JOURNAL OF
BIOLOGICAL
INSIGHTS

Vol. 1| Issue 1|pp:30-58| 2025 |
DOI: 10.4197/jbi.1-1.2

Sharing Knowledge, Shaping the Future

Published by Scientific Publishing Center, King Abdulaziz University

Research Article

Received: 2025/10/03| **Accepted**: 2025/11/19| **Published**: 2025/12/29|

Handling Editor: Irfan A. Rather | Department of Biological Sciences| King Abdulaziz University |Jeddah| Saudi Arabia

Comparative Analysis of Traditional and Molecular Methods for Detecting Human Papillomavirus in Histology and Cytology Samples

Almutiri Abdullah Bandar[1, 2]*, Fadhel Alotaibi[3], Mohammed Alsaeed[4,5], Sari S. Sabban[1], Mohamed Morsi M. Ahmed[1,6,7,8]*

[1]Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.
[2]Prince Sultan Military Medical City, Ministry of Defense Health Services, Riyadh, Saudi Arabia.
[3]Pathology Department, Security Forces Hospital, Riyadh, Saudi Arabia.
[4]Medicine Department, Infectious Diseases Division, Prince Sultan Military Medical City, Riyadh, Saudi Arabia.
[5]College of Medicine, Alfaisal University, Riyadh, Saudi Arabia.
[6]Princess Dr Najlaa Bent Saud Al Saud Ctr of Excellence, Jeddah, Saudi Arabia.
[7]Centre of Excellence in Bionanoscience, King Abdulaziz University, Jeddah, Saudi Arabia.
[8]Nucleic Acids Research Deprt., Genetic Engineering and Biotechnology Research Institute (GEBRI), City for Scientific Research and Technological Applications, Alexandria, Egypt.

*Corresponding author: abdullah.2003@yahoo.com (A.A.B) | mmmahmed@kau.edu.sa (M.M.A)

Abstract

Objective: Human Papillomavirus (HPV) is a critical factor in the pathology of various carcinomas, including cervical cancer, and warrants improved testing platforms for effective management and therapeutic outcomes. This study aims to compare the various traditional methods and molecular diagnostic platforms used for HPV screening in histological and cytological samples. **Method:** in this study, we identified the gap in the HPV screening methods being used currently, by systematically scrutinizing the strengths and limitations of traditional techniques such as the histological- cytological approaches, In-Situ Hybridization, Immunohistochemistry (IHC), and nucleic acid-based assays including the conventional Polymerase Chain Reaction (PCR) and then the Next-Generation Sequencing (NGS) as modern HPV genotyping method. **Results:** We found the traditional methods were the most widely used due to their low cost. While the NGS remains the unique and complex screening technique recommended by many medical and health bodies across the globe, most literature acknowledged a series of disadvantages using such approaches, and these include low sensitivity and optimization of probes; issues related to primer design, integrity of the specimen, impurities from the amplification, and discrepancies due to antibodies' variations. **Conclusion:** for an integrated screening approach, this study proposes a more complex, holistic framework for selecting and applying HPV detection methods to enhance clinical decision-making and improve patient outcomes in HPV-related disease management. Finally, the study stressed the development of AI-assisted approaches to improve the efficiency and consistency of HPV detection by enabling rapid triage of more complex cases.

Keywords: Human papillomavirus | polymerase chain reaction | in situ hybridization | immunohistochemistry | next-generation sequencing

1. Introduction

Human Papillomavirus (HPV) is well established as a primary causative agent of cancer, especially in the anogenital and oropharyngeal areas [1]. The most conspicuous impact can be attributed to the rise in cases related to cervical cancer, which develops due to the advancement of precursor tumors into invasive cancer, an event exacerbated by the presence of high-risk HPV [2]. Thus far, the emergence of over 200 types of HPV has been noted, defining a broad viral group [3]. It is important to note, however, that HPV types 16 and 18 pose a special threat due to their formidable role in oncogenesis and malignant transformation [4]. Minimizing cancer cases, especially among those with high-risk HPV types, demands a better understanding of the virus and better methods of early diagnostics [5]. With the established link of HPV with cervix and uterine cancer, present-day scientific emphasis has moved from only understanding the virus itself and its implications to improving diagnostic tools and techniques. Correct HPV typing is critical for implementing early control and prevention programming related to HPV infections. This is why pioneering advancements in both cytologic and histologic diagnostic methods, as well as newer molecular tests such as Polymerase Chain Reaction, *In-Situ* Hybridization (ISH), Immunohistochemistry, and Next-Generation Sequencing, have greatly improved HPV diagnostics by increasing sensitivity, specificity, and prognostic value.

Conventional screening is currently dominated by standard methodologies, such as cytologic evaluation (e.g., the Papanicolaou test, commonly called the Pap smear) and histologic evaluation. However, these existing methodologies are prone to some limitations, among which is the inability to screen effectively for the molecular alteration associated with HPV-induced cancer [6]. This is due to an unmet need for more effective, innovative molecular screening methodologies capable of targeting molecular markers and events related particularly to HPV and its malignant potential [6]. Due to their increased sensitivity, specificity, and ability to distinguish infection from induced cellular alterations, molecular methodologies have gained ready acceptance for the detection of HPV infection [6, 7]. As HPV has a propensity to colonize epithelial cells and induce genetic changes leading to cancer, attempts for virus isolation in histological and cytological specimens hold promise [8]. Moreover, it has been recognized that the initial screen for HPV-induced events is paramount in the diagnostic process before the establishment of cancer [9]. Here, cytological methods involve analyzing cellular anomalies consistent with precancerous changes, while histological analysis depicts tissue architecture to identify disease [9]. However, given the fact that a precursor stage of cervical cancer is always a dysplastic change leading to invasive carcinoma, its prevention with conservative treatment is always a favorable factor because the cancer itself takes a predictable pathway.

Notably, HPV types 16 and 18 have already established themselves as the most cancer-causing strains, being responsible for a large pool of cases related to cervical cancer worldwide

[2]. Over the past two decades, the diagnostic technologies used to detect HPV have significantly improved with the advent of molecular diagnostics platforms [6]. Today, these molecular diagnostic platforms permit the specific and precise typing of high-risk HPV strains, some of which may be missed with cytological/histological analyses alone [7]. Moreover, molecular diagnostics, namely polymerase chain reaction (PCR) and ISH, further enable mapping of viral genomic material within infected cells, thereby enabling precise diagnostic analysis [11]. Specifically, with regard to the molecular diagnostics employed in the analysis of infected tissue for HPV infection, especially at early stages, these diagnostics permit a level of diagnostic complexity previously unattainable with classical protein staining alone [12]. Moreover, other molecular diagnostics, namely immunohistochemistry (IHC) and next-generation sequencing (NGS), have increasingly been used in HPV disease diagnostic research and molecular analysis [13]. With these sophisticated molecular diagnostic platforms, including both IHC and NGS platforms, specific proteins associated with HPV infections can be detected and mapped within infected tissues, providing valuable subject information related to the functional activity of the virus within the subject tissue sample [13]. Concerning HPV virus molecular diagnostics within subject tissue, NGS technologies further enable broad-scale comparative genomics analysis of the virus within a subject sample, including specific mapping and tracking of virus genotypic variability related to virus integration within subject cellular genomic targets [14].

International standard operating procedures (SOPs) have been developed by organizations including the Centers for Disease Control (CDC, USA), the World Health Organization (WHO, UN), and the American Society for Colposcopy and Cervical Pathology (ASCCP, USA) [13]. These international health bodies have recommended molecular screening as the preferred testing method for HPV, shifting from cytological screening to nucleic acid-based assays to improve risk assessment and early diagnosis. Due to the high cost of molecular analyses, the shortage of experts and adequate infrastructure, these novel technologies are rarely used [14]. Therefore, this paper compares the primary and molecular diagnostic methods for HPV screening—thereby providing complementary evidence to advance the easy accessibility of the novel techniques.

The present study is designed to critically assess the effectiveness of traditional diagnostic platforms compared to molecular diagnostic paradigms, thereby establishing an evidence-based framework to guide method selection and the possible integration of various diagnostic platforms in HPV screening.

2. Traditional methods for HPV detection

2.1 Pap smear (cytology)

The Papanicolaou test, also known as the Pap smear, has been the primary platform for cytological screening for cervical cancer since the 1940s, when it was introduced by Dr George Papanicolaou [15,16,17]. This screening protocol is designed to identify aberrant distortion in exfoliated cervical cells, which may be suggestive of the occurrence of pre-cancerous lesions or invasive cervical tumors [18]. Although this method was not initially designed to screen for

HPV directly, it has been very useful for the detection of cellular aberrations triggered by a prolonged regimen of HPV infections [19].

During a Pap smear procedure, specimens of cells from the cervix are taken and scrutinized microscopically for evidence of dysplasia (pre-cancerous changes) and cancer [20]. With HPV infection, specific characteristic cellular changes may be visible, such as koilocytosis, a cell with a typical halo surrounding the nucleus, which is associated with high-risk HPV infection [21]. Additionally, these cellular characteristics play a key role in the diagnosis of precancerous changes, especially those with CIN, a potential precancerous stage that may progress to cancer if left untreated [22].

Moreover, the sensitivity of the Pap smear test is currently a subject of great concern. Even though this diagnostic tool shows a high level of specificity and accuracy in recognising cases with apparent cytological changes, its potential to overlook both hidden and deeper lesions is high, especially for some types of cancer, such as adenocarcinomas [23]. As a type of cancer developing from glandular tissue, adenocarcinomas mostly pose challenges in the process of being detected with the aid of cytologic tools such as the Pap smear tests [24]. According to evidence, about 50% of adenocarcinomas may be overlooked when Pap smear testing is independent [25], thereby underscoring its inadequacy, especially for non-squamous cell carcinoma [26].

These limitations in Pap smear sensitivity have led to increased interest in new molecular paradigms with potential for use in combination with cytologic exams [27]. Besides, in many screening programs, the concept of co-testing, which integrates HPV DNA testing protocols (which directly detect high-risk HPV genotypes) with cytological methods, has become a standard practice that promises to overcome the pitfalls of cytological examination [2]. Nevertheless, while the Pap smear has maintained its prominence as an indispensable diagnostic platform, it is now identified as part of a larger diagnostic strategy [28].

2.2 Histopathological examination

Histopathology, the microscopic examination of tissue samples to detect disease, has been used to diagnose cervical carcinoma. In the case of HPV-associated lesions, histological inspection has been useful for confirming cytological test results or evaluating aberrant tissue samples obtained by biopsy, colposcopy, or surgical excision [29]. This is especially efficient at identifying high-grade lesions, such as CIN2 and CIN3, and invasive carcinoma [30].

From a clinical standpoint, H&E staining is the most commonly used staining method in histopathology. This is a common staining technique used to observe cellular and tissue morphology and to preliminarily assess tissues for pathology [31]. As a diagnostic tool for HPV infection, histopathological evaluation can provide insights into the main features of the infection and its potential for cancerous transformation [32]. As a clinical tool, the observation of koilocytes, which is specific for infection with HPV, is straightforward [32]. As specific viewpoints within the context of a newer methodology for treating late infection, including cellular dysplasia and an increased Nuclear-to-Cytoplasmic ratio, among others, can be observed [33].

Histopathological analysis is highly beneficial for assessing tissue changes associated with HPV. However, just like the Pap smear test, histopathological analysis platforms cannot independently identify the virus [34]. Even though it helps assess the level of anomalies produced by the causative agent, histopathological analysis is incapable of distinguishing among HPV types or identifying specific high-risk HPV infections without the aid of other molecular diagnostic platforms [35]. However, a significant drawback of histopathological screening is its invasiveness, which often involves a biopsy procedure. This makes it unsuitable for general clinical use, as it is mostly indicated for screens with a previously established level of suspicion and for cases showing apparent pathological changes, including abnormal cytology results and lesions noted during colposcopic examination [36]. Moreover, when infections present with significant histological changes despite being early-stage, they can evade standard histological analysis, underscoring the importance of hybrid approaches that incorporate molecular platforms such as immunohistochemistry and in situ hybridisation [37].

2.3 Strengths and weaknesses of traditional methods

Traditional paradigms based on cytology and histology are associated with several strengths, as well as some weaknesses. Traditional diagnostic platforms have found wide application and a long history of utility in clinical practice, due to their accessibility and relatively low cost [38]. The Pap smear, especially, is non-invasive and, hence, could be easily performed as part of a clinical routine, and this is one of the advantages that gave it its initial utility as a screening platform [39]. Conversely, histopathology affords a more comprehensive and close examination of tissue architecture and is suitable for screening for high-grade lesions and confirming invasive cancers [40]. However, both diagnostic paradigms are associated with some notable limitations. Notably, the Pap smear screening tool is not sufficiently sensitive, especially for adenocarcinomas and early-stage lesions, as it can lead to missed cases of HPV-related malignancies [2]. Furthermore, both methods are not suitable for identifying the presence of HPV infection or deciphering between high-risk and low-risk genotypes, making them inferior in efficacy in determining the precise culprit of cellular abnormalities [41]. As a result, reliance on these conventional platforms alone may result in underdiagnosis or delayed identification of high-risk HPV infections [42].

Besides, the urgent need for more precise, early diagnosis has led to the introduction and incorporation of molecular diagnostic tools into testing programs. Besides, the Co-testing method, which incorporates molecular platforms, such as PCR or HPV DNA testing with primary cytological routines, has resulted in improved sensitivity and specificity of screening paradigms [43]. Interestingly, coupling the visual inspection afforded by cytology and histology with the molecular precision unlocked using modern diagnostic platforms has enabled clinicians to obtain a more detailed understanding of HPV infection and its potential to induce malignancies [44]. Table 1 highlights the various diagnostic methods and their utility.

Table 1 Comparison of HPV detection techniques in histology and cytology samples.

HPV Detection Technique	Sensitivity	Specificity	Sample Type Compatibility	Advantages	Limitations
Pap Smear (Cytology)	Moderate	Moderate	Cytology	Widely available and cost-effective, it detects cytological changes in exfoliated cells.	Lower sensitivity for adenocarcinomas can miss early HPV infection.
Histopathological Examination	Moderate	High	Histology	Provides a direct view of tissue architecture, good for diagnosing advanced lesions	Limited to visual changes, cannot confirm HPV presence without molecular markers
PCR (Polymerase Chain Reaction)	High	High	Cytology, Histology	Detects low viral loads, identifies high-risk HPV genotypes, fast results	Prone to contamination and false positives, it doesn't indicate viral integration status
In Situ Hybridization (ISH)	Moderate	High	Histology	Visualizes HPV DNA in tissue architecture, useful for studying viral localization	Less sensitive than PCR, it may miss infections with low viral loads.
Immunohistochemistry (IHC)	Moderate to High	High	Cytology, Histology	Identifies HPV-related proteins and markers (e.g., p16INK4a), easy to perform	Subjectivity in result interpretation may require a combination with other methods for higher accuracy.
Next-Generation Sequencing (NGS)	Very High	Very High	Cytology, Histology	Detects multiple HPV strains and viral mutations simultaneously, with precise viral integration analysis	High cost, complex technology, limited to research or specialized clinical settings

3. Molecular methods for HPV detection

3.1 Polymerase Chain Reaction (PCR)

The PCR is a molecular tool widely used in HPV screening [45,46]. By PCR, also known as a thermocycler, specific sequences of viral DNA are amplified, facilitating the detection of viral signature genetic material in samples with very low viral load [47]. Consequently, PCR has gained utility for detecting infections at an early stage or in conditions where the viral load is low but the risk of oncogenic transformation is high [48]. PCR functions by targeting certain characteristic regions of the HPV genome, such as the L1, E6, or E7 genes. These oncogenes have been shown to play key roles in establishing the viral replication processes and oncogenesis and, hence, represent a suitable genetic biomarker for identifying the presence

and activity of HPV [49]. PCR tool also enables the determination of the specific viral genotype involved in the infection, and this is useful in classifying the risk level of the infection and guiding therapeutic intervention [50]. Notably, the E6 and E7 oncoproteins are important molecular markers for screening for HPV infection, because their expression directly correlates with the virus's ability to initiate cancerous events in host cells [51]. The identification of these genes using the PCR platforms could be used to confirm the occurrence of high-risk HPV types, such as HPV-16 and HPV-18, which are notorious culprits associated with cervical cancers and other non-cervical malignancies [52].

Recently, Yerim et al. [53] and Khan et al. [54] found PCR to be the analytically accurate biomedical tool for HPV detection, with comprehensive genotyping and high sensitivity. Even though PCR is influenced by primer design, specimen integrity, and possible impurities introduced during amplification, it remains the most suitable method for detecting high-risk HPV types from small DNA quantities.

The PCR diagnostic tool is preferred for its high specificity and sensitivity. This diagnostic tool can be used to isolate HPV DNA from specimens when standard approaches, including histopathological examination of a Pap smear, may not detect anomalies, especially those with minimal changes [55]. As a direct consequence of this advantage, the PCR diagnostic tool has gained popularity and emerged as a reliable alternative for detecting subclinical infections, especially when the virus causes little change in cellular morphology [56]. Moreover, PCR can distinguish among different HPV strains [57]. However, some limitations related to PCR have been noted. For instance, the high sensitivity of the PCR procedure may, in some cases, lead to false positives because of contamination with trace amounts of the virus from the environment or from a previously positive sample [58]. This problem is particularly concerning in a clinical setting, where tight control over contamination may be impractical. Moreover, the procedure fails to provide data concerning the integration of the virus into the host genome, an important factor in oncogenic transformation [59]. The integration of the viral genome into the host genome may disrupt regulatory control over cell growth, leading to uncontrolled cellular growth and, thus, cancer [60].

Irrespective of its limitations, the PCR technique is currently a key standard in HPV diagnostics. Furthermore, when used in combination with other diagnostic tools, it significantly improves the accuracy of HPV diagnosis, especially concerning persons with a heightened predisposition to developing HPV-induced carcinomas [55].

3.2 *In Situ* Hybridization (ISH)

In-Situ Hybridisation (ISH) is a major molecular diagnostic tool used to localise HPV-related DNA/RNA within biological tissues [6]. Unlike PCR, which concentrates nucleic acids in solution, ISH can be used to analyse the spatial location of viral nucleic acids within tissues, thereby helping to understand the virus's topology within the tissue sample [61, 62]. It is worth noting that one of the key applications of ISH is distinguishing benign from malignant tissues, given the co-localization of HPV DNA within specific loci within a tissue due to potential integration of viral DNA into the host genome [63]. Although standard ISH can be used to correlate virus load with histological location, a host of new technologies, such as

RNA-based and chromogenic ISH (c-ISH), are currently being used to enhance sensitivity with the aid of newer tools for probe optimization.

The ISH diagnostic model uses 'labeled' probes that target specific DNA or RNA sequences associated with HPV in the tissue sample. When these probes hybridise with specific sequences, they can be detected with a microscope using a special staining technique, thereby identifying the specific location of the viral genetic material within the cellular environment [64]. The ability to identify regional spatial details of the virus is a significant advantage of the ISH diagnostic model [65]. Unlike PCR, ISH can identify the location of viral genetic material within the tissue sample and better aid in assessing risks associated with the causative pathogen [66].

Another significant use of ISH is the demonstration of the presence of high-risk HPV types in cases of cervical and oropharyngeal cancers. By precisely localizing HPV-related DNA within a tissue, ISH can determine viral load in areas of cellular dysplasia/malignancy [67]. This use makes ISH a valuable tool for analyzing and categorizing cases involving the integration of viral genetic material into the host genome, as viral integration is a key factor in malignant transformation [68]. Moreover, one significant advantage ISH has over other methods been its potential to differentiate episomal from integrated viral genomic content, due to its established direct link with oncogenic potential [69]. However, some limitations in the ISH technique have been identified. The most significant limitation is its lower sensitivity compared with PCR when a low viral load is present, although ISH offers high spatial resolution [6]. Thus, ISH can fail to detect the virus when viral load is low in infected cases [70]. In addition, the cost-effective and technically demanding nature of the ISH procedure compared to PCR limits its use in clinical routine practices [67]. Nevetheless, its potential to pinpoint the exact location and spatial distribution of the virus within host tissues makes it an indispensable tool for genomic and pathologic research aimed at elucidating the underpinnings of viral integration and its oncogenic potential [71].

3.3 Immunohistochemistry (IHC)

Of note, IHC is a widely used molecular method for detecting HPV-related proteins within tissue samples. Unlike PCR and ISH, which focus on viral nucleic acids, IHC detects protein expression, particularly the viral oncoproteins E6 and E7, and the cellular protein p16INK4a, which is a surrogate marker for high-risk HPV infection [71]. However, the presence of p16/Ki-67 and Ki-67 proteins indicates a semi-quantitative surrogate marker of HPV activity. Recently, von Knebel Doeberitz [72] confirmed that, for transforming diseases, the synergistic complex p16/Ki-67 increases diagnostic specificity. Even though results vary with antibody quality and scoring, the ease of interpreting tissue architecture remains the sole advantage of the IHC technique in HPV detection.

The E6 and E7 oncoproteins play a critical role in the progression of HPV infections to cancer by interfering with tumour suppressor proteins such as p53 and retinoblastoma (Rb), leading to uncontrolled cell growth [14]. The use of the IHC tool in screening for these proteins provides evidence of HPV involvement in oncogenic activity in the tissue. Besides, p16INK4a, a protein usually overexpressed in high-risk HPV-infected cells, represents a valuable

biomarker for detecting HPV-induced cancers, particularly in cervical, head, and neck carcinomas [7].

The IHC is relatively user-friendly and provides useful clues about the biological actions of cells infected with HPV. It could be used to determine the active participation of the viral proteins in oncogenesis, thereby unearthing the clinical relevance of the attendant HPV infection [6]. Besides, in cases where the presence of HPV-associated DNA has been screened using PCR, but the activity of E6 or E7 protein is not evident, it may be reasoned that the infection is rather latent than active, with no or less risk of cancer development [71]. However, some limitations of the IHC tool have been identified. One of which is the subjective nature of result interpretation, which largely depends on the level of expertise and judgment of the handling pathologist. Hence, variability in staining intensity and background noise can confound diagnostic outcomes, increasing the risk of false positives or negative results [73]. Besides, while IHC is an excellent detection platform for assessing the oncogenic activity of HPV, it may not provide information on the specific HPV genotype present in the tissue sample. Hence, IHC is often utilized in conjunction with other molecular platforms, such as PCR, to realize a more detailed diagnosis of the infection [74].

3.4 Next-Generation Sequencing (NGS)

Next-generation sequencing is one of the foremost molecular techniques for detecting Human Papillomavirus because it provides a detailed view of the HPV genome. Contrary to other molecular techniques, such as Polymerase Chain Reaction and In Situ Hybridization, which can only target specific parts of the HPV genome, next-generation sequencing is capable of sequencing the entire HPV genome and provides a detailed overview of the genetic variability, mutations, and integration of the virus itself with the host genome [75]. Moreover, next-generation sequencing can be used for the simultaneous characterization of multiple HPV strains, making it an ideal molecular technique for cases involving infections with coexistent HPV strains [76]. Moreover, next-generation sequencing can be used for the characterization of HPV strains with potential oncogenic mutations within the virus itself [76]. For the efficient genotyping of HPV, next-generation sequencing is the most advanced biomedical tool available currently for the detection of HPV infection. The most recent observations by Goulart et al. [68, 78] and Andersen et al. [79] clearly establish the ability of next-generation sequencing for the precise characterization of coexistent HPV strains with substantial accuracy. However, because of costs and longer turnaround times in a clinical setting with complexities in bioinformatics analysis, next-generation sequencing is rarely used for such purposes.

Even with the high specificity and vast amount of information generated, the use of NGS in a routine clinical setting is hampered by cost and complexity. It is mainly used for research and when other diagnostic approaches have failed [78]. The potential of NGS for identifying new HPV types and analysing viral integration sites establishes NGS as a crucial tool in unravelling HPV's role in cancer induction, although its use is restricted [79].

3.5 Novel and under-explored genetic biomarkers for the diagnosis of uterine cancers

Lately, scientists have gained interest in a variety of genetic markers to develop better diagnostic, prognostic, and therapeutic outputs for uterine and cervical cancers, especially those induced by HPV infection. Along with the continuous search for new and efficient genetic markers, relatively unexplored markers, including HPV-cDNA, PAX1, the long non-coding RNA MIR22HG, circulating tumour DNA, and CA242, have emerged [80, 90]. Each of these markers has shown great promise and received significant attention for their potential to improve diagnostic capabilities and enable personalised therapy. However, these markers have some limitations in common. A discussion about these markers is given in Table 2.

Table 2 Key genetic biomarkers and their roles in HPV-related uterine carcinomas

Biomarker	Type	Function/Significance	Clinical Applications	Current Challenges	References
HPV-cDNA	DNA-based	Detects active HPV infections and differentiates transient from high-risk infections	Early detection, predicting persistent infections, and treatment planning	Requires further clinical validation for routine screening	[87-90]
PAX1	Methylation-based DNA marker	Tumor suppressor gene; hypermethylation linked to high-grade CIN and cervical cancer	Early detection of cervical and endometrial cancer, risk assessment	Sensitivity and specificity across populations need further study	[91,92]
MIR22HG lncRNA	Long non-coding RNA	Involved in epigenetic regulation; affects tumor growth, invasion, and metastasis	Potential prognostic marker for tracking disease progression	Needs large-scale validation for clinical use	[93, 94]
Circulating Tumor DNA (ctDNA)	DNA fragments in the bloodstream	Non-invasive "liquid biopsy" detecting genetic alterations	Real-time monitoring of disease progression, treatment response	Standardization and sensitivity in early detection remain challenges	[95, 96]
CA242	Tumor-associated carbohydrate antigen	Elevated levels in some cervical and uterine cancers	Potential marker for tumor burden assessment and prognosis	Lacks specificity for HPV-related cancers, requiring further study	[97]

3.6 Comparative overview

Each molecular technique for analysing human papillomavirus (HPV) infection has unique strengths and weaknesses. Polymerase chain reaction is both sensitive and specific; therefore, it is ideal for analysing low numbers of viral deoxyribonucleic acid copies, although it does not provide information on viral integration [6]. In situ hybridisation is valuable because it provides information about the virus's location within tissue sections, though it is less

sensitive than PCR. It provides information about HPV biological activity because it targets viral proteins termed E6 and E7; however, it fails to identify the HPV type [71]. Finally, next-generation sequencing is the most thorough technique for analysing the HPV genome; however, its high cost and complexity limit its widespread use [79]. The use and diagnostic potential of HPV analysis platforms are demonstrated in Table 3.

Table 3 Applications and diagnostic relevance of HPV detection methods

HPV Detection Method	Primary Applications	Diagnostic Relevance	Biomarkers/HPV Genotypes Identified
Pap Smear (Cytology)	Screening for cervical dysplasia and pre-cancerous changes	Useful in initial screening to detect cellular abnormalities	General cellular changes; does not specify HPV genotypes
Histopathological Examination	Diagnosis of HPV-associated lesions in tissue biopsies	Essential for visualizing structural abnormalities in advanced lesions	Tissue architecture changes require IHC for protein markers
PCR (Polymerase Chain Reaction)	Detection of high-risk HPV genotypes and viral presence	High sensitivity and specificity for detecting HPV DNA	HPV-16, HPV-18, and other high-risk genotypes
In Situ Hybridization (ISH)	Localization of HPV DNA within tissue sections	Visualizes infection within specific cells, distinguishes benign/malignant lesions	HPV DNA, HPV-16, HPV-18
Immunohistochemistry (IHC)	Detection of HPV-related proteins and oncogenic markers	Indicates HPV oncogenic activity through specific protein expression	p16INK4a, E6, E7 proteins
Next-Generation Sequencing (NGS)	Comprehensive viral genome analysis	Identifies multiple HPV strains, mutations, and viral integration patterns	Multiple HPV strains, specific viral mutations

4. Comparative analysis of detection methods

4.1 Traditional methods *vs*. molecular methods

Early HPV tests were based on cytological observations carried out using the Pap smear in cervical cancer screening, followed by histopathological tests. It can be attributed to the

ingenious work of Dr George Papanicolaou in the early part of the twentieth century, who introduced the Pap smear, a tool for a preliminary diagnostic evaluation of cervical cells infected with HPV and displaying abnormal cells [91, 92]. As a historical diagnostic tool used extensively for decades, the Pap smear has its limitations, especially in the detection of high-risk HPV types and adenocarcinomas, a specific form of cervical cancer found in the glandular cells, which can often be elusive even with a cytological study [93]. Hematoxylin and Eosin staining is a common tool in histopathological analysis for cell characterisation. The procedure is performed by microscopic examination of dissected tissues [94]. This procedure, although slightly more invasive than a cell study, is a better modality for understanding tissue alteration [94]. It is deficient in molecular-level HPV detection, especially at the early stages of infection when cellular alterations are not yet apparent [94]. Molecular biology principles applied with increased accuracy—PCR, ISH, IHC, and NGS—took diagnostic accuracy significantly forward [95]. This is because these modalities can directly detect biological molecules, such as nucleic acids and proteins, with appreciably greater accuracy than cytological disruptions of altered cellular morphology in cancer diagnostics [95, 96].

4.2 Strengths of traditional methods

The Papanicolaou test, also known as the Pap smear, is an important part of general screening because of its costs, lack of invasiveness, and ease of performance. Its accessibility makes it amenable to wide uptake in public health campaigns aimed at lowering the prevalence of cervical cancer, especially in areas where cost is a significant factor and access to molecular tools is restricted [97]. Though the test is licensed with some drawbacks, it has made a great impact in lowering the rate of cervical cancer due to the timely diagnosis of abnormally growing cells. Histopathological analysis, which greatly aids comprehension of architectural details and abnormally growing cells, is the 'gold standard' for recognising serious cases of cancer [98]. This analysis is used by pathologists with the aid of staining tools such as Hematoxylin and Eosin to identify levels of both malignancy and dysplasia in cancerous cells and plays an important role in recognition when a specific cancer diagnosis is required, especially with the aid of molecular markers. However, both cytologic and histopathologic analyses have some serious limitations [99]. The Pap smear is seriously deficient in sensitivity; evidence shows that up to 50% of cases of adenocarcinoma can be missed by the screen [100]. This is actually a serious problem because, unlike the other main form of cervical cancer, namely Squamous cell carcinoma, the former may lack obvious cytologic changes [100]. Though the histopathologic screen is efficient for detecting structural anomalies, it is used only for obvious cell anomalies and cannot detect direct HPV infection [101].

4.3 Strengths of molecular methods

Molecular biology methods offer a more specific strategy for HPV infection. Of these, the most distinctive characteristic of PCR is its high sensitivity and specificity [102]. This methodology is especially useful in cases with a low biological load but a potentially high oncogenic risk, as it can detect trace levels of biological DNA [102]. Moreover, because it can amplify specific

regions of the HPV genome—such as the *L1*, *E6*, and *E7* genes—it can identify the HPV infection itself and, therefore, assess a person's risk individually [102]. This feature is especially valuable in cases of high-risk HPV types, including HPV 16 and 18, which have a broad correlation with the emergence of oral and cervical cancer [103].

There are some advantages of ISH, including the ability to localize HPV DNA within a tissue section. This assay is valuable for visualizing the virus's tissue distribution and distinguishing between benign and malignant lesions [65]. Unlike PCR, ISH allows researchers to visualize the location of the virus within specific cells or tissues rather than just detecting viral DNA in solution. This is important for understanding viral integration, an essential step in HPV infection's progression towards cancer [7].

Another advantage of immunohistochemistry is the additional insight it provides by detecting proteins within cells or viruses, such as p16INK4a, a marker for high-risk HPV infection, in combination with other viral proteins, such as *E6*/*E7*. This is especially useful in cases where proof of the virus's oncogenic potential is of prime importance, along with its detection [104]. For example, a strong link exists between the expression of high-risk HPV and the overexpression of p16INK4a protein.

Next-generation sequencing is currently capable of providing the most precise analysis of the virus genome, although its cost limits its use in clinical diagnostics [105]. This technology can identify multiple HPV strains simultaneously and provides a detailed overview of genomic changes, thereby aiding in understanding the pathogenesis induced by HPV infection [52]. When standard tests fail to yield conclusive results in complex cases, next-generation sequencing stands out as a valuable tool [52].

4.4 Comparative strengths and weaknesses

Every detection technique has its own advantages and disadvantages, and no single technique is sufficient for thorough HPV detection. For example, Pap smears are inexpensive and non-invasive, but they are not sensitive enough to identify HPV infections in their early stages, especially adenocarcinomas [106]. Even though histopathological tests are quite good at identifying invasive malignancies, they cannot detect HPV directly and instead depend on obvious structural alterations that may not appear until much later in the infection [11]. However, molecular techniques such as PCR and ISH offer high sensitivity and direct viral DNA detection. PCR is a potent method for early detection because it is particularly good at detecting low viral loads and genotype-specific information [107]. However, PCR cannot detect whether the virus has integrated into the host genome, a crucial step in cancer development, and is prone to contamination and false positives. By providing spatial resolution, ISH overcomes this limitation and enables localization of the virus within the tissue [108]. ISH is typically less sensitive than PCR, though, especially when viral levels are low. IHC detects biological proteins and substitutes for markers such as p16INK4a, which provide fundamental information on biological oncogenic activity, though it is less sensitive for viral detection than PCR [109]. Because of this, it is beneficial when determining the clinical importance of an HPV infection. IHC can be personal, though, and the pathologist's understanding greatly influences how it is interpreted [110]. Lastly, NGS provides a detailed

understanding of the biological genome, including the identification of mutations and patterns of viral incorporation; however, due to its high cost and complexity, its application is limited to specialist clinical cases or research settings [111]. A comparative analysis of the strengths and weaknesses of traditional and molecular diagnostic platforms is presented in Table 4.

Table 4 Strengths and weaknesses of traditional and molecular HPV detection methods.

HPV Detection Method	Diagnostic Strengths	Practical Challenges	Clinical Utility
Pap Smear (Cytology)	Detects cellular abnormalities indicating HPV-related dysplasia; widely accepted for early screening	Moderate sensitivity, especially for adenocarcinomas; subjective	Useful for routine HPV screening, low-cost, accessible
Histopathological Examination	Provides detailed tissue architecture; highly specific for advanced lesions and malignancies	Requires biopsy; cannot confirm HPV presence without adjunct tests	Effective in diagnosing advanced HPV-related cancers
PCR (Polymerase Chain Reaction)	High sensitivity and specificity; detects low viral loads and differentiates high-risk HPV genotypes	Contamination risks; false positives possible; no viral integration info	Ideal for high-risk HPV identification; rapid results
In Situ Hybridization (ISH)	Localizes HPV DNA in tissue samples, revealing infection in benign vs. malignant lesions	Lower sensitivity; less effective at detecting low viral loads	Used to confirm HPV presence in tissue for clinical correlation
Immunohistochemistry (IHC)	Detects HPV-associated proteins (e.g., p16INK4a), indicating viral activity; easy to use in clinical labs	Interpretation can be subjective and may require confirmatory tests	Supports HPV oncogenic activity assessment
Next-Generation Sequencing (NGS)	Highly specific; provides data on multiple HPV genotypes and viral mutations.	High cost, complex setup, mainly research-focused	Valuable for research on HPV variants and integration patterns

4.5 Importance of combined approaches

The best strategy for detailed and trustworthy HPV discovery, bearing in mind the advantages and disadvantages of each technique, is to combine the classical and molecular methods [16]. Co-testing using both HPV DNA testing and Pap smears has been established to greatly increase diagnostic sensitivity. While HPV DNA testing, particularly by PCR, can detect high-risk HPV strains even before cellular alterations become obvious, Pap smears can detect cellular abnormalities [2]. Additionally, a more complete picture of the infection can be obtained by incorporating molecular methods such as PCR, ISH, and IHC. For instance, the ISH can determine whether the virus is confined or incorporated into the host genome, whereas PCR can verify its presence and detect its genotype [112]. The IHC platform could also be used to assess viral oncogenic activity by identifying surrogate marker proteins, such as p16INK4a, E6, and E7. This complete strategy is exceptionally fundamental for detecting

high-risk individuals who might be more prone to malignancies linked to HPV [99]. Merging these methods in clinical practice ensures that no constituent of the infection is wasted, improving diagnostic precision and enabling better patient outcomes [87].

5. Challenges in HPV detection

5.1 Technical challenges

The prospect of false positives and false negatives is one of the main technical challenges in HPV detection. This problem is particularly evident in molecular methods such as IHC and PCR, which are prone to technical errors despite their high precision [65,113]. When samples are contaminated with foreign DNA, PCR can yield false-positive results. Because PCR is so sensitive and can amplify even minute amounts of biological DNA, a contaminated sample may lead to the identification of HPV when the tolerant does not truly have it [114]. This puts the patients at risk of getting an improper diagnosis, which could result in unnecessary follow-up care, nervousness, and even overtreatment [115]. Though they are essential, contamination prevention trials, including firm laboratory practices and the use of distinct workspaces for multiple sample dispensation stages, can increase operational costs and complexity [116]. On the other hand, when the viral burden is too low to detect, false negatives may occur. Even PCR may not adequately amplify the biological genome for identification in early-stage infections or in samples with damaged DNA [117]. Furthermore, personal interpretation of IHC results or disparities in staining measures may lead to false negatives. Because changes in tissue research, antibody superiority, and staining concentration can yield contradictory results, this bias depends on the pathologist's level of information [118].

Furthermore, the accuracy of detection methods can be significantly compromised by sample quality. Inaccurate test results may result from low-quality samples produced by improper collection methods, storage problems, or deterioration over time [119]. This is particularly true for cytological samples, such as Pap smears, where inadequate sampling or improper handling may prevent the detection of HPV DNA or abnormal cells. Low-quality samples might not yield sufficiently high-integrity DNA for amplification in molecular testing, leading to additional negative results [6, 93]. The challenge of detecting biological integration with the host genome presents another technical difficulty. Although tools like NGS can provide comprehensive information on biological integration, their high cost and complexity prevent their widespread use in standard clinical settings [120]. Although PCR is quite good at detecting HPV DNA, it cannot distinguish between integrated and non-integrated viral DNA, which is fundamental for assessing the virus's capacity to cause cancer (Dias et al., 2020). A fundamental step in the development of cancer is HPV integration into the host genome, particularly for high-risk HPV strains like HPV-16 and HPV-18 [4].

5.2 Clinical challenges

Discrimination between temporary and persistent HPV infection is one of the key issues in HPV detection. A large proportion of HPV infections can be treated as self-limiting infections, with an average clearance period of about two years because of the host's immune response

[122]. However, persistent infections with high-risk strains of HPV are causative in invasive cancers and precancerous diseases. Conventional HPV detection methods, including molecular techniques such as PCR, can only detect HPV DNA but fail to distinguish between transient and persistent infections [6]. As most cases of HPV infection do not progress to cancer, a lack of distinction is of clinical importance [123]. The main challenge is determining which cases, based on specific cancer risks, require closer observation and treatment [123]. There is value in using other markers, such as p16INK4a, which is overexpressed due to high-risk HPV-induced cellular changes, to better understand viral expression and cancer induction [124]. Moreover, false positives may be present due to a lack of oncogenic viral processes, although these do not pose a problem [125]. The other challenge in clinical practice is the identification of adenocarcinomas and other non-squamous malignancies related to HPV infection. Although the use of Pap smear tests has significantly lowered the prevalence of cervical squamous cell carcinoma, the technique is much less sensitive for the identification of adenocarcinomas [24]. The reason for this is that adenocarcinomas arise from the glandular cells within the cervix, which are located up high within the endocervical canal and therefore may be rarely sampled with a Pap smear [126]. Thus, a significant proportion of adenocarcinomas may be missed by common screening tests, and new technologies are needed to develop better diagnostic tools for these cancers [127]. Although molecular techniques, including HPV DNA tests, have significantly improved the potential for the identification of high-risk HPV types associated with adenocarcinomas, these tumors continue to create challenges in diagnostic tests due to the indistinct cytological and histological characteristics [93]. Moreover, co-infections with more than one HPV type and other sexually transmitted infections can add complexity to diagnostic tests [128]. However, distinguishing these strains, including high- and low-risk strains, is a significant factor because both strains may often be present within a patient [129]. Even though molecular techniques, including PCR and next-generation sequencing, can accurately identify HPV types, co-infections may further complicate analysis because of substantial variation in the quantity of virus present within a given infection [52].

5.3 Logistical and cost challenges

The expense and complexity of cultured molecular methods pose major problems to their acceptance in standard clinical practice in many parts, particularly in low- and middle-income countries (LMICs) [130]. Because of their affordability and ease of use, Pap smears remain the most commonly used screening method [131]. They are not very sensitive, though, particularly when it comes to detecting early-stage lesions or high-risk HPV infections. The use of subtle molecular methods, such as PCR or HPV DNA testing, in these settings is sometimes limited by a lack of funding, inadequate infrastructure, and a shortage of qualified staff [2]. Although NGS provides the most comprehensive information on viral genotypes and integration patterns, its high cost and requirement for advanced laboratory equipment and bioinformatics resources make it unrealistic for broad use [79]. NGS is primarily used for research or when current diagnostic techniques have not yielded conclusive results. On the other hand, though less costly techniques such as PCR and IHC are becoming more widely available, their uptake

remains limited in many regions of the world due to financial and practical constraints [120, 132]. Furthermore, both high- and low-income settings and many disadvantaged groups still lack access to frequent screening programs and high-quality healthcare. Cervical and other HPV-related cancer rates are higher in regions lacking formal screening programs because HPV infections often go undiagnosed until they reach more progressive stages [121, 122, 133, 134]. The uneven identification and treatment of HPV infections are also due to the lack of standardized screening measures and follow-up care [123, 134]. Finally, another practical matter is whether HPV testing is suitable, particularly in societies where infection is denounced. Even in regions where HPV testing is accessible, people may be reluctant to get screened because of confidentiality issues, apprehension about getting a diagnosis, or wrong information regarding HPV [133, 135]. To overcome these problems, public health efforts that inform people about the value of HPV screening and vaccination are important. Still, in some places, these programs often encounter opposition or a lack of funding [136, 137].

5.4 Future directions and solutions

To meet these challenges, an integrated, multimodal strategy that brings together advancements in technology, clinical practice, and infrastructure is required. Moving forward with the clinical management of HPV infection requires fundamental research on new biomarkers capable of distinguishing between transient and persistent infection [138–140]. For example, a molecular approach coupled with existing biomarkers, such as p16INK4a and E6/E7 mRNA expression levels, may help select those infected who are most susceptible to developing cancer [20, 141]. Figure 1 shows an integrated methodology for overall HPV detection, including efficient diagnostic and clinical management of HPV infection. It is also important to note that, with a view to offsetting errors due to technology, automated platforms working with sample handling and molecular analysis may alleviate contamination and errors due to human judgment in molecular analysis, with established methodologies related to sample handling and processing working towards alleviating errors associated with HPV infection detection [142].

Globally, a reduction in the disease burden of HPV-associated malignancies can be made possible by ensuring improved access to molecular tests in LMICs. This can be ensured by developing molecular tests for the Point-of-Care setting that are cost-effective and can be used in resource-poor settings [143]. Furthermore, improved accuracy and efficiency of screening programs can be ensured by educating medical professionals about the performance and interpretation of HPV tests. Finally, prevention of HPV-associated malignancies can be achieved by actively promoting routine screening and HPV immunization [144]. Widespread immunization can significantly reduce the need for advances in diagnostic technologies in the coming generations, given the established efficacy of the HPV vaccine in preventing infections with high-risk HPV types [145]. However, achieving a high level of widespread immunization will require overcoming significant socio-logistical hurdles [146]. Moreover, some very recent studies identify a need for a holistic approach to improving immunization xrates, which, according to many experts, may lay the foundation for an effective strategy [147–149].

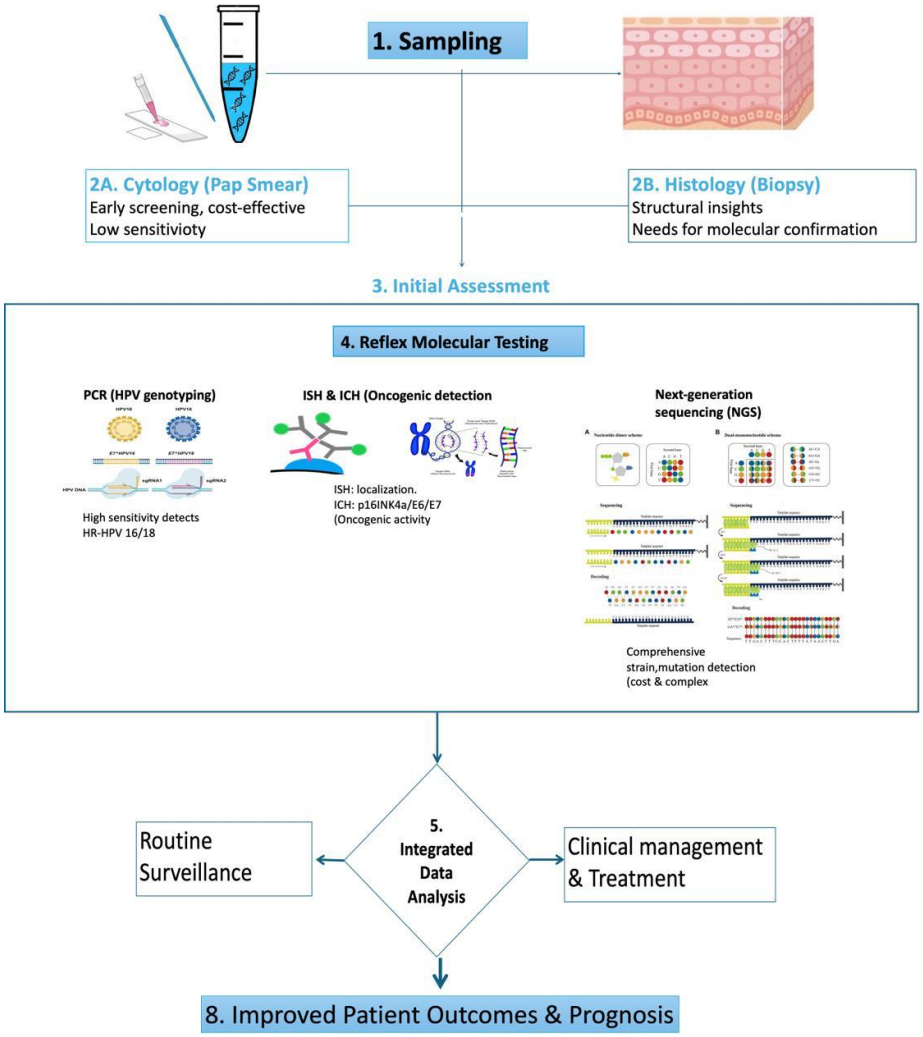

Figure 1. Itinerary workflow illustrating an evidence-based framework for an integrated HPV screening. This schematic representation depicts a complex detection strategy for HPV screening, starting from sample collection. Upon histo-cytological examinations of the collected sample, an initial assessment is done. Integrated data analysis then drives the clinician toward either routine surveillance or clinical management and treatment, with the ultimate goal of improving patient outcomes and prognosis.

5.5 Future perspectives

For future studies related to the pattern of HPV detection, a combination of validated molecular tests with histological and cytological markers of HPV needs to be pursued for enhanced accuracy of HPV testing, standardization of workflow protocols among different laboratories, and the use of cost-effective high-throughput technologies for facilitating rapid HPV screen testing and typing in clinical samples [150, 151]. Microfluidic technologies and portable devices may be designed to facilitate rapid on-site HPV testing [152]. For these purposes, these ideas and approaches would be cost-effective and can be applied worldwide for HPV screening and typing. Thus, cost-effective analyses, staffing and training of lab personnel, and establishment of consensus among researchers regarding the clinical

application of molecular HPV tests in routine histological and cytological tests would be important areas of future work [153]. Bioinformatic cloud computing may be used for an enhanced level of accessibility and inter-platform reproducibility of molecular data related to HPV testing. Standardised molecular reports may be designed according to existing frameworks for histological and cytological tests used for HPV testing. The synergistic approach to molecular HPV testing may require reconciling clinical sensitivity with cost-effectiveness to standardise molecular tests across respective pathology labs in accordance with cost-benefit standards [153]. Moreover, artificial intelligence would increase the efficiency of HPV testing by improving consistency across labs, facilitating rapid reevaluation of ambiguous HPV tests for rapid decision-making regarding clinical pathways and public health surveillance of HPV diseases [153].

6. Conclusion

This review highlights the importance of both conventional and molecular diagnostic techniques for the analysis of HPV infection in histological and cytological preparations. Pap smears and histopathological tests currently play a significant role in the diagnostic process and disease screening in the initial phases. However, these methods fail to identify the virus type and the form of integration with host cells. It is obvious that molecular techniques, such as PCR, ISH, IHC, and NGS, are much more sensitive and can precisely identify the virus type and elucidate the molecular mechanisms induced by HPV infection in cancer initiation and progression. An efficient diagnostic strategy often combines these methods and has a stronger impact on diagnostic validity, disease management, and the surveillance of HPV-induced cancer cases worldwide, especially in low- and middle-income countries, creating an urgent need to implement efficient molecular diagnostics in these settings.

Author Contributions

A.B. Almutiri performed the conceptualization, methodology design, investigation, data curation, formal analysis, visualization, and project administration, and wrote the original draft. F.Z. Alotaibi contributed to the investigation, pathological evaluation, validation, and provision of resources. M.A. Alsaeed contributed to clinical consultation, validation, and interpretation in the context of infectious diseases. S.S. Sabban contributed to conceptualization, methodology guidance, supervision, and validation. M.M.M. Ahmed contributed to conceptualization, supervision, scientific guidance, provision of resources, validation, and writing – review and editing.

Funding

No external funding was received for this study.

Institutional Review Board Statement

This study did not involve human participants or animal subjects.

Informed Consent Statement

Not applicable.

Data Availability Statement

The data supporting this study are included within the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Gribb JP, Wheelock JH, Park ES. Human papilloma virus (HPV) and the current state of oropharyngeal cancer prevention and treatment. Dela J Public Health. 2023;9(1):26-28. doi:10.32481/djph.2023.04.008
2. Okunade KS. Human papillomavirus and cervical cancer. J Obstet Gynaecol. 2020;40(5):602-608. doi:10.1080/01443615.2019.1634030
3. Dom-Chima N, Ajang YA, Dom-Chima CI, Biswas-Fiss E, Aminu M, Biswas SB. Human papillomavirus spectrum of HPV-infected women in Nigeria: an analysis by next-generation sequencing and type-specific PCR. Virol J. 2023;20(1):144. doi:10.1186/s12985-023-02106-y
4. Nelson CW, Mirabello L. Human papillomavirus genomics: understanding carcinogenicity. Tumour Virus Res. 2023;15:200258. doi:10.1016/j.tvr.2023.200258
5. Pathak P, Pajai S, Kesharwani H. A Review on the Use of the HPV Vaccine in the Prevention of Cervical Cancer. Cureus. 2022;14(9):e28710. doi:10.7759/cureus.28710
6. Williams J, Kostiuk M, Biron VL. Molecular detection methods in HPV-related cancers. Front Oncol. 2022;12:864820. doi:10.3389/fonc.2022.864820
7. Lechner M, Liu J, Masterson L, Fenton TR. HPV-associated oropharyngeal cancer: epidemiology, molecular biology and clinical management. Nat Rev Clin Oncol. 2022;19(5):306-327. doi:10.1038/s41571-022-00603-7
8. Sravani AB, Ghate V, Lewis S. Human papillomavirus infection, cervical cancer and the less explored role of trace elements. Biol Trace Elem Res. 2023;201(3):1026-1050. doi:10.1007/s12011-022-03226-2
9. Valones MA, Guimarães RL, Brandão LA, de Souza PR, de Albuquerque Tavares Carvalho A, Crovela S. Principles and applications of polymerase chain reaction in medical diagnostic fields: a review. Braz J Microbiol. 2009;40(1):1-11. doi:10.1590/S1517-83822009000100001
10. Yadav K, Cree I, Field A, Vielh P, Mehrotra R. Importance of cytopathologic diagnosis in early cancer diagnosis in resource-constrained countries. JCO Glob Oncol. 2022;8:e2100337. doi:10.1200/GO.21.00337
11. Wang R, Pan W, Jin L, et al. Human papillomavirus vaccine against cervical cancer: opportunity and challenge. Cancer Lett. 2020;471:88-102. doi:10.1016/j.canlet.2019.11.039
12. Cabibi D, Giannone AG, Quattrocchi A, et al. High-Risk HPV CISH Detection in Cervical Biopsies with Weak and/or Focal p16 Immunohistochemical Positivity. Int J Mol Sci. 2024;25(10):5354. doi:10.3390/ijms25105354.
13. Rayner M, Welp A, Stoler MH, Cantrell LA. Cervical cancer screening recommendations: now and for the future. Healthcare (Basel). 2023;11(16):2273.doi:10.3390/healthcare11162273

14. Gomes M, Provaggi E, Pembe AB, Olaitan A, Gentry-Maharaj A. Advancing cervical cancer prevention equity: innovations in self-sampling and digital health technologies across healthcare settings. Diagnostics (Basel). 2025;15(9):1176.doi:10.3390/diagnostics15091176.
15. Chrysostomou AC, Kostrikis LG. Methodologies of primary HPV testing currently applied for cervical cancer screening. Life (Basel). 2020;10(11):290. doi:10.3390/life10110290
16. Pal A, Kundu R. Human papillomavirus E6 and E7: the cervical cancer hallmarks and targets for therapy. Front Microbiol. 2020;10:3116. doi:10.3389/fmicb.2019.03116
17. Baamer WO, Anfinan N, Sait M, et al. The diagnosis of cervical dysplasia in a university hospital using pap smear and colposcopy in the western region of Saudi Arabia: a correlational study. Cureus. 2022;14(3):e23242. doi:10.7759/cureus.23242
18. Eun TJ, Perkins RB. Screening for cervical cancer. Med Clin North Am. 2020;104(6):1063-1078. doi:10.1016/j.mcna.2020.08.006
19. Boon SS, Luk HY, Xiao C, Chen Z, Chan PKS. Review of the standard and advanced screening, staging systems and treatment modalities for cervical cancer. Cancers (Basel). 2022;14(12):2913. doi:10.3390/cancers14122913
20. Molina MA, Carosi Diatricch L, Castany Quintana M, Melchers WJ, Andralojc KM. Cervical cancer risk profiling: molecular biomarkers predicting the outcome of hrHPV infection. Expert Rev Mol Diagn. 2020;20(11):1099-1120. doi:10.1080/14737159.2020.1835472
21. Molina MA, Carosi Diatricch L, Castany Quintana M, Melchers WJ, Andralojc KM. Cervical cancer risk profiling: molecular biomarkers predicting the outcome of hrHPV infection. Expert Rev Mol Diagn. 2020;20(11):1099-1120. doi:10.1080/14737159.2020.1835472
22. Wang X, Huang X, Zhang Y. Involvement of human papillomaviruses in cervical cancer. Front Microbiol. 2018;9:2896. doi:10.3389/fmicb.2018.0289
23. Wright, T.C., Ronnett, B.M., Kurman, R.J. Precancerous lesions of the cervix. in: Kurman, R., Hedrick Ellenson, L., Ronnett, B. (eds) Blaustein's pathology of the female genital tract. Springer, Cham; 2019.
24. Najib FS, Hashemi M, Shiravani Z, Poordast T, Sharifi S, Askary E. Diagnostic accuracy of cervical pap smear and colposcopy in detecting premalignant and malignant lesions of cervix. Indian J Surg Oncol. 2020;11(3):453-458. doi:10.1007/s13193-020-01118-2
25. Castanon A, Landy R, Sasieni PD. Is cervical screening preventing adenocarcinoma and adenosquamous carcinoma of the cervix?. Int J Cancer. 2016;139(5):1040-1045. doi:10.1002/ijc.30152
26. Maitanmi JO, Fabiyi TE, Eniola O. Knowledge and acceptability of cervical cancer screening among female undergraduates in Babcock University Ilishan-Remo, Ogun State, Nigeria. Ecancermedicalscience. 2023;17:1502. doi:10.3332/ecancer.2023.1502
27. Borkar S, Reche A, Paul P, Deshpande A, Deshpande M. Noninvasive technique for the screening and diagnosis of oral squamous cell carcinoma. Cureus. 2023;15(9):e46300. doi:10.7759/cureus.46300
28. Najib FS, Hashemi M, Shiravani Z, Poordast T, Sharifi S, Askary E. Diagnostic accuracy of cervical pap smear and colposcopy in detecting premalignant and malignant lesions of cervix. Indian J Surg Oncol. 2020;11(3):453-458. doi:10.1007/s13193-020-01118-2
29. Jafari A, Farahani M, Abdollahpour-Alitappeh M, Manzari-Tavakoli A, Yazdani M, Rezaei-Tavirani M. Unveiling diagnostic and therapeutic strategies for cervical cancer: biomarker discovery through proteomics approaches and exploring the role of cervical cancer stem cells. Front Oncol. 2024;13:1277772. doi:10.3389/fonc.2023.1277772
30. Nategh F, Mohit M, Saatian M, Farahmand Z, Soleimanjahi H. Histopathological characteristics and HPV status in cervical biopsy specimens diagnosed as flat condyloma. Iran J Microbiol. 2023;15(3):468-474. doi:10.18502/ijm.v15i3.12909

31. Ouh YT, Kim TJ, Ju W, et al. Development and validation of artificial intelligence-based analysis software to support screening system of cervical intraepithelial neoplasia. Sci Rep. 2024;14(1):1957. doi:10.1038/s41598-024-51880-4.
32. Javaeed A, Qamar S, Ali S, Mustafa MAT, Nusrat A, Ghauri SK. Histological stains in the past, present, and future. Cureus. 2021;13(10):e18486. doi:10.7759/cureus.18486
33. Haręża DA, Wilczyński JR, Paradowska E. Human papillomaviruses as infectious agents in gynecological cancers. oncogenic properties of viral proteins. Int J Mol Sci. 2022;23(3):1818. doi:10.3390/ijms23031818
34. Singh I, Lele TP. Nuclear morphological abnormalities in cancer: a search for unifying mechanisms. Results Probl Cell Differ. 2022;70:443-467. doi:10.1007/978-3-031-06573-6_16
35. Bedard MC, Chihanga T, Carlile A. Single cell transcriptomic analysis of HPV16-infected epithelium identifies a keratinocyte subpopulation implicated in cancer. Nat Commun. 2023;14(1):1975. doi:10.1038/s41467-023-37377-0
36. Yang D, Zhang J, Cui X, Ma J, Wang C, Piao H. Risk factors associated with human papillomavirus infection, cervical cancer, and precancerous lesions in large-scale population screening. Front Microbiol. 2022;13:914516. doi:10.3389/fmicb.2022.914516
37. Neuberger J, Patel J, Caldwell H. Guidelines on the use of liver biopsy in clinical practice from the British Society of Gastroenterology, the Royal College of Radiologists and the Royal College of Pathology. Gut. 2020;69(8):1382-1403. doi:10.1136/gutjnl-2020-321299
38. Oumarou Hama H, Aboudharam G, Barbieri R, Lepidi H, Drancourt M. Immunohistochemical diagnosis of human infectious diseases: a review. Diagn Pathol. 2022;17(1):17. doi:10.1186/s13000-022-01197-5
39. Mezei T, Kolcsár M, Joó A, Gurzu S. Image analysis in histopathology and cytopathology: from early days to current perspectives. J Imaging. 2024;10(10):252. doi:10.3390/jimaging10100252
40. Sachan PL, Singh M, Patel ML, Sachan R. A study on cervical cancer screening using pap smear test and clinical correlation. Asia Pac J Oncol Nurs. 2018;5(3):337-341. doi:10.4103/apjon.apjon_15_18
41. Maiques O, Georgouli M, Sanz-Moreno V. Recent advances in tissue imaging for cancer research. F1000Res. 2019;8:F1000 Faculty Rev-1980. doi:10.12688/f1000research.19037.1
42. Kabir A, Bukar M, Nggada HA, Rann HB, Gidado A, Musa AB. Prevalence of human papillomavirus genotypes in cervical cancer in Maiduguri, Nigeria. Pan Afr Med J. 2019;33:284. doi:10.11604/pamj.2019.33.284.18338
43. Daponte N, Valasoulis G, Michail G, et al. HPV-based self-sampling in cervical cancer screening: an updated review of the current evidence in the literature. Cancers (Basel). 2023;15(6):1669. doi:10.3390/cancers15061669
44. Tota JE, Bentley J, Blake J. Introduction of molecular HPV testing as the primary technology in cervical cancer screening: Acting on evidence to change the current paradigm. Prev Med. 2017;98:5-14. doi:10.1016/j.ypmed.2016.11.029.
45. Wai KC, Strohl MP, van Zante A, Ha PK. Molecular diagnostics in human papillomavirus-related head and neck squamous cell carcinoma. Cells. 2020;9(2):500. doi:10.3390/cells9020500.
46. Altindiş M, Kahraman Kilbaş EP. Managing viral emerging infectious diseases via current and future molecular diagnostics. Diagnostics (Basel). 2023;13(8):1421. doi:10.3390/diagnostics13081421.
47. Wai KC, Strohl MP, van Zante A, Ha PK. Molecular diagnostics in human papillomavirus-related head and neck squamous cell carcinoma. Cells. 2020;9(2):500. doi:10.3390/cells9020500
48. Wongsamart R, Bhattarakasol P, Chaiwongkot A. Multiplex recombinase polymerase amplification for high-risk and low-risk type HPV detection, as potential local use in single tube. Sci Rep. 2023;13(1):829. doi:10.1038/s41598-023-28038-9

49. Chavda VP, Valu DD, Parikh PK, et al. Conventional and novel diagnostic tools for the diagnosis of emerging SARS-CoV-2 variants. Vaccines (Basel). 2023;11(2):374. doi:10.3390/vaccines11020374
50. Dias TC, Longatto-Filho A, Campanella NC. Human papillomavirus genotyping as a tool for cervical cancer prevention: from commercially available human papillomavirus DNA test to next-generation sequencing. Future Sci OA. 2020;6(9):FSO603. doi:10.2144/fsoa-2019-0159
51. Li H, Yang Y, Hong W, Huang M, Wu M, Zhao X. Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects. Signal Transduct Target Ther. 2020;5(1):1. doi:10.1038/s41392-019-0089-y
52. Pal A, Kundu R. Human papillomavirus E6 and E7: the cervical cancer hallmarks and targets for therapy. Front Microbiol. 2020;10:3116. doi:10.3389/fmicb.2019.03116
53. Yerim SA, Khazraji YC, Bekkali R. Evaluating the performance of the Xpert HPV assay in detecting HPV positive cases in Morocco. Tumour Virus Res. 2025;19:200318. doi:10.1016/j.tvr.2025.200318
54. Khan A, Pillay M, Bipath R. Evolution of testing for the diagnosis of human papillomavirus (HPV) status in head and neck squamous cell carcinoma: Where from and where to?. Oral Oncol. 2025;162:107208. doi:10.1016/j.oraloncology.2025.107208.
55. Dom-Chima N, Ajang YA, Dom-Chima CI, Biswas-Fiss E, Aminu M, Biswas SB. Human papillomavirus spectrum of HPV-infected women in Nigeria: an analysis by next-generation sequencing and type-specific PCR. Virol J. 2023;20(1):144. doi:10.1186/s12985-023-02106-y
56. Li ZF, Jia XH, Ren XY, et al. Comparison of the performance of HPV DNA chip test and HPV PCR test in cervical cancer screening in rural China. Front Microbiol. 2022;13:1040285. doi:10.3389/fmicb.2022.1040285
57. Cassedy A, Parle-McDermott A, O'Kennedy R. Virus detection: a review of the current and emerging molecular and immunological methods. Front Mol Biosci. 2021;8:637559. doi:10.3389/fmolb.2021.637559
58. Huang Z, Yao S, Zou L, et al. Evaluation of HPV infection and presence of licensed HPV vaccine genotypes among genital warts in Foshan, China. Front Microbiol. 2024;15:1376141. doi:10.3389/fmicb.2024.1376141
59. Liu HY, Hopping GC, Vaidyanathan U, Ronquillo YC, Hoopes PC, Moshirfar M. Polymerase chain reaction and its application in the diagnosis of infectious keratitis. Med Hypothesis Discov Innov Ophthalmol. 2019;8(3):152-155
60. Bulcha JT, Wang Y, Ma H, Tai PWL, Gao G. Viral vector platforms within the gene therapy landscape. Signal Transduct Target Ther. 2021;6(1):53. doi:10.1038/s41392-021-00487-6
61. Dong W, Wang H, Li M, Li P, Ji S. Virus-induced host genomic remodeling dysregulates gene expression, triggering tumorigenesis. Front Cell Infect Microbiol. 2024;14:1359766. doi:10.3389/fcimb.2024.135976
62. Zhao Z, Jiang M, He C, et al. Enhancing specific fluorescence in situ hybridization with quantum dots for single-molecule RNA imaging in formalin-fixed paraffin-embedded tumor tissues. ACS Nano. 2024;18(14):9958-9968. doi:10.1021/acsnano.3c10216
63. Valones MA, Guimarães RL, Brandão LA, de Souza PR, de Albuquerque Tavares Carvalho A, Crovela S. Principles and applications of polymerase chain reaction in medical diagnostic fields: a review. Braz J Microbiol. 2009;40(1):1-11. doi:10.1590/S1517-83822009000100001
64. Singh MP, Galhotra S, Saigal K, Kumar A, Ratho RK. Quantitative nucleic acid amplification methods and their implications in clinical virology. Int J Appl Basic Med Res. 2017;7(1):3-9. doi:10.4103/2229-516X.198498

65. Wang Z, Liu C, Liu W, et al. Long-read sequencing reveals the structural complexity of genomic integration of HPV DNA in cervical cancer cell lines. BMC Genomics. 2024;25(1):198. doi:10.1186/s12864-024-10101-y
66. Monné Rodríguez JM, Frisk AL, Kreutzer R. European society of toxicologic pathology (pathology 2.0 molecular pathology special interest group): review of in situ hybridization techniques for drug research and development. Toxicol Pathol. 2023;51(3):92-111. doi:10.1177/01926233231178282
67. Cavalleri JV, Korbacska-Kutasi O, Leblond A. European college of equine internal medicine consensus statement on equine flaviviridae infections in Europe. J Vet Intern Med. 2022;36(6):1858-1871. doi:10.1111/jvim.16581
68. Goulart LR, Colombo BFM, Lima MIS, et al. Expanded HPV genotyping by single-tube nested-multiplex PCR may explain HPV-related disease recurrence. Microorganisms. 2024;12(11):2326. doi:10.3390/microorganisms12112326
69. Andersen K, Bonde J, Waldstrøm M, et al. Evaluation of targeted next-generation sequencing for detection of HPV genotypes and sublineages in cervical liquid-based cytology SurePath samples from the Danish screening program. Int J Cancer. 2026;158(1):193-201. doi:10.1002/ijc.70148
70. Normandin E, Rudy M, Barkas N, et al. High-depth sequencing characterization of viral dynamics across tissues in fatal COVID-19 reveals compartmentalized infection. Nat Commun. 2023;14(1):574. doi:10.1038/s41467-022-34256-y
71. Zito Marino F, Ronchi A, Stilo M, et al. Multiplex HPV RNA in situ hybridization/p16 immunohistochemistry: a novel approach to detect papillomavirus in HPV-related cancers. A novel multiplex ISH/IHC assay to detect HPV. Infect Agent Cancer. 2020;15:46. doi:10.1186/s13027-020-00310-x
72. von Knebel Doeberitz M. p16INK4a/Ki67 Dual-Staining Immunocytochemistry to Refer Women Infected by High-Risk Human Papillomavirus for Colposcopy. Acta Cytol. 2025;69(1):16-25. doi:10.1159/000542504
73. Balaji H, Demers I, Wuerdemann N, et al. Causes and consequences of HPV integration in head and neck squamous cell carcinomas: state of the art. Cancers (Basel). 2021;13(16):4089. doi:10.3390/cancers13164089
74. Myers, J. E., Guidry, J. T., Scott, M. L., Zwolinska, K., Raikhy, G., Prasai, K., Bienkowska-Haba, M., Bodily, J. M., Sapp, M. J., & Scott, R. S. (2019). Detecting episomal or integrated human papillomavirus 16 DNA using an exonuclease V-qPCR-based assay. Virology (Lond). 537, 149–156. https://doi.org/10.1016/j.virol.2019.08.021
75. Blaser N, Wettstein C, Estill J, et al. Impact of viral load and the duration of primary infection on HIV transmission: systematic review and meta-analysis. AIDS. 2014;28(7):1021-1029. doi:10.1097/QAD.0000000000000135
76. Xu S, Shi C, Zhou R. Mapping the landscape of HPV integration and characterising virus and host genome interactions in HPV-positive oropharyngeal squamous cell carcinoma. Clin Transl Med. 2024;14(1):e1556. doi:10.1002/ctm2.1556.
77. Yang D, Shi Y, Tang Y, et al. Effect of HPV infection on the occurrence and development of laryngeal cancer: a review. J Cancer. 2019;10(19):4455-4462. doi:10.7150/jca.34016.
78. Lifsics A, Groma V, Cistjakovs M, Skuja S, Deksnis R, Murovska M. Identification of high-risk human papillomavirus DNA, p16, and E6/E7 oncoproteins in laryngeal and hypopharyngeal squamous cell carcinomas. Viruses. 2021;13(6):1008. doi:10.3390/v13061008
79. Kim SW, Roh J, Park CS. Immunohistochemistry for Pathologists: Protocols, Pitfalls, and Tips. J Pathol Transl Med. 2016;50(6):411-418. doi:10.4132/jptm.2016.08.08

80. Simoens C, Gheit T, Ridder R. Accuracy of high-risk HPV DNA PCR, p16(INK4a) immunohistochemistry or the combination of both to diagnose HPV-driven oropharyngeal cancer. BMC Infect Dis. 2022;22(1):676. doi:10.1186/s12879-022-07654-2
81. Mühr LSA, Guerendiain D, Cuschieri K, Sundström K. Human papillomavirus detection by whole-genome next-generation sequencing: importance of validation and quality assurance procedures. Viruses. 2021;13(7):1323. doi:10.3390/v13071323
82. Ma Z, Gharizadeh B, Cai X, et al. A comprehensive HPV-STI NGS assay for detection of 29 HPV types and 14 non-HPV sexually transmitted infections. Infect Agent Cancer. 2022;17(1):9. doi:10.1186/s13027-022-00420-8.
83. Basiletti JA, Valls J, Poklépovich T, et al. Human papillomavirus genotyping using next generation sequencing (NGS) in cervical lesions: Genotypes by histologic grade and their relative proportion in multiple infections. PLoS One. 2022;17(11):e0278117. doi:10.1371/journal.pone.0278117
84. Di Resta C, Galbiati S, Carrera P, Ferrari M. Next-generation sequencing approach for the diagnosis of human diseases: open challenges and new opportunities. EJIFCC. 2018;29(1):4-14.
85. Mühr LSA, Guerendiain D, Cuschieri K, Sundström K. Human papillomavirus detection by whole-genome next-generation sequencing: importance of validation and quality assurance procedures. Viruses. 2021;13(7):1323. doi:10.3390/v13071323
86. Satam H, Joshi K, Mangrolia U. Next-generation sequencing technology: current trends and advancements. Biology (Basel). 2023;12(7):997. doi:10.3390/biology12070997
87. Gu Y, Wan C, Qiu J, Cui Y, Jiang T, Zhuang Z. Circulating HPV cDNA in the blood as a reliable biomarker for cervical cancer: A meta-analysis. PLoS One. 2020;15(2):e0224001. doi:10.1371/journal.pone.0224001
88. Callahan SC, Kochat V, Liu Z, et al. High enhancer activity is an epigenetic feature of HPV negative atypical head and neck squamous cell carcinoma. Front Cell Dev Biol. 2022;10:936168. doi:10.3389/fcell.2022.936168
89. Van Arsdale A, Turker L, Chang YC, et al. Structure and transcription of integrated HPV DNA in vulvar carcinomas. NPJ Genom Med. 2024;9(1):35. doi:10.1038/s41525-024-00418-8
90. Sivars L, Jylhä C, Crona Guterstam Y, et al. Cell-free human papillomavirus DNA is a sensitive biomarker for prognosis and for early detection of relapse in locally advanced cervical cancer. Clin Cancer Res. 2024;30(13):2764-2771. doi:10.1158/1078-0432.CCR-23-3941.
91. Li X, Liu H, Zhou X, et al. PAX1 hypomethylation as a prognostic biomarker for radioresistance of cervical cancer. Clin Epigenetics. 2023;15(1):123. doi:10.1186/s13148-023-01538-1
92. Huang M, Wang T, Li M, Qin M, Deng S, Chen D. Evaluating PAX1 methylation for cervical cancer screening triage in non-16/18 hrHPV-positive women. BMC Cancer. 2024;24(1):913. doi:10.1186/s12885-024-12696-7
93. Naseer QA, Malik A, Zhang F, Chen S. Exploring the enigma: history, present, and future of long non-coding RNAs in cancer. Discov Oncol. 2024;15(1):214. doi:10.1007/s12672-024-01077-y.
94. Zhang L, Li C, Su X. Emerging impact of the long noncoding RNA MIR22HG on proliferation and apoptosis in multiple human cancers. J Exp Clin Cancer Res. 2020;39(1):271. doi:10.1186/s13046-020-01784-8.
95. Gattuso G, Crimi S, Lavoro A, et al. Liquid biopsy and circulating biomarkers for the diagnosis of precancerous and cancerous oral lesions. Noncoding RNA. 2022;8(4):60. doi:10.3390/ncrna8040060
96. Bittla P, Kaur S, Sojitra V, et al. Exploring circulating tumor DNA (CtDNA) and its role in early detection of cancer: a systematic review. Cureus. 2023;15(9):e45784. doi:10.7759/cureus.45784
97. Dou H, Sun G, Zhang L. CA242 as a biomarker for pancreatic cancer and other diseases. Prog Mol Biol Transl Sci. 2019;162:229-239. doi:10.1016/bs.pmbts.2018.12.007

98. Ramírez AT, Valls J, Baena A, et al. Performance of cervical cytology and HPV testing for primary cervical cancer screening in Latin America: an analysis within the ESTAMPA study. Lancet Reg Health Am. 2023;26:100593. doi:10.1016/j.lana.2023.100593
99. Swailes AL, Hossler CE, Kesterson JP. Pathway to the papanicolaou smear: the development of cervical cytology in twentieth-century america and implications in the present day. Gynecol Oncol. 2019;154(1):3-7. doi:10.1016/j.ygyno.2019.04.004
100. Koliopoulos G, Nyaga VN, Santesso N, et al. Cytology versus HPV testing for cervical cancer screening in the general population. Cochrane Database Syst Rev. 2017;8(8):CD008587. doi:10.1002/14651858.CD008587.pub2
101. Morrison LE, Lefever MR, Lewis HN, Kapadia MJ, Bauer DR. Conventional histological and cytological staining with simultaneous immunohistochemistry enabled by invisible chromogens. Lab Invest. 2022;102(5):545-553. doi:10.1038/s41374-021-00714-2
102. Westra WH. Detection of human papillomavirus (HPV) in clinical samples: evolving methods and strategies for the accurate determination of HPV status of head and neck carcinomas. Oral Oncol. 2014;50(9):771-779. doi:10.1016/j.oraloncology.2014.05.004
103. Das S, Dey MK, Devireddy R, Gartia MR. Biomarkers in cancer detection, diagnosis, and prognosis. Sensors (Basel). 2023;24(1):37. doi:10.3390/s24010037
104. Jallah JK, Anjankar A, Nankong FA. Public health approach in the elimination and control of cervical cancer: a review. Cureus. 2023;15(9):e44543. doi:10.7759/cureus.44543
105. Sachan PL, Singh M, Patel ML, Sachan R. A study on cervical cancer screening using pap smear test and clinical correlation. Asia Pac J Oncol Nurs. 2018;5(3):337-341. doi:10.4103/apjon.apjon_15_18
106. Huang PW, Ouyang H, Hsu BY. Deep-learning based breast cancer detection for cross-staining histopathology images. Heliyon. 2023;9(2):e13171. doi:10.1016/j.heliyon.2023.e13171
107. Castanon A, Landy R, Sasieni PD. Is cervical screening preventing adenocarcinoma and adenosquamous carcinoma of the cervix?. Int J Cancer. 2016;139(5):1040-1045. doi:10.1002/ijc.30152
108. Chan PK, Picconi MA, Cheung TH, Giovannelli L, Park JS. Laboratory and clinical aspects of human papillomavirus testing. Crit Rev Clin Lab Sci. 2012;49(4):117-136. doi:10.3109/10408363.2012.707174
109. 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):6347-6354. doi:10.1002/jmv.27118
110. Siqueira JD, Alves BM, Castelo Branco ABC. Comparison of four different human papillomavirus genotyping methods in cervical samples: Addressing method-specific advantages and limitations. Heliyon. 2024;10(3):e25474. doi:10.1016/j.heliyon.2024.e25474.
111. Sarwath H, Bansal D, Husain NE, Mohamed M, Sultan AA, Bedri S. Introduction of p16INK4a as a surrogate biomarker for HPV in women with invasive cervical cancer in Sudan. Infect Agent Cancer. 2017;12:50. doi:10.1186/s13027-017-0159-0
112. Salmerón J, Torres-Ibarra L, Bosch FX. HPV vaccination impact on a cervical cancer screening program: methods of the FASTER-Tlalpan Study in Mexico. Salud Publica Mex. 2016;58(2):211-219. doi:10.21149/spm.v58i2.7790.
113. Vega-Crespo B, Neira VA, Maldonado-Rengel R. Barriers and advantages of self-sampling tests, for HPV diagnosis: a qualitative field experience before implementation in a rural community in ecuador. Int J Womens Health. 2024;16:947-960. doi:10.2147/IJWH.S455118
114. Kelesidis T, Aish L, Steller MA. Human papillomavirus (HPV) detection using in situ hybridization in histologic samples: correlations with cytologic changes and polymerase chain reaction HPV detection. Am J Clin Pathol. 2011;136(1):119-127. doi:10.1309/AJCP03HUQYZMWATP

115. Vandereyken K, Sifrim A, Thienpont B, Voet T. Methods and applications for single-cell and spatial multi-omics. Nat Rev Genet. 2023;24(8):494-515. doi:10.1038/s41576-023-00580-2
116. Simoens C, Gheit T, Ridder R. Accuracy of high-risk HPV DNA PCR, p16(INK4a) immunohistochemistry or the combination of both to diagnose HPV-driven oropharyngeal cancer. BMC Infect Dis. 2022;22(1):676. doi:10.1186/s12879-022-07654-2.
117. Burd EM. Human papillomavirus laboratory testing: the changing paradigm. Clin Microbiol Rev. 2016;29(2):291-319. doi:10.1128/CMR.00013-15
118. Qin D. Next-generation sequencing and its clinical application. Cancer Biol Med. 2019;16(1):4-10. doi:10.20892/j.issn.2095-3941.2018.0055
119. Aranda Flores CE, Gomez Gutierrez G, Ortiz Leon JM, Cruz Rodriguez D, Sørbye SW. Self-collected versus clinician-collected cervical samples for the detection of HPV infections by 14-type DNA and 7-type mRNA tests. BMC Infect Dis. 2021;21(1):504. doi:10.1186/s12879-021-06189-2
120. Fashedemi O, Ozoemena OC, Peteni S, et al. Advances in human papillomavirus detection for cervical cancer screening and diagnosis: challenges of conventional methods and opportunities for emergent tools. Anal Methods. 2025;17(7):1428-1450. doi:10.1039/d4ay01921k
121. Bartosik M, Moranova L, Izadi N, et al. Advanced technologies towards improved HPV diagnostics. J Med Virol. 2024;96(2):e29409. doi:10.1002/jmv.29409
122. Gupta P, Gupta M, Koul N. Overdiagnosis and overtreatment; how to deal with too much medicine. J Family Med Prim Care. 2020;9(8):3815-3819. doi:10.4103/jfmpc.jfmpc_433_20
123. Salam MA, Al-Amin MY, Pawar JS, Akhter N, Lucy IB. Conventional methods and future trends in antimicrobial susceptibility testing. Saudi J Biol Sci. 2023;30(3):103582. doi:10.1016/j.sjbs.2023.103582
124. Dronina J, Samukaite-Bubniene U, Ramanavicius A. Advances and insights in the diagnosis of viral infections. J Nanobiotechnology. 2021;19(1):348. doi:10.1186/s12951-021-01081-2
125. Magaki S, Hojat SA, Wei B, So A, Yong WH. An introduction to the performance of immunohistochemistry. Methods Mol Biol. 2019;1897:289-298. doi:10.1007/978-1-4939-8935-5_25
126. Moat SJ, George RS, Carling RS. Use of dried blood spot specimens to monitor patients with inherited metabolic disorders. Int J Neonatal Screen. 2020;6(2):26.doi:10.3390/ijns6020026
127. Beviere M, Reissier S, Penven M, et al. The role of next-generation sequencing (NGS) in the management of tuberculosis: practical review for implementation in routine. Pathogens. 2023;12(8):978. doi:10.3390/pathogens12080978
128. Dias TC, Longatto-Filho A, Campanella NC. Human papillomavirus genotyping as a tool for cervical cancer prevention: from commercially available human papillomavirus DNA test to next-generation sequencing. Future Sci OA. 2020;6(9):FSO603. doi:10.2144/fsoa-2019-0159
129. Alizon S, Murall CL, Bravo IG. Why human papillomavirus acute infections matter. Viruses. 2017;9(10):293. doi:10.3390/v9100293
130. Demarco M, Hyun N, Carter-Pokras O, et al. A study of type-specific HPV natural history and implications for contemporary cervical cancer screening programs. EClinicalMedicine. 2020;22:100293. doi:10.1016/j.eclinm.2020.100293
131. Wang H, Sun R, Lin H, Hu WH. P16INK4A as a surrogate biomarker for human papillomavirus-associated oropharyngeal carcinoma: consideration of some aspects. Cancer Sci. 2013;104(12):1553-1559. doi:10.1111/cas.12287
132. Molina MA, Carosi Diatricch L, Castany Quintana M, Melchers WJ, Andralojc KM. Cervical cancer risk profiling: molecular biomarkers predicting the outcome of hrHPV infection. Expert Rev Mol Diagn. 2020;20(11):1099-1120. doi:10.1080/14737159.2020.1835472
133. Kamal MM. The Pap smear in inflammation and repair. Cytojournal. 2022;19:29. doi:10.25259/CMAS_03_08_2021

134. Chacko N, Ankri R. Non-invasive early-stage cancer detection: current methods and future perspectives. Clin Exp Med. 2024;25(1):17. doi:10.1007/s10238-024-01513-x
135. Paula Almeida Cunha A, Kassandra Pereira Belfort I, Pedro Belfort Mendes F. Human papillomavirus and its association with other sexually transmitted coinfection among sexually active women from the northeast of Brazil. Interdiscip Perspect Infect Dis. 2020;2020:8838317. doi:10.1155/2020/8838317
136. Oktay Gultekin E, Can B. Prevalence of candida albicans in high-risk human papillomavirus-positive women: a study in diyarbakır province, Turkey. Can J Infect Dis Med Microbiol. 2023;2023:9945561. doi:10.1155/2023/9945561
137. Hull R, Mbele M, Makhafola T, et al. Cervical cancer in low and middle-income countries. Oncol Lett. 2020;20(3):2058-2074. doi:10.3892/ol.2020.11754.
138. Dasgupta S. The efficiency of cervical pap and comparison of conventional pap smear and liquid-based cytology: a review. Cureus. 2023;15(11):e48343. doi:10.7759/cureus.48343
139. Nong L, Zhang Z, Xiong Y, et al. Comparison of next-generation sequencing and immunohistochemistry analysis for targeted therapy-related genomic status in lung cancer patients. J Thorac Dis. 2019;11(12):4992-5003. doi:10.21037/jtd.2019.12.25
140. Vega Crespo B, Neira VA, Ortíz Segarra J. Barriers and facilitators to cervical cancer screening among under-screened women in Cuenca, Ecuador: the perspectives of women and health professionals. BMC Public Health. 2022;22(1):2144. Published 2022 Nov 22. doi:10.1186/s12889-022-14601-y
141. Lawson O, Ameyan L, Tukur Z, et al. Cervical cancer screening outcomes in public health facilities in three states in Nigeria. BMC Public Health. 2023;23(1):1688. doi:10.1186/s12889-023-16539-1
142. Adedimeji A, Ajeh R, Pierz A. Challenges and opportunities associated with cervical cancer screening programs in a low income, high HIV prevalence context. BMC Womens Health. 2021;21(1):74. doi:10.1186/s12905-021-01211-w
143. Chen J, Zhang R, Xu W, et al. Knowledge and practices regarding human papillomavirus and cervical cancer screening among women in low-income areas of China: a cross-sectional study. Cureus. 2024;16(3):e55930. doi:10.7759/cureus.55930
144. Bautista-Valarezo E, Vega Crespo B, Maldonado-Rengel R, Espinosa ME, Neira VA, Verhoeven V. Knowledge and perceptions about cervical cancer and HPV screening in women in rural areas of ecuador: a qualitative research study. Int J Environ Res Public Health. 2022;19(17):11053. doi:10.3390/ijerph191711053
145. Kutz JM, Rausche P, Gheit T, Puradiredja DI, Fusco D. Barriers and facilitators of HPV vaccination in sub-saharan Africa: a systematic review. BMC Public Health. 2023;23(1):974. doi:10.1186/s12889-023-15842-1
146. Zhao XL, Hu SY, Hu JW, et al. Tackling barriers to scale up human papillomavirus vaccination in China: progress and the way forward. Infect Dis Poverty. 2023;12(1):86. doi:10.1186/s40249-023-01136-6
147. Achimaș-Cadariu T, Pașca A, Nicoară D, Dumitrașcu DL. Exploring vulnerable, ethnic minority, and low socioeconomic children's knowledge, beliefs, and attitudes regarding HPV vaccination in Romania. Healthcare (Basel). 2025;13(16):2010. doi:10.3390/healthcare13162010
148. Skof AS, Rotenberg L, Hannemann PVF, Thies S, Boschetti-Grützmacher E, Kaufmann AM. Human papillomavirus E7 and p16INK4a mRNA multiplexed quantification by a quantiGeneTM proof-of-concept assay sensitively detects infection and cervical dysplasia severity. Diagnostics (Basel). 2023;13(6):1135. doi:10.3390/diagnostics13061135.
149. Cuschieri K, Fellner MD, Arroyo Mühr LS. Quality assurance in human papillomavirus testing for primary cervical screening. Int J Gynecol Cancer. 2023;33(5):802-811. doi:10.1136/ijgc-2022-004197

150. Afolabi EK, Olaogun AAE. Assessment of the quality of cervical cancer prevention services in Southwest Nigeria: a cross-sectional study using donabedian's quality framework. Cancer Control. 2025;32:10732748251333036. doi:10.1177/10732748251333036.
151. Kroneman M, Fermin A, Rechel B. Initiatives to increase childhood vaccination coverage: an international comparison. Health Policy. 2025;158:105351. doi:10.1016/j.healthpol.2025.105351
152. Vashisht V, Vashisht A, Mondal AK, Farmaha J, Alptekin A, Singh H, Ahluwalia P, Srinivas A, Kolhe R. Genomics for emerging pathogen identification and monitoring: prospects and obstacles. BioMedInformatics. 2023; 3(4):1145-1177. https://doi.org/10.3390/biomedinformatics3040069
153. Shinge SAU, Zhang B, Zheng B, et al. Unveiling the future of infective endocarditis diagnosis: the transformative role of metagenomic next-generation sequencing in culture-negative cases. J Epidemiol Glob Health. 2025;15(1):108. doi:10.1007/s44197-025-00455-1
154. Asgedom YS, Kebede TM, Seifu BL, et al. Human papillomavirus vaccination uptake and determinant factors among adolescent schoolgirls in sub-Saharan Africa: A systematic review and meta-analysis. Hum Vaccin Immunother. 2024;20(1):2326295. doi:10.1080/21645515.2024.2326295
155. Xiang J, Sun X. Socioeconomic disparities in HPV vaccine uptake: multivariable analysis of vaccination data from Tianjin (2018-2023). Front Public Health. 2025;13:1428267. doi:10.3389/fpubh.2025.1428267.
156. Tobaiqy M, MacLure K. A systematic review of human papillomavirus vaccination challenges and strategies to enhance uptake. Vaccines (Basel). 2024;12(7):746. doi:10.3390/vaccines12070746
157. Gouton CD, Bejide IO, Ope-Ewe OO. HIV-HPV co-infection and identification of novel high-risk HPV among women at two hospital centers in cotonou, republic of Benin. Viruses. Viruses. 2025;17(5):714. doi:10.3390/v17050714

How to cite this paper:

Almutiri Abdullah Bandar, Fadhel Alotaibi, Mohammed Alsaeed, Sari S. Sabban, Mohamed Morsi M. Ahmed. Comparative Analysis of Traditional and Molecular Methods for Detecting Human Papillomavirus in Histology and Cytology Samples. *J Biol Insights*. 2025;1(1):30–59.

Arabic translation of title page

التحليل المقارن للطرق التقليدية والجزيئية للكشف عن فيروس الورم الحليمي البشري في العينات النسيجية والخلوية

عبدالله بندر المطيري[1,2]، فاضل العتيبي[3]، محمد السعيد[4,5]، ساري صبان[1]، محمد مرسي محمد أحمد[1,6,7,8]

1قسم العلوم البيولوجية، كلية العلوم، جامعة الملك عبدالعزيز، جدة، المملكة العربية السعودية.
2مدينة الأمير سلطان الطبية العسكرية، الخدمات الصحية بوزارة الدفاع، الرياض، المملكة العربية السعودية.
3قسم علم الأمراض، مستشفى قوى الأمن، الرياض، المملكة العربية السعودية.
4قسم الطب، قسم الأمراض المعدية، مدينة الأمير سلطان الطبية العسكرية، الرياض، المملكة العربية السعودية.
5كلية الطب، جامعة الفيصل، الرياض، المملكة العربية السعودية.
6مركز التميز للأميرة الدكتورة نجلاء بنت سعود آل سعود، جدة، المملكة العربية السعودية.
7مركز التميز في علم النانو الحيوي، جامعة الملك عبدالعزيز، جدة، المملكة العربية السعودية.
8قسم بحوث الأحماض النووية، معهد بحوث الهندسة الوراثية والتقنية الحيوية(GEBRI) ، مدينة الأبحاث العلمية والتطبيقات التكنولوجية، الإسكندرية، مصر.

*المؤلف المسؤول عن المراسلات | abdullah.2003@yahoo.com :mmmahmed@kau.edu.sa

الملخص

الهدف: يُعد فيروس الورم الحليمي البشري (HPV) عاملًا محوريًا في التسبب بأنواع متعددة من السرطانات، وعلى رأسها سرطان عنق الرحم، مما يستدعي تطوير منصات تشخيصية أكثر دقة وفعالية لتحسين المتابعة والعلاج. يهدف هذا البحث إلى إجراء تقييم مقارن بين الطرق التقليدية والمنصات الجزيئية المستخدمة حاليًا في الكشف عن فيروس HPV في العينات النسيجية والخلوية. **المنهجية:** في هذا السياق، تم تحديد الفجوات المرتبطة بطرق الفحص الحالية من خلال تحليل منهجي لنقاط القوة والقصور في التقنيات التقليدية، بما في ذلك الفحوصات النسيجية والخلوية ، وتقنية التهجين الموضعي (In Situ Hybridization)، وصبغة المناعة النسيجية(IHC) ، بالإضافة إلى الاختبارات المعتمدة على الأحماض النووية مثل تفاعل البلمرة المتسلسل التقليدي (PCR) وتقنية التسلسل عالي الإنتاجية (NGS) بوصفها أحدث منصات تحديد الأنماط الجينية للفيروس. **النتائج:** أظهرت الدراسة أن الطرق التقليدية لا تزال الأكثر استخدامًا عالميًا لكونها منخفضة التكلفة وسهلة التطبيق. وفي المقابل، تُعد تقنية NGS من أكثر الطرق الحديثة تميزًا وتعقيدًا، وقد أوصت بها العديد من الهيئات الطبية والصحية حول العالم، رغم ما أشارت إليه عدة دراسات من تحديات مرتبطة باستخدامها، مثل انخفاض الحساسية، وصعوبة تحسين المجسات، ومشكلات تصميم البوادئ(Primers) ، وتأثر النتائج بسلامة العينة، واحتمالات التلوث أثناء التضخيم، إضافةً إلى التباينات الناتجة عن اختلاف الأجسام المضادة المستخدمة. **الخلاصة:** ومن أجل تطوير نهج تشخيصي متكامل، تقترح الدراسة اعتماد إطار شامل وأكثر تعقيدًا عند اختيار وتطبيق طرق الكشف عن فيروس HPV بهدف دعم اتخاذ القرار السريري وتحسين النتائج العلاجية للمرضى المصابين بالأمراض المرتبطة بالفيروس. وأخيرًا، شددت الدراسة على أهمية تطوير تقنيات مدعومة بالذكاء الاصطناعي لتعزيز كفاءة وثبات كشف الفيروس، خاصةً في التعامل مع الحالات المعقدة التي تتطلب فرزًا سريعًا وقراءة دقيقة.

الكلمات المفتاحية :فيروس الورم الحليمي البشري | تفاعل البوليميراز المتسلسل | التهجين الموضعي في الموقع | الكيمياء المناعية النسيجية | تسلسل الجيل التالي