OPTIMIZATION OF HPV DETECTION AND SCREENING STRATEGIES: CURRENT TECHNOLOGIES, CHALLENGES, AND FUTURE DIRECTIONS

[1]Rochelle Lawrence, [2]Dr. Noopur Khare
[1]Scientific Officer, [2]Director
[1,2]Cancer Research and Prevention Unit,
[1,2]Cancer Patients Aid Association, Mumbai, Maharashtra, India
Corresponding author email: noopur.khare@cancer.org.in

Abstract : Human Papillomavirus (HPV) infection remains a major global public health concern due to its strong association with cervical cancer and other HPV-related malignancies. Early and accurate detection of high-risk HPV genotypes is essential for effective screening, timely intervention, and reduction in disease burden. Although several HPV detection methods, such as Pap smear cytology, Hybrid Capture assays, polymerase chain reaction (PCR), real-time PCR, and next-generation sequencing (NGS), are currently available, variations in sensitivity, specificity, turnaround time, and accessibility continue to affect the efficiency of screening programs.

Optimization of HPV screening is therefore essential to improve diagnostic performance, enhance detection of persistent high-risk infections, and reduce false negative and false positive results. Improved workflows can streamline sample processing, broaden genotype coverage, lower operational costs, and enable scalable, population-based screening, particularly in resource constrained settings. Such advances are pivotal to strengthening cervical cancer prevention and supporting global elimination efforts.

Yet substantial barriers impede optimal implementation. These include inadequate laboratory infrastructure, absence of standardized testing protocols, limited technical expertise, the high cost of molecular assays, low public awareness and stigma surrounding HPV, and practical challenges in specimen collection and storage. Moreover, HPV genetic diversity and frequent co-infections can compromise assay sensitivity and complicate result interpretation.

This paper focuses on the HPV genome and its relevance in molecular diagnostics, reviews currently available information on HPV detection methods, and emphasizes the need for optimization of screening techniques to improve clinical and public health outcomes. The paper further discusses key barriers and challenges associated with HPV detection and highlights the importance of developing sensitive, cost-effective, and accessible screening strategies for wider implementation across diverse healthcare settings.

IndexTerms **- Human papillomavirus; HPV genotyping; molecular diagnostics; screening optimization; cervical cancer prevention; resource limited settings**

1. INTRODUCTION

1.1 Background of Human Papillomavirus (HPV)

1.1.1 Genotype and Life Cycle

Human Papillomavirus (HPV) is a non-enveloped, small, double-stranded DNA virus with an icosahedral capsid which consists of 72 capsomers. The virus genome is circular and 8kb in size. The basal cell layer of squamous epithelia is a common point of entry for the virus, the infection initiates through microabrasions in the tissue.[1, 2] It is recognized as one of the most consequential viral agents in human oncogenesis. With about 448 identified types in 2023[3], HPV is categorized into the genera Alpha, Beta, Gamma, Mu, and Nu based on their genetics and tissue tropism. The Alpha genus is most relevant to mucosal infections, and the Beta genus is associated with cutaneous infections. [4,5] The Alpha genus is further classified into low- and high-risk types based on their ability to cause transformation of epithelial cells, which can progress to warts or cancer, respectively.

Each strain of the virus is associated with specific diseases. Common warts usually involve HPV 2, 4 and 7, while flat warts are linked to HPV 3 and 10, and planar warts to HPV 1, 2 and 4. Epidermodysplasia verruciformis is mainly associated with HPV 3, 10, 5 and 8. Anogenital warts are most often caused by HPV 6 and 11, while Bowenoid papulosis is linked to HPV 16 and 55. Oral lesions are commonly associated with HPV types 2, 6, 7, 11, 16, 18, and 32. The HPV types 16, 18, 31, 33, 35, 52, 56, 58, 68, 73 and 82 are highly carcinogenic and are mostly responsible for anogenital cancers and oropharyngeal carcinoma. The high-risk HPV types are responsible for nearly 5% of all human cancers, with cervical cancer being the most prevalent.[6,1]

The life cycle of this virus is closely related to epithelial cell differentiation. The virus establishes infection in basal keratinocytes, where it maintains a low copy number, and only upon differentiation does it enter the productive phase, amplifying its genome and

assembling virions.[7,3] This strategic pattern allows the virus to escape any recognition by the host's immune system, which is further enhanced by its ability to suppress innate immune signalling pathways such as cGAS-STING, which plays an important role in the detection of viral pathogens and RIG-I and MDA5 receptors, primarily through the action of the HPV's early proteins E6 and E7.[8,4] Once tumour progression of the infection is initiated, the viral oncoproteins E6 and E7 disable two key tumour suppressors, namely p53 and the retinoblastoma protein (pRb). The E6 protein binds to the E3 ubiquitin ligase E6AP, forming a complex that targets p53 for proteasomal degradation.[9] This prevents p53 from initiating cell cycle arrest or apoptosis in response to DNA damage, allowing genetically unstable cells to survive and proliferate. E6 also blocks p53's transcriptional activity and interferes with other p53-related pathways, contributing to genomic instability and transformation.[10] Meanwhile, E7 binds to Rb and promotes its degradation, releasing E2 promoter-binding (E2F) transcription factors that drive uncontrolled cell proliferation. This deregulation of the cell cycle is further compounded by E7's inhibition of p21 and p27, which normally restrain Cyclin-Dependent Kinase 2 (CDK2) activity, hence increasing its activity. The loss of Rb function also leads to compensatory over-expression of p16INK4A, a cyclin-dependent kinase inhibitor, which is often used as a surrogate marker for HPV activity. However, in HPV-infected cells, elevated p16 fails to halt proliferation because functional pRb is absent, allowing continued cell cycle progression. Together, the suppression of p53 and pRb dismantles critical cellular checkpoints, enabling HPV-infected cells to accumulate mutations, evade apoptosis, and progress towards malignancy.[11,2]

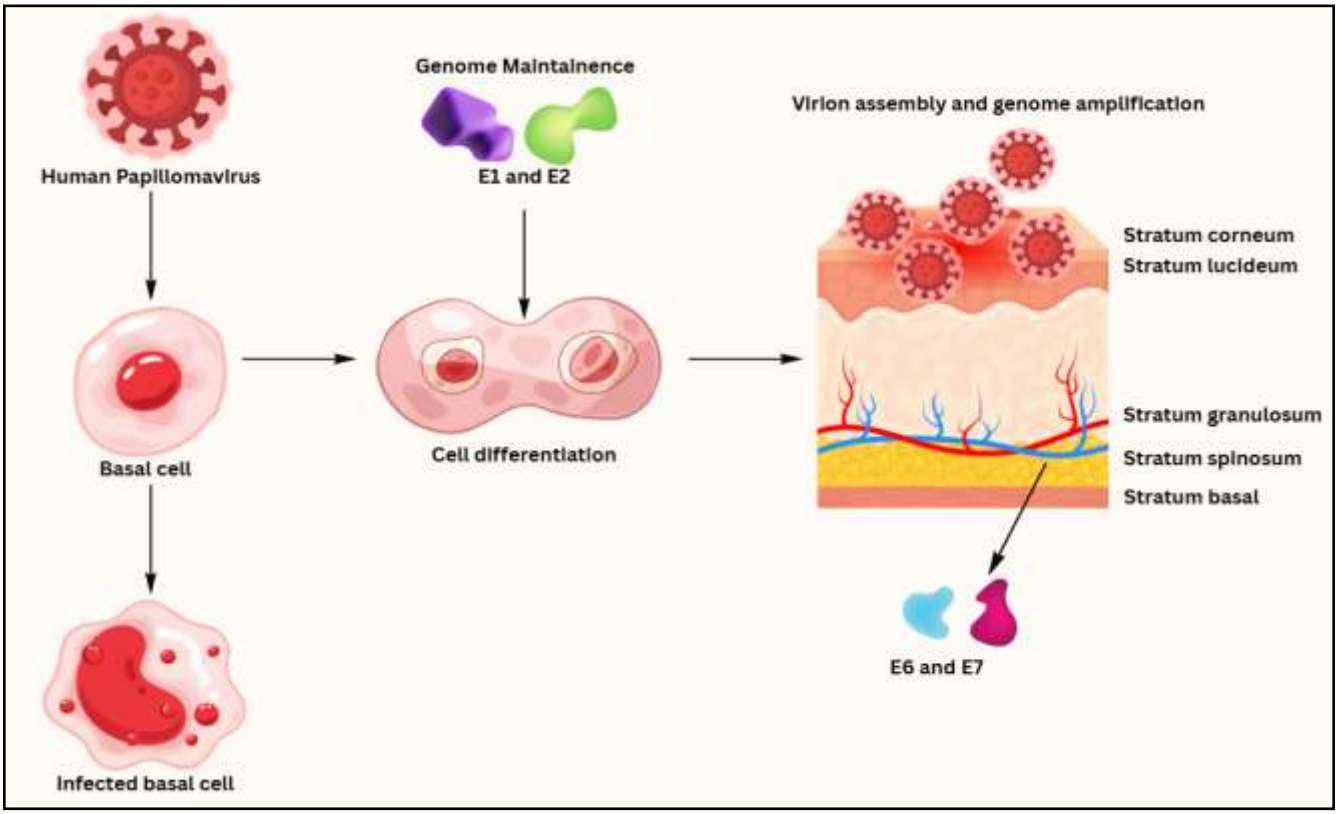

Fig 1: Initial infection of HPV and life cycle of the virus in the host cell.

Despite the high prevalence of the infection, in most cases it is transient and cleared by the host's immune system within a few years. However, persistent infection poses a significant risk for progression to precancerous lesions and invasive cancers, particularly in immunocompromised individuals.[4] Among the high-risk infection strains, HPV 16 and 18 are primarily linked to causing cervical cancer. These strains can integrate the viral genome into the host DNA, this process contributes to clonal evolution, epigenetic dysregulation, and the formation of fusion transcripts that enhance oncogene expression.[1] Genomic studies have revealed that even within a single HPV type, variant lineages and sublineages can differ in carcinogenic potential. For example, HPV 16 variants are classified into European, African, Asian and American lineages, each with distinct pathogenic profiles.[12,13] Advances in next-generation sequencing (NGS) have enabled deeper insights into intrahost diversity and viral evolution, highlighting the role of host-virus interactions and mutational processes, such as APOBEC-3-mediated editing, in shaping HPV's genomic landscape.[14,3]

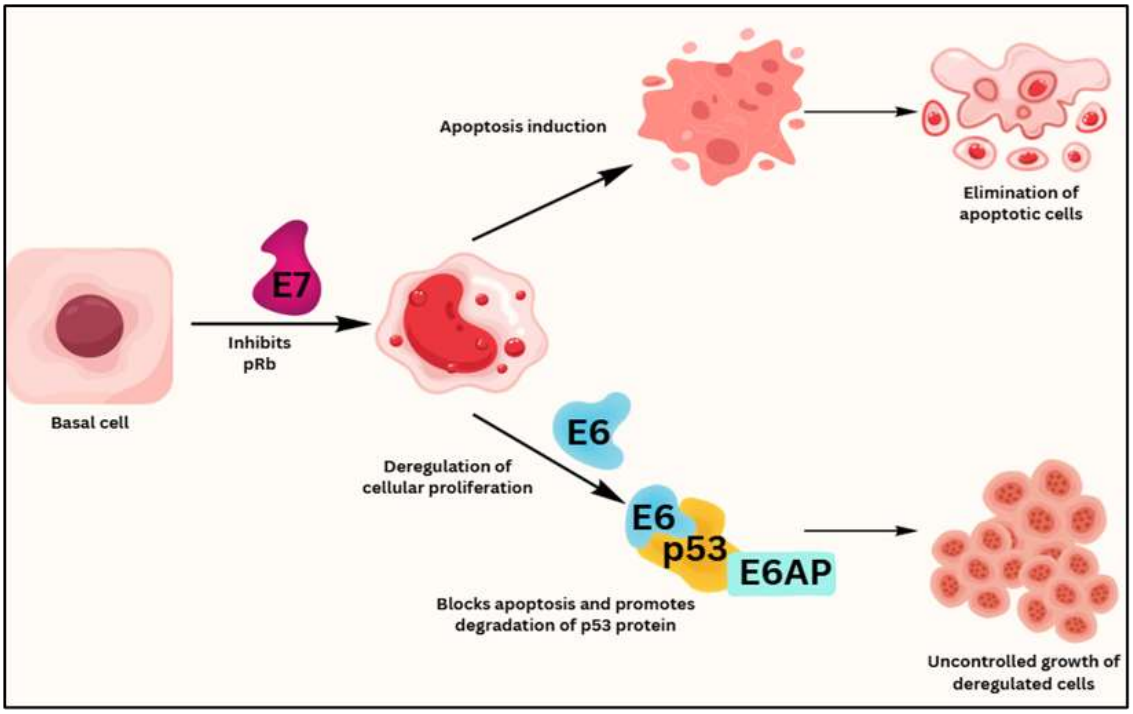

Fig 2: Mechanism action of E6 and E7 on p53 and pRb respectively.

1.1.2 Modes of Transmission

Transmission occurs primarily through close skin-to-skin contact, making individuals who engage in sexual activity particularly susceptible to the infection.[6] During the initiation of the process, virion particles permeate the basal layer of epithelial cells during the occurrence of microabrasions. The virus remains dormant for a long time once it enters the host's system. Hence, in many cases, the infection is asymptomatic and transient. [15] Apart from skin-to-skin contact transmission, there have been several studies done that reveal non-sexual means of transmission as well. Petca et al. discuss various aspects of transmission, which include horizontal transmission that occurs through routes such as fingers, mouth, fomites and non-sexual skin contact as well.[16] The virus possesses the ability to survive on various surfaces for a long period of time, as they are known to be resistant to heat and drying.[16] The spread of the infection could take place through fomites, commonly used instruments during gynecological examinations, gloves and fingernails of an infected person due to lack of hand hygiene. The author further discusses vertical transmission, in w hich the virus could infect a newborn child through amniotic fluid or contact with the genital area during birth.[16] Another crucial way of transmission is through a water transmission route. In multiple studies, it was observed that HPV was present in raw sewage waste. [17,18] In 2013, oncogenic strains of HPV were discovered in sewage sludge and multiple strains of HPV were also detected in swimming pool water.[18] This makes the waterborne route of transmission an important factor in the spread of the infection.[16-18]

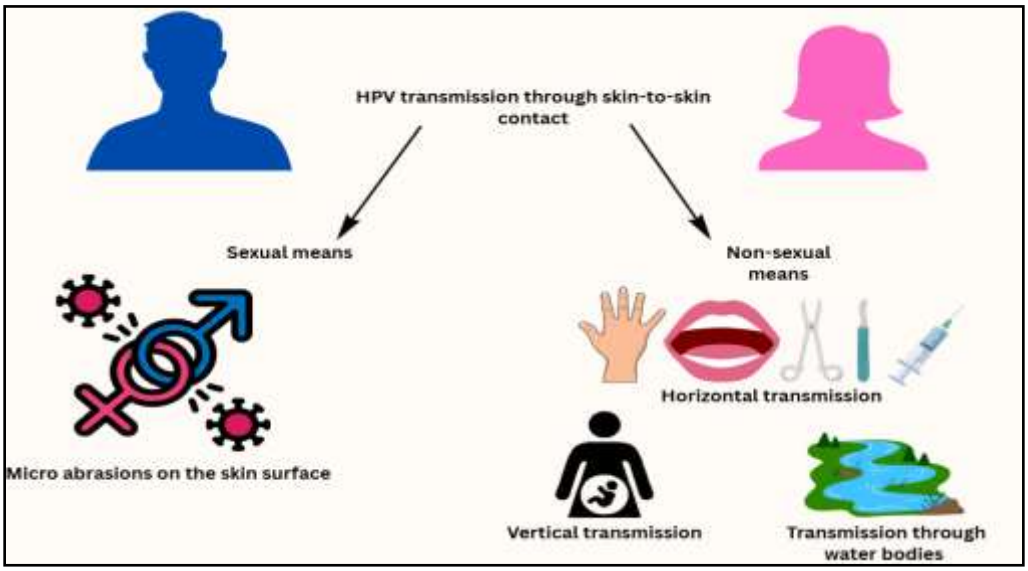

Fig 3: Multiple routes of virus transmission.

1.1.3 Global Epidemiology and Prevention of HPV infection and Cervical cancer

Persistent HPV infection is the etiological agent of cervical cancer, with nearly 95% of cases directly attributable to the virus. In 2022, cervical cancer accounted for 662,044 new cases worldwide and almost half of all HPV related cancer deaths, making it the single most important outcome of HPV infection.[19] It was observed that Africa carries the heaviest burden, mainly the sub-Saharan Africa region, where cervical cancer was the leading cause of female cancer mortality. This reflects the limited screening programs and access to vaccination for the population across the continent.[20] Cervical cancer to this date persists as a burden in regions of Latin America and the Caribbean. In a study done, it was observed that, in 2022, more than 56,000 women were diagnosed with cervical cancer, with around 28,000 deaths. The higher incidence and mortality rates in countries like this reflect limited access to healthcare. HPV prevention program coverage varies widely, leaving some nations without any national program despite regional progress towards elimination goals.[21] China and India together contribute to nearly one-third of global cervical cancer cases, yet their mortality rates differ markedly. In 2022, India recorded 79,906 deaths compared to China's 55,694, though China is projected to see a 9.5 % rise in new cases by 2050 alongside declining deaths. India, however, faces a sharper burden, with new cases expected to grow by almost 80% and deaths by 92% by 2050. The Southeast Asia region ranks second globally, accounting for nearly 30% of cases and over 34% of deaths, with India reporting 127,526 new cases in 2022.[22]

Even though the global incidence pattern of cervical cancer has significantly reduced, in several countries the incidence rates persist as they do not adhere to the World Health Organization (WHO) standard of having 4 cases in 1,00,000 people.[19,23] While developed countries like China experience an increase in the number of cases, they do have strategies which are being implemented to reduce the burden of cervical cancer. In low- and middle-income (LMIC) countries such as those in Africa, the implementation of screening and vaccination programs faces multiple barriers due to socio-economic factors.[23] To overcome such constraints, awareness of the infection must be spread through different programs and initiatives.[15] Ideal prevention strategies include screening for the virus and vaccinating the population to reduce the global burden of infection.[15,23] While vaccination programs play an important role, screening remains essential, especially in countries with high cervical cancer incidence. Vaccination alone may not achieve elimination in regions with very high infection rates; screening accelerates the detection of precancerous lesions and reduces mortality.[23]

1.1.4 Epidemiological Landscape of HPV Infection in India

While the global burden of HPV remains concerning, its impact varies across different regions. In India, it continues to pose a major public health challenge for women. Partha et al revealed that in 139 studies and over 607,000 participants, HPV infection prevalence among Indian women is variable but consistently significant.[24] In women, prevalence rates ranged from 0.4% to 40.6% in different regions across India, with high-risk types (HPV16 and 18) being the most frequently detected cases. The regional statistics of HPV prevalence among women ranged from 0–39.5% in the north, 0.4–67% in the south, 6.6–42.8% in the east, and 7.4–100% in the west. These disparities reflect uneven healthcare access and differences in testing methods across the country. HPV causes infection in the cervix, with prevalence alone reaching up to 36.8%, while oral cavity infections were reported as high as 58%. Women aged 30–39 years had prevalence rates of 7.4%-42%, while those aged 40–49 years had rates of 25.5%–33%. Infection persisted in older-aged women as well; for women over 60 years, the prevalence was around 37%, highlighting the risk of chronic or recurrent infection.[24] While the burden in women is well documented, men are highlighted mainly as carriers who sustain transmission cycles. HPV infection in and around the genital areas, prevalence in Indian males has been reported at 31%, with around 20% of infections being high-risk types.[24,25] Men also face morbidity through genital warts and head and neck cancers, but the data remain limited compared to those of women. The current situation in India demonstrates that HPV is not only a women's issue but a gender-neutral infection with disproportionate consequences for women's health.[25] Cervical cancer remains the most visible outcome, and there is an urgent need to expand awareness to HPV related malignancies. The variability in prevalence across regions and age groups highlights the urgency of standardized screening programs and wider vaccination coverage, especially targeting women in their reproductive and middle-aged years.[24,25]

1.1.5 Indian scenario of HPV infection reducing strategies

While the Indian society recognizes HPV as a global threat, the implementation of prevention strategies to eliminate the infection remains a major concern due to its limited availability to only certain states and regions of the country.[26] Current prevention strategies include vaccination drives and cytology-based screening. Despite these strategies, the burden of the infection still poses a threat to the population, as coverage remains extremely low. The reasons for this disparity include barriers such as high vaccine costs, limited access to healthcare, low public awareness, lack of physician recommendations, sociocultural constraints, inadequate infrastructure, shortage of trained providers, and hesitancy among the general population.[27] In 2020, the government of India commenced the National Strategic Plan, which included implementing guidelines aligned with WHO's global elimination targets, including a 90% HPV vaccination coverage target for girls aged 9–14 years and70% screening coverage for women aged 30–65 years.[28] Adoption of HPV molecular based testing for screening and managing treatment for 90% of the population who indicate positive results and show precancerous lesions.[28] The guidelines also emphasize partnerships between the government and private sector, social and behavioural change campaigns, and robust monitoring systems.[28] India has recognized cervical cancer elimination

as a national public health goal, so as to implement scaling up of vaccination drives, integrating molecular-based screening, and addressing barriers in the near future to reduce HPV infection and cervical cancer mortality.[26]

To prevent high-risk HPV from further progressing into cervical cancer and for risk stratification, early detection of the infection is necessary. Molecular testing techniques allow precise identification of HPV genotypes and monitoring of viral load, which is critical for assessing persistence and progression risk.[29] These tools, combined with cytological screening and vaccination strategies, form the foundation of global efforts to reduce the burden of HPV-associated diseases, which India has adopted in recent times to eliminate the infection.

1.2 The importance of early detection of Human Papillomavirus (HPV)

When detected early, the infection can be prevented from progressing to cervical malignancy, hence, it is essential to screen and receive treatment in time.[30,31] Currently, various screening methods and guidelines are used worldwide, ranging from cytology-based to molecular testing, which now serves as the primary screening technique for the WHO. It enables earlier and more accurate identification of individuals at risk.[32] Cervical cancer is one of the few cancers where early detection can almost entirely change the outcome. Unlike many malignancies that progress silently and unpredictably, cervical cancer develops slowly from HPV-driven precancerous lesions over years, offering a wide window for intervention.[31,32] Mishra et al. highlight that in India, where organized screening programs are lacking, most women are diagnosed at advanced stages when treatment options are limited, and survival rates drop sharply. Early detection through screening can identify morphological and precancerous changes that are mostly curable if treated promptly.[31]

Kakotkin et al. reveal how prevention strategies succeed or falter depending on national resources and health system design.[34] Without effective early detection, vaccination alone cannot protect women already at risk. Early detection bridges the gap between prevention and treatment, ensures that precancerous changes are caught before progression, and makes the WHO elimination strategy achievable across diverse health systems.[34] Monitoring and auditing of screening programs are ethical requirements, as early detection only fulfils its promise if abnormal results are followed up and treated.[34] Without the follow-up of screening results, cancers still develop despite screening, which undermines the preventive value. Shiraz et al explain that while HPV vaccination has reduced disease incidence in some high-income countries, screening continues to play a critical role because vaccines are not yet universally available, coverage is uneven, and herd immunity is far from guaranteed in vulnerable populations.[35] It is also effective to implement triage strategies, for example, combining HPV testing with cytology, genotyping, or emerging biomarkers such as DNA methylation and viral protein expression. By anchoring these innovations in the molecular biology of HPV pathogenesis, screening can move beyond simply detecting infection to predicting the disease life cycle.[34,35]

1.3 Current Techniques for Detecting HPV Infection

Cervical cancer screening protocols have undergone significant evolution in recent decades, driven by advances in molecular diagnostics and shifting public health priorities. The WHO continues to advocate for population-based screening programs as a major aspect of cervical cancer prevention, particularly in light of the global burden of disease, which accounted for over 342,000 deaths in 2020.[36] Nanda et al. showed in their study that cytology has long been the most common screening technique for cervical disorders, especially in countries with strong health systems.[37] It relies on examining cells from the cervix, usually through the Papanicolaou (Pap) smear test, in which samples are spread on a slide, stained, and then studied under a microscope. This method, however, depends heavily on the skill of the person preparing and reading the slides, and mistakes can occur due to poor sampling, debris, or inflammation, which often leads to false negatives.[37,38]

Liquid-based cytology (LBC) was introduced as an improvement, in which cells are placed in a liquid medium and processed into a thin layer, reducing unsatisfactory samples and allowing additional molecular tests, though its accuracy is similar to that of conventional cytology.[39] In low-income settings, visual inspection with acetic acid (VIA), visual acetic acid with Lugol's iodine (VILI) and visual inspection with acetic acid through magnification (VIAM) have been used as a simpler option. Here, the cervix is examined after applying diluted acetic acid, and changes are observed with the naked eye. Despite its limitations, VIA has helped countries with fewer resources begin building screening programs.[40] More recently, molecular based testing has become the most recommended method. This test detects the genetic material of high-risk HPV types, especially proteins such as E6 and E7 that are linked to cancer development. It is highly sensitive and reproducible, making it more accurate than cytology.[38] Women who test negative for HPV can safely wait for a long period before being screened again, which reduces unnecessary procedures.[41] Several commercial tests, such as Hybrid Capture 2, CareHPV, GeneExpert, Cervista, Abbott, and Cobas, are already in use, and many countries have adopted molecular-based testing as the main screening tool.[38]

Consequently, molecular-based screening methods have gained prominence in clinical practice. Quality assurance of the assays remains a critical concern in most countries, especially as molecular testing becomes central to primary screening.[32,36] Laboratories must adhere to rigorous internal and external quality control protocols, including participation in programs such as UK NEQAS, QCMD, and WHO HPV LabNet, which assess analytical accuracy and inter-laboratory consistency.[6] Moreover, the choice of specimen transport media, such as PreservCyt, SurePath, or STM, can influence test performance, particularly for RNA-based assays, necessitating careful validation and standardization.[6]

The clinical relevance of molecular-based screening assays will continue to be refined and optimized, while vaccination programs expand across multiple regions and become accessible to larger populations. To prevent HPV, vaccinations can be administered to

young adolescent girls aged 9-14 years, and screening should be performed for women aged 25-65 years.[41,42] The challenge lies in adapting screening protocols to maintain sensitivity while avoiding overdiagnosis, especially in low-prevalence settings.[38] This explains the need for ongoing surveillance, robust data systems, and flexible guidelines that can accommodate emerging evidence and technologies.

1.4 Challenges in HPV Screening and Follow-Up

Despite significant advances in HPV diagnostics and cervical cancer prevention, persistent challenges in screening and follow-up continue to undermine global efforts to reduce disease burden. One of the most critical issues is the disparity in access and adherence among underserved populations. In both high-income and low-resource settings, structural barriers, such as limited healthcare infrastructure, cost, and geographic isolation, impede the implementation of effective screening programs.[43,44] Even in countries with organized screening systems, such as the United States, marginalized groups, including racial minorities, rural residents, sexual and gender minorities, exhibit lower screening uptake and follow-up adherence due to systemic inequities and psychosocial barriers.[45]

Psychosocial factors play a critical role in follow-up adherence after abnormal cytology results. Low-income, inner-city women, for instance, often face knowledge deficits, emotional distress, and logistical challenges such as transportation and childcare, which collectively contribute to missed colposcopy appointments and delayed care. These barriers are compounded by limited health literacy and mistrust in the healthcare system, particularly among younger women and those with lower educational attainment. [46]

Cervical cancer screening, overall, faces broader constraints, including technological limitations in current testing procedures.[43] While molecular testing offers higher sensitivity than cytology, its specificity remains a concern, especially in distinguishing transient infections from clinically significant lesions.[47] Socio-economic factors in multiple regions all over the world, mostly LMICs, still persist, which is a major challenge that needs to be solved in order to reduce the worldwide burden of the disease.[43,46,47]

Ultimately, addressing these diverse challenges requires a combination of tailored interventions, improved diagnostic tools, and equitable healthcare delivery models. Strategies such as patient navigation, culturally sensitive education, and streamlined approaches have shown promise in enhancing follow-up adherence and reducing disparities.[44,46]

1.5 Need for optimization of screening protocols and detection pathways

The primary objective of HPV screening is to detect high-risk HPV infections before progression to cervical intraepithelial neoplasia (CIN) or invasive carcinoma.[48,50] Although screening has shifted from cytology-based methods to molecular approaches with improved sensitivity, significant limitations remain.[49] A major issue is the low specificity of HPV assays despite high sensitivity, particularly in younger women, where transient infections are common.[6,48] This results in overdiagnosis, unnecessary follow-up procedures, and increased patient anxiety.[47] To address this, current research focuses on molecular triage methods, such as mRNA testing, p16/Ki-67 dual staining, and DNA methylation analysis, to distinguish transient from clinically significant infections. [35, 50, 54, 55] These approaches show improved specificity, although challenges related to cost, standardization, and accessibility persist. Variability in assays, sample collection, and laboratory practices further contributes to inconsistent screening outcomes across settings.[49] This occurs due to differences in protocols and lack of uniform quality control. Optimization efforts therefore emphasize standardization and quality assurance, which have improved consistency in some settings but are not yet uniformly implemented, particularly in low-resource regions.[38,49] Cytology-based methods are limited because they rely on morphological changes, which may not be present in early or transformation zone lesions, reducing sensitivity. Molecular methods improve detection but remain prone to false positives and interpretive variability.[33,52]

Current strategies aim to overcome this through risk-based screening models that integrate biomarkers, HPV genotyping, and patient factors to improve accuracy. Differences in population risk profiles and healthcare infrastructure necessitate adaptable screening strategies, especially in low- and middle-income settings.[31] Resource constraints and limited access reduce screening uptake in underserved populations.[44,45] Research is addressing this through non-invasive sampling, point-of-care testing, and simplified algorithms, which improve accessibility but require further validation for large-scale implementation.[44] Finally, increasing HPV vaccination coverage is altering disease patterns, potentially reducing infection prevalence and affecting test predictive value.[6] This necessitates integration of vaccination and screening strategies and the development of risk-adapted protocols.

Overall, optimization is required to balance sensitivity and specificity, reduce unnecessary interventions, and ensure consistent and equitable screening. Current research is actively addressing these limitations; however, full effectiveness depends on improved standardization, accessibility, and integration across healthcare systems. [36,41,67,71]

2. CURRENT SCREENING APPROACHES

2.1. Cytology-based screening approaches

Cytology has been the foundation of cervical cancer screening for more than eight decades, forming the basis of one of the most successful cancer prevention strategies in medical history.[57] The origins of cytology-based screening date back to the pioneering work of George N. Papanicolaou, who developed the Pap smear in 1941, transforming the early detection of cervical cancer. His early observations in 1928 were initially met with several doubts, however, the subsequent demonstration of cytology's diagnostic potential led to broad recognition of its value.[56] The Pap test, designed to be simple, low-cost, and reproducible, enabled mass

screening and became accessible to women across diverse healthcare systems.[57] By 1945, the American Cancer Society endorsed cytological screening, marking a decisive shift in cervical cancer prevention policy.[57] The public health impact of cytology-based screening has been profound.

Countries that implemented organized screening programs observed substantial declines in cervical cancer incidence and mortality. In multiple regions globally, where population-based registries, quality assurance, and routine follow-up were integrated, mortality reductions and incidence declines were reported.[58] These successes highlight that the benefit of cytology arises not only from the test itself but from systematic implementation, including screening intervals, coverage, and follow-up protocols.[57,58]

Researchers also discovered various visual screening methods for cervical cancer, such as VIA and VILI. This uses acetic acid to highlight abnormal cells, offering immediate, low-cost results with good sensitivity but lower specificity, making training and quality control essential.[40,59] VIAM adds magnification but has not shown clear benefits over VIA and may reduce specificity. VILI, using Lugol's iodine, demonstrates higher sensitivity than both VIA and VIAM, with easier lesion recognition, though its specificity remains limited and staining delays subsequent procedures.[59]

A major technological advance occurred with the emergence of LBC in the 1990s. It was designed to address the core deficiencies of conventional smears by improving sample adequacy, producing cleaner preparations, and minimizing cell loss.[39,60] Systems such as ThinPrep® and SurePath™ suspend collected cells in preservative fluid, remove obscuring elements, and deposit a thin monolayer onto slides.[60] This standardization improves visualization and reduces the prevalence of unsatisfactory samples, one of the most consistent weaknesses of conventional cytology. Numerous studies have shown that LBC enhances sensitivity and maintains or improves specificity relative to conventional Pap smears.[60] It was also reported that aggregated sensitivities of 71.5% for conventional cytology versus 80.1% for LBC, reflecting a significant improvement in the detection of clinically meaningful lesions.[61] Large technology assessments by the U.S. Agency for Healthcare Policy and Research and the American College of Obstetricians and Gynecologists further supported the transition to LBC, citing improved detection of high-grade lesions and reductions in false negatives.[60,61]

Comparative clinical studies reinforce these advantages. Patel et al, in a study of 600 women in Maharashtra, found that LBC significantly reduced unsatisfactory smears (1.4% vs. 5% with conventional Pap smears) and provided cleaner backgrounds with fewer artifacts, facilitating interpretation.[62] Similarly, in a study, Pakaj et al reported unsatisfactory rates of 1.6% for LBC compared with 7.2% for conventional smears in a large study from Bihar. These findings reflect the consistent pattern across global research.[63] LBC provides more adequate cellular samples and fewer technically limited slides. Beyond improved adequacy, LBC also enhances detection.[62,63] Patel et al found that LBC identified slightly more epithelial abnormalities than conventional smears (11% vs. 9.7%), while maintaining comparable accuracy for high-grade lesions. Although conventional cytology occasionally identifies organisms more effectively, this does not offset the diagnostic advantages conferred by LBC.[62] In contrast, Pankaj et al demonstrated that LBC detected abnormalities in 5.3% of cases, marginally higher than conventional cytology at 5%, while HPV DNA testing detected abnormalities in 6.9%, highlighting LBC's intermediate positioning between traditional cytology and HPV-based screening.[63]

Cost-effectiveness analyses have also supported LBC. It was also shown that LBC not only improves detection but also yields optimal economic outcomes by reducing cancer incidence, mortality, and the need for invasive procedures such as hysterectomies.[61] These findings influenced updated recommendations that many national programs adopted in subsequent years. Furthermore, it emphasized that it supports automated screening platforms such as FocalPoint™ and PrepStain™, which enhance laboratory productivity and reduce interpretive workload.[60]

The integration of cytology with HPV testing further strengthened cytology's role in modern screening frameworks.[52] HPV testing offers higher sensitivity, approximately 90–100% in detecting CIN2+ lesions, but lower specificity, particularly in younger women with transient infections.[64] Cytology, therefore, remains critical for triage. For example, HPV-positive women with normal cytology require repeat testing or colposcopic evaluation depending on age, risk factors, and persistence of infection.[64,65]

At the same time, cytology plays a key role in settings where HPV testing is unavailable or impractical. In many LMICs, including parts of sub-Saharan Africa and South Asia, cytology remains the primary screening tool due to its low cost, established infrastructure, and lower technical requirements than HPV assays.[65] Even in resource-rich settings, cytology remains an essential adjunct to HPV testing, particularly in co-testing algorithms widely used in the United States. Valentine et al noted that cytology retains higher specificity than HPV testing and continues to identify some cancers, especially adenocarcinomas, that may be missed by HPV testing alone. Co-testing, which pairs the sensitivity of HPV screening with the specificity of cytology, minimizes missed disease and provides the strongest negative predictive value.[66]

Recent evaluations comparing cytology, HPV molecular testing and co-testing reinforce its continued relevance. Woo et al, analyzing a Korean cohort, found that while co-testing achieved the highest sensitivity (97.5%), LBC demonstrated the best specificity (53.5%) and required fewer follow-up tests than primary HPV screening.[67] This balance of sensitivity, specificity, and efficiency supports the ongoing role of cytology alongside HPV-based strategies. Although HPV testing is increasingly central to screening programs, cytology remains indispensable, particularly for triage, follow-up, and risk stratification.[67,68]

Importantly, successful cytology-based screening depends on more than test performance alone. Participation remains the decisive determinant of program effectiveness. While conducting a study on women in Iran, it was found that knowledge gaps, fear, embarrassment, and cultural barriers were major determinants of low uptake of Pap testing.[69] The findings observed globally identified broader patterns and strategies. High coverage, accessible services, and public education are essential to achieving the

mortality reductions observed in countries with organized programs.[58] Hence, cytology's effectiveness is as dependent on social and systemic factors as on technical performance.[66-69]

India does not yet have a nationwide cervical cancer screening program, and most testing is opportunistic.[26] To guide practice, FOGSI has adopted global recommendations to suit both well-resourced and limited-resource settings.[68] In better-equipped facilities, HPV testing is preferred as the primary method or in combination with cytology, while VIA remains an option where resources are constrained. Women who are immunocompromised are recommended to undergo screening more frequently, and HPV testing is also used to monitor treatment outcomes. This stepwise approach reflects the need to balance effectiveness with feasibility across diverse healthcare environments.[68]

Cytology-based screening has undergone substantial evolution from the original conventional Pap smear to advanced liquid-based preparations and automated systems. Although HPV testing now surpasses cytology in sensitivity, cytology remains central to global screening efforts due to its specificity, diagnostic utility, adaptability to diverse healthcare settings, and ongoing role in triage and co-testing algorithms. Its historical contributions are undeniable, and its continued integration with molecular diagnostics ensures that cytology will remain a vital component of cervical cancer prevention for the foreseeable future.

2.2 Molecular-based assays for screening of HPV

HPV molecular testing plays a central role in both the prevention and management of cervical cancer, serving purposes ranging from diagnosing infections and triaging cytological abnormalities to monitoring persistence and guiding treatment follow-up.[70,73] This method demonstrates superior sensitivity compared to cytology, enabling earlier detection of precancerous lesions and reducing false negatives.[70,73,74] Its strong negative predictive value supports extended screening intervals, optimizing healthcare resources while offering significantly greater protection against invasive cervical cancer and adenocarcinomas. It also provides reassurance for HPV-negative women, as their risk of developing cervical cancer or CIN3+ remains very low for 5–10 years, which enables more efficient follow-up care.[75] Persistent infection with high-risk HPV DNA serves as a key marker for pre-cancer, while negative results after abnormalities or treatment allow safe return to routine screening. Over time, HPV-based screening programs detect fewer new cases than cytology, emphasizing their superior effectiveness.[70,72-75]

Several trials confirmed that molecular diagnosis of HPV alone could identify more women at risk, with negative predictive values exceeding 99% for both methods. These findings suggest that molecular diagnosis provides a more reliable primary screening tool, though cytology testing still contributes valuable specificity. Together, the evidence supports a shift in screening strategies toward integrating molecular-based testing more centrally.[71]

Molecular-based assays to detect the virus are becoming central to cervical cancer screening, offering both diagnostic and prognostic insights for women at risk. Emerging DNA-based assays show promise, but many still need rigorous clinical validation.[72] Their utility depends on performance measures such as sensitivity, specificity, and negative predictive value. Hybrid Capture II (HC2) was the first FDA-approved assay for cervical screening and ASC-US triage, serving as the benchmark against which newer HPV DNA tests are often evaluated, given its sensitivity comparable to PCR methods.[72,73] In contrast, RT-PCR platforms such as the Cobas® 4800 enable automated processing and simultaneous detection of 13 high-risk HPV types, with specific identification of HPV16 and HPV18. Cobas® has undergone clinical validation and received FDA approval for diagnosis in women over 30.[70,72,73]

Understanding how the infection leads to malignancy has helped shape modern screening and prevention strategies.[74] HPV infection is extremely common, and in most people, it clears on its own. Problems arise when high-risk HPV types stay in the body for several years.[74] The virus produces proteins called E6 and E7, which interfere with two important protective proteins in our cells, p53 and retinoblastoma protein (pRb).[75] When these protective proteins are damaged, cells begin to grow uncontrollably, are not destroyed, and start accumulating genetic mutations. These early changes lay the foundation for cancer development.[75,76] As infection continues, HPV DNA may integrate into the host's genome. This integration causes the virus to produce even more E6 and E7, pushing infected cells further toward cancer.[76] This helps explain why cervical cancer develops slowly, moving from persistent infection to low-grade lesions, then to high-grade precancerous lesions, and eventually invasive cancer. Since this process takes years, early detection is extremely important.[75,76]

As a result, modern molecular tests for HPV have improved greatly. Studies comparing different PCR-based methods show that multiplex PCR is the most sensitive, detecting more HPV types and mixed infections than older primer sets such as MY09/MY11. These older methods often miss infections or detect fewer genotypes, which highlights the importance of using updated molecular techniques in screening programs.[77] As HPV testing technology advances, new assays must be evaluated using updated standards, such as second-generation comparator tests. All of them focus specifically on the 12 HPV types known to cause cervical cancer and meet strict requirements for accuracy and reproducibility. They serve as modern benchmarks for approving novel testing methods.[78,79] Most laboratory methods worldwide have been developed to improve detection and genotype identification. The Hybrid Capture 2 (HC2) assay has long been one of the most widely used tests, and early studies showed that it performs slightly better than PCR for cervical cancer screening because it is more sensitive in detecting high-grade lesions while still maintaining excellent negative predictive value, meaning it reliably rules out disease when the test is negative.[79] Later research found that the HC2 method can do more than detect infections. It can be repurposed for whole-genome sequencing of HPV by capturing viral DNA, which can then be analyzed by next-generation sequencing.[78-80]

NGS has transformed HPV genome analysis by enabling highly parallel sequencing with minimal DNA input. In HPV typing, it offers accuracy, reproducibility, and sensitivity, enabling the simultaneous detection of multiple infections.[81] By using barcoded pooled samples, NGS can process large volumes without compromising sensitivity, while also identifying previously unknown HPV types. Importantly, its bioinformatic pipeline can distinguish closely related variants and filter out chimeric sequences, ensuring reliable characterization of novel HPV genomes.[81,82] The NGS workflow for HPV detection uses barcoded primers that assign unique identifiers to each sample during PCR, enabling accurate post-sequencing analysis.[83] These barcodes enable multiplexing, allowing multiple samples to be pooled and processed in a single run. Library preparation involves adapter ligation, which supports fragment binding, enrichment, and further multiplexing across flow cells.[83,84] After sequencing, data are filtered, demultiplexed, and mapped to HPV reference genomes, with multiple assays improving accuracy across different HPV types. Overall, barcoded NGS provides high sensitivity and scalability, making it a powerful tool for clinical HPV diagnosis, prognosis, and risk stratification.[83,84]

A range of assays for HPV testing and typing are being developed, incorporating various primer designs, sequencing platforms, and bioinformatic approaches. NGS methods can detect subtle mutations, making them valuable for epidemiological research, vaccine monitoring, and tracking variants that may evade immune protection.[81-83] Importantly, differences among high-risk HPV variants, such as infectivity, persistence, and oncogenic potential, can shape the trajectory of cervical cancer progression.[81,82] Research from the past two decades makes it clear that HPV testing offers major advantages over traditional cytology. It is more sensitive, more reproducible, and capable of supporting longer screening intervals. It works well for primary screening, triage and post-treatment follow-up. With ongoing advances in mRNA testing, biomarkers, AI tools, and self-collection methods, HPV testing is now widely recognized as the central concept of modern cervical cancer prevention and a crucial tool in achieving the WHO's global goal of eliminating cervical cancer as a public health problem.[85,86]

These molecular-based assays, on their own, are sensitive and specific in detecting cervical changes. When combined with cytology, this co-testing approach achieves nearly 90% sensitivity and minimizes the 5-year risk of developing precancerous lesions. HPV DNA testing, with its high sensitivity and standardized biochemical approach, has become central to primary screening, highlighting the urgent need for better risk stratification strategies in HPV-positive women.[70,73] This approach also supports large-scale surveillance, offering valuable data for public health planning and global strategies. In doing so, it strengthens both individual patient care and population-level prevention strategies.[87]

2.3 Principle and applications of using co-testing as a screening method for HPV screening globally.

Co-testing is a cervical cancer screening strategy that integrates two diagnostic modalities, high-risk HPV DNA testing and cytological examination of cervical cells. This dual approach is designed to improve the detection of pre-cancerous lesions and early-stage cervical cancer, particularly in women aged 30 years and older.[68,88] The principle behind co-testing lies in the complementary strengths of the two methods. HPV testing offers high sensitivity for detecting oncogenic viral infections, while cytology provides morphological assessment of cellular abnormalities that may indicate disease progression.[68] The principle of co-testing is based on the natural history of cervical cancer, which is almost always preceded by persistent infection with high-risk HPV types, especially HPV 16 and 18. HPV DNA testing identifies the presence of these oncogenic viruses, often before any cytological changes are visible.[88] Cytology, on the other hand, detects cellular abnormalities, such as atypical squamous cells or high-grade lesions, that may signal progression toward cervical intraepithelial neoplasia or invasive carcinoma.[68,88] By combining these two methods, co-testing achieves greater diagnostic accuracy than either test alone. HPV testing is used first to identify people at risk, while cytology is then used to assess the severity of infection in those who test positive. This step-by-step approach helps classify risk more accurately and reduces the chances of missing a diagnosis.[89]

In clinical settings, co-testing typically involves collecting a single cervical sample using a liquid-based cytology medium. This sample is then processed for both HPV DNA testing and cytological evaluation. If both tests are negative, the woman is considered at very low risk for cervical cancer and may safely extend her screening interval to five years, as recommended by several guidelines.[68] If the HPV DNA test is positive but cytology is negative, the patient may be monitored more closely or undergo HPV genotyping to assess for high-risk strains such as HPV 16 or 18.[88] If cytology is abnormal, regardless of HPV DNA test status, then colposcopic evaluation is typically advised. This risk-based management approach helps avoid overdiagnosis while ensuring timely intervention for high-risk cases.[88,89] Co-testing is also used in follow-up protocols for women previously treated for CIN or those with prior abnormal results. Cytology helps monitor cellular changes over time, while HPV testing assesses viral persistence or clearance. This dual control is particularly valuable in post-treatment scenarios where recurrence risk is a possibility.[90]

Several factors contribute to the preference for co-testing in countries with established screening programs. Enhanced Sensitivity and Early Detection - Co-testing has been shown to detect more CIN2+ and CIN3+ cases than cytology alone. In a large U.S. study, HPV testing identified a significant proportion of women with high-grade lesions who had normal cytology, demonstrating the added value of molecular screening.[90] Cost-Effectiveness - Modelling studies have demonstrated that co-testing is economically favourable over time. Co-testing prevented more cancer cases and deaths than primary HPV testing alone, while also saving healthcare costs and increasing quality-adjusted life years (QALYs).[91] Technological Advancements - The transition from older signal-amplified assays to newer target-amplified platforms has improved test accuracy and workflow efficiency. These platforms enable partial genotyping and are compatible with automated cytology systems, facilitating large-scale implementation.[92] Preservation of Cytology Expertise - In countries with well-established cytology infrastructure, co-testing helps maintain

cytotechnologist proficiency and supports diagnostic confirmation. The importance of retaining cytology, especially in early screening rounds and in unvaccinated populations with higher disease prevalence, is often emphasized.[88] Adaptability Across Health Systems - Co-testing is flexible and can be tailored to different resource settings. In Argentina, a study was conducted that implemented a co-testing program that successfully stratified risk and guided follow-up, despite challenges with insurance coverage and patient retention.[89] In India, co-testing is gaining traction due to its balance of sensitivity and feasibility, supported by improvements in LBC and HPV DNA based assay accessibility.[68]

While co-testing remains a preferred strategy in many regions, some guidelines are shifting toward primary DNA testing as the sole screening method. This transition is based on evidence that molecular assays alone offer comparable sensitivity and may reduce unnecessary colposcopies.[92] However, successful implementation of molecular-based screening requires robust follow-up systems, provider training, and public education, which may not be uniformly available.

Table 1: Comparative Overview of HPV Screening and Diagnosis

Category	Strength	Limitations	Future potential	Examples
Cytology based screening	Cost effective, widely available, long history in screening[56]	Low sensitivity and specificity, risk of missed lesions and subjective interpretation[56,59]	Serves as a baseline screening but increasingly supported by molecular assays[88]	Pap smear, Liquid based cytology[56,59,60]
Signal amplification assays	High sensitivity, validated for mass screening and FDA approved.[79]	Cannot differentiate HPV subtypes, cross reactivity, false positives/negatives[92]	Widely used but limited in genotype specific risk stratification[92]	Hybrid capture 2, Cervista[79]
PCR based assays	High sensitivity, can genotype HPV 16 and 18; quantify viral load [70,72]	High cost and reduced specificity for low grade lesions[70,73]	Valuable for monitoring persistence and high risk infections[70,72,73]	Cobas, BD Onclarity, Abbott real time[72,73]
Next- generation sequencing	High throughput, able to detect mutations and subtypes[81]	Expensive because of it being a specialised equipment and not fully used commercially.[81]	Enables comprehensive genomic profiling and research translation.[83]	Whole genome sequencing.[80,83]

2.4. Novel approaches: current research scenarios

2.4.1 Technological Advances in HPV Infection Detection and Screening

The trajectory of HPV screening has shifted dramatically over the past decades. Initially, cytology-based methods such as the Pap smear and LBC dominated clinical practice, relying on morphological changes in cervical cells to infer disease risk.[93] While these approaches were cost-effective and widely implemented, their sensitivity was limited, leading to missed precancerous lesions and delayed intervention.[94] The integration of molecular diagnostics marked a turning point, with assays targeting HPV DNA and RNA offering far greater accuracy and enabling earlier detection of high-risk infections. This transition has been pivotal in reducing cervical cancer incidence and mortality, particularly when combined with vaccination programs.[93,94]

The rationale for advancing next-generation screening tools lies in overcoming barriers while expanding accessibility. Emerging technologies such as droplet digital PCR (ddPCR), NGS, CRISPR/Cas-based diagnostics, and isothermal amplification techniques (IAT) promise enhanced sensitivity, rapid turnaround, and scalability.[95] These innovations not only improve analytical performance but also open the door to point-of-care testing, reducing dependence on centralized laboratories.[94,95] Parallel advances in sampling strategies, such as urine-based assays and liquid biopsies using extracellular vesicles, are reshaping screening into a more patient-centred process. By combining molecular precision with non-invasive sampling, these approaches aim to broaden coverage, improve compliance, and ultimately reduce the global burden of HPV-related cancers.[95]

HPV diagnostics have evolved from conventional cytology and PCR-based assays toward emergent molecular and biosensing technologies. Bartosik et al emphasize the transition from commercial PCR-based assays to advanced isothermal amplification techniques, CRISPR-Cas systems, and lab-on-a-chip devices, highlighting their potential for rapid, low-cost, and decentralized testing. These innovations are particularly suited for point-of-care applications, where sensitivity and specificity must be balanced with affordability and ease of use.[96] Their review underscores how multiplex assays and genotype-specific detection are reshaping HPV diagnostics by enabling broader coverage and more precise risk stratification.[96] Fashedemi et al, on the other hand, situate these advances within the global public health context, noting the limitations of conventional methods such as Pap smears and standard PCR in resource-limited settings.[95] They highlight the promise of droplet digital PCR (ddPCR), DNA microarrays, and electrochemical biosensors, which not only improve sensitivity and reproducibility but also reduce reliance on highly specialized

infrastructure.[97] Importantly, their review emphasizes the role of point-of-care molecular diagnostics in achieving the WHO's 90-70-90 targets, particularly in LMICs where cervical cancer burden is highest.[96,97]

These perspectives illustrate a dual trajectory in HPV diagnostics. High-throughput multiplex assays are advancing laboratory-based screening by enabling simultaneous detection of dozens of genotypes with greater efficiency, while genotype-specific and biosensor-driven tools are expanding access to sensitive, low-cost diagnostics in decentralized settings, bridging the gap between innovation and equity. This synthesis shows that the field of study is moving beyond detection toward precision molecular profiling and scalable implementation, aligning technological innovation with global health priorities. The convergence of these approaches, automation, multiplexing, and point-of-care biosensing, marks a paradigm shift in HPV diagnostics, offering both clinical precision and public health impact.

Table 2: Current scenario of research to optimize HPV diagnosis

Technique	Principle	Advantages	Uses
Droplet Digital PCR (ddPCR)	Absolute quantification of viral load; superior sensitivity; detects co-infections; reproducible[95]	Labor-intensive; requires specialized equipment; not yet widely available in LMICs[95]	Research and advanced diagnostics; promising for genotype-specific monitoring[95]
Isothermal Amplification Techniques (LAMP, RPA)	Rapid amplification (20–30 min); constant temperature; tolerant to inhibitors; suitable for crude lysates[95]	Risk of contamination; primer design complexity; limited large-scale validation [95]	Point-of-care testing in decentralized or resource-limited settings [95]
CRISPR-Cas based assays	Ultra-specific detection; adaptable to multiplexing; potential for portable biosensing[96,99]	Still experimental; requires optimization for clinical workflows [96,99]	Next generation biosensing; potential for rapid triage and precision diagnostics[96]
DNA Microarrays	High-throughput genotyping; simultaneous analysis of thousands of sequences; integration with microRNA profiling [95, 97]	Signal instability (ozone effect); requires specialized scanners; cost-intensive[97]	Comprehensive molecular profiling; triage for HPV-positive women[97]
Electrochemical Biosensors	Low-cost and portable; rapid readout; high specificity; adaptable to multiplex formats[97]	Early-stage development; requires validation in large cohorts[97]	Decentralized screening; strong candidate for LMIC implementation[95,97]
Spectroscopy-based tools (FTIR, Raman, SERS)	Non-invasive biochemical profiling; rapid and accurate; suitable for automation[95]	Limited clinical validation; requires advanced instrumentation[95]	Potential adjunct diagnostics; early detection of molecular changes[95]

2.4.2 Paper-based hybrid capture assay: novel technique

Smith et al. describe a low-cost, paper-based assay for detecting high-risk HPV DNA at the point of care, particularly in settings with limited lab infrastructure. While HPV DNA testing is the most sensitive screening method, existing commercial options are expensive, require batch processing, and require trained staff. To overcome these barriers, the team developed a two-dimensional paper network (2DPN) that combines sample preparation and detection into a simple workflow, producing results in under an hour for less than $3 per test. The assay uses RNA probes to capture HPV DNA, followed by antibody-driven signal amplification on a paper strip. Lyophilized reagents allow storage without refrigeration, and only a basic heater is needed. The seven-step process from swab collection to visual readout was designed for use by minimally trained workers. In lab testing using provider-collected samples from El Salvador and Mozambique, the assay demonstrated strong accuracy relative to the careHPV standard in El Salvador. However, field trials in Mozambique with self-collected samples revealed reduced specificity, likely due to higher cell content, leading to false positives.[98]

Healthcare workers in both countries found the test easy to use after brief training. The authors highlight the need for protocol refinements, especially for dense cell samples, and the inclusion of internal controls. Overall, this innovation offers a promising, affordable tool to expand cervical cancer screening in underserved regions.

2.4.3. Detection assay based on CRISPR/Cas12a technology

Liu et al. present a multiplex point-of-care platform that combines recombinase polymerase amplification (RPA) with CRISPR/Cas12a to detect and type high-risk HPV. Conventional assays often rely on complex equipment and long processing

times, limiting their use in low-resource settings.[99] The new system, called H-MRC12a, integrates isothermal amplification with Cas12a's collateral cleavage activity, enabling rapid, sensitive detection without thermal cycling. Unlike tests that only confirm HPV presence, this assay can distinguish multiple genotypes in a single reaction, which is a key advantage for guiding treatment and vaccination strategies.[99] Validation showed high accuracy with minimal cross-reactivity, comparable to PCR but with faster turnaround. Its portability and simple visual readout make it well-suited for field use. By lowering cost and complexity, H-MRC12a offers a practical tool for cervical cancer screening programs, supporting early intervention and equitable access in underserved regions.[99]

2.4.4. Detection of the infection based on Methylation markers

Recent advances in cervical cancer screening have focused on DNA methylation as a triage tool to improve accuracy and reduce unnecessary interventions. While HPV DNA testing is highly sensitive, it has limited specificity. This often leads to excessive colposcopy referrals and overtreatment, particularly in low-resource settings.[55] Methylation analysis of host and viral genes offers a more refined approach. It distinguishes transient infections from those with malignant potential. Zhang et al. detail the molecular mechanisms by which persistent high-risk HPV infections drive carcinogenesis through epigenetic changes.[55] These involve hypermethylation of tumor suppressor genes and viral CpG sites. They highlight several promising biomarkers, including CADM1, MAL, FAM19A4, and miR-124. These biomarkers demonstrate strong associations with lesion severity and progression risk. Panels combining multiple methylation markers consistently outperform single-gene assays. They offer higher specificity and sensitivity for the detection of CIN2+ and CIN3+.[55] Importantly, these markers are lesion-specific and adaptable to self-sampling, making them particularly valuable in low- and middle-income countries. Burdier et al. further developed this framework by presenting consensus from an international expert meeting. They review the performance of established assays, including QIAsure (FAM19A4/miR124-2), GynTect (six host genes), and the S5 classifier (EPB41L3 plus HPV L1 genes). All these show robust sensitivity for CIN3+, though specificity varies. Their report underscores the heterogeneity of CIN2/3 lesions.[100] Methylation-positive cases are more likely to progress, while methylation-negative lesions often regress spontaneously.[100] This predictive capacity positions methylation testing as a powerful triage tool. It can guide clinical management and reduce overtreatment.[55,100]

2.4.5. HPV mRNA Testing as a Marker of Oncogenic Activity

Initial research on HPV infection has tested mRNA testing for its potential to detect E6/E7 oncogene transcripts, which are directly associated with malignant transformation. Benevolo et al demonstrated that the PreTect HPV-Proofer assay offered higher specificity than DNA testing, particularly in triage settings for ASC-US and L-SIL cytology.[101] Although sensitivity was lower, the assay reduced unnecessary colposcopies and improved the positive predictive value, highlighting its clinical utility in distinguishing transient infections from those with oncogenic activity.[101] Further, Burger et al conducted a systematic review comparing PreTect Proofer/EasyQ and APTIMA assays.[102] Their findings reinforced the specificity advantage of mRNA testing, with PreTect Proofer showing specificity up to 97% but lower sensitivity, while APTIMA demonstrated higher sensitivity (90–95%) but lower specificity.[102] Importantly, both assays consistently yielded fewer false positives than DNA tests, suggesting that mRNA detection could refine triage strategies by identifying clinically relevant lesions more accurately.[101,102] Regional studies further validated these observations. Wang et al, in a Chinese cohort, reported that while DNA assays detected a higher prevalence of HPV (79.9%), mRNA assays had superior specificity (88.6% vs. 48.1% in normal cytology).[103] Genotype distribution analysis confirmed HPV-16 as the dominant type, followed by HPV-53, HPV-33, HPV-58, and HPV-18, underscoring the importance of mRNA testing in identifying high-risk genotypes associated with progression.[103] Their findings suggested that mRNA assays could serve as a sensitive and specific tool for screening, while also informing vaccine development by highlighting prevalent oncogenic types beyond HPV-16 and 18.[101,102,103]

Most recently, Arbyn et al provided a large-scale systematic review and meta-analysis, focusing primarily on the APTIMA assay. They confirmed that mRNA testing had comparable sensitivity to DNA assays (relative sensitivity ~0.98) but slightly higher specificity (relative specificity ~1.03). Longitudinal data over 4–7 years showed no significant difference in safety outcomes between mRNA and DNA testing, supporting the use of APTIMA in primary screening programs with 5-year intervals. However, they noted reduced sensitivity in self-collected samples, limiting their applicability in self-sampling strategies. This meta-analysis consolidated earlier findings, positioning mRNA testing as a validated alternative to DNA assays in clinician-collected screening contexts.[104] Taken together, these studies illustrate the evolution of evidence. From early triage-focused evaluations, through systematic reviews highlighting specificity and predictive value, to regional genotype distribution studies, and finally to global meta-analyses confirming non-inferior accuracy and safety.[101,102,103,104] The phenomenon across this trajectory is that HPV mRNA assays provide a biologically meaningful marker of oncogenic activity, improving specificity and predictive value while maintaining comparable sensitivity to DNA-based methods.[101,102,103,104]

2.4.6. Evolution of Evidence on Urine-Based HPV Testing

Early work on urine HPV-DNA detection emphasized its potential as a non-invasive alternative but highlighted significant limitations. Viral loads in urine were consistently lower than those in cervical scrapes, sometimes up to 50-fold lower, and PCR inhibitors further reduced sensitivity, leading to poorer detection of high-grade lesions compared to clinician-collected cervical

samples.[105-108] Despite these challenges, concordance for invasive cancers was reported as high as 79–100%, suggesting urine testing could still capture clinically significant disease under certain conditions.[105,106]

A systematic review and meta-analysis provided quantitative validation of urine testing, pooling data from 14 studies and over 1,400 women. Sensitivity for detecting any HPV was 87% and specificity 94%, while detection of high-risk HPV achieved 77% sensitivity and 88% specificity.[106] Importantly, accuracy was significantly higher when first-void urine was used, underscoring the need for standardized collection protocols. Specificity for HPV16/18 reached 98%, reducing false positives and unnecessary follow-up procedures.[106] Clinical studies in Asian populations further demonstrated urine's utility. In Korea, paired vaginal discharge and urine samples from 203 women showed positivity rates of 17.2% and 15.8%, respectively, with an overall concordance of 84.8%.[107] These findings confirmed urine's validity as a non-invasive medium, particularly for younger women who often avoid gynaecological visits due to discomfort, and highlighted the prevalence of high-risk genotypes such as HPV16, 52, and 58 in the region.[107] In the UK, a cross-sectional study optimized urine processing by demonstrating that preservative-fixed samples maintained DNA stability for up to one month.[108] Concordance between urine and cervical swab samples was substantial (82–92%), and sensitivity for CIN2+ detection was 83–88%, only modestly lower than cervical and vaginal swab samples (~89%). Acceptability surveys revealed women preferred urine sampling over vaginal or cervical methods, citing greater confidence and comfort, reinforcing urine's potential to improve uptake among non-attenders.[108]

Subsequent reviews highlighted improvements based on biomarker use, advances such as methylation markers and nanowire assays improved sensitivity and concordance, while meta-analyses confirmed PCR-based urine assays approached parity with clinician-collected samples.[108.109] Signal amplification and mRNA assays, however, remained less reliable, clarifying that assay type is the critical determinant of accuracy. These developments positioned urine testing as a feasible adjunct with robust diagnostic performance when optimized protocols are applied.[109]

Most recently, an updated meta-analysis incorporating 21 studies and over 11,000 women confirmed that the overall sensitivity of urine testing was lower than clinician-collected samples (ratio 0.84, 95% CI 0.78–0.91). Yet PCR-based assays such as GP5+/6+ and SPF10 achieved near-equivalent sensitivity, demonstrating that with standardized protocols, urine testing can match cervical sampling for CIN2+ detection.[110] Specificity remained high across both sample types, reinforcing urine's reliability in ruling out disease. The authors concluded that standardized PCR-based assays and validated collection devices and media are essential for urine testing to serve as a viable alternative or complement to clinician sampling, particularly to expand coverage in underserved populations.[110]

Novel HPV screening approaches, such as optimisation in molecular assays and advanced triage algorithms, are steadily reshaping the diagnostic landscape. These innovations promise greater sensitivity and specificity, while also broadening the potential for equitable public health outcomes. Their impact extends beyond laboratory performance, influencing how populations engage with preventive care and how systems allocate resources. Yet, as promising as these developments appear, their translation into routine practice requires careful consideration of feasibility, sustainability, and integration. In this way, the focus gradually shifts from anticipation of discovery to the practical realities and optimization of screening strategies across diverse health contexts.

3. OPTIMISATION OF STRATEGIES IN SCREENING: ADDRESSING KEY CHALLENGES

Optimizing cervical cancer screening protocols globally requires aligning technological advances with system-level strategies that ensure accessibility, accuracy, and sustainability. In high-income settings, HPV screening benefits from advanced triage tools like E6/E7 mRNA and p16 biomarkers, supported by digital systems and extended screening intervals.[111] To make these protocols accessible in low-resource settings, validated HPV DNA tests should be paired with simplified triage methods and community-based follow-up strategies.[112] Integrating education and mobile health tools can further support scalable screening programs.[111,112,113] Streamlining algorithms, enabling sample collection, and quality assurance are the keys to improving reach and equity in cervical cancer prevention.

3.1. Role of Quality assurance in optimization of screening

The evolution of quality assurance (QA) in cervical cancer screening reflects a progressive shift from cytology-based methods to molecular HPV assays and, more recently, NGS, each stage requiring stronger measures to ensure results are accurate, consistent, reproducible, and patient-safe.

Pap smear cytology, introduced in the mid-20th century, was foundational in reducing cervical cancer incidence but was limited by false positives and negatives.[157] To address these shortcomings, quality control guidelines, such as the Bethesda System for Reporting Cervical Cytology, were developed, alongside indicators such as ASCUS/SIL (atypical squamous cells of undetermined significance / squamous intraepithelial lesion) ratios, cytology-histology concordance, and positivity rates.[158] Modern approaches further incorporated Six Sigma metrics and Lean principles to minimize variability and streamline workflows, embedding continuous training and audits to maintain diagnostic precision.[114] These innovations positioned cytology quality assurance as a foundation of early detection, but the transition to molecular testing required a broader, more dynamic framework.

HPV molecular assays introduced higher sensitivity and longer negative predictive intervals, but their reliability hinged on rigorous validation and verification. Internationally recognized criteria, such as those proposed by Meijer and expanded through validation of HPV GENotyping tests (VALGENT), established benchmarks for sensitivity, specificity, and reproducibility.[159] Laboratories were tasked not only with adopting clinically validated assays but also with embedding ongoing verification, internal quality control

(IQC), and external quality assessment (EQA) schemes to sustain reproducibility across diverse settings.[115] Carozzi et al emphasized that quality assurance must advance beyond simple validation, becoming a sustained assurance system across the workflow. This requires centralized infrastructure, specialized personnel and alignment of operational practices with external regulatory standards. This scenario envisioned laboratories as hubs of longitudinal monitoring, where reproducibility checks and proficiency schemes converge to anticipate the demands of large-scale screening.[49] Cuschieri et al further highlighted the multi-level nature of quality assurance, shaped by international validation criteria, national program oversight, and local laboratory practices, ensuring consistency and patient safety across contexts.[116]

The emergence of NGS represents the next frontier in quality control. NGS enables novel insights into HPV genomics, including detection of variants, integration sites, and persistence markers, but its complexity demands rigorous validation at every step from sample preparation and nucleic acid extraction to enrichment, sequencing, and bioinformatics.[81,160,161] Mühr et al stressed that without standardised QA protocols, results may be inconsistent or clinically unreliable.[117] Embedding pilots, internal controls, proficiency testing, and inter-laboratory comparisons into NGS workflows positions this technology as a robust complement to existing HPV screening, enabling precision diagnostics while maintaining patient outcomes. [116, 117] A critical advancement in quality control has been the recognition of sample adequacy as a determinant of test accuracy. Unlike cytology, molecular-based assays lacked standardized cellularity thresholds, yet studies demonstrated that low nucleated cell counts, reflected by late β-globin amplification, significantly reduce HPV positivity rates and reduce the risk of false negatives.[118, 162, 163] d'Avenia et al proposed integrating quantitative cellularity assessment alongside internal controls to strengthen confidence in negative results, envisioning future frameworks in which adequacy thresholds are standardized, collection practices harmonized, and atrophic samples in older women are carefully managed.[118] This shift embedded sample quality as the basis of HPV prevention, ensuring that negative results truly reflect the absence of disease rather than inadequate sampling.

Collaborative quality assurance models have also emerged as vital to sustaining program credibility. Edvardsson and Dillner outlined the importance of audits of HPV-negative samples before high-grade squamous intraepithelial lesion (HSIL) or cancer, combined with blinded proficiency panels, to verify screening effectiveness.[119] Extended genotyping, biobanking, and registry linkages were identified as critical tools for monitoring long-term outcomes and detecting emerging strains of the virus.[123,164,165] Multidisciplinary collaboration- cytologists, pathologists, and experts jointly interpreting results was emphasized as a means to strengthen communication and ensure accurate follow-up. Such networked systems of audits and registries safeguard reproducibility and comparability across laboratories and countries.[119,166,167] Country-specific implementation models further illustrate the adaptability of guidelines. In India, where resource variability and population scale present unique challenges, HPV-based screening must be strengthened through structured strategies and measurable outcomes.120 The emphasis lies on validated assays, standardized sample collection, and integration of internal and external quality programs, while adapting to regional disparities.[168,169] Collaborative models linking laboratories, registries, and public health authorities are proposed to ensure reproducibility, equitable access, and sustainability, positioning quality assurance as the backbone of national cervical cancer elimination strategies.[119,120]

3.2. Enhancing screening: optimization of test methods, follow up and triage strategies.

Cervical cancer screening requires a broad approach that integrates advances in diagnostic technology with evidence-based follow-up and triage strategies to ensure both effectiveness and efficiency across diverse healthcare settings.

Conventional methods such as Pap smears, visual inspection, and standard PCR have historically reduced disease burden but remain limited by low sensitivity, specificity, and reproducibility, particularly in low-resource environments.[170] To overcome these shortcomings, emergent tools such as droplet digital PCR, DNA microarrays, and biomarker assays targeting E6/E7 oncogenes have been proposed, alongside electrochemical detection platforms and point-of-care devices that promise rapid, sensitive, and cost-effective screening adaptable to LMICs. These innovations are positioned to help programs meet the WHO's 90-70-90 elimination targets by expanding access and reducing overtreatment.[97]

Long-term randomized evidence from Sweden confirms the durability of protection offered by HPV screening, showing that HPV-negative women maintain consistently lower risks of CIN2+ and CIN3+ over 13 years compared to cytology-negative women. These findings demonstrate that HPV's higher sensitivity reflects earlier detection rather than overdiagnosis, supporting the extension of screening intervals to five years or more and reinforcing HPV testing as the preferred primary tool. Double testing with cytology provides only a limited benefit and does not strengthen the case for streamlined HPV-based protocols that improve efficiency and reduce unnecessary procedures.[121] Complementary evidence from the large U.S. IMPACT trial provides benchmarks for genotype-specific risk stratification, showing markedly elevated cumulative risks of CIN3+ among HPV16-positive women compared to those testing negative, while HPV negative women had extremely low risk. These results validate high-throughput molecular assays as safe and effective for routine screening and highlight the importance of genotype-specific triage in guiding follow-up intensity.[122]

Evolving triage strategies for HPV positive women emphasize the limitations of Pap cytology and the promise of alternatives. Current standards rely on HPV16/18 genotyping with reflex cytology for other high-risk types, but newer approaches, such as p16/Ki-67 Dual Stain, extended genotyping, and methylation assays, offer improved sensitivity and specificity in many settings.[171] Dual Stain has shown superior performance in large cohorts, though cost and complexity remain barriers, while methylation testing and detailed genotyping provide molecular pathways that may streamline triage, particularly in vaccinated populations and self-collection contexts. These innovations underscore the need to balance sensitivity, specificity, and resource use as HPV screening

becomes the global standard.[123, 172] Large, randomized trials demonstrate that DNA-based assays enable extended screening intervals and later initiation ages without compromising safety, while triage methods, such as cytology or molecular assays, refine specificity and guide referrals.[173]

Next-generation molecular approaches, such as advanced genotyping, further enhance participation among women who are less likely to attend routine programs, highlighting the importance of tailoring follow-up protocols to risk profiles and embedding them within national guidelines.[124] Survivorship care adds another dimension to optimization, as follow-up after cervical cancer treatment must balance recurrence monitoring with psychosocial needs. Risk-stratified models recommend structured HPV based surveillance for early-stage survivors and symptom-driven imaging with MRI or PET/CT scans for advanced disease, while Patient-Initiated Follow-Up (PIFU) offers a promising pathway for low-risk groups by reducing unnecessary clinic visits.[125] However, gaps in psychosocial and psychosexual support remain, highlighting the need for standardized protocols that integrate mental health care and sexual health counselling to ensure valuable outcomes of survival.[125]

Post-treatment strategies further illustrate the optimization of follow-up, with HPV assays outperforming cytology in detecting residual or recurrent CIN2+. Negative HPV results provide strong reassurance and allow less intensive monitoring, while persistent positivity, especially type-specific infections such as HPV16, signals higher recurrence risk.[47,125] Although co-testing marginally increases sensitivity, it reduces specificity and adds complexity, reinforcing the need for streamlined protocols. Global disparities in assay choice, timing, and intervals underscore the importance of further research into genotyping, mRNA assays, and lesion management to refine post-treatment surveillance.[47,125]

Optimization of screening is achieved through a multi-layered strategy, adopting HPV testing as the primary modality, extending intervals for women who test negative, employing genotype and biomarker-based triage for positives, and integrating risk-stratified follow-up in both post-treatment and survivorship care. By combining technological innovation with tailored protocols, programs can reduce unnecessary interventions, expand access, and ensure that screening translates into meaningful cancer prevention across diverse populations.

3.3 Population based screening: optimization of strategies and accessibility

Evidence from population-based studies demonstrates that HPV screening efficiency varies substantially by genotype, underscoring the importance of adapting follow-up strategies to the real-world prevalence and oncogenic potential of each genotype. For instance, Swedish data reveal that HPV16 and HPV18 yield far greater preventive impact compared to lower-risk types, which demand disproportionately high resources, especially in younger women. Universal follow-up of all HPV-positive results risks overwhelming health systems with false positives, whereas genotype-specific approaches allow a rational allocation of care, ensuring that high-risk groups are prioritized without straining resources.[126]

The challenge of ensuring equal access to screening procedures globally makes optimization difficult, as strategies must adapt to varied healthcare systems and diverse population needs. While DNA testing is more sensitive and reliable than cytology or VIA, access remains limited in LMICs, where the burden of cervical cancer is highest. Technical barriers include the lack of standardized collection devices, transport media, and validated point-of-care platforms.[93,174] Opportunities, however, lie in innovations such as non-invasive sample collection, near-patient testing, and validation of dry swab transport methods in terms of stability, where the DNA sample remains intact during storage and transportation. This can significantly expand reach in underserved populations. Sustainable implementation requires not only technical innovation but also procurement strategies, regulatory support, and integration of triage tools to ensure continuity of care. Addressing the mentioned concerns is essential to advancing equitable access to and sustainable HPV testing worldwide.[127]

Beyond technical and logistical considerations, psychosocial factors play a critical role in screening uptake. A synthesis of 22 evidence-based studies highlights that knowledge gaps, behavioural approaches, beliefs, and emotional reactions strongly influence women's acceptability of HPV testing. Misunderstandings often arise from the limited awareness regarding HPV transmission, misconceptions that it affects only sexually active individuals and confusion about its direct causal link to cervical cancer. Most people struggle to differentiate the type of HPV testing available, from DNA testing to cytology, and its significance. All these factors contribute to resistance towards getting tested. [128]

Concerns about extended screening intervals and delayed initiation ages also act as barriers, while reassurance from negative results, perceived benefits, and trust in healthcare providers serve as facilitators. Importantly, psychosocial influences extend beyond traditional health behaviour models, encompassing embarrassment, fear, and cultural norms. Thus, optimization requires not only clinical precision but also tailored education, culturally sensitive messaging, and supportive interventions to build confidence and acceptance among women. [128]

Pilot studies in low-resource settings illustrate how HPV-based screen-and-treat models can be practically implemented. Indian states have adopted diverse strategies to overcome barriers in rural cervical cancer screening, optimizing approaches based on population realities and accessibility.[129] Maharashtra led with the Barshi rural cancer registry (1987), later expanded to Sevagram and Sindbudberg, embedding case-finding directly into villages and doubling early detection rates over time. Camp-based approaches in Barshi Tehsil revealed low Pap smear acceptance (8.3%), especially among older women, emphasizing the need for pre-camp education and counselling to optimize uptake.[175]

Gujarat's Ahmedabad district registry (2007) and the Ratnagiri registry under Tata Memorial Centre (2009) showed how registries can systematically manage dispersed populations.[129] In Tamil Nadu, training village health nurses to collect Pap smears

decentralized services and overcame workforce shortages, while Kerala's Karindalam village demonstrated the value of community mobilization in camp-based programs.[176, 177]

Uttar Pradesh's Kakori and Malihabad blocks raised Pap smear acceptance to 32% through counselling and pamphlets, while Dadri studies compared VIA, VILI, and Pap, recommending VIA as a feasible primary method where cytology infrastructure was lacking. Eastern and northeastern states explored cost-effective solutions.[178, 179] West Bengal implemented large-scale HPV testing in over 44,000 women, proving feasibility but highlighting poor follow-up compliance, and evaluated HPV vaccination for genotype-specific protection.[180, 181]

Advancements in screening and sample collection further enhanced accessibility by enabling non-invasive collection methods, reducing laboratory dependence and expanding the reach to a more diverse population. Telangana and Andhra Pradesh identified HPV prevalence in peri-urban women, linking high-risk types to abnormal cytology.[182] Tribal regions in Madhya Pradesh, Chhattisgarh, and Jharkhand adopted urine-based HPV testing, overcoming cultural resistance and logistical barriers.[183]

These state-led initiatives and strategic recommendations demonstrate that optimizing rural cervical cancer screening in India depends on aligning test methods with population demographics and accessibility, implementing registries for systematic surveillance, conducting camps and nurse-led services to mobilize, and using HPV testing or tumour markers for effective triage. This approach ensures that screening is scalable, cost-effective, and responsive to the barriers faced by real rural populations. [129]

Economic modelling further strengthens the case for tailored strategies by demonstrating how resource distribution, cost effectiveness and population specific outcomes can guide more efficient screening implementation. In China, hybrid models integrating demographic trends, HPV type distribution, and healthcare resource constraints predict that initiating routine bivalent vaccination in 2021, scaling catch-up vaccination to age 25, and later transitioning to a 9-valent vaccine, combined with HPV-based screening every five years, would avert over 7.5 million cases and 2.5 million deaths between 2021 and 2100.[130] Elimination, defined as fewer than four new cases per 100,000 women, could be achieved by 2047, with net economic savings compared to the status quo. Differences between urban and rural areas highlight the need for risk-stratified strategies, but overall nationwide feasibility indicates the importance of aligning vaccination scale-up with optimized screening intervals to achieve WHO elimination targets sustainably.[130]

Another modelling study in Hong Kong demonstrates how HPV vaccination reshapes the economics of cervical screening. For unvaccinated women, cytology every three years with reflex HPV triage was more cost-effective than cytology alone. In vaccinated cohorts, frequent screening lost cost-effectiveness once vaccine uptake reached 85% and protection lasted at least 20 years. Under these conditions, HPV testing with genotyping triage became the most efficient approach, but only when screening intervals were extended to 10–15 years or initiated later at ages 30–35.[131] This reflects the reduced prevalence of high-risk HPV in vaccinated populations, lowering the predictive value of intensive screening. The study emphasizes that cost-effectiveness can be tailored to vaccination coverage, helping health authorities design screening strategies that can be combined with vaccine uptake and aligned with the population's risk profiles.[131, 184]

These studies highlight that optimizing HPV screening requires a multifaceted approach that integrates genotype-specific risk stratification, technical innovation, psychosocial acceptability, and economic feasibility. Population-level modelling and real-world pilot programs demonstrate that strategies must be adapted to local epidemiology, resource availability, and vaccination coverage. Sustainable elimination of cervical cancer depends on integrating screening techniques with vaccination, ensuring that prevention programs are both effective and equitable across diverse populations.

3.4. Cultural and socio-economic barriers: impacts on screening optimization

Development in screening methods may inadvertently reinforce inequalities if not carefully implemented. Women from lower socio-economic backgrounds often struggle with limited knowledge about HPV, heightened anxiety upon receiving positive results, and reduced ability to afford opportunistic testing.[132, 185, 186] Participation patterns differ by education and cultural background, with disadvantaged women less likely to adopt new methods. Communication emerges as a critical determinant, poorly tailored messaging risks widening disparities, while effective counselling can mitigate them. Without systematic program design, the benefits of HPV testing may be unevenly distributed, reinforcing rather than reducing socioeconomic gaps. [132, 186]

Targeted interventions have shown promise in breaking down these barriers. Lay health advisors (LHAs), trusted community members trained to provide culturally tailored education and navigation support, consistently improve uptake, particularly among ethnic minority groups.[133] Outreach strategies, such as personalized letters and phone calls, yield mixed results, while complex or generic invitations often fail to reach disadvantaged populations. The broader perspective suggests that interventions rooted in community trust, simplicity, and accessibility are most effective in reducing inequalities in screening participation.[133] Beyond participation, socioeconomic differences also shape women's anticipated emotional and behavioural reactions to testing HPV positive. The challenges are even more pronounced in LMICs, where women face a complex web of barriers. At the personal level, limited knowledge and pervasive misconceptions, such as associating cancer with death or unnatural causes, discourage screening.[134] Individual-level barriers include lack of awareness, fear of pain or infertility, and stigma linking screening to "immoral" behaviour.[135, 189] Patriarchal norms restrict women's autonomy, with many requiring spousal or elder permission to attend clinics. Modesty concerns further discourage participation, especially when male providers are involved.[189] India provides an example of how socioeconomic and systemic barriers intersect. Despite national programs promoting screening for cervical cancer, the uptake in rural areas remains low.[129, 135, 189] Tackling these barriers requires coordinated action, clear national policies,

stronger health system capacity, gender sensitive approaches, and community-driven education that engages local leaders to dispel myths. Without such holistic strategies, inequities in screening uptake will persist, perpetuating the disproportionate burden of cervical cancer mortality in LMICs.[134, 188]

Evidence from diverse community-based interventions underscores that barriers such as uninsured status, language isolation, geographical distance, and entrenched gender norms must be addressed through community-centred strategies that embed cultural resonance and family involvement. Cultural and religious norms often prohibit gynaecological exams or require spousal permission, while community stigma reinforces silence around reproductive health.[134]

For example, the Es Tiempo campaign in Los Angeles demonstrated how culturally tailored outreach, co-created with local actors, reframed screening from a stigmatized act into a family-centred responsibility. By using culturally significant symbols like the jacaranda tree and emphasizing family values, the campaign successfully increased screening intent among Hispanic women, showing that dismantling perceptions of shame and linking prevention to collective well-being can bridge structural inequities.[138] This highlights the importance of situating interventions in everyday community spaces and leveraging trusted partnerships to normalize screening behaviours.

Beyond traditional outreach, technological innovations such as multi-cancer early detection (MCED) tests offer new pathways to overcome barriers. MCED tests, which use non-invasive samples such as blood or urine, could overcome logistical hurdles in remote areas where travel to clinics is difficult. Their less invasive nature also addresses the cultural stigma associated with pelvic exams, while multilingual instructions and digital translation tools could mitigate language barriers.[139, 193]

Importantly, MCED's potential to reduce resistance in communities bound by modesty or religious taboos positions it as a culturally acceptable alternative. However, implementation challenges remain significant, including high costs, logistical distribution, ethical concerns about communicating results, and ensuring follow-up care in low-resource settings. To ensure equitable adoption, strategies such as government subsidies, pilot programs, and community partnerships are essential.[139]

In rural India, where cervical cancer incidence and mortality are disproportionately high, mobile screening programs have shown promise in addressing infrastructural and awareness gaps. A mobile initiative in Mysore district brought services directly into community spaces, supported by peer educators and task-shifting to nurses.[140, 194] This approach achieved high acceptance of screening but faced challenges such as refusal of same-day treatment, loss to follow-up, and perceptions that asymptomatic women did not require care. By leveraging local women's groups, adapting school and Anganwadi centre infrastructure, and integrating culturally tailored education, the program demonstrated how community engagement can overcome barriers. Yet, future improvements must focus on strengthening linkage to treatment, including men in outreach to address gendered decision-making, and adopting HPV DNA testing to improve accuracy.[140, 194]

Family-centred interventions highlight the importance of addressing gender norms alongside technical solutions. A pilot study in rural Maharashtra combined HPV self-sampling with sexual health literacy interventions, engaging both women and supportive male relatives through gender-specific education sessions and storytelling-based communication.[141] By normalizing communication around HPV and cervical cancer, the program aimed to reduce stigma and empower women to choose self-sampling in the privacy of their homes.[120]

Community pioneers such as Accredited Social Health Activists (ASHAs) played a crucial role in recruitment and trust-building, while family involvement ensured collective decision-making and sustained uptake. This protocol emphasized that tackling gender norms and stigma is as essential as introducing new technologies, and that equitable access to screening requires both cultural sensitivity and systemic support.[141] This integrated approach would simultaneously reduce stigma, improve accessibility, and strengthen follow-up care, ensuring that cervical cancer screening becomes both equitable and sustainable across diverse populations.[120, 141]

Health systems in low-income settings are frequently under-resourced, with weak policies, poor implementation, and limited workforce capacity. Structural obstacles such as geographic isolation, financial constraints, and low literacy compound these issues, leaving women underserved.[134, 187] The rural primary health centres often lack trained female staff, privacy, and basic equipment. Referral systems are weak, leading to high rates of loss to follow-up. Economic and logistical barriers, long travel distances, unreliable transport, and wage loss make even free screening inaccessible.[135]

Supply-side barriers further constrain screening procedures. A cross-sectional study of primary health centres in Pondicherry revealed that, while basic infrastructure such as examination rooms and infection-control supplies was present, advanced diagnostic capacity was almost entirely absent. None of the centres had HPV test kits, colposcopes, or pathology facilities, and only one had staff trained for cytology. Supply chain systems were functional for routine items like gloves and acetic acid, but critical HPV specific consumables and equipment were missing. Data management was weak, with no health information system in place, and referral mechanisms, though documented, lacked integration with higher-level care.[136]

Effective HPV screening thus requires investment in infrastructure, personnel training, reliable supply chains, and robust health information systems, alongside stronger adherence to policies and stakeholder collaboration to bridge the gap between national guidelines and rural service delivery.[136, 169] Solutions must therefore be multi-pronged, including community education to challenge myths, empowering frontline health workers, integrating screening into routine maternal care, deploying mobile outreach units, and strengthening accountability in national programs. Importantly, culturally sensitive interventions designed with local community input are essential to bridge the gap between policy and practice.[135]

The lessons from HPV vaccination programs in LMICs further illuminate the socio-economic and cultural dynamics at play. Knowledge gaps and misinformation remain persistent obstacles, with rumours about infertility or sexual stigma undermining trust.[137, 190] Societal values and stigma, particularly linking HPV to sexual activity, discourage open discussion and make vaccination appear inappropriate for young girls. Caregiver consent is crucial, and their attitudes are shaped by education, income, and the credibility of health providers.[137, 191] Facilitators of awareness include school-based vaccination programs, which consistently achieve higher coverage than facility-based ones, especially when teachers are trained as health coordinators. Gender neutral vaccination reduces stigma, spreads responsibility across both boys and girls, and builds herd immunity more quickly.[137] Community mobilization, engaging parents, religious leaders, and peer networks, helps normalize vaccination and counter misinformation.[191] Health worker training ensures providers can confidently recommend the vaccine, which strongly influences caregiver acceptance.[192] Sustainable HPV vaccination in LMICs thus requires national policy buy-in, integration into routine immunization, and culturally sensitive communication strategies.[137]

4. POST - SCREENING TREATMENT AND CLINICAL MANAGEMENT

4.1. Treatment of Low-Risk HPV Infections

Pharmacological therapies for low-risk HPV infections primarily involve topical antivirals, cytotoxic agents, and immune response modifiers. Imiquimod, a toll-like receptor agonist, stimulates local cytokine induction and enhances immune clearance of viral lesions. Clinical studies show moderate clearance rates, though recurrence remains possible, and side effects such as erythema or flu-like symptoms can limit tolerability. [142, 143] Podophyllotoxin, a purified extract of podophyllin, acts as an antimitotic agent leading to necrosis of infected cells. It is self-applied and generally more effective and safer than crude podophyllin resin, which is now discouraged due to systemic toxicity and inconsistent formulations.[142, 195] Chemical cauterization agents such as trichloroacetic acid (TCA) are widely used in clinical practice, particularly for vaginal warts, as they coagulate tissue proteins and destroy lesions without systemic absorption. TCA is considered safe even during pregnancy, though its efficacy is limited to small lesions.[143]

Another notable pharmacological option is Sinecatechins, a standardized green tea extract rich in catechins with antiviral and immunomodulatory properties. Applied as an ointment, Sinecatechins demonstrate favourable clearance rates and low recurrence, though prolonged treatment duration and local irritation are common challenges.[143] Collectively, these drug-based therapies provide non-invasive options, but their success depends on patient adherence, lesion characteristics, and tolerance to side effects. Direct and targeted procedural interventions remain central to managing low-risk HPV lesions, especially when rapid clearance is desired. Cryotherapy, using liquid nitrogen or nitrous oxide, induces cytolysis through freezing and is effective for small to moderate lesions. While clearance rates are high, recurrence is frequent, and pain during application can reduce patient acceptance.[144,196] Electrocautery removes warts immediately by thermal destruction, often requiring anesthesia. It is efficient for bulky lesions but carries risks of scarring and discomfort.[145] Laser ablation, particularly carbon dioxide laser therapy, provides precise tissue vaporization and is favoured for extensive or multifocal disease. Its advantages include controlled depth of destruction and preservation of anatomy, though specialized equipment and training are required.[144, 145]

Overall, these procedural approaches deliver rapid lesion clearance and are often combined with pharmacological agents to reduce recurrence, forming the basis of proactive, sequential therapy in clinical practice.

4.2 Treatment of High-Risk HPV Infections (Pre-Malignant Lesions)

High-risk HPV infections, particularly those involving oncogenic strains such as HPV16 and HPV18, are strongly associated with the development of CIN and other pre-malignant lesions. Current management strategies for these lesions largely rely on drug-based therapies and surgical excision techniques, such as the loop electrosurgical excision procedure (LEEP), cold knife conization, and ablative methods such as cryotherapy and laser therapy.[144, 146] These approaches are effective in removing abnormal tissue but do not directly target the underlying viral persistence, which remains the root cause of disease progression. Importantly, while excisional and ablative procedures achieve high clearance rates, they also carry risks such as cervical insufficiency and preterm birth, underscoring the need for adjunctive therapies that address viral oncogene drug-based therapies.[146]

Imiquimod, an immune response modifier, has been explored as an off-label topical therapy for high-grade HPV-related lesions. Acting through Toll-like receptor 7 (TLR7), it stimulates local innate immunity and enhances adaptive responses by inducing cytokines such as interferons and interleukins.[197] Clinical studies of vulvar intraepithelial neoplasia (VIN) and cervical lesions have shown variable response rates, ranging from 30–90%, with regression associated with robust Th1-type immune activation and cytotoxic T-cell infiltration. However, treatment tolerance can be challenging due to local inflammation, and outcomes are often influenced by the balance between effector T cells and regulatory T cells within the lesion microenvironment. [144, 197] Interferon-α has been investigated as both topical and intralesional therapy, leveraging its broad antiviral and immunomodulatory properties. By enhancing macrophage and T-cell activity, interferon can suppress viral replication and promote clearance of dysplastic cells. Clinical outcomes, however, have been inconsistent, with clearance rates varying widely and recurrence remaining a concern. Its utility is further limited by systemic side effects such as flu-like symptoms and fatigue, which reduce patient adherence. Despite these drawbacks, interferon remains a valuable model for understanding immune-based interventions in HPV-related disease.[144, 146, 198]

Persistent high-risk HPV infection is the common thread driving progression across cervical, vaginal, and anogenital lesions. While spontaneous clearance is possible, persistence predicts recurrence and malignant transformation. LEEP is widely used for cervical dysplasia due to accessibility and ease of performance.[147] However, multiple studies by Bogani et al. show higher rates of positive margins and recurrence than with laser conization. Five-year recurrence rates were nearly double with LEEP (8.1% vs. 4%), while effective in removing lesions and providing tissue for histology, its limitations lie in margin control and long-term recurrence risk.[147] Cold knife conization remains a gold standard for histological diagnosis and treatment of high-grade cervical lesions, particularly when glandular involvement or occult invasion is suspected. Laser conization and CO_2 laser ablation demonstrated superior margin control and lower recurrence rates compared to LEEP, with large retrospective cohorts confirming cure rates above 90% for CIN2 lesions.[148] However, outcomes for CIN3 are less optimal, and recurrence remains a concern. In vaginal intraepithelial neoplasia (VaIN), CO_2 laser ablation was shown to be equivalent to excision in long-term effectiveness, with recurrence driven primarily by HPV persistence.[147, 148]

4.3. Treatment of High-Risk HPV–Associated Cervical Cancer

Surgery remains the foundation of treatment for an early-stage disease. Hu and Ma emphasized radical hysterectomy as a standard, while Kusakabe et al highlighted fertility-sparing procedures such as conization or trachelectomy for HSIL and Adenocarcinoma in situ (AIS).[150] Luttmer et al underscored the importance of triage to avoid overtreatment, noting that not all HPV-positive women require excision. [151] Moreno-Acosta et al provided a cautionary case where refusal of hysterectomy at the preinvasive stage led to progression to stage IIIB carcinoma.[152] Khairkhah et al reinforced that surgical excision and radical hysterectomy remain definitive, but recurrence risk is similar across techniques, allowing tailoring to patient needs.[146] Chemotherapy has consistently been positioned as essential for advanced or recurrent disease. Hu and Ma, Kusakabe et al., both identified cisplatin as the backbone, with carboplatin, paclitaxel, and topotecan as key agents.[149, 146] Luttmer et al. framed chemotherapy within the context of triage, stressing that early identification of transforming infections reduces the need for systemic therapy.[151] Moreno-Acosta et al. linked molecular biomarkers (IGF1R and TP53 polymorphisms) to potential chemo responsiveness, suggesting personalized regimens.[152] Khairkhah et al expanded the chemotherapy landscape, noting that multi-agent combinations (vinorelbine, pemetrexed, irinotecan, capecitabine) are increasingly used to improve survival.[146]

5. NOVEL THERAPEUTIC STRATEGIES IN HPV ASSOCIATED MALIGNANCIES

Advances in nanotechnology have enabled precise drug delivery systems that overcome the limitations of conventional chemotherapy and radiotherapy. Liposomes, dendrimers, micelles, and gold nanoparticles have been shown to enhance bioavailability, reduce systemic toxicity, and improve tumour-specific targeting.[153] Nanoparticle-mediated radio sensitization, particularly with gold-based systems, has been shown to enhance apoptosis and improve radiotherapy outcomes in cervical and head-and-neck cancers.[153, 199] Moreover, nano-enabled immunotherapy and gene therapy, such as siRNA and CRISPR/Cas9 delivery, directly silence HPV oncogenes E6/E7, restore tumour suppressor pathways, and promote immune clearance.[153] Gene-editing platforms, especially CRISPR/Cas9, have demonstrated preclinical success in disrupting E6 and E7, thereby reactivating p53 and Rb. While delivery challenges remain, nanoparticle and liposome vectors are under investigation to improve specificity.[154, 200]

Epigenetic drugs, such as Histone deacetylase (HDAC) and DNA methyltransferase (DNMT) inhibitors, complement these approaches by reversing HPV-driven gene silencing and sensitizing tumours to immunotherapy. Therapeutic vaccines like VGX-3100 and Papzimeos (PRGN-2012) further integrate into precision oncology frameworks, eliciting robust T-cell responses against HPV antigens. Patient-derived organoids and AI-driven drug screening platforms add a translational dimension, enabling personalized therapy stratification and biomarker discovery.[154] Structural biology has provided insights into the molecular interactions of HPV proteins, offering rational targets for drug development.[155] Capsid proteins L1 and L2 inform vaccine design, while replication proteins E1 and E2 provide opportunities for small-molecule inhibitors that disrupt viral DNA replication.[155, 201, 202] Oncoproteins E6 and E7 remain central, with structural studies revealing critical motifs for p53 degradation and pRb disruption.[203] Inhibitory ligands targeting E6–E6AP–p53 complexes, or stabilizers of Rb–E2F interactions, represent promising avenues for therapeutic intervention.[155] E5, though less studied, contributes to immune evasion and oncogenic signalling, highlighting the need for therapies that restore antigen presentation and counteract the epidermal growth factor receptor (EGFR) driven proliferation.[155]

Prophylactic vaccines such as Gardasil, Cervarix and Cervavac remain the foundation of HPV prevention, effectively reducing infection incidence rates and precancerous lesions.[206] However, they cannot treat established infections, which has driven the development of therapeutic vaccines targeting E6/E7.[156] These vaccines aim to stimulate cytotoxic T-cell responses and are increasingly combined with checkpoint inhibitors to overcome tumour immune evasion. Immune checkpoint blockade, particularly PD-1/PD-L1 inhibitors like nivolumab and pembrolizumab, has shown efficacy in cervical and head-and-neck cancers.[204] Adoptive cell therapies and therapeutic vaccination are being explored as synergistic modalities. Importantly, regional disparities in vaccine access and screening highlight the need for equitable implementation of these strategies, especially in high-burden countries.[204, 205] Nanotechnology provides precision delivery, genome editing offers direct viral gene disruption, structural biology informs rational drug design, and immunotherapy strengthens host defences.[156] The future of HPV and cervical cancer management lies in integrating

these modalities into tailored multimodal regimens, supported by organoid-based functional screening and biomarker-driven stratification.

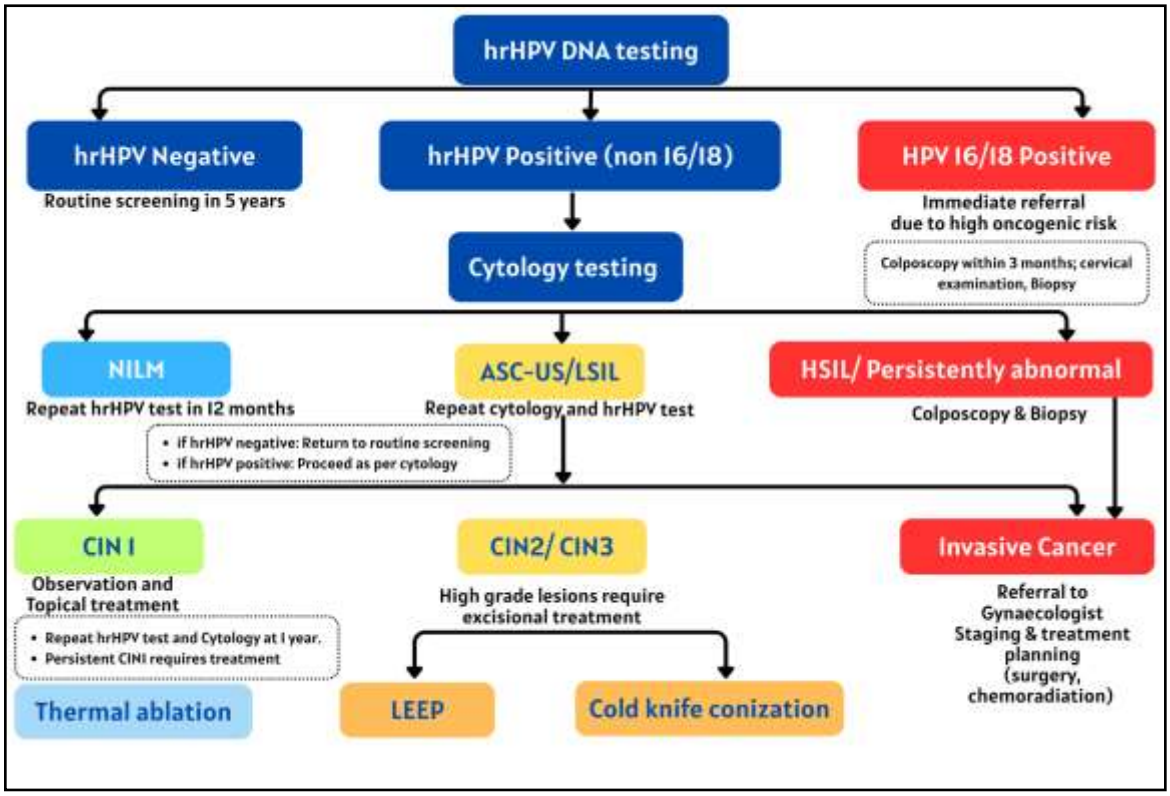

Fig 4: Risk-based human papillomavirus (HPV) screening and management algorithm:

The flowchart outlines a risk-based approach beginning with hrHPV DNA testing. Individuals who are hrHPV negative return to routine screening. Those positive for non-16/18 hrHPV undergo cytology triage, categorized as negative for intraepithelial lesion or malignancy (NILM), atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion (LSIL), or high-grade squamous intraepithelial lesion (HSIL). HPV types 16 and 18–positive individuals are referred directly for colposcopy. Colposcopy with biopsy determines the grade of cervical intraepithelial neoplasia (CIN). CIN 1 is managed conservatively, whereas CIN 2 and CIN 3 require excisional treatment such as loop electrosurgical excision procedure (LEEP) or cold knife conization. Invasive carcinoma requires referral for specialized oncologic management.

6. DISCUSSION AND FUTURE DIRECTIONS

The path toward HPV elimination and cervical cancer prevention requires not only technological optimization but also equitable implementation.[28] Advances in screening protocols must achieve an optimal balance between sensitivity and specificity, ensuring early detection while minimizing unnecessary interventions.[95,97] Importantly, these protocols should be adaptable to diverse populations, the strategies that are effective in well-resourced urban centres would require modification to suit rural or low-income settings.[112,132] Another way is to expand access to co-testing approaches that integrate cytology with molecular HPV testing, alongside non-invasive sample collection, representing a critical step in broadening coverage. In resource-limited communities, such innovations are not merely technical upgrades, they determine the level of awareness and participation of the population in prevention programs.[68,88,95] Embedding psychosocial support that includes counselling and education along with screening initiatives, can further enhance adherence, recognizing that a test without follow-up cannot fulfil its preventive purpose.[45,46] At the health system level, inconsistent screening guidelines give rise to inequities.[184,188] Standardization through international collaboration, coupled with rigorous quality-control frameworks, is essential to ensure uniform access to prevention.[45,116] Linking screening programs with HPV vaccination initiatives offers synergistic protection, vaccination reduces new infections, while screening identifies cases beyond vaccine coverage.[131,137,181] Prospective future research should prioritize multicenter, longitudinal studies evaluating the effectiveness of integrated screening and vaccination strategies across diverse populations. Emerging technologies such as portable HPV DNA assays, AI-assisted cytology, and digital health platforms with systematic assessment for scalability in low-resource settings.[85,86] Equally important is the incorporation of survivorship care, addressing psychosocial and sexual health needs to provide holistic support beyond initial detection.[125,128]

7. CONCLUSION

By integrating current advances, this review provides a foundation for the prevention of HPV that causes cervical cancer through optimization of screening methods and vaccination programs by implementing them throughout the population. It emphasizes that the consistent application of existing knowledge and research studies is the most effective strategy for reducing the burden of the

infection. Ultimately, cervical cancer is a largely preventable disease. Early detection remains the fundamental aspect of reducing morbidity and mortality, but the decisive factor lies in the fair, creative, and universal application of existing knowledge. Sustained commitment to innovation, equity, and collaboration will be pivotal in transforming the vision of cervical cancer elimination into a global reality.

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