ORIGINAL RESEARCH

Cobas® 4800 HPV test is accurate for detecting high risk Human Papillomavirus from urine samples at dr. Cipto Mangunkusumo National Central General Hospital, Jakarta, Indonesia

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Article Info
Received Aug 3, 2022
Revised Oct 17, 2022
Accepted Nov 15, 2022
Published Dec 1, 2022

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Keywords:
Papillomavirus
Urine
4800 Cobas®
Genotyping
Maternal health

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ABSTRACT

Objective: To find the accuracy, sensitivity, specificity, as well as predicted values, both positive and negative, of urine samples using Cobas® 4800 in detecting high risk Human Papillomavirus.

Materials and Methods: This study was a cross-sectional study with a total of 72 samples taken from medical records of hrHPV DNA examination with Cobas® 4800 in 2017-2020. Study subjects were called for re-examination of urine samples and cervical samples using Cobas® 4800. Samples with positive hrHPV DNA in the cervix, urine, or both were examined for cervical fluid-based cytology (LBC). Data were analyzed using Chi-square.

Results: Overall, 84.72% agreement was detected through specimens of urine and cervical mucus tested of hrHPV DNA with Cobas® 4800. In all samples, a significant rate of concordance detection of hrHPV DNA with Cobas® 4800 was reported (κa = 0.62; 95% IC: 39-84). In this population, in determining the presence of hrHPV DNA in cervical and urine specimens, it was found that the sensitivity, specificity, positive predictive value and negative predictive value were respectively 87.5% (95% IC: 64–97%), 84% (95% IC: 72–91%), 60.9% (95% IC: 40.8–77.8%), and 96 % (95% IC: 86.3–98.9%).

Conclusion: The presence of hrHPV infection in the cervix can be determined by detecting hrHPV DNA in the urine. According to these findings, urine samples subjected to the Cobas® 4800 HPV test may be helpful for the clinical treatment of HPV infection.

Cite this as: Wityawan, I., Andrijono, & Kekalih, A. (2022). Cobas® 4800 HPV test is accurate for detecting high risk Human Papillomavirus from urine samples at dr. Cipto Mangunkusumo National Central General Hospital, Jakarta, Indonesia. Majalah Obstetri & Ginekologi, 30(3), 116-121. https://doi.org/10.20473/mog.V30I32022.116-121

INTRODUCTION

Cervical cancer has the highest prevalence and it is the fourth leading cause of death from all cancers. Human papillomavirus (hrHPV) is a virus that poses a high risk of infection, considered the main etiology of cervical cancer.[1] According to WHO, one of the global goals is to provide a technique for expediting the eradication cervical carcinoma, which has become a concern of global health-related services. It is intended that 70% of women be screened at the age of 35 and then again at the age of 45 by 2030.[2-5] The hrHPV DNA test has to be the most sensitive screening procedure for CIN 2+ with sensitively value of 98.3% and a specificity values of 85.3-86.2%.[6]

According to Indonesia's 2018 health profile data, women who have screened for cervical cancer with acetate visual inspection were 7.34%.[4,5] However, this low achievement was because the screening methods needed pelvic examination, which is invasive, painful for the patients, time-consuming for healthcare profes-

sionals, and difficult to do in settings with limited resources. Therefore, the development of non-invasive self-sample collection techniques might potentially have the benefit of raising the acceptability of the screening processes. Cytology-based screening is being replaced by primary screening or co-testing based on the human papillomavirus (HPV). For this, the use of urine, which is simple to collect, would be beneficial. Furthermore, molecular techniques to identify DNA in urine samples are already regularly used to diagnose prevalent sexually transmitted diseases, including *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.[7]

HPV does not have a preference for the urinary system, in contrast to its preference for the cervix and other anogenital anatomical regions.[8-11] Therefore, the presence of HPV in urine is very certainly due to secondary cervix exfoliation or other anogenital lesions. The purpose of this study was to assess urine testing for high risk HPV in a group with a high prevalence of the disease. The clinical effectiveness of self urine-based sampling was also assessed in comparison to cervical sampling, and the results were compared to the presence of cervical disease as determined by histology. The Cobas® 4800 HPV test, an FDA-approved real-time PCR assay created for the simultaneous genotyping of HPV-16 and HPV-18 and the detection of high-risk HPV (HR-HPV), was utilized for this purpose.[12,13]

MATERIALS AND METHODS

This study used a cross-sectional design of a diagnostic test to compare urine hrHPV DNA to cervical hrHPV DNA preparations, which was the gold standard that carried out at the Kencana Women Health Cluster from January to December 2020, at dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia. Patients aged 30-60 years were enrolled on this study. Pregnant women and those who had given birth within the past three months of the examination date were excluded. The patients were recalled for cervical and urine hrHPV DNA examinations on the same day and a cytology examination with liquid-based cytology (LBC) was performed if the results of the hrHPV examination were positive, either in cervical swabs, urine, or both.

Cervical samples were taken using a cervical brush by the doctors and deposited in five days in a real-time polymerase chain Cell Cobas® medium bottle (Roche Diagnostic). Cervical specimens taken for PCR cell collection at Cobas® media are stable for up to 6 months at 2–30º C for use with the Cobas® 4800 test for HPV testing.[5] In sterile containers, we collected urine specimens identically dated as the pelvic examination and delivered to a laboratory for further processing within five days. A minimum of each urine sample was diluted to a volume of 20 milliliters, and centrifuged for 10 minutes at 4000 x g. The solid pellet was resuspended in water following centrifugation of five milliliters supernatant into a vial of PCR collect media. The Cobas® 4800 test was used to process urine samples, despite the fact that it had not been verified for use for this type of specimen.[12,13]

In a single system, the Cobas® 4800 system combines completely real-time PCR technique, enabling automated sample preparation. In addition to hrHPV genotypes, the test identified globin gene of human cells. In a single tube, amplification and detection of polymerase chain reaction took place, with probes containing four separate dyes that act as reporters tracking the multiplex reaction's distinct targets. There were four different dyes: Reporter dye 1 observing the hrHPV pool containing 12 human papillomavirus (HPV) targets (HPV-31, -33; -35; 39; 45; 51), while 2 and 3 dyes observing two forms of HPV, the type16 and type 18. Dye 4 examined the b-globin protein to determine the adequacy of the cells, their extraction, and amplification. The examination was conducted according to the producer's recommendations for the extraction of DNA with Cobas® X 480 and real-time PCR with the Cobas® Z 480.[11,12]

Sensitivity, PPV (positive predictive value), specificity, NPV (negative predictive value) are the terms used to describe the prediction accuracy of hrHPV detection in urine speciments, which is measured as percentages with confidence intervals of 95% in comparison to cervical sample detection (gold standard) (95% CI). The Kappa statistic (Cohen's Kappa) was utilized to ascertain if test concordance was poor (κ=0), slight ($0.01<\kappa<0.20$), fair ($0.21<\kappa<0.40$), moderate ($0.41<\kappa<0.60$), substantial ($0.61<\kappa<0.80$), nearly perfect ($0.81<\kappa<1$), or perfect (κ=1). Differences between paired proportions were calculated using the McNemar's test. $P<0.05$ was utilized as the significance level.[14]

RESULTS AND DISCUSSION

The b-globin gene was detected in every 72 cervix and urinary specimens used in this study. Preliminary data from 2017-2020 tests showed that 42 women had previously tested positive for hrHPV DNA, while 30 women had previously tested negative. A prevalence of 34.7% was found in 25 women who had positive test results of hrHPV using a minimum one of the two specimens examined. In urine samples, the prevalence of carcinogenic type hrHPV was 31.9%, while in cervical samples, it was 22.2% (Table 1).

Table 1. Concordance and agreement in detecting cervical and urine samples for HPV DNA analysis.

		Cervical Samples		% Agreement	κ^a (95% IC)
		Positive	Negative		
Urine samples	Positive	14	9	84.72%	0.62 (39-84)
	Negative	2	47		

a: Cohen's Kappa

There was an overall concordance of 84.72% between hrHPV identification in urine and cervical secretions specimens. The identification of hrHPV DNA in both samples had a high concordance rate (κ= 0.62; 95 % IC: 39–84). For both types of samples, there were 11 disparities in the results. In two of them, hrHPV was detected in the cervix but not in the urine. However, nine positive samples were obtained for hrHPV in the urine but negative in the cervix. Overall, for detecting hrHPV DNA extracted from urine compared to cervical samples, sensitivity, PPV (positive predictive value), specificity, and NPV (negative predictive value) were 87.5% (95% IC: 64–96), 83.9% (95 % IC: 72–91), 95.9% (95 % IC: 86–99), and 60.9% (95% IC: 41–78), respectively. For the pool of HR-genotypes, the prevalence of hrHPV samples of urine was higher compared to cervical samples (without HPV16 and HPV 18).

Table 2 compares the sensitivity, PPV (positive predictive value), specificity and NPV (negative predictive value) of particular PCR analysis (HPV type 16, HPV type 18, and another hrHPV) comparing those found in urine specimens to those found in cervical specimens. The sensitivity of any hrHPVgenome was 84.62% (95% IC = 58–96), and the Cohen's Kappa was moderate.

Twenty-five women received cytological results, with 17 (68%) being negative for intraepithelial lesions, 5 (20%) having LSIL (Low Grade Squamous Intraepithelial Lesions), 2 (8%) having ASCH (Atypical Squamous Cells, could not exclude HSIL), and 1 (4%) having ASCUS (Atypical Squamous Cells of Uncertain Significance) (Table 3). To find out the factors that influence the results of the urine DNA hrHPV examination, a relationship test was carried out between the factors that might affect the examination results (age, education, and parity) with the suitability of the urine DNA hrHPV results to the cervix as shown in Table 4.

Table 2. Specific genotyping sensitivity, specificity, NPV, and PPVurinalysis specimens (IC 95%).

	HPV type 16	HPV type 18	12 HR-HPV[a]
Sensitivity (95 IC%)	-	100% (21.100)	84.62% (58.96)
Spesifisity (95 IC%)	-	100% (95.100)	84.75% (73.92)
PPV[b] (95 IC%)	-	100% (21.100)	55% (34.74)
NPV[c] (95 IC%)	-	100% (95.100)	96% (87.99)
κ (95 IC%)	-	1 (77-123)	0.57 (35.79)

a hrHPV other than type 16 and type 18
b Predictive values that are positive.
c Predictive values that are negative.

Table 3. hrHPV infection prevalence cervical and urinary examinations sample method compared to cytology results

Cytology	*n*	12 hrHPV		HPV 16		HPV 18	
		Cervical	Urine	Cervical	Urine	Cervical	Urine
Negative[a]	17 (68%)	7/13 (54%)	14/20 (54%)	0/0	0/0	0/1	0/1
ASCUS[b]	1 (4%)	1/13 (8%)	1/20 (5%)	0/0	0/0	0/1	0/1
LSIL[c]	5 (20%)	3/13 (23%)	3/20 (15%)	0/0	0/0	1/1	1/1
ASCH[d]	2 (8%)	2/13 (15%)	2/20 (10%)	0/0	0/0	0/1	0/1
Total	25	13	20	0	0	1	1

Table 4. Age, level of education, parity, and concordance between urine and cervical samples

	Concordance		Total	P
	Yes	No		
Age				
≤40 yo	40 (86.9%)	6 (13.1%)	46 (63.9%)	0.353
>40 yo	21 (80.8%)	5 (19.2%)	26 (36.1%)	
Level of education				
Basic (< high school)	6 (66.7%)	3 (33.3%)	9 (12.5%)	0.134
> high school	55 (87.3%)	8 (12.7%)	63 (87.5%)	
Parity				
Nulli/Primiparous	54 (83.1%)	11 (16.9%)	65 (90.3%)	0.296
Multiparous	7 (100%)	0 (0%)	7 (9.7%)	

Some data suggest that using self-collected urine, rather than cervical samples, as an option for HPV testing could be beneficial. First, HPV genotypes urinalysis and cervix lesions tissue specimens are agreed significantly. Urine contaminated with exfoliated cervical cells infected with HPV is known to rise as the severity of cervical lesions increases. Thus, HPV presence in urine, that resembles an HPV infection in the cervix, requires constant monitoring.[15]

Urine sample for the diagnosis of oncogenic HPV has been studied in several research. Because various factors, such as the urine sample technique, conditions of storing, centrifuging, and extracting DNA, and HPV DNA test, might adversely influence HPV DNA detection, HPV DNA testing in the urine has a variety of limits applicable to this purpose.[15]

We examined 72 matched cervix and urine specimens from women who have been referred to gynecology unit by community physicians for examination due to abnormal results of Pap smear or VIA screening outcomes. Due to the fact that the research was done on a selected sample a group of women previously possessed an abnormality VIA or pap smear test, HPV infection was quite prevalent (>50%), as expected. Urine had a higher prevalence (31.9%) than cervical specimens (22.2%). The presence of hrHPV-DNA from epithelial tissues of the urethra and vulva, which are similarly vulnerable to hrHPV infection, could be one explanation. In 11 samples, there was a discrepancy, with two samples having positive hrHPV in the cervix but negative in urine sample and nine specimens having positive hrHPV in the urine but negative in the cervix. These 11 samples had normal cervical cytology examinations using liquid-based cytology (LBC). Some explanations on this may be the possibility of a faster regression in the cervix than in the urine. Another possibility is that there was previous sexual contact so that hrHPV transmission occurred, while the cervix had no infection process yet. In this group, the possibility of primary hrHPV infection originating from the urinary tract required further investigation. Some literature mentions the possibility of hrHPV infection as a cause of urinary tract cancer. The most common is the bladder cancer (transitional cell cancer) of 22%.[9-11]

We discovered a significant concordance rate of HR-HPV DNA detection and an overall agreement of 84.72%. (κ=0.62; 95% IC: 0.39–0.84). The sensitivity was 87.5% and the specificity was 83.9%. According to Pathak et al., HPV infection detection in urine had a pooling sensitivity of 87% and specificity of 94%, while hrHPV had a 77% sensitivity, 88% specificity in a meta-analysis and systematic review on precision of urine HPV testing for cervical HPV infection.[16] The HPV types 16 and 18 detection through urine exhibited a combined sensitivity and specificity of 73% and 98%, respectively.[16] When urine samples were obtained as first voiding instead of random or midstream urine samples, meta-regression revealed a 22-fold increase in overall accuracy.[16]

The sensitivity of first voiding urine is significantly higher than random or intermediate urine samples, resulting in a considerable difference in accuracy. The results of this study were better than those of Oliveira et al. (sensitivity of 50.0% with a Kappa value of 0.55), of Tranberg et al. with sensitivity of 63.9% with a Kappa value of 0.66), and of Sahasrabuddhe et al. with sensitivity of 51.6% with a Kappa value of 0.55 with CLART assay (κ=0.55).[17,18]

These findings were in contrast with those of Bernal et al. (sensitivity 88%, Kappa value 0.76), of Ostensson et al. (with Abbot RealTime sensitivity 33-100%, Kappa ranged from 0.36 to 0.85), and of Ornskov et al. with Abbot RealTime sensitivity 33-100%, Kappa ranged from 0.66 to 0.77).[19,20] The sensitivity of 12 hrHPV other than HPV types 16 and 18 was calculated to be 84.62%. Because there were no samples with a single infection with HPV type 16 and just one specimen having only one infection of HPV type 18, it was not done for HPV types 16 and 18. Using bivariate analysis

on the variables in the research subjects, the researchers looked for factors that could influence the outcome of a urine hrHPV DNA examination. The investigation found no research variables in terms of age, education level, or parity that were connected to the outcomes of the HPV DNA urine screening. With respect to alternative HPV detection and/or genotyping assays, we chose the Cobas® 4800 HPV test for our investigation. This real-time PCR-based assay test offers a number of benefits. First of all, the test simultaneously gives partial HPV genotyping for HPV-16 and HPV-18, in addition to reporting the existence of HR HPV genotypes. Second, since the test has been modified for primary specimens, it is highly automated and simple to use. Finally, the findings will be available around 4 hours after receiving the materials.

CONCLUSION

The findings of this research encourage the use of urine in conjunction with Cobas® 4800 HPV testing among women who have severe cervical lesions. Validation should be performed before using urine/Cobas® 4800 as a diagnostic test. More research is needed to show their equal performance in a group undergoing initial screening. If the findings are validated, urine collection may be used within populations with limited availability of healthcare providers or where, for example, pelvic examination is not practical. Additionally, when molecular methods are used in conjunction with initial tests for primary cervical cancer screening, urine samples may be used as a suitable substitute for cervical specimens. Besides for vaccination trials, disease surveillance, and epidemiological investigations, urine can also be utilized to evaluate HPV prevalence.[21-23]

DISCLOSURES

Acknowledgment

Authors like to express their appreciation to all patients who consented to take part for this research. The authors appreciate our parents and relatives for their intellectual and psychological assistance.

Conflict of interest

This study's authors claim no conflicts of interest.

Funding

This research has received no external funding.

Author contribution

All authors have contributed to all process in this research, including preparation, data gathering and analysis, drafting and approval for publication of this manuscript.

REFERENCES

1. de Sanjosé S, Brotons M, Pavón MA. The natural history of human papillomavirus infection. Best Pract Res Clin Obstet Gynaecol. 2018;47:2-13. doi: 10.1016/j.bpobgyn.2017.08.015. Epub 2017 Sep 6. PMID: 28964706.
2. Global strategy to accelerate the elimination of cervical cancer as a public health problem [Internet]. World Health Organization 2020. Available from: https://www.who.int/publications/i/item/9789240014107
3. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6): 394-424. doi: 10.3322/caac.21492. Epub 2018 Sep 12. Erratum in: CA Cancer J Clin. 2020 Jul; 70(4):313. PMID: 30207593.
4. Kementerian Kesehatan Republik Indonesia. Komite Penanggulangan Kanker Nasional. Panduan penatalaksanaan kanker serviks [Guidelines for cervical cancer management]. Jakarta; 2018.
5. Kementrian Kesehatan Republik Indonesia. Profil kesehatan Indonesia 2018. Gambar 6.66. Persentase pemeriksaan deteksi dini kanker leher rahim dan payudara pada perempuan usia 30-50 tahun menurut provinsi s/d tahun 2018 [Percentage of early detection of cervical and breast cancer in women aged 30-50 by province to 2018]. Jakarta; 2018. p. 250.
6. Melnikow J, Henderson JT, Burda BU, et al. Screening for cervical cancer with high-risk human papillomavirus testing: A systematic evidence review for the U.S. Preventive Services Task Force [Internet]. Rockville (MD): Agency for Healthcare Research and Quality (US); 2018 Aug. Report No.: 17-05231-EF-1. PMID: 30256575.
7. Bernal S, Palomares JC, Artura A, et al. Comparison of urine and cervical samples for detecting human papillomavirus (HPV) with the Cobas 4800 HPV test. J Clin Virol. 2014;61(4):548-52. doi: 10.1016/j.jcv.2014.10.001. Epub 2014 Oct 12. PMID: 25453566.
8. Senkomago V, Henley SJ, Thomas CC, et al. Human papillomavirus-attributable cancers - United States, 2012-2016. MMWR Morb Mortal Wkly Rep. 2019;68(33):724-28. doi: 10.15585/

mmwr.mm6833a3. PMID: 31437140; PMCID: PMC6705893.
9. Li N, Yang L, Zhang Y, et al. Human papillomavirus infection and bladder cancer risk: a meta-analysis. J Infect Dis. 2011;204(2):217-23. doi: 10.1093/infdis/jir248. PMID: 21673031; PMCID: PMC3114469.
10. Abdollahzadeh P, Madani SH, Khazaei S, et al. Association between human papillomavirus and transitional cell carcinoma of the bladder. Urol J. 2017;14(6):5047-50. doi: 10.22037/uj.v14i6.3748. PMID: 29101759.
11. Cai T, Di Vico T, Durante J, et al. Human papilloma virus and genitourinary cancers: a narrative review. Minerva Urol Nefrol. 2018;70(6): 579-587. doi: 10.23736/S0393-2249.18.03141-7. Epub 2018 Aug 29. PMID: 30160386.
12. Roche Molecular Systems I. Cobas® PCR Urine Sample Kit. 2013 [Internet]. Available from: http://ekstern.infonet.regionsyddanmark.dk/Files/Formularer/Upload/2013/09/cobas® PCR Urine Sample Kit (05170621001-01 Doc Rev 6 0).pdf
13. Roche. Cobas®. Short guide for cervical sample collection using the Rovers. 2015 [Internet]. Availabe from: htttp://chumontreal.com/laboratoires/images/stories/documents/nouvelles/Short_guide_prelevement_col_avec_Rovers_Cervex_Brush_anglais_20 15-02-27.pdf
14. Dahlan, MS. Penelitian diagnostik. Dasar-dasar teoritis dan aplikasi dengan program SPSS dan stata. [Diagnostic research, Theory and application with SPSS ans stata]. Seri Evidence Based Medicine 5. Jakarta: Salemba Medika; 2009.
15. Chatzistamatiou K, Vrekoussis T, Tsertanidou A, et al. Acceptability of Self-Sampling for Human Papillomavirus-Based Cervical Cancer Screening. J Womens Health (Larchmt). 2020;29(11):1447-56. doi: 10.1089/jwh.2019.8258. Epub 2020 Jul 30. PMID: 32757997.
16. Pathak N, Dodds J, Zamora J, et al. Accuracy of urinary human papillomavirus testing for presence of cervical HPV: systematic review and meta-analysis. BMJ. 2014;349:g5264. doi: 10.1136/bmj.g5264. PMID: 25232064; PMCID: PMC4166201.
17. Oliveira CM, Musselwhite LW, de Paula Pantano N, et al Detection of HPV E6 oncoprotein from urine via a novel immunochromatographic assay. PLoS One. 2020;15(4):e0232105. doi: 10.1371/journal.pone.0232105. PMID: 32320451; PMCID: PMC7176116.
18. Tranberg M, Jensen JS, Bech BH, et al. Urine collection in cervical cancer screening - analytical comparison of two HPV DNA assays. BMC Infect Dis. 2020;20(1):926. doi: 10.1186/s12879-020-05663-7. PMID: 33276740; PMCID: PMC7716507.
19. Daponte A, Michail G, Daponte AI, et al. Urine HPV in the Context of Genital and Cervical Cancer Screening-An Update of Current Literature. Cancers (Basel). 2021;13(7):1640. doi: 10.3390/cancers13071640. PMID: 33915878; PMCID: PMC8036396.
20. Ørnskov D, Jochumsen K, Steiner PH, et al. Clinical performance and acceptability of self-collected vaginal and urine samples compared with clinician-taken cervical samples for HPV testing among women referred for colposcopy. A cross-sectional study. BMJ Open. 2021;11(3):e041512. doi: 10.1136/bmjopen-2020-041512. PMID: 33674367; PMCID: PMC7939007.
21. Lee EH, Um TH, Chi HS, et al. Prevalence and distribution of human papillomavirus infection in Korean women as determined by restriction fragment mass polymorphism assay. J Korean Med Sci. 2012;27(9):1091-7. doi: 10.3346/jkms.2012.27.9.1091. Epub 2012 Aug 22. PMID: 22969258; PMCID: PMC3429829.
22. Zhou HL, Zhang W, Zhang CJ, et al. Prevalence and distribution of human papillomavirus genotypes in Chinese women between 1991 and 2016: A systematic review. J Infect. 2018;76(6): 522-8. doi: 10.1016/j.jinf.2018.02.008. Epub 2018 Mar 1. PMID: 29477803.
23. Gultekin M, Dundar S, Keskinkilic B, et al. How to triage HPV positive cases: Results of four million females. Gynecol Oncol. 2020;158(1):105-11. doi: 10.1016/j.ygyno.2020.04.698. Epub 2020 Apr 30. PMID: 32362567.