection was 5.9% based on the COBAS® 6800 test on the cervical swab as the gold standard. However, the test kit (CerviScan) on urine samples resulted a prevalence of 6.8%.

Agreement between CerviScan on urine samples and standard test on cervical swab is shown in Table 1a and 1b. Additional seven results were detected during sequencing on urine samples which were initially invalid, hence the total sample is change from 826 (table 1a) to 833 (table 1b).

Table 1a. Agreement between CerviScan (Bio Farma, Indonesia) on Urine Samples and COBAS® 6800 on Cervical Swab on the detection of high-risk Human Papiloma Virus (n= 826).

CerviScan	COBAS® 6800	
	HR-HPV +	**HR-HPV-**
HR-HPV+	38	21
HR-HPV-	14	753

Kappa statistics = 0.662; *p* < 0.001

Table 1b. Agreement between CerviScan (Bio Farma, Indonesia) on Urine Samples and COBAS® 6800 + Direct Sequencing on Cervical Swab on the detection of high-risk Human Papiloma Virus (n= 833).

CerviScan	COBAS® 6800 + Direct Sequencing	
	HR-HPV +	**HR-HPV-**
HR-HPV+	50	5
HR-HPV-	13	765

Kappa statistics = 0.828; *p* < 0.001

CerviScan showed >95% accuracy against COBAS® 6800 as the standard method, both on urine and cervical swab specimens as shown in table 2.

Table 2. Diagnostic Performance of CerviScan (Bio Farma, Indonesia) on the Detection of High-risk Human Papiloma Virus Compared to COBAS® 6800.

	Cervical Swab (n=838)		Urine (n=658)	
	%	**95% CI**	**%**	**95% CI**
Sensitivity (%)	87.8	75.2 – 95.4	84.3	71.4 – 93.0
Specificity (%)	98.6	97.5 – 99.3	97.5	96.0 – 98.6
PPV (%)	79.6	66.5 – 89.4	74.1	61.0 – 84.7
NPV (%)	99.2	98.3 – 99.7	98.7	97.4 – 99.4
Accuracy (%)	98.0	96.8 – 98.8	96.5	94.8 – 97.8

PPV: positive predictive value, NPV: negative predictive value

Results of CerviScan on urine samples had 95.8% of accuracy compared to the current gold standard, i.e. COBAS® 6800 on cervical swab specimens (95.8%). The accuracy was even better (97.8%) when additional direct sequencing was performed for dispute results as shown in Table 3.

Table 3. Diagnostic Performance of CerviScan (Bio Farma, Indonesia) on the detection of high-risk Human Papiloma Virus on Urine Compared to COBAS® 6800 on Cervical Swab Specimens.

	COBAS® 6800 (n=826)		COBAS® 6800 plus sequencing (n=833)	
	%	**95% CI**	**%**	**95% CI**
Sensitivity (%)	73.1	58.9 – 84.4	79.4	67.3 – 88.5
Specificity (%)	97.3	95.9 – 98.3	99.3	98.5 – 99.8
PPV (%)	64.4	50.9 – 76.4	90.9	80.0 – 96.9
NPV (%)	98.2	96.9 – 99.0	98.3	97.2 – 99.1
Accuracy (%)	95.8	94.2 – 97.0	97.8	96.6 – 98.7
LR-positive	26.9	17.1 – 42.4	122.2	50.6 – 295.5
LR-negative	0.28	0.2 – 0.4	0.21	0.1 – 0.3

PPV: positive predictive value, NPV: negative predictive value; LR: likelihood ratio

DISCUSSION

We showed that the accuracy of urine-based HPV-DNA testing using CerviScan was very high, above 95%, compared to standard method on cervical swab-based method. Specimens that tested negative on standard method but positive on CerviScan were proved to harbor hr-HPV on amplicon sequencing. This showed that specimens were true positive and that CerviScan was superior to the standard method of HPV DNA testing. The higher failure rate of the COBAS® 6800 on urine sample were in concordance with the claim of the manufacturer that the system was not validated for urine samples.

There are not many studies comparing diagnostic performance of urine-based HPV detection with the standard test on cervical swab. Our study showed a substantial agreement between CerviScan and the standard method with Kappa value of 0.662 (p<0.001). But the agreement was improved when sequencing was added as comparing method, with Kappa value of 0.828 (p<0.001). A study in Thailand showed comparable results with our study, they reported a substantial agreement of hr-HPV detection between urine and cervical samples with κ=0.65. Diagnostic test results were in 68.6% sensitivity, 93.2% specificity, 80.0% PPV, and 88.2% NPV. The gold standard used in the study was Cobas 4800® system.[15] Another study in southern Mexico on 108 pairs of urine and cervical samples from an indigenous population found 68.3% concordance of HPV positivity and 64.5% concordance for hr-

HPV. The sensitivity to detect hr-HPV was 89.7% but the specificity was only 25.7%. The method of HPV genotyping in this study was INNO-LiPA HPV assay.[16]

In our study, we did not look in to the clinical relevances of the HPV infection with regard to cervical intraepithelial lesion (CIN). As HPV infection usually precedes the cervical lesions and has a very long asymptomatic phase of infection. However, recent meta-analysis reported that urinary HPV test was less sensitive than common cervical HPV tests (including COBAS) even though the difference was not statistically significant to detect CIN 2 or worse.[17]

The results of this study may have direct implication on cervical cancer screening program in Indonesia in parallel with a nationwide vaccination programme. As the performance was comparable with the standard method, and due to its convenience, self-sampled urine specimens would be preferable for most women and therefore may increase the rate of HPV-DNA testing by expanding the scope of screening. Moreover, urine sample collection generally requires less consumables, trained personnel, and special facilities, that may result in more affordable test for public heath settings

CONCLUSIONS

In conclusion, urine-based HPV-DNA testing with CerviScan is a reliable tool to detect high-risk HPV subtypes. It is an alternative choice of method for HPV-DNA testing and cervical cancer screening program.

REFERENCES

1. World Health Organization International Agency for Research on Cancer. The Global Cancer Observatory: Indonesia 2020. 2020.
2. Bruni L, Albero G, Serrano B, Mena M, Collado JJ, Gómez D, et al. Human papillomavirus and related diseases report: Indonesia. ICO/IARC Information Centre on HPV and Cancer (HPV Information Centre): Barcelona. 2021.
3. Scarth JA, Patterson MR, Morgan EL, MacDonald A. The human papillomavirus oncoproteins: a review of the host pathways targeted on the road to transformation. J Gen Virol. 2021;102(3):001540. doi: 10.1099/jgv.0.001540.
4. Ronco G, Dillner J, Elfstr€om KM, et al. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. Lancet. 2014;383(9916):524–32.
5. Mittal S, Basu P, Lucas E. Atlas of visual inspection of the cervix with acetic acid for screening, triage, and assessment for treatment: IARC CancerBase No. 16 Lyon, France: International Agency for Research on Cancer. 2020.
6. Melnikow J, Henderson JT, Burda BU, Senger CA, Durbin S, Weyrich MS. Screening for cervical cancer with high-risk human papillomavirus testing: updated evidence report and systematic review for the us preventive services task force. JAMA. 2018;320:687–705.
7. Racey CS, Withrow DR, Gesink D. Self-collected HPV testing improves participation in cervical cancer screening: a systematic review and meta-analysis. Can J Public Health. 2013;104(2):e159-6.
8. Wilding S, Wighton S, Halligan D, West R, Conner M, O'Connor DB. What factors are most influential in increasing cervical cancer screening attendance? An online study of UK-based women. Health Psychol Behav Med. 2020;8:314–28. doi: 10.1080/21642850.2020.1798239.
9. Sellors JW, Lorincz AT, Mahony JB, Mielzynska I, Lytwyn A, Roth P, et al. Comparison of self-collected vaginal, vulvar and urine samples with physician-collected cervical samples for human papillomavirus testing to detect high-grade squamous intraepithelial lesions. CMAJ. 2000;163:513–8.
10. Shin HY, Lee B, Hwang SH, Lee DO, Sung NY, Park JY, et al. Evaluation of satisfaction with three different cervical cancer screening modalities: clinician-collected Pap test vs. HPV test by self-sampling vs. HPV test by urine sampling. J Gynecol Oncol. 2019;30:e76.
11. Pathak N, Dodds J, Zamora J, Khan K. Accuracy of urinary human papillomavirus testing for presence of cervical HPV: systematic review and meta-analysis. BMJ 2014;349:g5264.
12. Buderer NM. Statistical methodology: I. Incorporating the prevalence of disease into the sample size calculation for sensitivity and specificity. Acad Emerg Med. 1996;3(9):895–900.
13. Negida A, Fahim NK, Negida Y. Sample Size Calculation Guide - Part 4: How to calculate the sample size for a diagnostic test accuracy study based on sensitivity, specificity, and the area under the ROC Curve. Adv J Emerg Med. 2019;3(3):e33. doi: 10.22114/ajem.v0i0.158.
14. Handayani S, Dewi RM, Wibowo HA, Dany F, Kipuw NL, Adelina R, et al. Cervical Cancer Risk Factor: HPV Infection Among Indonesian Urban Women; Adv Health Sci Res. 2019;22:452–5.
15. Khunamornpong S, Settakorn J, Sukpan K, Lekawanvijit S, Katruang N, Siriaunkgul S. Comparison of human papillomavirus detection in urine and cervical samples using high-risk HPV DNA testing in Northern Thailand. Obstet Gynecol. 2016;2016: 6801491. doi: 10.1155/2016/6801491.
16. Torres-Rojas FI, Mendoza-Catalán MA, Alarcón-Romero LC, Parra-Rojas I, Paredes-Solís S, Leyva-Vázquez MA, et al. HPV molecular detection from urine versus cervical samples: an alternative for HPV screening in indigenous populations. Peer J. 2021; 9:e11564. doi:10.7717/peerj.11564.
17. Cho H-W, Shim SR, Lee JK, Hong JH. Accuracy of human papillomavirus tests on self-collected urine versus clinician-collected samples for the detection of cervical precancer: a systematic review and meta-analysis. J Gynecol Oncol. 2022;33(1):e4. doi: 10.3802/jgo.2022.33.e4.