https://doi.org/10.34883/PI.2024.12.1.016
UDC 618.146:616.974-006.6-022-084:578

Tillyashaykhov M.[1, 2], Zakhirova N.[1]✉, Akin D.[3], Egamberdiev D.[1], Akhmedov O.[1], Djanklich S.[1], Imamov O.[1]
[1] Republican Specialized Scientific-Practical Medical Center of Oncology and Radiology, Tashkent, Uzbekistan
[2] Tashkent Pediatric Medical Institute, Tashkent, Uzbekistan
[3] Swansea University, Wales, UK

Review of HPV Testing and New Approaches in Cervical Cancer Screening

Conflict of interest: nothing to declare.
Authors' contribution: study concept and design, editing – M. Tillyashaykhov, N. Zakhirova, D. Egamberdiev; concept and design of the study, collection of material, processing, writing the text – Deniz Akin, Akhmedov O., Djanklich S., Imamov O.
The article is published in the author's edition.

Submitted: 15.02.2024
Accepted: 21.03.2024
Contacts: nematovna2023@mail.ru

Abstract

Human Papillomavirus (HPV) stands as the most prevalent sexually transmitted infection globally. More than 70% of cervical cancer cases occurring worldwide are caused by two types of HPV – 16 and 18. Another four oncogenic HPV types – 31, 33, 45 and 58 – are less commonly associated with the development of this disease. Uzbekistan, like many nations, faces a substantial burden of HPV-related diseases, notably cervical cancer. Essential prevention strategies encompass HPV vaccination, safe sexual practices, and advanced HPV-DNA testing. The lack of effective prevention programs, as well as limited access to modern treatments for precancer and early stages of cervical cancer in low- and middle-income countries (LMIC), will lead, according to WHO forecasts, to an increase in the incidence of cervical cancer by 2030 to 700,000 new cases and 400 000 deaths per year.Today, in world practice, there are mainly two methods used to detect HPV DNA: amplified and non-amplified. The innovative vNAT (Nucleic acid testing) method with specific tubes for transferring material plays a key role in simplifying diagnosis and increasing biosafety, ensuring rapid and accurate detection of HPV infection.Collaborative global efforts, extensive research, and healthcare capacity strengthening are paramount to combat HPV's significant public health impact, with vNAT tubes offering a promising tool in this battle.
Keywords: HPV, cervical cancer, screening, prevention, diagnostics, vNAT

Тилляшайхов М.Н.[1, 2], Захирова Н.Н.[1], Акин Д.[3], Эгамбердиев Д.М.[1], Ахмедов О.М.[4], Джанклич С.М.[1], Имамов О.А.[1]
[1] Республиканский специализированный научно-практический медицинский центр онкологии и радиологии, Ташкент, Узбекистан
[2] Ташкентский педиатрический медицинский институт, Ташкент, Узбекистан
[3] Университет Суонси, Уэльс, Великобритания

Обзор тестирования на ВПЧ и новые подходы к скринингу рака шейки матки

Конфликт интересов: не заявлен.
Вклад авторов: концепция и оформление исследования, редактирование – Тилляшайхов М.Н., Захирова Н.Н., Эгамбердиев Д.М.; концепция и оформление исследования, сбор материала, обработка, написание текста – Акин Д., Ахмедов О.М., Джанклич С.М., Имамов О.А.
Статья опубликована в авторской редакции.

Подана: 15.02.2024
Принята: 21.03.2024
Контакты: nematovna2023@mail.ru

Резюме

Вирус папилломы человека (ВПЧ) – самая распространенная инфекция, передающаяся половым путем, в мире. Более чем 70% случаев рака шейки матки, возникающих в мире, вызывают два типа ВПЧ – 16 и 18. Еще четыре онкогенных типа ВПЧ – 31, 33, 45 и 58 – реже связаны с развитием этого заболевания. В Узбекистане, как и во многих других странах, существует высокая распространенность заболеваний, вызванных ВПЧ, в частности рак шейки матки. Основные стратегии профилактики включают в себя вакцинацию против ВПЧ, соблюдение безопасных сексуальных практик и расширенное тестирование на ДНК ВПЧ. Отсутствие эффективных программ профилактики, а также ограниченный доступ к современным методам лечения предрака и ранних стадий рака шейки матки в странах с низким и средним уровнями доходов (СНСД) приведут, по прогнозам ВОЗ, к 2030 г. к росту заболеваемости раком шейки матки до 700 000 новых случаев и 400 000 смертей в год.
На сегодняшний день в мировой практике применяются в основном два метода выявления ДНК ВПЧ: амплифицированные и неамплифицированные. Инновационный метод vNAT (Nucleic acid testing) со специфическими пробирками для переноса материала играют ключевую роль, упрощая диагностику и повышая биобезопасность, обеспечивая быстрое и точное выявление инфекции ВПЧ. Совместные усилия в масштабах мира, исследования и укрепление системы здравоохранения имеют первостепенное значение для преодоления влияния ВПЧ на общественное здоровье, с пробирками vNAT в качестве перспективного инструмента.
Ключевые слова: ВПЧ, рак шейки матки, скрининг, профилактика, диагностика, vNAT

■ INTRODUCTION

Human Papillomavirus (HPV), encompassing a diverse family of over 200 related viruses, ranks as the most prevalent sexually transmitted infection (STI) on a global scale [16, 41]

HPV transmission primarily occurs through direct skin-to-skin contact, predominantly via sexual activities such as vaginal, anal, and oral intercourse [54, 61]. The outcomes of HPV infection vary according to the specific viral strain involved. Notably, one-third of HPV types specifically target the genital tract and a subset of these is implicated in anogenital cancers [13, 44]. These HPV strains are categorized into low-risk types (e.g., 6, 11, 26, 40, 42, 43, 44, 53, 54, 61, 69, 70, 73, and 82) and high-risk types (e.g., 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) (Figure 1) [5, 25, 38, 63]. Low-risk HPV strains predominantly give rise to conditions like genital warts, characterized by the emergence of small, cauliflower-shaped growths.Contrary, high-risk strains have the potential to lead to various cancers, including anogenital cancer, i.e., penial, vaginal, vulvar, anal [5, 25, 38,63], and head and neck cancers (even though oropharyngeal cancers traditionally are the result of alcohol and tobacco, current studies have illustrated that over 70% of oropharynx cancers are linked to HPV) [36]. Globally, high-risk HPVs account for approximately 5% of total cancer cases, resulting in an estimated 570,000 infections in women and 60,000 in men annually [63]. Cervical cancer is the second most common cause of cancer-related deaths worldwide [56], and HPV is the second most prominent cancer-causing infectious agent [21, 55]. In 2018, cervical cancer claimed the lives of approximately 311,000 women globally, with over 85% of these fatalities occurring in low- and middle-income nations, where cervical cancer ranks as the leading cause of cancer-related deaths among women [21, 62]. This global burden is mirrored in Uzbekistan, where HPV poses a significant threat, particularly in relation to cervical cancer. This form of cancer is a pressing public health concern within the country;cervical cancer ranks as the second most common cancer among Uzbek women, affecting a population of 12.2 million females aged 15 and above [10]. Based on the statistical data obtained from the Republican Cancer Centre, this resulted in an annual diagnosis of approximately 1,827 cases of cervical cancer in women, leading to 997 deaths in 2021 [34, 35]. In response to the increasing HPV burden, countries and the World Health Organization have implemented comprehensive prevention and management strategies [68]. These strategies encompass primary prevention through HPV vaccination of children aged 9–14 and educational initiatives promoting condom use, secondary prevention involving the detection and treatment of pre-cancerous lesions through screening programs, and tertiary prevention focusing on diagnosing and treating advanced-stage invasive cervical cancer. Additionally, palliative care is provided to enhance the quality of life for individuals facing the challenges of cervical cancer [30, 63, 39]. Nevertheless, screening methods should be emphasised to effectively address this challenge and mitigate the risk of HPV-related cancer and associated fatalities.

Table 1
Summary of key HPV types and associated disease [45]

	Low Risk	High Risk
HPV Strains	6, 11, 26, 40, 42, 43, 44, 53, 54, 61, 69, 70, 73, 82	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
Associated Diseases	Anogenital warts; Cutaneous warts; Recurrent respiratory papillomatosis; Heck's disease	Intraepithelial neoplasia; Invasive carcinoma: Squamous Cell Carcinoma of the Head and Neck, Cervical cancer, Anogenital cancers, non-melanoma skin cancer

HPV Overview

Understanding HPV Biology

HPV is a member of the Papillomaviridae family, which is among the earliest known viral families, with the capability to invade epithelial cells found in the skin, as well as the mucous membranes of the oral and genital areas [3, 64, 66]. HPV virion, i.e., viral particle, display a consistent icosahedral shape, with a 50–55 nm diameterand a molecular weight of 5×10^6 Da. The genetic material of this virus consists of a circular double-stranded DNAcontaining roughly 8,000 base pairs, with associated proteins similar to histones [3, 42, 67]. The HPV genome is divided into three segments: the early (E) region, housing genes such as E1, E2, E4, E5, E6 and E7; the late (L) region, encoding the major (L1) and minor (L2) capsid proteins; and a non-coding region (NCR), commonly referred to as the long control region (LCR) (Fig. 1) [3, 64].

Exploring the HPV Genome: Genes, Function and Implications

The late region, L1 and L2, encodes vital proteins essential for viral infection and assembly. While L1, the primary capsid protein, facilitates infection by binding virions to heparin sulfate receptors in the basal membrane, L2 enables infection and assembly by aiding DNA packaging. These proteins are expressed in later stages, primarily in differentiated epithelium layers [3, 28]

In the early region, E1, E2, and E4 proteins are pivotal for viral replication. E1, forming a di-hexameric complex, binds to the viral Ori and attracts essential factors. E2, with transactivation domains, remodels chromatin, regulates E6 and E7 ORFs, and influences transcription factor recruitment and viral copy numbers. It also interacts with epigenetic regulators, impacting TP53 activity. The E4 protein family, modified through splicing, is abundant in specific epithelial layers, aiding virion release via keratin-associated amyloid fibers[3, 14, 32, 46, 58]. The remaining early region of the viral genome, E5, E6 and E7, encodes oncoproteins that may promote cancer progression. E5, a hydrophobic transmembrane protein, promotes cell proliferation by interacting with various cellular components [2, 24, 57]. E6 plays a central role in cell immortalization by activating the human telomerase reverse transcriptase (hTERT), promotes proliferation, metabolic deregulation and inhibits p53, a key tumor suppressor. E6 can also interact with numerous proteins, leading to genetic instability and cytogenetic damage [11, 50]. Similarly, E7 enhances cell proliferation andinduces genomic instability via induction of DNA breaks and reduction of histone γ-H2 AX levels. Together, E6 and E7 downregulate proteins involved in matrix remodelling and immune response, aiding cancer progression and HPV persistence. These oncoproteins collectively contribute to papillomavirus-driven carcinogenesis [2, 47, 57].

HPV16 contains a circular genome comprised of the early (E) and late (L) genes and the long control region (LCR). The HPV genome consists of eight major genes, organized based on their expression order during the viral life cycle. The image depicts the key functions of each HPV protein. These proteins have been validated as crucial for viral pathogenesis and represent authentic targets for small molecule-based approaches aimed at treating HPV-associated diseases (D'Abramo & Archambault, 2011; da Silva Reiset al., 2011; Evande et al., 2023)

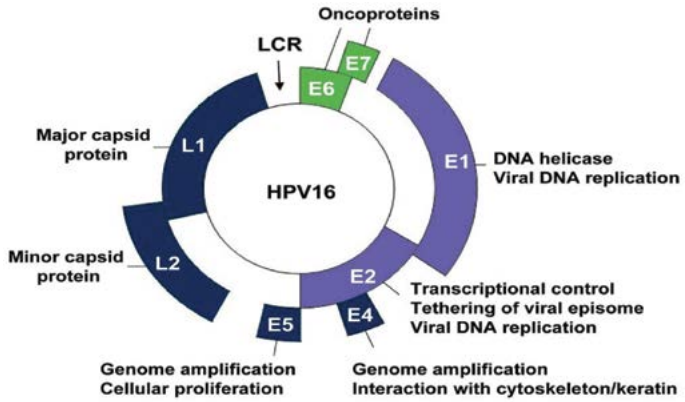

Fig. 1. Diagram illustrating the organization of the HPV genome

HPV Pathogenesis

The HPV infection pathway typically begins in mucosal or skin tissue, where the virus gains access to basal keratinocytes through tissue lesions. Once inside, the L1 protein binds to heparin sulfate proteoglycans (HSPGs) or other integrins like α6, 332, and α6β4, resulting in a conformational change in the viral capsid. This change exposes the N-terminal region of the L2 protein, enabling the virus to bind to cell receptors, including α6β4 integrin. The virus is then internalized through endocytosis, andin acidic phagolysosomes, the viral capsid disassembles, releasing the viral genome. Since HPV cannot replicate independently due to the lack of polymerases, early proteins (E) interact with host cells to induce DNA polymerase-mediated DNA replication during the S phase, which can lead to DNA replication stress and genomic instability, contributing to cancer

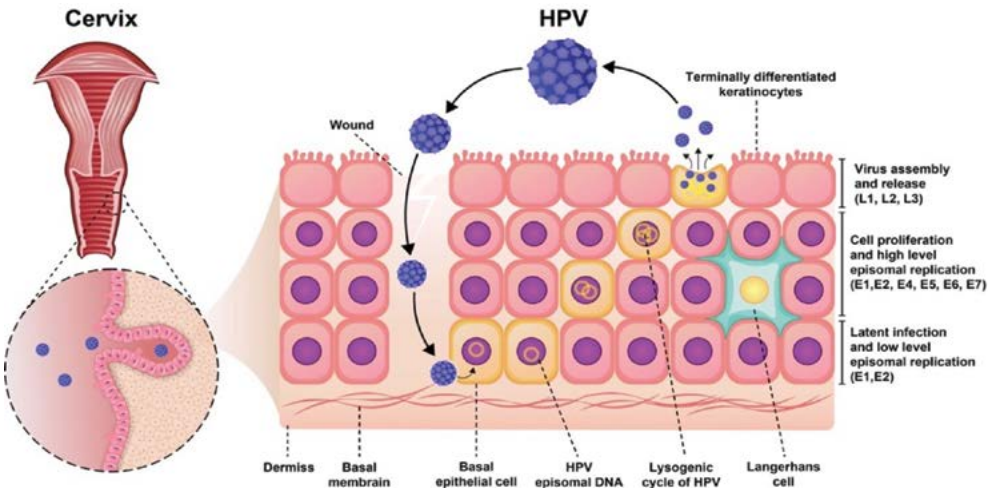

Fig. 2. HPV Pathogenesis

initiation (figure 2). Viral assembly mainly occurs in differentiated epithelium layers, and virion release is facilitated by the degeneration of differentiated cells, forming koilocytes. These cells are destined for apoptosisdue to macromolecule synthesis inhibition [2, 27, 31, 71].

In the initial stages, HPV remains dormant within epithelial cells, exhibiting a low replication rate. However, its replication rate surges as the virus transitions into the lysogenic cycle. Ultimately, mature viruses are assembled and released from keratinocytes, allowing them to initiate new infection cycles [71].

Clinical Manifestations

According to Cancer Research UK [15], 8 out of 10 people will get infected with HPV at some point during their sexually active life. As mentioned, there are two main classifications: low-risk HPV and high-risk HPV. HPV 6 and HPV 11 are the most prevalent low-risk strains, responsible for approximately 90% of genital warts cases. It is important to note that in most cases, low-risk HPV infections remain subclinical, i.e., they do not manifest as any noticeable health issues, and the infection disappears within two years with no remaining abnormalities [25, 38, 63]. In certain instances, HPV infection has the potential to endure, and specific HPV genotypes exhibit a greater tendency for persistence than others [51]. HPV 16 and HPV 18 are the most common high-risk strains, responsible for over 90% of anal cancers and about 70% of vulvar and vaginal [5, 25, 38, 63]. However, the most common HPV-related disease is cervical cancer, with roughly 99.7% of cervical cancer instances attributed to the continual presence of high-risk HPV infection in the genital region [52]. Cervical cancer, which typically progresses gradually, often initiates with alterations in the cells of the cervix. These cell modifications are categorized utilizing the Cervical Intraepithelial Neoplasia (CIN) scale, where CIN1 is mild dysplasia, CIN 2 is moderate dysplasia, and CIN3 is severe dysplasia and carcinoma in situ (figure 3)

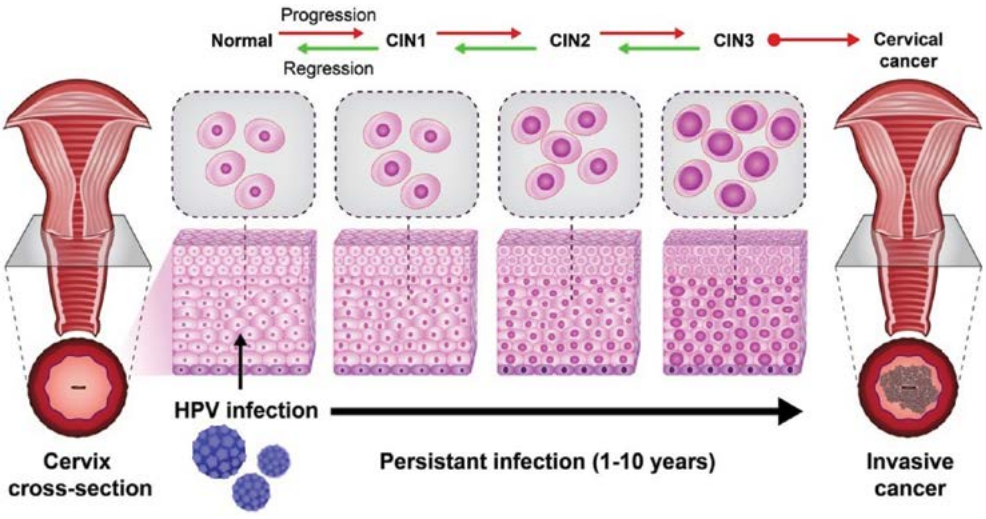

Fig. 3. Clinical Manifestations

[52, 71].According to Loopiket al. [74] around 10% of women who receive treatment for CIN lesions exhibit high-grade disease within the initial two years of follow-up, characterized as persistent or remaining disease. This high-grade disease represents a heightened risk of developing cervical cancer [74].

Understanding the sequence of events from an initial HPV infection to the various stages on the CIN scale plays a pivotal role in preventing cervical cancer. Consistent cervical cancer screenings, including Pap smears and HPV tests, are indispensable for early identification and timely intervention, as they can detect abnormal cell alterations at an early phase when treatment yields the best results.

HPV infections occurring naturally can progress to cervical cancer. To assess the severity of cellular changes, medical professionals use the Cervical Intraepithelial Neoplasia (CIN) scale, which categorizes abnormalities into three grades: CIN1 (representing mild dysplasia), CIN2 (indicating moderate dysplasia), and CIN3 (signifying severe dysplasia and carcinoma in situ). These classifications aid in understanding the risk and potential progression of cervical abnormalities towards cancer [71].

■ HPV SCREENING AND DETECTION METHODS

Initially, the primary method for cervical cancer screening relied on the Pap smear test, but it has since been replaced by liquid-based cytology. It is well-established that HPV plays a pivotal role in most instances of cervical cancer, leading to the inclusion of testing for high-risk HPV strains in many screenings. This necessitates a method capable of differentiating between various HPV types. Some countries, including Uzbekistan, are now in the process of transitioning from emphasizing microscopic examination to making HPV-DNA testing the central screening method. Nevertheless, adopting this new screening method requires significant changes, including revising age-related guidelines and screening schedules, reallocating resources, and educating healthcare providers and patients [1, 9, 22].

The Pap Smear Test

Until recently, in developed nations, cervical cancer screening relied on cytological examination of a cervical smear, commonly known as the Pap test. This procedure involves visualizing the cervix using a speculum and collecting a sample with a spatula or brush, which is then applied to a glass slide (Figure 4A) [40, 37]. The interpretation of the Pap tests is typically done using the Bethesda classification system to report cervical cytologic findings. This system categorizes smears as negative for intraepithelial lesions or malignancy, atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells that cannot exclude high-grade lesions (ASC-H), low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL), squamous cell carcinoma, atypical glandular cells (ACG), adenocarcinoma in situ (AIS), or adenocarcinoma. Women with abnormal Pap test results are recommended for further evaluation, including repeat cytology, HPV testing, or colposcopy [40, 49].

The field of cervical cancer screening is still evolving, with ongoing reassessment of Pap smear screening practices and the development of new screening technologies.It is important to note that Pap smear is primarily designed as a screening test for asymptomatic individuals rather than a diagnostic tool for confirming or ruling out diseases. Reports on the sensitivity and specificity of this test vary widely. Current literature illustrates

that the Pap testing has a sensitivity between 47–55% and the specificity between 64–96% [22, 48, 53].

Liquid Based Cytology

Liquid-based cytology was introduced as an alternative approach to the Pap test due to the conventional test's tendency to produce inaccurate results, both false negatives and false positives. These inaccuracies stem from various factors, including inadequate sampling and preparation (such as interference from blood or inflammation, improper cell fixation, and uneven cell distribution) and errors in detection and interpretation. In liquid-based cytology, cervical cells are collected using a standard sampling device and placed in a vial containing a preservation solution rather than directly applied to a slide (Figure 4B). This approach offers benefits, including the preservation of cellular material, prevention of air drying, facilitation of even cell dispersion, improvement in the accuracy of detection, and the ability to conduct multiple tests on a single sample [40, 60].

A vaginal speculum is used to gently separate the vaginal walls, allowing for a clear view of the cervix. Subsequently, a sample of cervical cells is gathered using a small, cone-shaped brush and a miniature plastic spatula (steps 1 and 2). These collected samples of cells are then either smeared on a microscope for a conventional Pap test (A) or rinsed in a vial filled with liquid (B), and the vial is sent to a laboratory for liquid-based cytology test [40, 49].

HPV Testing

Detecting HPVinfection has been challenging due to the virus's inability to be cultured conventionally and limited sensitivity in serological assays. Diagnosis involved identifying its genome in cellular samples from the affected site, particularly cervical infections.

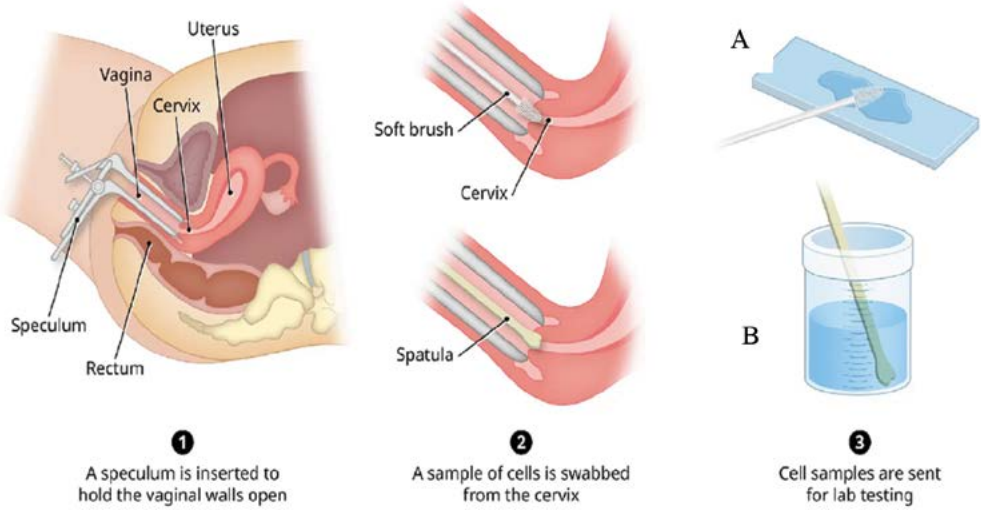

Fig. 4. Illustration of sample collection

Healthcare providers can administer this test during pelvic examinations or through convenient self-sampling at home. Molecular technologies for HPV DNA detection fall into two broad categories: amplified and non-amplified methods. In clinical research, amplified approaches dominate and further divide into signal-amplified and target-amplified techniques. Notable methods in each category include the FDA-approved hybrid capture 2 (HC2) assay and polymerase chain reactions (PCR), respectively [22, 40].

HC2 can detect any of the 13 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) without distinguishing between them. It requires a minimum of 5,000 viral copies per sample to yield a positive result. HC2 replaced an earlier test, the hybrid capture tube, which detected four fewer high-risk types and demanded a higher viral copy threshold (50,000 per sample). This led to lower sensitivity and eventual discontinuation [22, 40].

In contrast, PCR generates numerous target HPV DNA copies through a chemical reaction, making it suitable for testing with minimal cell samples, limited DNA quantities, or a small number of viral copies. The process involves two main stages: amplifying target DNA using thermocycling and oligonucleotide primers. Primers are generally consensus or general, designed for broad-genotype amplification, with a primary focus on the L1 region of the genome. Some PCR assays target E genes, while type-specific primers for a specific HPV genotype are rarely employed. Various general primer designs are available, varying in the size of the amplified DNA region and measures to address intertypic sequence variations. The second stage of PCR involves detecting and analysing the amplified DNA sequence, with multiple methods available, including agarose gel electrophoresis and type-specific analyses using techniques like restriction fragment length polymorphism, Southern blotting, microtiter plate hybridization, direct sequence analysis, and reverse hybridization. Several new HPV assays can identify specific HPV genotypes, ranging from the two primary oncogenic types, HPV 16 and HPV 18 to a complete spectrum of high-risk HPV types, as well as a limited or expanded selection of low-risk HPV types [17, 22, 40].

Innovations in HPV Testing:vNAT transfer tubes

Numerous diagnostic laboratories have progressively embraced PCR-based HPV detection owing to its precision and efficiency. Nonetheless, PCR-based HPV testing, despite its commendable sensitivity and specificity, generally entails several hours to complete due to its meticulous steps, encompassing DNA isolation, purification, amplification, and detection [17]. Consequently, extensive endeavours have been undertaken to continually refine and enhance this method.

One of these ground-breaking innovations is the vNAT transfer tubes, initially introduced during the COVID-19 pandemic to enhance preservation of the samples until they undergo qPCR testing. These vNAT transfer tubes are now gaining significant attention worldwide for their potential application in HPV detection. In essence, vNAT transfer tubes contain vNAT (viral nucleic acid transport) reagents. When they come into contact with the sample, they swiftly lyse the cells within few minutes, facilitating the release of viral nucleic acid and ensuring preservation until RT-qPCR analysis. This step expedites DNA isolation significantly, saving multiple hours of work. Moreover, vNAT reagents deactivate viral pathogens within a minute of contact, enhancing biosafety measures. Importantly, within vNAT transfer tubes, viral nucleic acid can be stored at room temperature for up to 72 hours, simplifying logistics.

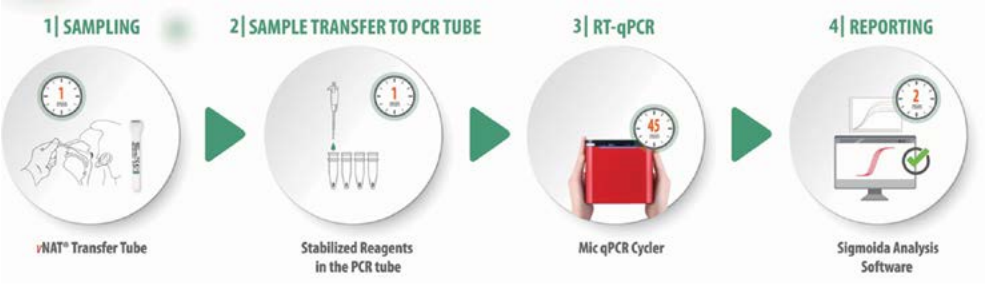

Fig. 5. Pathogen Detection Using vNAT Transfer Tubes

Since the viral nucleic acid is released and stabilized, it can be directly utilized in qPCR reactions. To simplify, the workflow can be summarized as follows (Figure 5):

1. Collection of the specimen (e.g., cervical, vaginal, penile swab samples) into the vNAT transfer tubes.
2. Release, stabilization, and inactivation of viral nucleic acid.
3. Transfer of the sample to the RT-qPCR tubes.
4. Loading RT-qPCR tubes into the RT-qPCR machine and initiating the process.
5. Automatic analysis and report generation.

The RT-qPCR process takes approximately 45 minutes, with results automatically analyzed and reports generated. As previously mentioned, this innovative solution played a pivotal role in efficient and accurate COVID-19 detection, streamlining the testing process and marking a transformative milestone in virology and diagnostics beyond the pandemic context. It's worth noting that during the COVID-19 pandemic, this product obtained World Health Organization Emergency Use Listing and U.S. Food and Drug Administration Emergency Use Authorization [6, 7, 23, 65].

A concise summary of all the HPV screening methods can be viewed in Figure 5.

The process begins with the (1) collection of specimens, for HPV detection this includes cervical, vaginal, or penile swab samples (not shown on image), into vNAT transfer tubes. These tubes serve to stabilize and inactivate viral nucleic acid. Once prepared, (2) the samples are transferred into RT-qPCR tubes for analysis. (3) These tubes are then loaded into the RT-qPCR machine, where the testing process is initiated. Following the testing, (4) the machine automatically analyses the results and generates reports, streamlining the pathogen detection procedure.

■ PREVENTING HPV INFECTION

Preventing HPV infections is of utmost importance for maintaining one's well-being. Adopting safe sexual practices, such as consistently and correctly using condoms, significantly reduces the risk of HPV transmission during intimate encounters. Moreover, limiting sexual partners and avoiding individuals with a history of multiple sexual relationships further lowers the chances of exposure to the virus [63].

Vaccination plays a pivotal role in preventing HPV infections. Highly effective HPV vaccines, such as Gardasil 9 and Cervarix, offer protection against both high-risk and low-risk HPV types linked to various cancers and genital warts. Gardasil 9 is a 9-valent vaccine,

Table 2
Comparison of HPV Detection Methods and Techniques [22, 37, 40, 43, 60, 73]

	Pap Smear Test	Liquid-Based Cytology	Conventional HPV DNA Testing	vNAT Transfer Tubes and RT-qPCR for HPV Detection
Sample Collection Process	Involves scraping cells from the cervix using a spatula or brush and smearing the sample on glass slide	Involves collecting cells from the cervix using a brush, and the sample is preserved in a liquid medium	Involves collecting cells from the cervix using a brush, and the sample is processed for DNA extraction.	Specimens collected in vNAT transfer tubes by using cervical, vaginal, and penile swab samples
DNA Isolation Step	No DNA isolation step: cells are directly examined under a microscope	DNA isolation is not required as cells are suspended in a liquid medium	Requires DNA extraction from collected cells, which can be time-consuming	vNAT reagents lyse cells and stabilize viral nucleic acid, eliminating the need for a separate DNA isolation step
Time Required for HPV Detection	The collection of cells is a quick process; however, the results may take a few days to couple of weeks	Cell collection is relatively quick and the time it takes to receive the results may be similar or slight faster than Pap test	Quick sample collection, however, the process itself is time-consuming due to DNA isolation and purification steps	Quick sample collection, rapid process, with viral nucleic acid ready for qPCR within minutes
Sensitivity and Specificity	Limited sensitivity; may miss some HPV infections	Improved sensitivity compared to Pap smear	High sensitivity and specificity for detecting HPV infections	High sensitivity and specificity for HPV detection
Biosafety Measures	No specific inactivation of viral pathogens	No specific inactivation of viral pathogens	No specific inactivation of viral pathogens	vNAT reagents deactivate viral pathogens within a minute, enhancing biosafety
Storage of Specimens	Not applicable; samples examined immediately	Liquid-based samples can be stored for a short period	DNA samples may require refrigeration or freezing	Viral nucleic acid can be stored at room temperature for up to 72 hours
Automation and Speed	Manual examination under a microscope	Semi-automated processing, still requires manual intervention	May require automated DNA extraction machines	Automation possible for qPCR step, significantly reducing manual labour
Regulatory Approvals	Generally well-established, part of the routine screening	Generally well-established	Established regulatory approvals for specific HPV DNA testing systems	Obtained WHO Emergency Use Listing and FDA Emergency Use Authorization during the COVID-19 pandemic
Overall Advantages	Established screening method, can detect precancerous changes and early-stage cervical cancer, cheap	Improved sample quality and laboratory workflow, Ability to Perform Additional Tests from the Same Sample	High sensitivity and specificity, additionally genotypes	Rapid sample processing, biosafety, and potential for room temperature storage
Overall Limitations	Limited sensitivity: subjective interpretation, does not tell specific strain of HPV	Limited sensitivity: subjective interpretation, does not tell specific strain of HPV	Time-consuming DNA extraction; potential for false negatives, logistic issue, expensive	Limited supporting information on HPV testing

i.e., it guards against nine high-risk HPV types, 6, 11, 16, 18, 31, 33, 45, 52, and 58 and is typically administered in two doses for those under 15 or three doses for individuals aged 15 to 26. Conversely, Cervarix primarily targets HPV types 16 and 18, significant contributors to cervical cancer, and is typically administered in three doses over six months. Adhering to the recommended vaccination schedule is crucial to ensure maximum protection against these high-risk HPV types, ultimately contributing to comprehensive HPV prevention strategies [8, 63]. The Ministry of Health of the Republic of Uzbekistan advocates a two-dose vaccination schedule for girls aged 9 and 13. Nevertheless, this strategy faces several challenges, including parental vaccine hesitancy, misinformation, and disengagement among teachers and healthcare workers, along with negative media coverage [68].

In addition to vaccination and safe sexual practices, regular gynecological check-ups are vital for women's health. Women should generally undergo routine gynecological examinations and HPV tests, typically commencing at age 21 and continuing at intervals recommended by their healthcare providers. These screenings are essential for the early detection of cervical abnormalities and facilitate timely management of any concerning findings, further fortifying HPV prevention efforts [4, 33].

■ CONCLUSION

A comprehensive examination of HPV underscores its profound impact on global public health. HPV, with its extensive family of viruses, has emerged as one of the most prevalent global sexually transmitted infections, necessitating a multifaceted approach to address both its transmission and the diseases it causes. Distinguishing between high-risk and low-risk HPV strains highlights the critical importance of allocating resources to mitigate the impact of high-risk types, which are associated with various cancers, with cervical cancer being the most prevalent among them. The global response to HPV, encompassing vaccination, education, and the advancement of screening methods, not only serves as a testament to international cooperation but also holds relevance for nations like Uzbekistan, which is among the six demonstrative countries selected by the World Health Organization for the Global Strategies for Cervical Cancer Elimination. Many countries are transitioning from traditional Pap smear tests to advanced HPV-DNA testing. The emergence of innovative testing technologies, such as vNAT transfer tubes, promises to streamline diagnostics and enhance biosafety, ultimately contributing to more effective HPV management. A foundational understanding of HPV biology and its role in replication and carcinogenesis is essential for taking action to combat the nationwide spread of HPV infection. Challenges like vaccine hesitancy and healthcare infrastructure gaps persist, emphasizing the need for concerted efforts in research, awareness, and healthcare capacity building. Effective preventive strategies, including vaccination, regular screenings, and screening programs, are equally critical within Uzbekistan's public health framework in the ongoing battle against HPV-related diseases. Ensuring access to full treatment for cervical cancer patients, as stipulated by the WHO's 90-70-90 strategy, as well as access to palliative care, represents the main strategic approach for eliminating cervical cancer. This applies to all developing countries where cervical cancer remains highly prevalent.

REFERENCES

1. Andersen B., Njor S.H., Jensen A.M.S., Johansen T., Jeppesen U., Svanholm H. (2020) HrHPV testing vs liquid-based cytology in cervical cancer screening among women aged 50 and older: a prospective study. *International Journal of Gynecologic Cancer*, 30(11).
2. Araldi R.P., Assaf S.M.R., Carvalho R.F.D., Carvalho M.A.C.R.D., Souza J.M.D., Magnelli R.F., Módolo D.G., Roperto F.P., Stocco R.D.C., Beçak W. (2017) Papillomaviruses: a systematic review. *Genetics and molecular biology*, 40, pp. 1–21.
3. Araldi R.P., Sant'Ana T.A., Módolo D.G., de Melo T.C., Spadacci-Morena D.D., de Cassia Stocco R., Cerutti J.M., de Souza E.B. (2018) The human papillomavirus (HPV)-related cancer biology: An overview. *Biomedicine & pharmacotherapy*, 106, pp. 1537–1556.
4. Athanasiou A., Bowden S., Paraskevaidi M., Fotopoulou C., Martin-Hirsch P., Paraskevaidis E., Kyrgiou M. (2020) HPV vaccination and cancer prevention. *Best Practice & Research Clinical Obstetrics & Gynaecology*, 65, pp. 109–124.
5. Ault K.A. (2007) Human papillomavirus vaccines and the potential for cross-protection between related HPV types. *Gynecologic oncology*, 107(2), pp. S31–S33.
6. Aydoğan O., GözünŞaylan E., Güven Ö., Ayaz A., Yiğitbaşı T. (2022) COVID-19 pozitifhastalarda SARS-CoV-2 varyantlarınınprevalansı. *KlimikDergisi*.
7. Bakir A., Hosbul T., Cuce F., Artuk C., Taskin G., Caglayan M., Guney M., Kurkcu M.F., Yildiz F., Erdal H., Erdem G. (2021) Investigation of Viral Load Cycle Threshold Values in Patients with SARS-CoV-2 Associated Pneumonia with Real-Time PCR Method. *The Journal of Infection in Developing Countries*, 15(10), pp. 1408–1414.
8. Bedell S.L., Goldstein L.S., Goldstein A.R., Goldstein A.T. (2020) Cervical cancer screening: past, present, and future. *Sexual medicine reviews*, 8(1), pp. 28–37.
9. Bhatla N., Singhal S. (2020) Primary HPV screening for cervical cancer. *Best Practice & Research Clinical Obstetrics & Gynaecology*, 65, pp. 98–108.
10. Bolormaa E., Yusupov B., Akhmedova M., Yoo Y.J., Choe S.A. (2023) Human Papillomavirus Knowledge and Cervical Cancer Screening Practice among Uzbekistan Women. *Clinical and Experimental Obstetrics &Gynecology*, 50(1), p. 4.
11. Boon S.S., Tomaić V., Thomas M., Roberts S., Banks L. (2015) Cancer-causing human papillomavirus E6 proteins display major differences in the phospho-regulation of their PDZ interactions. *Journal of virology*, 89(3), pp. 1579–1586.
12. Buck C.B., Day P.M., Trus B.L. (2013) The papillomavirus major capsid protein L1. *Virology*, 445(1–2), pp. 169–174.
13. Burd E.M. (2003) Human papillomavirus and cervical cancer. *Clinical microbiology reviews,* 16(1), pp. 1–17.
14. Burk R.D., Harari A., Chen Z. (2013) Human papillomavirus genome variants. *Virology*, 445(1–2), pp. 232–243.
15. Cancer Research UK (2023). Does HPV cause cancer?, *Cancer Research UK*. Available at: https://www.cancerresearchuk.org/about-cancer/causes-of-cancer/infections-eg-hpv-and-cancer/does-hpv-cause-cancer (Accessed: 09 September 2023).
16. Centres for Disease Control and Prevention. (2022) *Std Facts – Human papillomavirus (HPV)*, *Centres for Disease Control and Prevention*. Available at: https://www.cdc.gov/std/hpv/stdfact-hpv.htm (Accessed: 07 September 2023).
17. Chrysostomou A.C., Kostrikis L.G. (2020) Methodologies of primary HPV testing currently applied for cervical cancer screening. *Life*, 10(11), p. 290.
18. D'Abramo C.M., Archambault J. (2011) Small molecule inhibitors of human papillomavirus protein-protein interactions. *The open virology journal*, 5, p. 80.
19. D'Abramo C.M., Archambault J. (2011) *Genomic organization of the HPV genome. Research Gate*. Available at: https://www.researchgate.net/publication/51502367_Small_Molecule_Inhibitors_of_Human_Papillomavirus_Protein_-_Protein_Interactions (Accessed: 08 September 2023).
20. Da Silva Reis A.A., da Cruz A.D. (2011) The impact of human papillomavirus on cancer risk in penile cancer. *Human papillomavirus and related diseases*, p. 319.
21. De Martel C., Georges D., Bray F., Ferlay J., Clifford G.M. (2020) Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. *The Lancet Global Health*, 8(2), pp. e180–e190.
22. Delpero E., Selk A. (2022) Shifting from cytology to HPV testing for cervical cancer screening in Canada. *CMAJ*, 194(17), pp. E613-E615.
23. Dinç H.Ö., Karabulut N., Alaçam S., Uysal H.K., Daşdemir F.O., Önel M., Tuyji Tok Y., Sirekbasan S., Agacfidan A., Gareayaghi N., Çakan H. (2023) Evaluation of the diagnostic performance of a SARS-CoV-2 and influenza A/B combo rapid antigen test in respiratory samples. *Diagnostics*, 13(5), p. 972.
24. Disbrow G.L., Hanover J.A., Schlegel R. (2005) Endoplasmic reticulum-localized human papillomavirus type 16 E5 protein alters endosomal pH but not trans-Golgi pH. *Journal of virology*, 79(9), pp. 5839–5846.
25. Egawa N., Doorbar J. (2017) The low-risk papillomaviruses. *Virus research*, 231, pp. 119–127.
26. Evande R., Rana A., Biswas-Fiss E.E., Biswas S.B. (2023). Protein–DNA Interactions Regulate Human Papillomavirus DNA Replication, Transcription, and Oncogenesis. *International Journal of Molecular Sciences*, 24(10), p. 8493.
27. Florin L., Sapp M., Spoden G.A. (2012) Host-cell factors involved in papillomavirus entry. *Medical microbiology and immunology*, 201, pp. 437–448.
28. García-Vallvé S., Alonso Á., Bravo I.G. (2005) Papillomaviruses: different genes have different histories. *Trends in microbiology*, 13(11), pp. 514–521.
29. Gleneagles Hospital (2023). Papanicolaou (Pap) Smear Test, *Gleneagles Hospital.* Available at: https://www.gleneagles.com.sg/tests-treatments/pap-smear-test (Accessed: 09 September 2023).
30. Grandahl M., Rosenblad A., Stenhammar C., Tydén T., Westerling R., Larsson M., Oscarsson M., Andrae B., Dalianis T., Nevéus T. (2016) School-based intervention for the prevention of HPV among adolescents: a cluster randomised controlled study. *BMJ open,* 6(1), p. e009875.
31. Greber U.F. (2016) Virus and host mechanics support membrane penetration and cell entry. *Journal of virology*, 90(8), pp. 3802–3805.
32. Harari A., Chen Z., Burk R.D. (2014) Human papillomavirus genomics: past, present and future. In Human papillomavirus (vol. 45, pp. 1–18). *Karger Publishers.*
33. Hathaway J.K. (2012) HPV: diagnosis, prevention, and treatment. *Clinical obstetrics and gynecology*, *55*(3), pp. 671–680.
34. ICO/IARC (2023) *ICO/IARC Information Centre on HPV and cancer uzbekistan*, *HPV Information Centre*. Available at: https://hpvcentre.net/statistics/reports/UZB_FS.pdf (Accessed: 03 September 2023).
35. ICO/IARC (2023) *Human Papillomavirus and Related Cancers, Fact Sheet 2023* , *ICO/IARC Information Centre on HPV and Cancer*. Available at: https://hpvcentre.net/statistics/reports/UZB_FS.pdf (Accessed: 01 September 2023).
36. Jatinder S. (2004). O. Reference Centers for Disease Control and Prevention. *Indian Journal of Pharmacology,* 36, pp. 268–269.
37. Jayapalan S., Bindu R.S. (2020) Papanicolaou smear: A diagnostic aid in sexually transmitted infections. *Indian Journal of Sexually Transmitted Diseases and AIDS*, 41(2), p. 143.
38. Jin J. (2018) HPV infection and cancer. *Jama*, 319(10), pp. 1058–1058.
39. Kassa H.N., Bilchut A.H., Mekuria A.D., Lewetie E.M. (2021) Practice and associated factors of human papillomavirus vaccination among primary school students in minjar-shenkora district, north shoa zone, amhara regional state, ethiopia, 2020. *Cancer Management and Research*, pp. 6999–7008.

40. Koliopoulos G., Nyaga V.N., Santesso N., Bryant A., Martin-Hirsch P.P., Mustafa R.A., Schünemann H., Paraskevaidis E., Arbyn M. (2017) Cytology versus HPV testing for cervical cancer screening in the general population. *Cochrane database of systematic reviews,* 8.
41. KombeKombe A.J., Li B., Zahid A., Mengist H.M., Bounda G.A., Zhou Y., Jin T. (2021) Epidemiology and burden of human papillomavirus and related diseases, molecular pathogenesis, and vaccine evaluation. *Frontiers in public health*, 8, p. 552028.
42. Leto M.D.G.P., Santos Júnior G.F.D., Porro A.M., Tomimori J. (2011) Human papillomavirus infection: etiopathogenesis, molecular biology and clinical manifestations. *Anais brasileiros de dermatologia*, 86, pp. 306–317.
43. Liang L.A., Einzmann T., Franzen A., Schwarzer K., Schauberger, G., Schriefer D., Radde K., Zeissig S.R., Ikenberg H., Meijer C.J., Kirkpatrick C.J. (2021) Cervical cancer screening: comparison of conventional Pap smear test, liquid-based cytology, and human papillomavirus testing as stand-alone or cotesting strategies. *Cancer Epidemiology, Biomarkers & Prevention*, 30(3), pp. 474–484.
44. Longworth M.S., Laimins L.A. (2004) Pathogenesis of human papillomaviruses in differentiating epithelia. *Microbiology and molecular biology reviews,* 68(2), pp. 362–372.
45. Medda A., Duca D., Chiocca S. (2021) Human papillomavirus and cellular pathways: Hits and targets. *Pathogens*, 10(3), p. 262.
46. Mirabello L., Clarke M.A., Nelson C.W., Dean M., Wentzensen N., Yeager M., Cullen M., Boland J.F., NCI HPV Workshop, Schiffman M., Burk R.D. (2018) The intersection of HPV epidemiology, genomics and mechanistic studies of HPV-mediated carcinogenesis.
47. Moody C.A., Laimins L.A. (2010) Human papillomavirus oncoproteins: pathways to transformation. *Nature Reviews Cancer*, 10(8), pp. 550–560.
48. Najib F.S., Hashemi M., Shiravani Z., Poordast T., Sharifi S., Askary E. (2020) Diagnostic accuracy of cervical pap smear and colposcopy in detecting premalignant and malignant lesions of cervix. *Indian journal of surgical oncology*, 11, pp. 453–458.
49. National Cancer Institute (2023) *Cervical cancer screening*, *National Cancer Institute*. Available at: https://www.cancer.gov/types/cervical/screening (Accessed: 08 September 2023).
50. Nominé Y., Masson M., Charbonnier S., Zanier K., Ristriani T., Deryckère F., Sibler A.P., Desplancq D., Atkinson R.A., Weiss E., Orfanoudakis G. (2006) Structural and functional analysis of E6 oncoprotein: insights in the molecular pathways of human papillomavirus-mediated pathogenesis. *Molecular cell*, 21(5), pp. 665–678.
51. O'Rorke M., Anderson L., Jamison J., Gavin A., Wilson R. (2015) HPV prevalence and type-distribution in cervical cancer and premalignant lesions of the cervix: a population-based study from Northern Ireland. In National Cancer Intelligence Network: Cancer Outcomes Conference 2015: United Against Cancer.
52. Okunade K.S. (2020) Human papillomavirus and cervical cancer. *Journal of Obstetrics and Gynaecology,* 40(5), pp. 602–608.
53. Özalp S.S., Us T., Arslan E., Öge T., Kaşifoğlu N. (2012) HPV DNA and Pap smear test results in cases with and without cervical pathology. *Journal of the Turkish German Gynecological Association*, 13(1), p. 8.
54. Petca A., Borislavschi A., Zvanca M.E., Petca R.C., Sandru F., Dumitrascu M.C. (2020) Non-sexual HPV transmission and role of vaccination for a better future. *Experimental and therapeutic medicine*, 20(6), pp. 1–1.
55. Plummer M., de Martel C., Vignat J., Ferlay J., Bray F., Franceschi S. (2016) Global burden of cancers attributable to infections in 2012: a synthetic analysis. *The Lancet Global Health,* 4(9), pp. e609–e616.
56. Rana J.S., Khan S.S., Lloyd-Jones D.M., Sidney S. (2021) Changes in mortality in top 10 causes of death from 2011 to 2018. *Journal of general internal medicine,* 36, pp. 2517–2518.
57. Scarth J.A., Patterson M.R., Morgan E.L., Macdonald A. (2021) The human papillomavirus oncoproteins: A review of the host pathways targeted on the road to transformation. *The Journal of general virology*, 102(3).
58. Schuck S., Stenlund A. (2015). A conserved regulatory module at the C terminus of the papillomavirus E1 helicase domain controls E1 helicase assembly. *Journal of Virology*, *89*(2), pp. 1129–1142.
59. Siebers A.G., Klinkhamer P.J., Grefte J.M., Massuger L.F., Vedder J.E., Beijers-Broos A., Bulten J., Arbyn M. (2009) Comparison of liquid-based cytology with conventional cytology for detection of cervical cancer precursors: a randomized controlled trial. *Jama*, 302(16), pp. 1757–1764.
60. Smith E.M., Parker M.A., Rubenstein L.M., Haugen T.H., Hamsikova E., Turek L.P. (2010) Evidence for vertical transmission of HPV from mothers to infants. *Infectious diseases in obstetrics and gynecology*, 2010.
61. Sung H., Ferlay J., Siegel R.L., Laversanne M., Soerjomataram I., Jemal A., Bray F. (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*, 71(3), pp. 209–249.
62. Szymonowicz K.A., Chen J. (2020) Biological and clinical aspects of HPV-related cancers. *Cancer biology & medicine*, 17(4), p. 864.
63. Tommasino M. (2014) The human papillomavirus family and its role in carcinogenesis. In *Seminars in cancer biology* (vol. 26, pp. 13–21). *Academic Press.*
64. Toptan H., Koroglu M., Olmez M., Zengin M., Umaroglu M.M., Karabay O. (2022) Evaluation of Using Direct Viral Transport Medium Samples without Nucleic Acid Isolation for SARS-CoV-2 Diagnosis by RT-PCR. *Clinical laboratory*, 68(10).
65. Van Doorslaer K. (2013) Evolution of the papillomaviridae. *Virology*, 445(1–2), pp. 11–20.
66. Varsani A., van der Walt E., Heath L., Rybicki E.P., Williamson A.L., Martin D.P. (2006) Evidence of ancient papillomavirus recombination. *Journal of General Virology,* 87(9), pp. 2527–2531.
67. Warsi S.K., Nielsen S.M., Franklin B.A., Abdullaev S., Ruzmetova D., Raimjanov R., Nagiyeva K., Safaeva K. (2023) Formative Research on HPV Vaccine Acceptance among Health Workers, Teachers, Parents, and Social Influencers in Uzbekistan. *Vaccines,* 11(4), p. 754.
68. World Health Organisation (2023) *Human papillomavirus and cancer*, *World Health Organization*. Available at: https://www.who.int/news-room/fact-sheets/detail/human-papilloma-virus-and-cancer (Accessed: 01 September 2023).
69. Y Yousefi Z., Aria H., Ghaedrahmati F., Bakhtiari T., Azizi M., Bastan R., Hosseini R., Eskandari N. (2023) FIGURE 2 The natural HPV infection can lead to cervical cancer, Frontier. Available at: https://www.frontiersin.org/files/Articles/805695/fimmu-12-805695-HTML/image_m/fimmu-12-805695-g002.jpg (Accessed: 09 September 2023).
70. Yousefi Z., Aria H., Ghaedrahmati F., Bakhtiari T., Azizi M., Bastan R., Hosseini R., Eskandari N. (2022) An update on human papilloma virus vaccines: history, types, protection, and efficacy. *Frontiers in Immunology*, 12, p. 805695.
71. Yousefi Z., Aria H., Ghaedrahmati F., Bakhtiari T., Azizi M., Bastan R., Hosseini R., Eskandari N. (2023) *FIGURE 1 Pathogenesis of HPV infection*, *Frontier*. Available at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.805695/full (Accessed: 08 September 2023).
72. Kolukirik M., Korukluoglu G., Bayrakdar F., Ozgumus G.G., Altas A.B., Cosgun Y., KetreKolukirik C.Z. (2021) 40 minutes RT-qPCR Assay for Screening Spike N501Y and HV69-70del Mutations. *BioRxiv*, pp. 2021–01.
73. Loopik D.L., IntHout J., Ebisch R.M., Melchers W.J., Massuger L.F., Siebers A.G., Bekkers R.L. (2020) The risk of cervical cancer after cervical intraepithelial neoplasia grade 3: A population-based cohort study with 80,442 women. *Gynecologic Oncology*, 157(1), pp. 195–201.