Sakarya Tıp Dergisi
Sakarya Med J

e-ISSN: 2146-409X
Publisher: Sakarya University

Vol. 15, No. 2, 164-172, 2025
DOI: http://doi.org/10.31832/smj.1669557

Research Article

Performance of DiaRD-HPV Rt-PCR Kit for Detection of High-Risk Human Papilloma Virus Genotypes: A Preliminary Study

Bengul Durmaz[1], Hande Toptan[2], Mehmet Koroglu[2], Riza Durmaz[3*], Ulker Cuhaci[4]

[1] Faculty of Medicine, Department of Medical Microbiology, Lokman Hekim University. Ankara, Türkiye
bengul.durmaz@lokmanhekim.edu.tr, ror.org/04v8ap992

[2] Faculty of Medicine, Department of Medical Microbiology, Sakarya University. Sakarya, Türkiye
handecakar@hotmail.com, mkoroglu@sakarya.edu.tr, ror.org/04ttnw109

[3] Faculty of Medicine, Department of Medical Microbiology, Yıldırım Beyazıt University, Ankara, Türkiye
rdurmaz@aybu.edu.tr, ror.org/05ryemn72

[4] Faculty of Medicine, Department of Medical Microbiology, Yuksek İhtisas University, Ankara, Türkiye
ulkerabdulmecit@gmail.com, ror.org/04fbjgg20

* Corresponding Author

Received: 03.04.2025
Accepted: 28.05.2025
Available Online: 12.06.2025

Objectives: Rapid detection and identification of high-risk human papillomavirus (hrHPV) genotypes in cervical specimens has a crucial importance for management of patients. In this study, we evaluated the performance of the novel DiaRD-HPV Rt-PCR kit developed for screening HPV in cervical samples and identifying hrHPV types 16 and 18.

Materials and Methods: The performance of the novel DiaRD-HPV Rt-PCR kit was tested on a total of 243 clinical samples, including 65 HPV positive (genotype 16, n=23; genotype 18, n=2; other genotype, n=40) and 178 HPV negative samples previously tested with the QIAscreen HPV PCR test kit. DNA was isolated from cervical specimens in PreservCyt transport medium using the Diarex Viral DNA/RNA extraction kit. Multiplex PCR was performed following the DiaRD-HPV Rt-PCR kit protocol.

Results: The results of the novel kit were compared to those of the QIAscreen HPV PCR test kit using a comparator test. Of the 65 samples that were positive with the comparator kit, 62 (95.4%) were also positive with the novel kit. All 178 HPV DNA negative samples were also negative with the novel kit. The sensitivity, specificity, and accuracy of the novel kit were 95.4%, ≥99.9%, and 98.8%, respectively. According to the cycle threshold (Ct) values determined in the reproducibility studies, the intra-assay and inter-assay coefficients of variations (CVs) were less than 1%.

Conclusion: The high concordance between the results of the novel kit and comparator kit supports the use of the DiaRD HPV Rt-PCR kit in clinical samples.

Keywords: Human papillomavirus, Rt-PCR, DiaRD HPV kit

1. INTRODUCTION

Human papillomaviruses are small, double-stranded DNA viruses in the *Papillomaviridae* family.[1] Among more than 200 HPV genotypes identified, the high-risk HPV (hrHPV) genotypes, including HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 are strongly associated with cervical cancer.[2,3] Two of these, genotypes 16 and 18 are responsible for approximately 70% of cervical cancer cases worldwide.[3,4] In addition to cervical cancer, there is growing evidence that HPV is associated with head and neck cancers and other anogenital cancers such as anus, vulva, vagina and penis cancer.[5,6] Cervical cancer is the fourth most common cancer among women worldwide. It is estimated that 604,127 women are diagnosed with cervical cancer each year and 341,831 women died from this disease in 2020.[5] It is thought that >90% of cervical cancers are caused by HPV.[6]

The main objective of cervical cancer screening is to detect easily treatable precursor lesions to prevent progression to invasive cancer.[7] To screen cervical cancer, three approaches including polymerase chain reaction (PCR) for detection of hrHPV genotypes, Papanicolaou (Pap) smear for screening cervical cell changes, and both test together have been used.[2,8] Recent data showed that using molecular methods to detect nucleic acids of hrHPV types is more effective than cytology[9,10] International guidelines recommend HPV detection as primary screening test.[11]

The DiaRD-HPV screening kit is a novel real-time multiplex PCR (Rt-PCR) kit developed for identifying genotypes 16 and 18, and screening 18 other common hrHPV genotypes (6, 11, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 67, 68, 73, and 82). The kit contains specific primers for genotypes 16 and 18, and fluorescent dye-labeled TaqMan probes to differentiate each of these genotypes. In

Cite as: Durmaz B, Toptan H, Koroglu M, Durmaz R, Cuhacı U. Performance of DiaRD-HPV Rt-PCR kit for detection of high-risk human papilloma virus genotypes: A preliminary study. *Sakarya Med J.* 2025;15(2):164-172. doi:10.31832/smj.1669557

addition, there are genotype-specific primers and TaqMan probes labeled with a common fluorescent dye for amplification and detection of the DNA of each of the other 18 HPV genotypes. To control any PCR inhibitor, the novel kit also includes primers and probe specific for beta-actin gene. The aim of this study was to compare performance of a novel DiaRD-HPV Rt-PCR screening kit with the comparator QIAscreen HPV PCR kit in detecting common HPV genotypes in cervical samples and identifying hrHPV genotypes 16 and 18.

2. METHODS

2.1. Study population

In this retrospective study, a total of 243 samples, 65 of which were HPV positive including genotypes 16 (n:23), genotype 18 (n:2), and other genotypes (n:40) and 178 HPV negative were randomaly selected to evaluate the performance characteristics and comparability of a novel DiaRD-HPV Rt-PCR screening kit (Diagen Inc, Ankara, Türkiye) with a comparator test. All samples previously tested with the QIAScreen HPV PCR test (comparator test) for routine detection and genotyping of the hrHPV genotypes during 2023 were provided by the Medical Microbiology Laboratory of Sakarya University Education and Research Hospital in Sakarya/Türkiye. Cervical swab samples in PreservCyt (Hologic Corp.) transport medium were transferred in tightly sealed, break-resistant, screw-capped tubes and stored at 2-8°C without freezing until DNA isolation. Before testing by the novel kit, patient's information such as the name, surname, ID number, or phone number was anonymized. The study was approved by the ethics committee of Sakarya University, Medical Faculty Ethical Committee (Ethical Committee number: E-16214662-050.01.04-305620-153 and date of approval: 14.11.2023).

2.2. DNA Extraction

The DiaRD-HPV Rt-PCR screening kit was validated with DNA samples extracted using Diarex Viral DNA/RNA extraction kit (Cat. No: 5295-50 VDR-8786, Diagen AS, www.diagen.com.tr). DNA extraction was performed by following manufacturer's instruction. Briefly, after vortexing of cervical specimen in PreservCyt transport medium, 200 µl of the sample was mixed with 25 µl of proteinase K and 300 µl of lysis buffer. After spinning for 15 s, the tubes were incubated at 56°C for 5 min. Then, 300 µl of absolute ethyl alcohol was added and vortexed for 15 s. The entire lysate was transferred to a spin column placed in a collection tube and centrifuged at 8000 x g for 1 min. The spin column was placed back into the collection tube and 500 µL of wash buffer -1 was added and centrifuged at 8000 x g for 1 min. This step was repeated for wash buffer -2. The spin column was placed in a new collection tube and 60 µL of elution buffer was added and left for 2 min. Then it was centrifuged to 8000 x g for 1 min.

2.3. PCR amplification

Total reaction volume and amount of target DNA for the novel kit were 20 µl and 5 µl, respectively. An amplification program was constructed with an initial denaturation at 95°C for 10 min, following 50 cycles including denaturation at 95°C for 10 s, annealing and extension at 58°C for 50 s. After amplification, HPV DNA positivity and identification of genotypes 16 and 18 were done according to the criteria in the kit's instruction. Briefly, if cycle of threshold (Ct) values were ≤45 for HPV type 16 and/or for HPV type 18, and/or for the other HPV types, and ≤35 for beta-actin gene, sample was evaluated as HPV DNA positive. In positive samples, according to signal detected probe, the genotype was identified. When there was no fluorescent signal from HPV probes, but the Ct value of beta-actin gene was ≤35, the result was negative.

2.4. Reproducibility of the novel kit

Three HPV positive clinical samples, genotype 16, genotype 18, and other genotype were used to test inter- and intra- assay reproducibility of the novel kit. For each genotype, eight Rt PCR replicates were done over three separate days. The precision data was calculated based on the Ct values.

2.5. Statistical analysis

For statistical analysis, SPSS Software Version 17 was used. The sensitivity, specificity, positive predictive value (PPV), negative predictive value

(NPV), and accuracy of the novel kit were estimated by the comparison of the novel kit results to those of the comparator kit. Ct values of the samples used in reproducibility experiments were used to calculate the mean, standard deviation, and coefficient of variation (CV) values.

3. RESULTS

The performance studies of the DiaRD-HPV Rt-PCR screening kit on 243 clinical specimens showed a diagnostic sensitivity of 95.4%, specificity of >99.9%, PPV of >99.9%, and NPV of 98.3%. Of the 65 samples that were positive with the comparator kit, 62 (95.4%) were also positive with the novel kit. Positive samples had Ct values between 19.40 and 31.30 (mean: 24.65) for genotype 16, between 29.15 and 39 (mean:31.20) for genotype 18, between 16.61 and 26.54 (mean: 21.07) for other genotypes. Three samples having positive signals with the probe of beta-actin gene did not have any signal with HPV probes. Of these three samples having false negative results with the novel kit, one was genotype 18, two were the other HPV genotypes. All 178 HPV negative samples were also negative with the novel kit (Table 1).

Table 1.

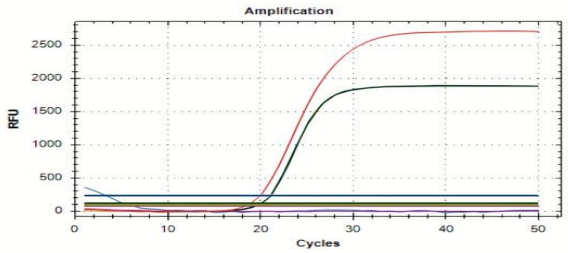
Diagnostic performance of the DiaRD-HPV Rt-PCR screening kit

	QIAscreen HPV PCR kit (comparator kit)		
DiaRD-HPV Rt-PCR kit	Positive	Negative	Total
Positive	62	0	62
Negative	3	178	181
Total	65	178	243
Sensitivity (%)	95.4	-	-
Specificity (%)	-	100	-
Positive predictive value (%)	100	-	-
Negative predictive value (%)	-	98.3	-
Accuracy (%)	-	98.8	-

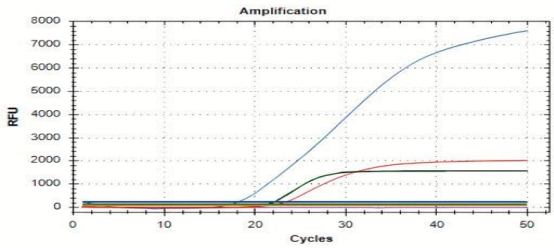
All positive samples yielded fluorescent signals with relative fluorescence units (RFU) more than 1000. Cycles of threshold values for positive samples ranged from 18 to 29. In all clinical samples, Ct values of internal control were about 22. Representative results for Rt-PCR positive and negative clinical samples were shown in Figure 1.

Figure 1.

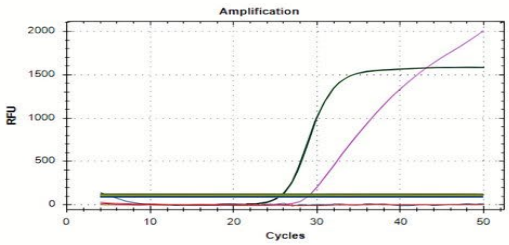
Rt-PCR results of positive and negative clinical samples. a: belongs to a clinical sample positive with genotype 16 (red color), b: represents the positive results for genotype 16 (red color) and other genotype (blue color), c: represents the positive results for genotype 18, d: belongs to a sample positive with any of the other 18 genotypes, and e: represents negative sample. All samples have positive signal with the beta-actin gene probe



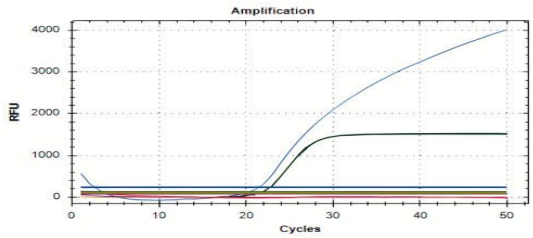
a: Genotype 16



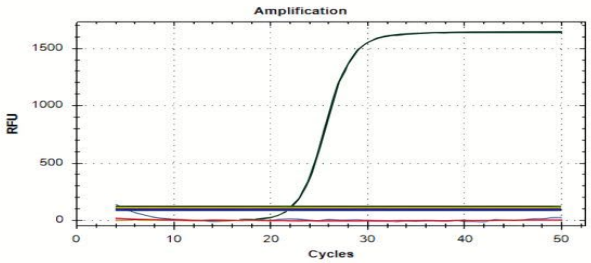
b:Genotype 16 and other genotype



c:Genotype 18



d: other genotype



e: Negative sample having signal with only beta-actin probe.

Reproducibility of the novel kit was evaluated by intra-assay, inter-assay, and overall variations. According to Ct values, the intra-assay, inter-assay, and overall coefficient of variations (CVs) for genotype 16, 18, and others were 0.01%, 0.03-0.06%, and 0.01%, respectively. The Ct values for the genotype 16 in 24 repeats were between 23.46 and 24.04 (nean:23.8). These values for genotype 18 and genotype other ranged from 32.38 to 39.17 (mean: 35.2), and 26.37 to 27.52 (mean: 27), respectively (Table 2).

Table 2.

Reproducibility of the DiaRD HPV Rt-PCR screening kit according to Ct values

HPV genotypes	16	18	Other
	Ct values	Ct values	Ct values
First day	23.94	34.16	27.52
	23.83	34.77	27.32
	24.02	34.10	26.76
	23.98	33.84	27.13
	23.76	35.24	27.02
	24.04	36.10	27.02
	23.88	33.54	27.07
	24.03	35.43	27.16
Second day	23.78	34.95	26.86
	23.59	35.39	26.71
	23.67	37.00	27.09
	23.89	34.67	27.03
	23.85	35.04	26.37
	23.67	35.70	27.01
	23.79	37.69	26.66
	23.98	36.06	26.81
Third day	23.88	35.22	26.88
	23.57	34.57	26.83
	23.71	33.61	27.18
	23.80	32.76	27.22
	23.71	32.38	26.67
	23.46	36.92	27.21
	23.64	39.17	26.97
	23.97	35.34	27.27
Total variations			
Means (Range)	23.80 (23.46-24.04)	35.15 (32.38-39.17)	26.99 (26.37-27.52)
Standard deviations	0.162	1.488	0.252
Coefficient of variation (%)	0.01	0.04	0.01

4. DISCUSSION

Polymerase chain reaction-based nucleic acid detection methods provide rapid and accurate results for detection of HPV at low level in clinical samples and can differentiate highly oncogenic genotypes, thereby helping clinicians for optimized patient management and understanding of the infection progression after treatment.[12] They become preferable tools in many counties instead of cytology-based cervical screening.[13-16] For these purposes several PCR-based tests having advantages and disadvantages have been developed. They use different genes as target and can separate different numbers of hrHPV genotypes. These tests also show a wide range of analytical sensitivity, diagnostic sensitivity, specificity, and accuracy.[3, 7, 10] Therefore, selecting the most accurate methos for cervical cancer screening purposes is a very critical issue.

The current study showed that the novel kit had a diagnostic performance comparable to many commercially available HPV DNA screening kits. It gave true positive results in 95.4% of the HPV positive cervical samples. Getting false-negative results in three samples, one of which was a high-

risk genotype 18 and two of these were in the other common genotypes, is an important point that should be considered. False negativity may result from the difference between analytical sensitivities of the novel and comparator kits. According to kit's instruction, the DiaRD HPV Rt-PCR screening kit had an analytical sensitivity of 207 copies/reaction (41 copies/μL) for genotype 16, 181 copies/reaction (36.2 copies/μL) for genotype 18, and 205.8 copies/reaction (41.2 copies/μL) for other genotypes (https://diagen.com.tr/human-papilloma-virus-rt-pcrkiti). The analytical sensitivity of the comparator kit was reported as 206 copies/reaction for genotype 16, 69 copies/reaction for genotype 18, and ≥617 copies/reaction for other genotypes.[17] The other reason might be the difference in DNA extraction method. Both comparator and the novel kits use the manual DNA extraction protocol with different companies' products, QIAamp®DSP virus spin kit versus Diarex Viral DNA/RNA extraction kit, respectively. The sample volume used in DNA isolation is different in the comparator and the novel kit, 500 μL versus 200 μL, respectively. These technical variations might be the reason for the false negative results.

Discrepancy between the novel and comparator kits may result from difference among their covered genotypes. The novel kit had been developed to identify genotypes 16 and 18 and to screen 18 different genotypes (6, 11, 26, 31, 33, 35, 39, 45, 51, 52, 58, 59, 66, 67, 68, 73 and 82). The number of HPV genotypes screened in this kit is higher than those of many commercial screening kits. For instance, AmpFire HPV screening 16/18/HR kit includes genotype 16,18 and a bulk of 13 hrHPV types, Abbott RealTime high risk HPV test includes genotype 16, 18, and 12 other hrHPV types, Cobas 4800 HPV Test includes genotype 16,18 and 12 other hrHPV types, and Alinity m HR HPV Assay detects individual genotype 16,18, 45, and 11 other hrHPV genotypes.[7] The QIAScreen HPV PCR kit used as comparator test is able to identify genotypes 16 and 18 and to screen genotype 31, 33, 35, 39, 45, 51, 52, 58, 59, 66, 67, and 68.[17] The novel kit includes all genotypes in the comparator kit. Genotypes in the novel kit also covered the HPV types commonly identified in Turkish population.[18-22]

The novel kit did not result in any false positivity, having a specificity more than 99.9% and NPV of 98.3%. These results agreed with those of several commercial kit. For instance, clinical sensitivity and specificity of the comparator test were reported as 97.1% and 94.3% for cervical intraepithelial neoplasia grade 2 (CIN2).[23] The relative clinical sensitivity and specificity values of the Cobas HPV 6800 platform for CIN2 were reported as ≥ 0.95 and ≥ 0.98 respectively.[24] Sensitivity for ≥CIN2 and specificity for ≤CIN1 of BD Onclarity HPV assay on self-collected samples were found as 100% and 83%, respectively.[25] The clinical sensitivity and specificity of Abbott RealTime High Risk HPV assay for CIN2+ were detected as 92% and 95%, respectively.[26] Using Xpert HPV assay as comparator kit, it showed that AmpFire® Multiplex HPV Assay had a sensitivity of 91.2%, and specificity of 79.3%.[27] These data indicate that performances of the DiaRD HPV Rt-PCR screening kit were like those of existing commercial kits used in routine diagnosis of HPV infection.

Reproducibility of the diagnostic test results is a critical issue, which should be addressed. The novel kit showed a total coefficient of variation (CV) of ≤ 0.1% indicating excellence reproducibility. This CV value is at a similar level to existing commercial kits. In the kit instruction, the total CV value for Cobas HPV kit was reported less than 2.7% for genotype 16 and 18, between 2.1 and 5.6% for pooled genotypes (https://www.accessdata.fda.gov/cdrh_docs/pdf10/p100020s017c.pdf. Accessed date: June 10, 2025). The total CV for BD Onclarity HPV Assay ranges from 2.1% to 5.63% (https://www.accessdata.fda.gov/cdrh_docs/pdf16/P160037C.pdf. Accessed date: June 10, 2025). CV values of the High-Risk HPV multiplex real-time PCR Kit (NZYtech) were reported between 0.61 and 1.46% (https://www.nzytech.com/media/dds/brochurescertificates/md0492_im_en_v2401.pdf. Accessed date: June 10, 2025).

The current study has some limitations. First, the study population, mainly the number of HPV

positive cases is relatively small. Second, this study carried out on the patients admitted to a single hospital, therefore these results might not be generalized. Third limitation, since this retrospective study is performed on the stored specimens, we could not discuss our results with Pap smear, demographic and epidemiological characteristics of study population.

5. CONCLUSION

The results of the current study showed that diagnostic sensitivity and specificity of the novel kit were comparable with the IVD/CE approved kits, together with high agreement between its reproducibility studies. One can conclude that DiaRD HPV Rt-PCR screening test is a promising option to identify genotype 16 and 18 and to screen other 18 common genotypes in cervical specimens. A larger prospective study including PCR, cytology, and clinical data will be useful for providing more comprehensive data for diagnostic performance of the novel kit.

Article Information Form

Authors' Contribution

Ulker Cuhaci (UU): experimental study, analysis of the data, writing – original draft. Hande Toptan (HT), Mehmet Koroglu (MK): sample collection, and experimental study, formal analysis, review & editing, Bengul Durmaz (BD): formal analysis, project administration, review & editing of the manuscript, Rıza Durmaz (RD): supervision, writing – original draft, review & editing of the manuscript.

The Declaration of Conflict of Interest/ Common Interest

No conflict of interest or common interest has been declared by authors.

Artificial Intelligence Statement

No artificial intelligence tools were used while writing this article.

Copyright Statement

REFERENCES

1. World Health Organization (WHO). Human Papillomavirus (HPV). Last accessed date: 2025 Fab 20. Available from: https://www.who.int/teams/health-product-policy-and-standards/standards-and-specifications/norms-and-standards/vaccine-standardization/human-papillomavirus
2. National Cancer Institute. HPV and Cancer. Last accessed date: 2025 Fab 20. Available from: https://www.cancer.gov/about-cancer/causes-prevention/risk/infectious-agents/hpv-and-cancer#what-is-hpv-human-papillomavirus
3. Sua P, Maa I, Yua L, Tanga S, Sun P. Clinical significance of extended high-risk human papillomavirus genotyping and viral load in cervical cancer and precancerous lesions. *Gynecol Obstet Clin Med.* 2023;3:22–29.
4. Perkins RB, Wentzensen N, Guido RS, Schiffman M. Cervical cancer screening: A review. *JAMA.* 2023;330(6):547-58.
5. Bruni L, Albero G, Serrano B, Mena M, Collado JJ, Gómez D, et al. ICO/IARC Information Centre on HPV and Cancer (HPV Information Centre). *Human Papillomavirus and Related Diseases in the World. Summary Report 10 March 2023.* [Accessed: 21 February 2025]
6. Centers for Disease Control and Prevention. *Basic Information about HPV and Cancer*. Last accessed date: 2025 Fab 20. Available from: https://www.cdc.gov/cancer/hpv/basic-information.html
7. Arbyn M, Simon M, Peeters E, Xu L, Meijer CJLM, Berkhof J, et al. 2020 list of human papillomavirus assays suitable for primary cervical cancer screening. *Clin Microbiol Infect.* 2021;27(8):1083-95.
8. Williams J, Kostiuk M, Biron VL. Molecular detection methods in HPV-related cancers. *Front Oncol*. 2022;12:864820.
9. Ouyang Y, Abuduxikuer G, Abulimiti T, Abudurexiti G, Zhuo Q, Li W, et al. Clinical performance of the SureX PCR HPV test versus hybrid capture assays (DH2/careHPV) in primary cervical cancer screening. *Tumour*

Virus Research. 2025;200319, ISSN 2666-6790.

10. Feldstein O, Gali-Zamir H, Schejter E, Feinberg T, Yehuda-Shnaidman E, Bornstein J, Levy T. High-risk HPV testing vs liquid-based cytology for cervical cancer screening among 25- to 30-year-old women: A historical cohort study. *Acta Obstet Gynecol Scand*. 2023;102(2):226-33.
11. Maver PJ, Poljak M. Primary HPV-based cervical cancer screening in Europe: Implementation status, challenges, and future plans. *Clin Microbiol Infect*. 2019;26(5):579-83.
12. Pereira AR, Redzic N, Van Vooren S, Pelak K, Broekmans A, Desloovere G, et al. Development, validation, and implementation of an augmented multiwell, multitarget quantitative PCR for the analysis of human papillomavirus genotyping through software automation, data science, and artificial intelligence. *J Mol Diagn*. 2024;26(9):781-91.
13. Santos FLSG, Invenção MCV, Araújo ED, Barros GS, Batista MVA. Comparative analysis of different PCR-based strategies for HPV detection and genotyping from cervical samples. *J Med Virol*. 2021;93(11):6347-54
14. Cheung LC, Egemen D, Chen X, Katki HA, Demarco M, Wiser AL, et al. ASCCP risk-based management consensus guidelines: methods for risk estimation, recommended management, and validation. *J Low Genit Tract Dis*. 2020;24(2):90-101.
15. Yan C, Ma J, Zheng X, Shu J, Wang X, Chen X, et al. Diagnostic accuracy of human papillomavirus testing on self-collected samples among women referred for colposcopy. *Sci Rep.* 2025;15(1):2513.
16. Lindquist S, Kjær SK, Frederiksen K, Ørnskov D, Munk C, Waldstrøm M. Comparative analysis of HPV testing versus cytology in Danish cervical cancer screening: Insights from a large-scale implementation study. *Gynecol Oncol*. 2024;191:45-55.
17. QIAscreen® HPV PCR Test Instructions for Use (Handbook). [Last accessed February 20, 2025]. Available from: https://self-screen.nl/wp-content/uploads/2024/06/HB-2579-004_1132289_R3_QIAScreen_PCR_CE_0623_EMEA.pdf
18. Taskin MH, Nursal AF, Oruc MA, Kariptas E. Genotype distribution and prevalence of high-risk human papillomavirus infection among women in Samsun province of Turkey. *Asian Pac J Cancer Prev*. 2022;23(7):2477-82.
19. Alacam S, Bakir A. Human papillomavirus prevalence and genotype distribution in cervical swab samples in Istanbul, Turkey. *J Infect Dev Ctries.* 2021;15(8):1190-1196.
20. Beyazit F, Sılan F, Gencer M, Aydin B, Paksoy B, Unsal MA, Ozdemir O. The prevalence of human papillomavirus (HPV) genotypes detected by PCR in women with normal and abnormal cervico-vaginal cytology. *Ginekol Pol.* 2018;89(2):62-67.
21. Çalişkan E, Coşkun SK, Öztürk CE, Cangür Ş, Önail B. Analysis of HPV genotypes and liquid-based cervical cytology: Results from a tertiary academic center in northwestern Turkey. *Jpn J Infect Dis*. 2021;74(1):69-72.
22. Tekkesin N, Goktas S, Alkis V, Tekkesin E, Goktas P. Male Human papillomavirus infection and genotyping in Turkey. *Asian Pac J Cancer Prev.* 2023;24(12):4187-93.
23. Hesselink AT, Berkhof J, van der Salm ML, van Splunter AP, Geelen TH, van Kemenade FJ, et al. Clinical validation of the HPV-risk assay, a novel real-time PCR assay for detection of high-risk human papillomavirus DNA by targeting the E7 region. *J Clin Microbiol*. 2014;52(3):890-896.
24. Keung MHT, Mehta N, Lynn E, Pineda E, Fetene D, Lee A, et al. Clinical validation of the Roche cobas HPV test on the Roche cobas 6800 system for the purpose of cervical screening and qualification as a second-generation comparator test. *J Virol Methods*. 2025;335:115145.
25. Martinelli M, Latsuzbaia A, Bonde J, Pedersen H, Iacobone AD, Bottari F, et al. Extended Valhudes Study Group. Performance of BD Onclarity HPV assay on FLOQSwabs vaginal self-samples. *Microbiol Spectr*. 2024;12(3):e0287223.
26. Latsuzbaia A, Vanden Broeck D, Van Keer S, Weyers S, Tjalma WAA, Doyen J, et al. Clinical

performance of the RealTi*m*e high risk HPV assay on self-collected vaginal samples within the VALHUDES framework. *Microbiol Spectr.* 2022:10(5):e0163122.

27. Moyo S, Ramogola-Masire D, Moraka NO, Tawe L, Noubary F, Motsumi K, et al. Comparison of the AmpFire® multiplex HPV assay to the Xpert® HPV assay for detection of human papillomavirus and cervical disease in women with human immunodeficiency virus: A pragmatic performance evaluation. *Infect Agent Cancer.* 2023;18(1):29.