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Research Article

PCR-Based Molecular Diagnostics of Human Papillomavirus (HPV)

Aysu Guluzade[1], Ayaz Mammadov[1], and Elin Aliyev[2]

[1]Department of Natural Sciences, School of Innovative Engineering and Natural Sciences, Western Caspian University, Baku, AZ1072, Azerbaijan

[2] Baku Medical Plaza, International Medical Center Closed Joint Stock Company, Babek Avenue 92n, Baku, Azerbaijan

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Abstract

Human papillomavirus (HPV) is one of the most common sexually transmitted viruses in the world. HPV is a key etiological factor in the development of cervical cancer and other anogenital and oropharyngeal malignancies. Early detection and accurate genotyping of this virus are crucial for disease prevention and screening programs. Among current molecular diagnostic methods, the polymerase chain reaction (PCR) has become the gold standard due to its high sensitivity and specificity, as well as its ability to detect low viral loads. This review summarizes the principles, methodologies, and diagnostic applications of PCR-based HPV detection. The review also discusses traditional PCR, real-time PCR, multiplex PCR, and other PCR-based approaches. The main advantages, limitations, and clinical importance of these methods are explained. Furthermore, it has been determined that there are factors affecting diagnostic accuracy, including sample collection and DNA extraction, primer selection, and result interpretation. The role of PCR in HPV genotyping, epidemiological surveillance, and the prevention of cervical cancer is also being investigated. The obtained results indicate that PCR-based molecular diagnostics ensure the reliable and highly sensitive detection of HPV infection. Furthermore, it supports public health strategies aimed at the early diagnosis of the infection, patient management, and the reduction of HPV-related diseases.

Keywords: molecular diagnostics, cervical cancer, DNA amplification, viral detection, screening, diagnostic accuracy.

1. Introduction

Human papillomavirus (HPV) belongs to the Papillomaviridae family. It is a non-enveloped virus (lacking a lipid envelope) with a genome consisting of circular, double-stranded DNA approximately 7,000–8,000 base pairs in length. More than 200 genotypes of HPV have been identified. They are classified into low- and high-risk groups based on their oncogenic potential.

Low-risk types, particularly HPV6 and HPV11, are primarily associated with benign growths such as condyloma acuminata (genital warts). High-risk types, such as HPV16, HPV18, HPV31, HPV33, HPV45, HPV52, and HPV58, are associated with cervical intraepithelial neoplasia (CIN) and several cancer types. Persistent infection with high-risk HPV is a major risk factor for malignant transformation. For instance, HPV16 and HPV18 cause more than 70% of cervical cancer cases worldwide [1], [2], [3], [4], [5].

CIN represents precancerous changes in the cervical epithelium. Depending on the severity of the dysplasia, it is classified as CIN1, CIN2, or CIN3. Since high-grade CIN progresses to invasive cervical cancer if not treated in

time, this underscores the importance of the early detection of high-risk HPV. Due to its high sensitivity and specificity, the polymerase chain reaction (PCR) is widely used for the detection of HPV DNA [1], [5].

The HPV virion has a diameter of approximately 50–55 nm. They possess an icosahedral capsid composed of L1 and L2 proteins. As the primary structural component, L1 forms the basis of prophylactic HPV vaccines through the formation of virus-like particles (VLPs). L2, on the other hand, aids in capsid assembly and the entry of the viral genome into host cells. The absence of a lipid envelope increases the virus's environmental stability [4]. The HPV genome consists of early (E), late (L), and long control region (LCR) functional regions. The early region includes the E1, E2, E4, E5, E6, and E7 genes, which are involved in regulating viral replication and the interaction with the host cell. The E6 and E7 genes encode key oncoproteins that disrupt the function of tumor suppressor proteins such as p53 and pRb. The activity of these oncoproteins leads to uncontrolled cell proliferation, the development of genomic instability, and, ultimately, the promotion of carcinogenesis. The late region encodes the L1 and L2 capsid proteins, while the LCR regulates viral replication and transcription [4].

Molecular and phylogenetic analyses have demonstrated the existence of significant genetic diversity among HPV genotypes, as well as within high-risk genotypes such as HPV16. Genomic variations can significantly influence the virus's persistence, ability to evade the immune response, pathogenicity, and oncogenic potential. Phylogenetic analyses have not only enabled a more precise classification of HPV variants but have also facilitated a better understanding of their evolutionary characteristics and clinical significance [1], [3], [4]. The development of molecular diagnostics and next-generation sequencing technologies has expanded the study of HPV genomic diversity and created new opportunities in the fields of epidemiological surveillance, risk assessment, vaccine development, and disease management. Therefore, the accurate identification of HPV genotypes via PCR-based molecular approaches is crucial for ensuring the effectiveness of modern cervical cancer screening and prevention strategies [1], [4], [5].

Routes of Transmission and Epidemiology of Human Papillomavirus (HPV)

Human papillomavirus (HPV) is an epitheliotropic DNA virus that infects the basal epithelial cells of the skin and mucous membranes. The virus typically enters the body through microscopic lesions in the epithelial tissue, resulting in infection of the basal cell layer. The primary route of HPV transmission is sexual contact. The infection can be transmitted during vaginal, anal, or oral sexual contact. However, penetrative sexual intercourse is not strictly required for the transmission of the virus, as direct skin-to-skin contact between the anogenital areas can also lead to the spread of the infection. Main risk factors include having multiple sexual partners, early onset of sexual activity, oral-genital contact, and immunosuppressive conditions. The fact that HPV infection is asymptomatic in most cases can enable infected individuals to transmit the virus to others without being aware of the infection [5].

Although its epidemiological significance is more limited compared to sexual transmission, non-sexual transmission of HPV is also considered possible. The virus's DNA was detected on contaminated surfaces and medical instruments. Furthermore, vertical transmission from mother to child can occur during pregnancy and, particularly, during vaginal delivery. In rare cases, perinatal HPV infection is associated with the development of recurrent respiratory papillomatosis in newborns [5], [6].

The potential effects of HPV infection on reproductive health have also been investigated. The detection of HPV DNA in semen samples has been associated with increased sperm DNA fragmentation and reduced sperm motility. These changes suggest that HPV infection could potentially affect male reproductive function. However, the molecular and cellular mechanisms underlying this connection have not yet been fully elucidated.

HPV is the most common sexually transmitted viral infection worldwide. More than 200 HPV genotypes are known, with approximately 40 infecting the anogenital tract. High-risk types, especially HPV16 and HPV18, cause most HPV-related cancers and more than 70% of cervical cancer cases. Persistent HPV infection is detected in approximately 95–99% of cervical cancers [5], [6].

Most sexually active individuals acquire HPV at least once during their lifetime. Approximately 90% of infections clear spontaneously within one to two years. However, persistent infection with high-risk genotypes increases the

risk of malignant transformation. Cervical cancer usually develops 15–20 years after persistent infection but may progress within 5–10 years in immunocompromised individuals, particularly those living with HIV [5].

The prevalence of high-risk HPV varies geographically. The highest rates are reported in sub-Saharan Africa, followed by Latin America, the Caribbean, Eastern Europe, and Southeast Asia. HPV is most common among women of reproductive age but also remains prevalent in women over 50 years, highlighting the importance of continued HPV screening throughout adulthood [5].

Recent epidemiological studies have demonstrated that HPV infection is highly prevalent in men as well as women. According to the World Health Organization [7], approximately one in three men worldwide is infected with the human papillomavirus (HPV), and one in five carries a high-risk HPV genotype. It is reported that HPV16 is the most frequently detected genotype among men. These results highlight the significant global public health burden associated with HPV infection and demonstrate that the virus affects both men and women. Although comprehensive nationwide epidemiological data on HPV infection in Azerbaijan are limited, recent studies indicate a significant prevalence of HPV among women of reproductive age.

A clinical study was conducted in Baku between 2021 and 2023 among women aged 18 to 65. High-risk HPV was detected in a significant proportion of the samples that tested positive. Among the most frequently encountered genotypes, HPV16, HPV18, HPV36, and HPV45 predominated. HPV infection was most frequently detected in women aged 30–45, and a significant proportion of the infections were asymptomatic; this underscores the importance of regular cervical cancer screening. Limited vaccination coverage, low levels of public awareness, and limited access to organized screening programs remain significant public health issues in Azerbaijan [6].

Preventive vaccination is one of the most effective strategies for preventing HPV infection and related diseases. Currently available vaccines (including Gardasil 9, Gardasil, and Cervarix) protect against several high-risk HPV genotypes, particularly HPV16 and HPV18. Vaccination is usually recommended before the onset of sexual activity—that is, at a time when the likelihood of prior HPV infection is low. Different dosing schedules are applied depending on the age at vaccination and the applicable immunization guidelines. For older age groups, however, a three-dose schedule is usually recommended [8].

The Association Between HPV Infection and Oncological Diseases

Human papillomavirus (HPV) is one of the most significant oncogenic viruses in humans and is estimated to cause approximately 5% of all cancer cases worldwide. Persistent infection caused by high-risk HPV genotypes is strongly associated with cervical cancer. It plays a significant role in the development of anal, vulvar, vaginal, penile, and oropharyngeal cancers. Most HPV infections are temporary and are effectively cleared by the body's immune system. However, persistent infection with high-risk genotypes significantly increases the risk of malignant transformation [6], [9], [10].

The process of carcinogenesis caused by HPV is primarily mediated by the virus's E6 and E7 oncoproteins. Following the integration of the viral DNA into the host genome, the E6 protein promotes the degradation of the tumor suppressor protein p53, while the E7 protein disrupts the function of the retinoblastoma protein (pRb). These molecular changes disrupt the normal regulation of the cell cycle, increase genomic instability, and lead to the uncontrolled proliferation of cells. Recent studies also indicate that oxidative stress caused by HPV can contribute to DNA damage and further accelerate the process of carcinogenesis. Furthermore, the body's genetic susceptibility factors can influence the persistence of the virus [6], [9], [10].

Conventional Methods for HPV Diagnosis and Their Limitations

Since HPV infection is asymptomatic in most cases, persistent infections may go undetected until precancerous lesions or invasive cancer develop. For this reason, accurate laboratory diagnostics are of great importance for ensuring effective screening and early intervention. For many years, the Papanicolaou (Pap) smear has been one of the primary screening methods based on the detection of cytological changes in cervical epithelial cells. However, this method does not directly detect HPV DNA and may have limited sensitivity, particularly in the early stages of infection. Colposcopy allows for the visual assessment of the cervical epithelium. It helps identify suspicious areas for biopsy following the application of acetic acid or Lugol's iodine solution. However, this

method also does not directly detect HPV infection, and its diagnostic results depend significantly on the experience of the specialist performing the examination [11], [12], [13], [14].

The development of molecular diagnostic methods, particularly PCR, has significantly expanded the capabilities for detecting and genotyping HPV infections. PCR enables the highly sensitive detection of viral DNA and the identification of specific HPV genotypes. Modern approaches such as real-time PCR, multiplex PCR, and digital PCR enable the rapid, sensitive, and reliable detection of high-risk HPV genotypes. These characteristics have made PCR-based methods one of the important molecular approaches for HPV screening, laboratory diagnostics, and genotyping [12], [15].

Importance of Early Diagnosis of HPV Infection

Persistent high-risk HPV infection is one of the key risk factors for the development of cervical cancer. The fact that HPV infection is often asymptomatic and can remain in a latent state for many years makes early detection difficult. For this reason, early diagnosis is crucial for identifying infected individuals before precancerous lesions or invasive cancer develop. Timely detection of the infection enables adequate clinical management and prevents the progression of the disease. Ultimately, this approach contributes to a significant reduction in the incidence and mortality rates of cervical cancer [13], [16].

Molecular diagnostic methods have significantly expanded the possibilities for early diagnosis by detecting HPV DNA before cytological changes appear. PCR-based tests make it possible to detect even very small amounts of viral DNA. This feature facilitates the detection of infections, particularly at an early stage or in the absence of clinical symptoms, as well as the precise identification of high-risk HPV genotypes. Early molecular diagnostics enable risk stratification and personalized patient monitoring. This approach can contribute to the timely implementation of clinical interventions, the improvement of treatment outcomes, and a reduction in the need for invasive procedures [13], [16].

Limitations of Conventional Diagnostic Methods

The Papanicolaou (Pap) smear has formed the basis of cervical cancer screening for many years and has contributed to a significant reduction in deaths from this disease. However, the Pap test primarily detects the cytological changes caused by HPV infection, rather than the infection itself. For this reason, its sensitivity in detecting early or latent infections may be limited. Cytological findings may be normal in high-risk HPV carriers. This situation can lead to false-negative results and a delay in diagnosis [11], [13], [16].

The diagnostic effectiveness of the Pap test depends on the quality of the sample, the preparation of the specimen, and the experience of the cytologist. These factors can lead to increased inter-observer variability in the assessment of results. Given its relatively low sensitivity, repeat examinations may be required to improve diagnostic accuracy. This leads to an increase in healthcare costs and the burden on the patient. Furthermore, the Pap test does not allow for the identification of the HPV genotype or the differentiation between low-risk and high-risk virus types.

Advances in the field of molecular diagnostics have made it possible to overcome several limitations of the Pap test. PCR-based HPV DNA tests—including real-time PCR, multiplex PCR, and digital PCR platforms—provide high sensitivity and specificity while also enabling accurate genotyping of the virus. For this reason, several countries have incorporated HPV DNA testing into their primary cervical cancer screening programs. However, the approach taken has varied: in some instances, these tests have been implemented to replace cytological screening, while in others, they serve to complement it. The combination of PCR-based HPV tests with cytological examination holds high diagnostic value for the early detection, risk assessment, and clinical management of HPV infection [11], [12], [13], [15], [16].

2. Materials and Methods

Study Population and Clinical Samples

This study included cervical samples collected from 149 patients aged 18–60 years who had undergone HPV testing. Cervical smear samples were collected by qualified gynecologists using sterile cervical brushes and immediately placed into a liquid-based transport medium. Subsequently, the samples were delivered to the

Molecular Genetics Laboratory of "Baku Medical Plaza" (Babek branch) for molecular analysis. All laboratory procedures were performed according to the laboratory's standard operating procedures (SOPs).

Sample Collection, Storage, and Transportation

Cervical samples were obtained from the cervical transformation zone, the anatomical site where HPV-associated lesions most frequently develop. After collection, specimens were placed into sterile transport tubes containing preservation medium and transported to the laboratory under recommended conditions. Samples were processed immediately or stored at 2–8°C for short-term preservation until DNA extraction. All procedures complied with standard biosafety and laboratory quality-control guidelines [11], [17], [18].

DNA Extraction and Quality Assessment

Genomic DNA was extracted from cervical specimens using a commercial silica-based DNA extraction kit according to the manufacturer's instructions. The extraction procedure included cell lysis, nucleic acid adsorption onto a silica sorbent, washing to remove contaminants, and DNA elution. All pre-PCR procedures were carried out in a laminar airflow cabinet to minimize the risk of contamination. Negative controls were included throughout the extraction process to monitor possible contamination.

The concentration and purity of the extracted DNA were assessed before PCR analysis to ensure adequate template quality. DNA samples meeting the quality requirements were used for subsequent molecular testing, whereas extracted DNA was stored at −20°C until analysis, when immediate amplification was not performed [19], [20].

PCR Amplification & Statistical Analysis

HPV DNA detection was performed using a real-time polymerase chain reaction (Real-Time PCR) assay following the manufacturer's protocol. The reaction mixture contained template DNA, sequence-specific primers, fluorescent probes, nucleotides, reaction buffer, magnesium ions, and thermostable DNA polymerase. Amplification was carried out in a real-time PCR thermal cycler under optimized cycling conditions. Appropriate positive and negative controls were included in each PCR run to validate assay performance and exclude contamination.

PCR results were analyzed using standard laboratory software. Descriptive statistical methods were applied to summarize the frequency and distribution of HPV-positive samples according to age group and HPV genotype. Statistical analyses were performed using appropriate software, and results were considered statistically significant at $p < 0.05$.

HPV DNA was detected using a real-time polymerase chain reaction (Real-Time PCR) assay. This method relies on the exponential amplification of target HPV DNA sequences through repeated steps such as denaturation, primer annealing, and DNA chain extension. Fluorescence-labeled probes included in the reaction allow for real-time monitoring of the amplification process, facilitating the qualitative detection and accurate genotyping of HPV. These features enable the sensitive and specific identification of HPV DNA in clinical samples [21].

PCR analysis was performed using a commercially available "master mix" containing Taq DNA polymerase, reaction buffer, Mg^{2+} ions, deoxynucleotide triphosphates (dNTPs), genotype-specific forward and reverse primers, and fluorescence-labeled oligonucleotide probes. DNA purified from clinical samples was used as the template for amplification. To minimize the risk of cross-contamination, all reagents were prepared and used in a dedicated work area designated for the pre-PCR stage.

The primer pairs were designed to specifically target conserved regions of the HPV genome, enabling the sensitive detection of clinically significant high-risk HPV genotypes while minimizing non-specific amplification. Positive and negative controls were included in each amplification run to monitor assay performance and detect potential contamination. PCR amplification and fluorescence detection were performed using a real-time thermal cycler in accordance with the manufacturer's instructions. The resulting fluorescent signals were analyzed to determine the presence of HPV DNA and identify the specific HPV genotype [15], [19], [20], [22], [23], [24].

The PCR amplification protocol consisted of an initial denaturation step followed by repeated cycles of denaturation, primer annealing, and extension. Initial denaturation was performed at 94–95°C for 3–5 minutes to

ensure complete separation of the double-stranded DNA and activation of the DNA polymerase. Following this stage, 35–40 amplification cycles were performed. In each cycle, denaturation was performed at 94–95°C for 15–30 seconds to separate the DNA strands. Subsequently, the primer annealing step, which allows sequence-specific primers to hybridize with the target HPV DNA sequences, was performed at a temperature of 50–65°C for 30–60 seconds. Subsequently, an extension step was performed at 72°C for 30–60 seconds. At this stage, Taq DNA polymerase synthesized complementary DNA strands by incorporating deoxyribonucleotides. After the amplification cycles were completed, a final extension step was performed at 72°C for 5–10 minutes to ensure the complete synthesis of the amplified products. Subsequently, the reaction mixture was maintained at 4°C until the PCR tubes were removed from the instrument. Depending on the thermal cycler used and the specific amplification protocol, the total amplification time under these conditions was approximately 1.5–2 hours (Table 1).

Table 1. PCR setup

PCR Step	Temperature (°C)	Time	Purpose
Initial denaturation	94–95	3–5 min	DNA strand separation and enzyme activation
Denaturation	94–95	15–30 s	Separation of DNA strands
Annealing	50–65	30–60 s	Primer binding to target DNA
Extension	72	30–60 s	DNA synthesis by Taq polymerase
Final extension	72	5–10 min	Completion of DNA synthesis
Hold	4	Until sample removal	Preservation of PCR products

3. Results and Discussion

Interpretation of Real-Time PCR Results

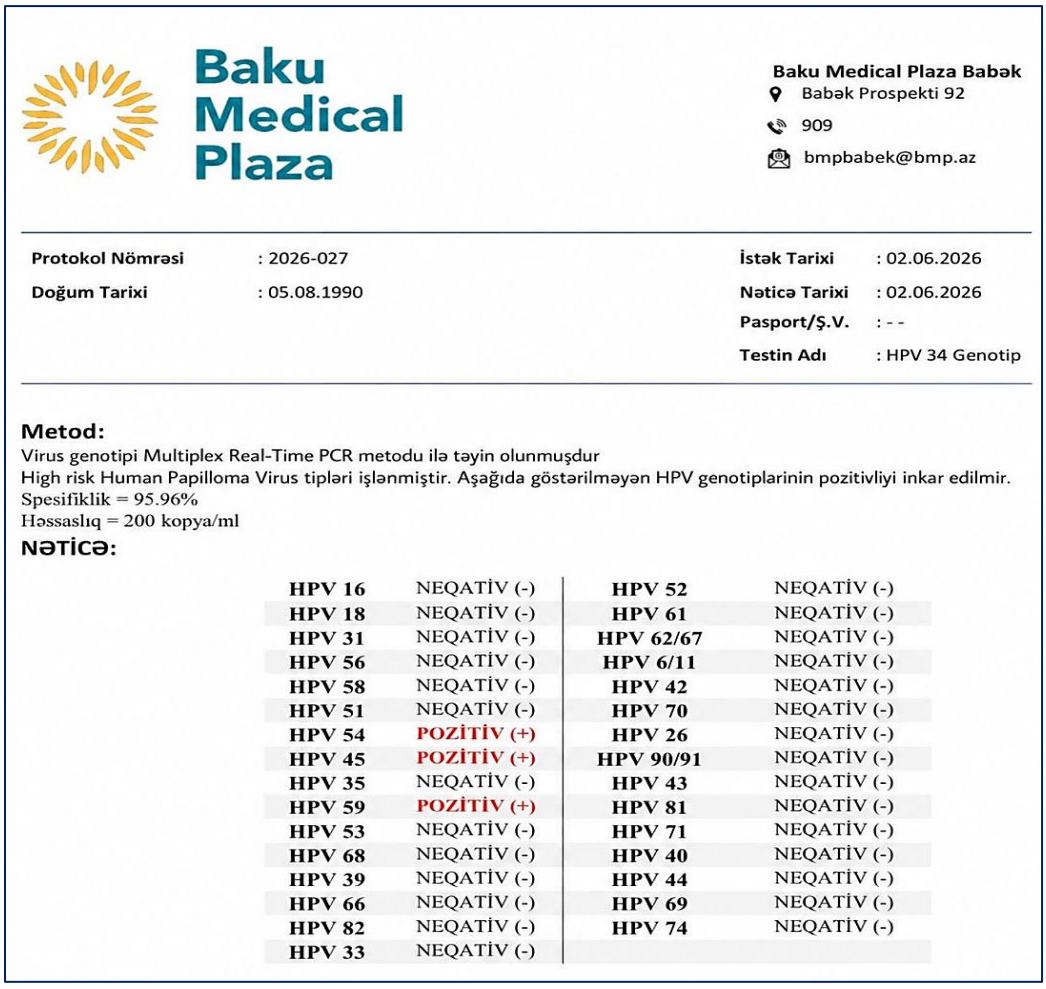
Real-time PCR results were interpreted in accordance with the manufacturer's instructions and established laboratory quality control criteria. Internal controls were used to verify successful DNA extraction and monitor for potential PCR inhibition, while external positive and negative controls were included in each amplification run to assess assay performance and monitor for potential contamination [25], [26].

Amplification curves and cycle threshold (Ct) values were evaluated to determine the presence or absence of HPV DNA. Samples showing amplification within the predetermined Ct range were considered positive. Provided that the corresponding internal control sample yielded a valid result, samples showing amplification outside the established threshold value or exhibiting no amplification were considered negative.

To ensure the reliability and diagnostic accuracy of the results, all findings were interpreted in conjunction with relevant quality control parameters and available clinical data (Figure 1). Real-time PCR provides a highly sensitive and specific method for the detection and genotyping of HPV, supporting early diagnosis, risk stratification, and appropriate clinical management [25], [26].

Analysis of overall HPV positivity rates

During the study, PCR analysis results of individuals who applied to the laboratory of the Baku Medical Plaza clinic between February 16 and May 25 for the detection of HPV infections were analyzed. During the study period, samples taken from a total of 149 individuals were examined. Of those examined, 135 were women, and 14 were men. In the vast majority of the analyzed samples, a test panel covering 14 high-risk HPV genotypes was used, and in only one patient was an extended HPV test covering 34 genotypes performed (Figure 2).



Baku Medical Plaza

Baku Medical Plaza Babək
Babək Prospekti 92
909
bmpbabek@bmp.az

Protokol Nömrəsi	: 2026-027	**İstək Tarixi**	: 02.06.2026
Doğum Tarixi	: 05.08.1990	**Nəticə Tarixi**	: 02.06.2026
		Pasport/Ş.V.	: - -
		Testin Adı	: HPV 34 Genotip

Metod:
Virus genotipi Multiplex Real-Time PCR metodu ilə təyin olunmuşdur
High risk Human Papilloma Virus tipləri işlənmiştir. Aşağıda göstərilməyən HPV genotiplarinin pozitivliyi inkar edilmir.
Spesifiklik = 95.96%
Həssaslıq = 200 kopya/ml

NƏTİCƏ:

HPV 16	NEQATİV (-)	**HPV 52**	NEQATİV (-)
HPV 18	NEQATİV (-)	**HPV 61**	NEQATİV (-)
HPV 31	NEQATİV (-)	**HPV 62/67**	NEQATİV (-)
HPV 56	NEQATİV (-)	**HPV 6/11**	NEQATİV (-)
HPV 58	NEQATİV (-)	**HPV 42**	NEQATİV (-)
HPV 51	NEQATİV (-)	**HPV 70**	NEQATİV (-)
HPV 54	**POZİTİV (+)**	**HPV 26**	NEQATİV (-)
HPV 45	**POZİTİV (+)**	**HPV 90/91**	NEQATİV (-)
HPV 35	NEQATİV (-)	**HPV 43**	NEQATİV (-)
HPV 59	**POZİTİV (+)**	**HPV 81**	NEQATİV (-)
HPV 53	NEQATİV (-)	**HPV 71**	NEQATİV (-)
HPV 68	NEQATİV (-)	**HPV 40**	NEQATİV (-)
HPV 39	NEQATİV (-)	**HPV 44**	NEQATİV (-)
HPV 66	NEQATİV (-)	**HPV 69**	NEQATİV (-)
HPV 82	NEQATİV (-)	**HPV 74**	NEQATİV (-)
HPV 33	NEQATİV (-)		

Figure 1. Evaluation of HPV results.

The results obtained showed that HPV infection was detected in the majority of the patients who underwent the analysis. In addition, a positive HPV result was recorded in 45 women. In addition, weak negative results were recorded in 2 women and 1 man. Weak negative results are usually associated with a low viral load or indicators close to the detection limit during the analysis. Such results are considered cases requiring clinical monitoring, and re-examination may be recommended after a certain period of time.

Based on the results of the study, HPV positivity was observed only in women. This indicator may be because women are more likely to be examined for the purpose of diagnosing HPV infection and the widespread use of cervical cancer screening programs. The lack of positive results among men may be due to the small number of male patients included in the study.

Various high-risk genotypes were identified among the HPV positive results. As a result of the genotyping, the highest frequency was observed in the HPV39 genotype. This genotype was detected in 13 people. The second highest was the HPV66 genotype, which was detected in 12 people. HPV16 and HPV52 were both detected in 7 people. HPV35 and HPV68 genotypes were recorded in 6 people, respectively.

Among other genotypes, HPV45, HPV56, and HPV59 genotypes were detected in 4 individuals each, HPV18 and HPV33 genotypes in 3 individuals each, and HPV31 and HPV51 genotypes in 2 individuals each. The lowest incidence was observed in the HPV58 genotype and was detected in only 1 individual.

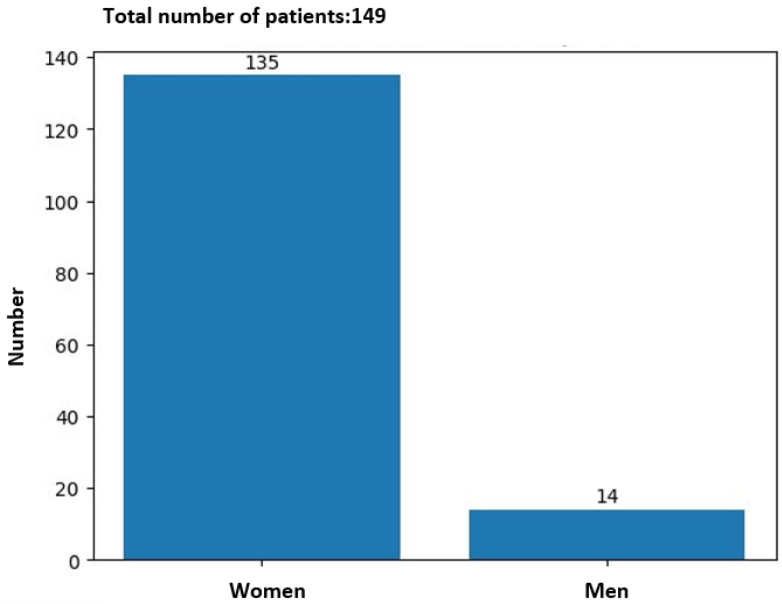


Figure 2. Total number of patients who underwent the analysis, separately for women and men.

Analysis of the distribution of genotypes shows that not only the classical high-risk HPV16 and HPV18, but also other high-risk genotypes such as HPV39, HPV66, HPV35, and HPV68 were widely distributed in the study group. This result indicates the genetic diversity of HPV infection and the importance of using a wide genotype panel in molecular diagnostics (Figure 3).

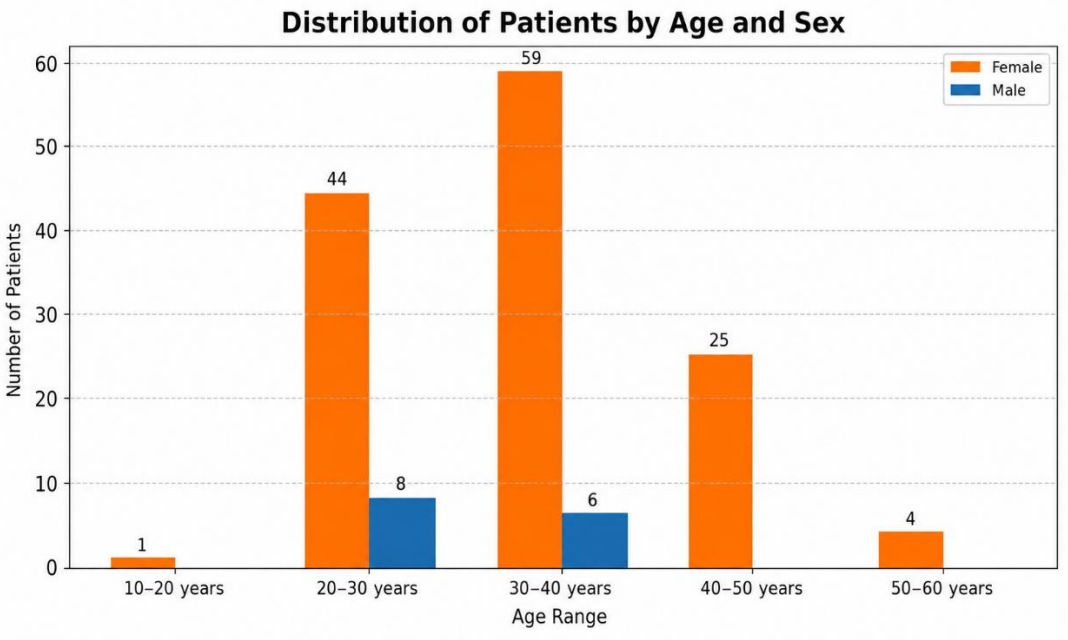


Figure 3. Distribution of those infected by age and gender.

It was found that in each of the patients who received a positive result, a minimum of 1 and a maximum of 3 genotypes were positive. This fact indicates that several genotypes can be positive in one patient at the same time. Cases of infection with multiple genotypes are of particular interest from a clinical point of view, since various

studies have shown that the presence of more than one high-risk HPV genotype in the same person can increase the risk of persistence and the likelihood of developing oncological changes.

Overall, the results obtained show that PCR technology is highly effective in accurately detecting HPV infection and its various genotypes. Due to the high sensitivity of the method, cases of infection with both single and multiple genotypes were identified. The analyses conducted show that HPV infection is quite widespread in the studied group, especially as high-risk genotypes prevail, and molecular diagnostics are of great importance in terms of early detection.

Thus, the study results confirmed the high informativeness of PCR technology in the diagnosis of HPV infection, allowing for the assessment of both overall positivity rates and the distribution of high-risk genotypes. The data obtained are of great importance for future epidemiological studies and the prevention of HPV-related diseases.

Comparative Analysis of HPV Prevalence Across Age Groups

The examined individuals were divided into different age groups and evaluated. This approach made it possible to determine the age group in which HPV infection is most common and to assess the epidemiological characteristics of the infection. According to the data obtained, the majority of the individuals participating in the study were between 20 and 40 years of age. A total of 52 individuals were examined in the 20-30 age group, of which 44 were women and 8 were men. This age group accounts for the majority of the total applicants. A total of 65 individuals in the 30-40 age group provided analysis. Of these individuals, 59 were women, and 6 were men. Thus, the largest number of applications was in the 30-40 age group. The number of people examined in the 40-50 age group was somewhat lower than the previous age group. In this age range, 26 women submitted an application, and no male patients were registered. In the 50-60 age group, only 4 women applied for examination. The least number of applications was in the 10-20 age range, and 1 woman in this age group submitted an analysis.

Analysis of the results by age groups showed that HPV positive results were observed only in people aged 21-36. This result indicates that HPV was detected in patients of reproductive age. As is known, HPV is one of the most common sexually transmitted infections, and the highest prevalence rates of infection are usually recorded among young and middle-aged people. The results obtained during the study also confirmed these epidemiological features. The higher number of positive results in the 20-30 age group is associated with the more active reproductive and social lifestyle of individuals in that age group. At the same time, the detection of positive cases in the 30-36 age group indicates that the viral infection remains relevant not only in young people, but also in the later reproductive period. In addition, the reason for the lack of positivity in people over 40 years of age is due to the low number of applicants in that age group.

The study results show that the 21-36 age group can be considered a risk group in terms of detecting infection. In this regard, regular gynecological examinations, participation in HPV screening programs and the use of molecular diagnostic methods in this age group can help in the early detection of infection and prevent possible complications.

4. Conclusion

This study demonstrated the high diagnostic performance of real-time polymerase chain reaction (PCR) for the detection and genotyping of human papillomavirus (HPV). The implementation of internal and external quality control methods has strengthened the robustness of the molecular diagnostic process by ensuring the reliability of DNA extraction, amplification, and result interpretation. Among the 149 individuals analyzed, HPV infection was detected exclusively in female participants, with positive results observed primarily in the 21–36 age group. The absence of HPV-positive results among male participants should be interpreted with caution, given the limited number of men included in the study. Genotype analysis revealed significant diversity. HPV39 and HPV66 were the most frequently detected high-risk genotypes, followed by HPV16, HPV52, HPV35, and HPV68. These results indicate that, alongside HPV16 and HPV18, other high-risk genotypes may play a significant role in the development of HPV infection within the studied population.

Both single and multiple genotype infections were identified, underscoring the importance of comprehensive HPV genotyping. The observed genotype distribution supports the use of broad-spectrum PCR tests capable of detecting multiple high-risk HPV types. HPV infection was most frequently detected in women of reproductive age, highlighting the importance of appropriate screening and early molecular diagnostics.

The study is limited by a relatively small sample size, a limited number of male participants, and a single-center design. Larger-scale, multicenter studies are needed to more accurately characterize HPV epidemiology and genotype distribution in Azerbaijan. Such studies can contribute to the optimization of screening strategies, vaccination policies, and other preventive measures.

Author Contributions

The sole author is responsible for conceptualizing, collecting, and analyzing data, and writing and editing the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

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References

[1] Katabathina, V. S., Ghannam, S., Chen, M., Desalme, B., Gabos, R., Emejulu, I., & Prasad, S. R. (2024). Update on pathologic conditions, imaging findings, prevention, and management of human papillomavirus–related neoplasms. *Radiographics*, *44*(8), e230179. https://doi.org/10.1148/rg.230179

[2] Li, Y., & Wu, X. (2025). Vaginal microbiome distinction in women with HPV+, cervical intraepithelial neoplasia, and cervical cancer, a retrospective study. *Frontiers in Cellular and Infection Microbiology*, *14*, 1483544. https://doi.org/10.3389/fcimb.2024.1483544

[3] Nikmanesh, N., Hosseini, S., Mirbagheri, F., Asadsangabi, K., Fattahi, M. R., Safarpour, A. R., ... & Nikmanesh, Y. (2024). Knowledge on human papillomavirus infections, cancer biology, immune interactions, vaccination coverage and common treatments: a comprehensive review. *Viral Immunology*, *37*(5), 221-239. https://doi.org/10.1089/vim.2023.0144

[4] Prétet, J.L., Charlot, J.F., Mougin, C. (2007). Aspects virologiques et carcinologiques des papillomavirus humains HPV [Virological and carcinogenic aspects of HPV]. *Bull Acad Natl Med., 191*(3):611-623.

[5] Wolf, J., Kist, L. F., Pereira, S. B., Quessada, M. A., Petek, H., Pille, A., & Nasi, L. A. (2024). Human papillomavirus infection: Epidemiology, biology, host interactions, cancer development, prevention, and therapeutics. *Reviews in Medical Virology*, *34*(3), e2537. https://doi.org/10.1002/rmv.2537

[6] Malagon, T., Franco, E. L., Tejada, R., & Vaccarella, S. (2024). Epidemiology of HPV-associated cancers past, present and future: towards prevention and elimination. *Nature Reviews Clinical Oncology*, *21*(7), 522-538. https://doi.org/10.1038/s41571-024-00904-z

[7] WHO (2023) One in three men worldwide are infected with genital human papillomavirus. https://www.who.int/news/item/01-09-2023-one-in-three-men-worldwide-are-infected-with-genital-human-papillomavirus

[8] Goldstone, S. E. (2023). Human papillomavirus (HPV) vaccines in adults: Learnings from long-term follow-up of quadrivalent HPV vaccine clinical trials. *Human vaccines & immunotherapeutics*, *19*(1), 2184760. https://doi.org/10.1080/21645515.2023.2184760

[9] Lu, Z., Haghollahi, S., & Afzal, M. (2024). Potential therapeutic targets for the treatment of HPV-associated malignancies. *Cancers*, *16*(20), 3474. https://doi.org/10.3390/cancers16203474
[10] Bhatia, R., Alcaniz Boada, E., Bonde, J. H., Quint, W. G., Xu, L., Ejegod, D. M., ... & Arbyn, M. (2023). Papilloplex HR-HPV test has non-inferior clinical performance for detection of human papillomavirus infection: assessment using the VALGENT framework. *Journal of Clinical Pathology*, *76*(3), 172-176. https://doi.org/10.1136/jclinpath-2021-207864
[11] Castle, P. E., Stoler, M. H., Wright, T. C., Sharma, A., Wright, T. L., & Behrens, C. M. (2011). Performance of carcinogenic human papillomavirus (HPV) testing and HPV16 or HPV18 genotyping for cervical cancer screening of women aged 25 years and older: a subanalysis of the ATHENA study. *The Lancet Oncology*, *12*(9), 880-890.
[12] Arbyn, M., Simon, M., Peeters, E., Xu, L., Meijer, C. J., Berkhof, J., & Poljak, M. (2021). 2020 list of human papillomavirus assays suitable for primary cervical cancer screening. *Clinical Microbiology and Infection*, *27*(8), 1083-1095. https://doi.org/10.1016/j.cmi.2021.04.031
[13] Perkins, R. B., Guido, R. S., Castle, P. E., Chelmow, D., Einstein, M. H., Garcia, F., & Schiffman, M. (2020). 2019 ASCCP risk-based management consensus guidelines for abnormal cervical cancer screening tests and cancer precursors. *Journal of lower genital tract disease*, *24*(2), 102. https://doi.org/10.1097/LGT.0000000000000525
[14] Schiffman, M., Hyun, N., Raine-Bennett, T. R., Katki, H., Fetterman, B., Gage, J. C., ... & Wentzensen, N. (2016). A cohort study of cervical screening using partial HPV typing and cytology triage. *International journal of cancer*, *139*(11), 2606-2615. https://doi.org/10.1002/ijc.30375
[15] Arbyn, M., Verdoodt, F., Snijders, P. J., Verhoef, V. M., Suonio, E., Dillner, L., & Anttila, A. (2014). Accuracy of human papillomavirus testing on self-collected versus clinician-collected samples: a meta-analysis. *The Lancet Oncology*, *15*(2), 172-183. https://doi.org/10.1016/S1470-2045(13)70570-9
[16] Nayar, R., & Wilbur, D. C. (2017). The Bethesda system for reporting cervical cytology: a historical perspective. *Acta Cytologica*, *61*(4-5), 359-372. https://doi.org/10.1159/000477556
[17] Arbyn, M., Cuschieri, K., Bonde, J., Schuurman, R., Cocuzza, C., Broeck, D. V., & Poljak, M. (2024). Criteria for second-generation comparator tests in validation of novel HPV DNA tests for use in cervical cancer screening. *Journal of Medical Virology*, *96*(9), e29881. https://doi.org/10.1002/jmv.29881
[18] Perkins, R. B., Wentzensen, N., Guido, R. S., & Schiffman, M. (2023). Cervical cancer screening: a review. *Jama*, *330*(6), 547-558.
[19] Fashedemi, O., Ozoemena, O. C., Peteni, S., Haruna, A. B., Shai, L. J., Chen, A., & Ozoemena, K. I. (2025). Advances in human papillomavirus detection for cervical cancer screening and diagnosis: challenges of conventional methods and opportunities for emergent tools. *Analytical Methods*, *17*(7), 1428-1450. https://doi.org/10.1039/d4ay01921k
[20] Bruni, L. B. R. L., Barrionuevo-Rosas, L., Albero, G., Serrano, B., Mena, M., Gómez, D., & De Sanjosé, S. (2019). Human papillomavirus and related diseases report. *ICO/IARC Information Centre on HPV and Cancer (HPV Information Centre)*, *7*, 26-42.
[21] Thornton, B., & Basu, C. (2011). Real-time PCR (qPCR) primer design using free online software. *Biochemistry and Molecular Biology Education*, *39*(2), 145-154. https://doi.org/10.1002/bmb.20461
[22] Ali, N., Rampazzo, R. D. C. P., Costa, A. D. T., & Krieger, M. A. (2017). Current nucleic acid extraction methods and their implications to point-of-care diagnostics. *BioMed Research International*, *1*, 9306564.
[23] Arya, M., Shergill, I. S., Williamson, M., Gommersall, L., Arya, N., & Patel, H. R. (2005). Basic principles of real-time quantitative PCR. *Expert review of molecular diagnostics*, *5*(2), 209-219. https://doi.org/10.1586/14737159.5.2.209
[24] Bustin S, Nolan T. (2017). Talking the talk but not walking the walk: RT-qPCR as a paradigm for the lack of reproducibility in molecular research. *Eur J Clin Invest., 47*:756–74
[25] Mackay, I. M., Arden, K. E., & Nitsche, A. (2002). Real-time PCR in virology. *Nucleic Acids Research*, *30*(6), 1292-1305. https://doi.org/10.1093/nar/30.6.1292
[26] Mackay, I. M. (2004). Real-time PCR in the microbiology laboratory. *Clinical microbiology and infection*, *10*(3), 190-212. https://doi.org/10.1111/j.1198-743X.2004.00722.x