Santé et Innovation
Laboratoire de Recherche UFAS

Setif 1 University - Ferhat ABBAS

Vol. 01 Num. 04 (2026) 386–403

Type of the Paper (Article)

HUMAN PAPILLOMAVIRUS (HPV) TESTING IN THE SURVEY OF HPV-ASSOCIATED CANCERS: A FOCUS ON NORTH AFRICA AND ALGERIALE TITRE

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Citation: To be added by editorial staff during production.

Academic Editor: First name Last name

Received: date: 11/07/2025
Revised: date : 02/08/2025
Accepted: date: 22/12/ 2025
Published: date:03/01/2026

Abstract

Molecular testing for HPV has become fundamental in the prevention of cervical cancer, often replacing or enhancing traditional cytological methods due to its superior sensitivity and specificity for oncogenic strains. This assay focuses on detecting viral DNA and RNA transcripts, which facilitates early diagnosis and allows for a risk assessment specific to different genotypes. In North Africa and Algeria, the incorporation of this technology is vital for improving screening and vaccination approaches. Local studies emphasize the importance of understanding the regional prevalence of high-risk HPV types and the intricate co-factors, such as the Epstein-Barr virus (EBV), that play a role in cancer development. Utilizing centralized laboratories and affordable platforms is critical for expanding HPV-based screening initiatives in areas with limited resources.

Keywords:

HPV DNA, molecular diagnostics, cervical cancer, Algeria, North Africa, genotyping, PCR, E6/E7 mRNA, hybrid capture, screening, co-infection, EBV

1. Introduction :

Human papillomaviruses (HPVs) are small, non-enveloped viruses characterized by their double-stranded DNA and classified within the Papillomaviridae family, primarily targeting epithelial cells in both skin and mucosal tissues. To date, over 220 distinct genotypes have been identified, with at least 14 of these classified as high-risk (HR) types due to their recognized potential to cause cancer **[1]**. Notably, HPV16 and HPV18 are accountable for roughly 70% of cervical cancer cases globally **[2]**, while other genotypes such as HPV31, 33, 45, 52, and 58 also contribute to the incidence, influenced by geographic and ethnic factors.

The presence of HPV infection is a crucial factor in the development of cervical cancer, which continues to be one of the most prevalent cancers among women in low- and middle-income countries (LMICs). The most recent GLOBOCAN 2024 projections indicate that cervical cancer results in over 660,000 new diagnoses and approximately 350,000 fatalities each year, with the most severe mortality rates found in Sub-Saharan Africa, South Asia, and North Africa **[3]**. The global impact of this disease is significantly shaped by disparities in vaccination rates, access to screening, and the availability of diagnostic facilities. In Algeria and its neighboring regions, the implementation of cytology-based screening programs has been inconsistent, and the integration of molecular HPV testing into clinical practice has only recently started to gain traction **[4]**.

The oncogenic process is primarily driven by the expression of two viral oncoproteins, E6 and E7, which inactivate the tumor suppressor proteins p53 and pRb, respectively. This deregulation leads to uncontrolled cell cycle progression, genomic instability, and eventual malignant transformation **[5]**. Persistent infection with high-risk HPV genotypes is thus a critical determinant of cancer risk. Molecular diagnostics that detect HPV DNA or RNA exploit these mechanisms, providing both analytical sensitivity and biological relevance to clinical outcomes.

Recent research also highlights the role of co-infections with other oncogenic viruses — such as Epstein–Barr virus (EBV) — in modulating HPV-mediated carcinogenesis. In Algerian women, Khenchouche et al. **[6]** demonstrated a significant rate of co-infection between HPV and EBV in cervical carcinoma samples, suggesting synergistic effects on viral persistence and oncogenic signaling pathways. Such molecular interactions may contribute to region-specific variations in disease progression and clinical response.

For decades, cervical cancer screening relied on the Papanicolaou (Pap) test, which, although effective in high-resource settings, shows reduced sensitivity for detecting early high-grade lesions in many low-income contexts. The development of HPV DNA-based assays in the 1990s revolutionized screening by directly identifying viral presence rather than morphological abnormalities. The Hybrid Capture 2 (HC2) test (Qiagen) became one of the first widely adopted molecular platforms, targeting 13 high-risk genotypes with high sensitivity **[7]**. Subsequently, polymerase chain reaction (PCR)-based and real-time quantitative PCR assays were introduced, enabling genotyping and viral load quantification **[8]**.

More recently, mRNA-based tests that detect E6/E7 oncogene transcripts, such as the Aptima HPV Assay (Hologic), have gained clinical significance for triaging women with ambiguous cytology results. These assays provide better specificity by

identifying transcriptionally active infections linked to neoplastic transformation [9]. Novel technologies, including next-generation sequencing (NGS), microarrays, and point-of-care isothermal amplification, further expand diagnostic potential and enable genotype surveillance.

The clinical utility of molecular HPV testing extends beyond screening to include triage, risk stratification, treatment monitoring, and vaccine policy planning. The World Health Organization [10] now recommends high-risk HPV detection as the primary screening technique for women aged 30-65. Furthermore, molecular assays are critical for monitoring cervical intraepithelial neoplasia (CIN) after treatment and mapping circulating genotypes.

However, in North Africa, clinical integration of molecular testing is hampered by high costs, a lack of laboratory infrastructure, and insufficient training. Pilot studies in Algeria, Morocco, and Tunisia revealed varying HPV prevalence and genotype distribution, with HPV16 prevailing but HPV33, 52, and 58 also present [11, 12]. Understanding these molecular and clinical dynamics is essential for refining diagnostic algorithms and creating cost-effective screening programs tailored to the regional environment.

2. Epidemiology of HPV-Associated Cancers: Global and Regional Overview

2.1 Global Burden of HPV-Associated Malignancies

Human papillomavirus infection is now recognized as the most prevalent viral cause of cancer worldwide. According to GLOBOCAN 2024 [3], HPV is responsible for approximately 5% of all cancers globally, corresponding to nearly 690,000 new cases annually (table 1). Cervical carcinoma remains the dominant HPV-associated malignancy, but oropharyngeal, anal, vulvar, vaginal, and penile cancers are increasingly reported, particularly in developed regions where vaccination and screening have altered disease patterns [13].

Table 1 : Global burden of HPV-associated malignancies: compiled from different references [3, 10, 13].

Cancer type	Predominant HPV genotypes	Estimated global cases (2024)	Proportion HPV-positive	Comments
Cervical carcinoma	HPV 16, 18, 31, 33, 45, 52, 58	660,000	99%	Highest burden in LMICs; screening-preventable
Anal cancer	HPV 16, 18	70,000	90%	Rising incidence in both sexes
Vulvar/vaginal cancer	HPV 16, 18, 33	45,000	45–60%	Often coexists with vulvar intraepithelial neoplasia
Penile cancer	HPV 16, 18	36,000	50%	Linked to poor hygiene and uncircumcision
Oropharyngeal cancer	HPV 16	130,000	30–60%	More common in men in high-income countries

Despite remarkable advances in vaccination and molecular testing, the geographical heterogeneity of HPV-driven disease remains striking. High-income regions have seen steady declines in cervical cancer incidence, while rates in many low- and middle-income regions, including North Africa, remain static or increasing. This disparity mirrors gaps in molecular diagnostic coverage and organized screening.

2.2. HPV-Related Cancers in North Africa

North African countries, including Algeria, Morocco, Tunisia, Libya, and Egypt, share sociocultural and healthcare characteristics that shape HPV epidemiology. Most maintain opportunistic, rather than population-based, screening programs, with cytology still predominant and HPV molecular testing introduced only in pilot phases **[4, 14]**.

In Algeria, cervical cancer ranks second among cancers in women, with an estimated 2,500 new cases and about 1,200 deaths annually **[3]**. The age-standardized incidence rate is approximately 14–16 per 100,000 women, comparable to Morocco and Tunisia but higher than in Europe (< 7/100,000). Studies from Algiers, Oran, and Constantine show HPV DNA positivity in 85–98% of invasive cervical cancers, confirming its etiological dominance **[11, 12]**.

While cervical cancer dominates, HPV has also been detected in anal and oropharyngeal squamous cell carcinomas in regional studies **[15]**. However, diagnostic confirmation of these non-cervical HPV-related cancers remains rare due to limited access to genotyping and molecular pathology laboratories.

2.3. Disparities in Screening and Diagnostic Infrastructure

The lack of organized molecular screening remains a central limitation in North Africa. Cytology (Pap smear) coverage rarely exceeds 20–25% of eligible women **[4]**, and HPV testing is mainly restricted to tertiary hospitals or private laboratories. Most molecular assays in use are imported commercial kits such as Hybrid Capture 2 or Cobas 4800 (Roche), with high costs (USD 30–50 per test) relative to average monthly income.

Pilot initiatives using PCR-based and Aptima RNA assays have shown superior sensitivity for CIN2+ lesions compared with cytology **[14]**, supporting WHO recommendations for HPV-first screening. Nevertheless, supply chain issues, lack of trained molecular technologists, and inconsistent reimbursement policies hinder national implementation. Algeria’s current screening strategy relies primarily on cytology and visual inspection with acetic acid (VIA), with HPV molecular testing

proposed as a next-generation diagnostic layer under the national cancer control plan **[16]**.

2.4. Clinical Implications and Molecular Epidemiology

The molecular detection of HPV provides not only diagnostic confirmation but also prognostic information. Persistent infection with specific genotypes (e.g., HPV16, 18, 33) correlates with higher risk of high-grade lesions and progression to invasive carcinoma **[5]**. Molecular epidemiology thus informs clinical triage, women positive for HPV16/18 are referred for immediate colposcopy, while those harboring other HR types may undergo repeat testing after 12 months.

In Algeria, the predominance of HPV16 and the detection of multiple high-risk co-infections (HPV 33, 52, 58) have clinical implications for follow-up and vaccination strategy design **[11, 12]**. Importantly, co-infection with Epstein–Barr virus (EBV) — documented in Algerian cervical carcinoma by Khenchouche et al.**[11],** may potentiate oncogenic signaling through activation of NF-κB and modulation of the host immune response, underscoring the necessity of molecular co-testing where feasible.

3. Epidemiology and Genotype Distribution of HPV: Global and Regional Perspectives

3.1. Global burden of HPV-associated cancers

HPV infection is responsible for approximately 690,000 new cancer cases annually worldwide, accounting for nearly 5% of all human cancers **[17]**. The highest incidence is linked to cervical carcinoma, which remains the fourth most common cancer in women globally, with 604,000 new cases and 342,000 deaths estimated in 2020 **[18]**. Beyond the cervix, HPV contributes to a significant proportion of anal (88%), vulvar/vaginal (43%), penile (50%), and oropharyngeal cancers (30–60%) **[19]**).

High-risk HPV (HR-HPV) genotypes—particularly HPV16 and HPV18—are detected in approximately 70% of cervical carcinomas, while genotypes 31, 33, 45, 52, and 58 contribute another 20% **[20]**. The distribution, however, varies geographically, reflecting differences in sexual behavior, vaccination coverage, and population genetics **[21]**.

3.2. Epidemiology in North Africa

In North Africa, cervical cancer remains a major public health challenge, ranking among the top two cancers in women in Algeria, Morocco, and Tunisia (IARC, 2023). Estimated age-standardized incidence rates (ASR) per 100,000

women are 17.2 in Algeria, 20.0 in Morocco, and 12.5 in Tunisia, compared with 7.5 in Europe and 5.0 in North America **[21]**.

Molecular studies from the region reveal HPV16 as the most prevalent genotype, followed by HPV18, 33, 45, 52, and 58 (table 2), consistent with global trends but with higher representation of HPV33 and 45 **[22]**. Importantly, the study by Khenchouche et al. **[11]** demonstrated co-infection with Epstein-Barr virus (EBV) in Algerian cervical carcinoma tissues, suggesting viral interactions that may potentiate oncogenesis, an observation unique to North African cohorts.

Table 2. HPV genotype distribution in selected regions of North Africa (summarized from key molecular studies)

Country	Sample type	N	Most prevalent HR-HPV genotypes (%)	Co-infections	Reference
Algeria	Cervical carcinoma	80	HPV16 (47.5), HPV18 (17.5), HPV33 (12.5), HPV45 (7.5)	HPV + EBV (24%)	**[11]**
Morocco	Cervical samples	150	HPV16 (49), HPV18 (20), HPV45 (10), HPV58 (5)	Multiple HR-HPV (21%)	**[22]**
Tunisia	Cervical biopsies	100	HPV16 (40), HPV31 (12), HPV33 (9), HPV52 (8)	Rare	**[23]**
Egypt	Cervical cytology	200	HPV16 (38), HPV18 (19), HPV58 (9), HPV45 (6)	HPV + HSV (8%)	**[24]**

3.3. Vaccine-preventable genotypes and coverage gaps

The currently available bivalent (Cervarix®), quadrivalent (Gardasil®), and nonavalent (Gardasil 9®) vaccines cover HPV16 and HPV18, with the latter extending protection to HPV31, 33, 45, 52, and 58 **[25]**. This broader coverage is highly relevant for North African populations, where HPV33, 45, and 52 are relatively common **[22, 23]**.

However, vaccine uptake across North Africa remains below 5%, largely due to limited programmatic implementation and public awareness **[26]**. Molecular surveillance, therefore, remains essential to monitor emerging genotypes and guide cost-effective screening strategies.

3.4. Genotype diversity in extra-cervical cancers

Other studies report HPV16 dominance in oropharyngeal squamous cell carcinoma (OPSCC) across both high- and low-income regions **[27, 28]**. In Algeria and Morocco, small case series confirm HPV DNA in ~30–35% of oropharyngeal cancers, primarily genotype 16 **[23]**. However, systematic testing is rare due to the lack of validated FFPE-compatible assays and limited reimbursement.

3.5. Epidemiological insights from molecular testing

The shift from cytology to HPV-based molecular screening not only increases sensitivity but also enables genotype surveillance—a critical tool for identifying

high-risk populations and evaluating vaccination impact [29]. Integrating molecular diagnostics into regional health systems in Algeria and neighboring countries could improve early detection, reduce cervical cancer mortality, and support molecular epidemiology of HPV-driven cancers.

4. Clinical Application of HPV Molecular Testing: From Screening to Management

4.1. Transition from cytology to molecular screening

The traditional Pap smear, while pivotal in reducing cervical cancer incidence in developed countries, has limitations in sensitivity (~55–60%) and inter-observer variability [30]. Molecular testing for high-risk HPV (HR-HPV) DNA or RNA now represents a paradigm shift, offering greater sensitivity (>95%) for detecting cervical intraepithelial neoplasia grade 2 or higher (CIN2+).

Randomized trials such as ATHENA [31] and VALGENT [30] established the non-inferiority and clinical superiority of molecular assays compared to cytology. Consequently, HPV testing is now recommended as the primary screening tool by the WHO and many national programs.

In Algeria and neighboring North African countries, HPV testing has been introduced in pilot screening projects [26]. However, cytology remains predominant, with molecular tests mainly used in research centers and private laboratories due to higher cost and limited reimbursement.

4.2. Clinical algorithms and triage strategies

HPV testing serves several functions throughout the clinical care continuum. It is generally used for primary screening, with the first-line test detecting high-risk HPV (HR-HPV) DNA to identify women at risk of cervical precancer and cancer. In triage testing, HPV genotyping or cytology is used to further stratify women who test positive, assisting in determining the necessity for additional diagnostic examination. Molecular detection of HPV is used as a cure test after treating CIN2+ lesions to guarantee viral clearance and treatment success. Furthermore, HPV testing is used as an additional test in cases of atypical squamous cells of uncertain significance (ASC-US) cytology, helping to clarify equivocal cytological findings.

Women positive for HPV16 or 18 have the highest risk of CIN3+, justifying direct referral to colposcopy [20]. Other HR-HPV positive women undergo cytology triage or repeat testing after 12 months (figure 1).

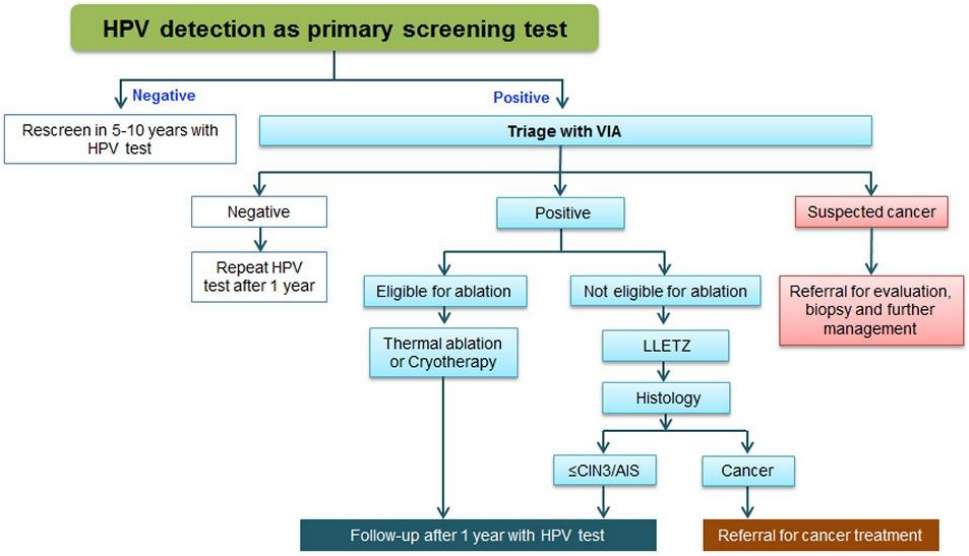

Figure 1 : Flowchart of clinical management integrating HPV molecular testing (screening → triage → treatment → follow-up) **[32]**.

4.3. HPV testing in extra-cervical cancers

While cervical screening is the most established application, HPV testing is increasingly used for non-cervical cancers. Oropharyngeal squamous cell carcinoma is distinguished by the presence of HPV16 DNA and p16 overexpression, both of which are prognostic indicators linked with improved survival **[28]**. In cases of anal intraepithelial neoplasia, PCR-based HPV DNA testing combined with cytology is widely used, especially in high-risk groups such as HIV-positive people and men who have sex with men **[33]**. HPV DNA and mRNA identification is critical for validating viral etiology and supporting molecular classification in penile and vulvar malignancies **[34]**. However, molecular confirmation of HPV in oropharyngeal and anogenital malignancies in North African nations is rare, with in-house PCR assays or imported test kits used in academic and research institutes **[23]**.

4.4. Principles of molecular HPV testing

Modern HPV diagnostics rely on nucleic acid detection rather than cytomorphology alone. Three major molecular approaches dominate. HPV DNA testing detects viral DNA, which is frequently targeted at the L1 area, as demonstrated in assays like Hybrid Capture 2 and Cobas 4800. This approach has good sensitivity and extensive genotyping coverage, but it cannot distinguish between transitory and transforming infections. In contrast, HPV RNA testing detects E6/E7 mRNA transcripts, which indicate active carcinogenic transcription, as demonstrated by the Aptima HPV assay. While this technique is more selective for clinically important infections, it may have slightly poorer analytical sensitivity. Finally, HPV diagnostic methods include DNA, RNA, and genotyping assays, each

with unique advantages. HPV DNA testing identifies the presence of viral DNA, generally focusing on the L1 region, as seen in the Hybrid Capture 2 and Cobas 4800 tests. It has good sensitivity and genotyping coverage but cannot distinguish between transitory and transforming infections. HPV RNA testing, on the other hand, detects E6/E7 mRNA transcripts that indicate active carcinogenic transcription, as demonstrated by the Aptima HPV assay. This technique has higher specificity for clinically severe infections, but it may have somewhat poorer analytical sensitivity. HPV genotyping separates distinct high-risk genotypes, as seen with the Anyplex II HPV28 and Abbott RealTime HPV assays, which is critical for epidemiological research and patient follow-up. The most widely used assays have been validated for cervical cancer screening and are increasingly applied in HPV-driven head and neck cancers **[27, 29]**. Table 3 summarizes selected platforms (table 3).

Table 3. Comparison of major HPV molecular diagnostic assays

Assay	Target	Genotypes detected	Detection method	Sensitivity (%)	Specificity (%)	Automation	Availability (North Africa)
Hybrid Capture 2 (Qiagen)	DNA (L1)	13 HR types	Signal amplification (chemiluminescence)	95	85	Moderate	Available in tertiary labs
Cobas 4800 (Roche)	DNA (L1, E6/E7)	14 HR (HPV16,18 separately)	Real-time PCR	98	88	Fully automated	Limited availability (Algeria, Morocco)
Abbott RealTime HPV	DNA (L1)	14 HR (16/18 separately)	PCR + fluorescence	97	90	Fully automated	Rare, used in regional research centers
Aptima HPV (Hologic)	RNA (E6/E7)	14 HR	Transcription-mediated amplification	94	92	Moderate	Increasing use in pilot programs
Anyplex II HPV28 (Seegene)	DNA	28 genotypes (HR + LR)	Multiplex real-time PCR	96	87	Semi-automated	Available in Tunisia, under evaluation in Algeria
INNO-LiPA HPV Genotyping	DNA	32 genotypes	Reverse hybridization	93	88	Manual	Used in epidemiological studies

4.5. Advantages and clinical limitations of HPV testing

The molecular HPV testing offers significant advantages for cervical cancer screening, including high sensitivity, early detection of pre-cancerous lesions, and the ability to perform genotype-specific risk assessment (table 4). It's also valuable for test-of-cure and vaccine monitoring, and facilitates integration with automation. However, its limitations include higher cost and the need for specialized equipment, leading to limited availability in low-resource regions.

In Algeria and Tunisia, laboratories face reagent import delays, limited access to high-throughput instruments, and non-standardized protocols, hindering large-

scale screening [26]. Therefore, cost-effective pooled-sample PCR methods and portable real-time PCR systems are under evaluation as scalable alternatives.

4.6. Future directions in clinical diagnostics

Emerging molecular technologies have greatly improved HPV detection and characterisation. Next-Generation Sequencing (NGS) allows for complete whole-genome HPV profiling as well as co-infection analysis [1]. Digital PCR allows for highly exact absolute quantification of viral load and aids the detection of viral integration events.

Furthermore, point-of-care isothermal amplification assays like as loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA) provide quick, sensitive, and cost-effective alternatives for low-resource settings [35].

Table 4: Evaluating Molecular HPV Testing: Advantages, Limitations, and Implementation Barriers

Advantages of molecular HPV testing	Limitations and challenges
High sensitivity and reproducibility	Cost and need for specialized equipment
Early detection before cytological abnormalities	Limited availability in low-resource regions
Enables genotype-specific risk assessment	Risk of overtreatment due to transient infections
Useful for test-of-cure and vaccine monitoring	Logistical barriers to sample collection and transport
Integration with automation and digital systems	Lack of clinician and public awareness

Furthermore, multiplex assays capable of detecting HPV alongside other sexually transmitted pathogens such as Epstein-Barr virus (EBV), herpes simplex virus (HSV), and Chlamydia trachomatis are of particular interest in North Africa, where viral co-infection rates are high [11]. Integration of these molecular innovations into national screening programs could significantly enhance early diagnosis and reduce HPV-related cancer burden.

4.7. Regional landscape and accessibility in North Africa

Despite the global shift toward molecular primary screening, implementation in North Africa remains limited due to cost, infrastructure, and reagent availability [26]. In Algeria, HPV testing is mainly available in academic laboratories and private clinics, often using manual or semi-automated assays such as Anyplex II or INNO-LiPA. The national screening strategy still relies on Pap smears, with molecular tests used as confirmatory or research tools.

Cross-sectional studies in Algeria, Morocco, and Tunisia have identified HPV16 and HPV18 as predominant genotypes, but HPV33, 45, 52, and 58 also show notable prevalence **[7, 11]**. These patterns mirror Sub-Saharan and Southern European distributions, supporting the need for regional test validation and cost-adjusted implementation.

4.8. Clinical relevance beyond cervical cancer

Molecular HPV testing has extended to anal, penile, and oropharyngeal squamous cell carcinomas, where HPV DNA or p16 immunostaining are prognostic markers **[28]**. Cobas 4800 and in-house PCR assays have been adapted for FFPE tissue analysis in head and neck cancers. Yet, in North Africa, such applications remain sporadic and research-limited, underscoring disparities between high-income and low-middle-income settings.

5. Cost, Accessibility, and Implementation Challenges in North Africa

5.1. Global perspectives on cost-effectiveness

From a health economics standpoint, HPV molecular screening has proven to be cost-effective in many countries when implemented at national scale. Compared with cytology-based programs, HPV testing every 5 years can achieve similar or better outcomes than annual Pap smears at a lower long-term cost **[36]**.

WHO modeling studies estimate that introducing HPV-based screening in low- and middle-income countries (LMICs) could reduce cervical cancer mortality by up to 60% over 30 years **[37]**. However, the initial investment in instrumentation, cold-chain reagents, and laboratory infrastructure remains prohibitive in many developing regions, including North Africa, where laboratory capacity is unevenly distributed and centralized in urban university hospitals.

5.2. Cost and accessibility in North Africa

The introduction of HPV molecular testing in Algeria, Morocco, Tunisia, and Egypt is constrained by considerable direct and indirect expenses. Direct costs include consumables and reagents, which range between $20 and $40 per test depending on the assay platform employed. Indirect costs include logistics, personnel training, quality assurance, and sample transportation, all of which add to the overall financial and operational load. Furthermore, health-care concerns such as the lack of national reimbursement codes and varying levels of patient knowledge impede the general adoption and sustainability of HPV testing programs in these countries.Most national programs in the Maghreb still rely on

opportunistic cytology. Pilot molecular screening projects, supported by WHO and regional oncology centers, have shown promising feasibility but lack sustainable funding [26].

In Algeria, university hospitals in Algiers, Constantine, and Oran have adopted semi-automated platforms (Seegene Anyplex II, Qiagen HC2) primarily for research or confirmatory diagnostics (table 5).

Table 5. Comparative overview of HPV molecular testing costs and implementation status in North Africa

Country	Dominant test types	Mean cost/test (USD)	Reimbursement	Laboratory availability	National screening program	Reference
Algeria	HC2, Anyplex II, INNO-LiPA	25–40	No	Limited to major cities	Pilot only	[11, 26]
Morocco	Cobas 4800, HC2	25–35	Partial (private)	Moderate	Pilot (Rabat–Casablanca)	[22]
Tunisia	Anyplex II, Aptima	20–30	No	Limited	Research-based	[23]
Egypt	Abbott RealTime, HC2	25–45	Partial	Broad in tertiary centers	Under discussion	[24]

5.3. Infrastructure and logistical barriers

5.3.1. Laboratory infrastructure

Most North African laboratories lack automated nucleic acid extraction platforms, biosafety facilities, and continuous cold-chain reagent supply. Sample referral networks are weak, leading to delays and loss of integrity—particularly in rural areas [26]. Routine molecular diagnostics are generally limited to university hospitals, private clinics, or research institutes.

5.3.2. Training and quality assurance

Personnel training is inconsistent across the region. Few laboratories participate in external quality assessment (EQA) schemes or proficiency panels like those conducted by WHO and QCMD [33].

This results in variable analytical performance and reduced comparability between studies.

5.3.3. Cultural and policy barriers

Cervical cancer screening remains stigmatized in many North African communities, and awareness of HPV's role in carcinogenesis is limited. Public

health messaging and school-based vaccination programs are only partially implemented, further limiting demand for molecular testing.

5.4. Policy and implementation opportunities

Several significant projects are now being implemented to improve access and assure sustainability. These include the formation of regional laboratory networks, such as the Maghreb Molecular Oncology Network, which aim to improve diagnostic capacity and collaboration across borders. Pooled procurement agreements for reagents are also being established as part of the WHO's Global HPV Laboratory Initiative (GHLI) to cut costs and increase supply chain efficiency. These initiatives are further integrated with global cervical cancer elimination programs associated with the WHO's 90-70-90 targets, which aim to achieve 90% HPV vaccination coverage, 70% screening coverage, and 90% access to treatment for precancerous lesions and invasive tumors.

The Algerian National Cancer Plan (2021–2030) recognizes HPV vaccination and molecular screening as strategic priorities but notes the need for technology transfer, training, and pilot evaluation before full-scale roll-out.

5.5. Toward equitable access and diagnostic integration

To achieve equal access and efficiency, North African molecular screening must be carried out using tiered laboratory models that integrate central reference laboratories with satellite PCR hubs. The adoption of portable and low-cost molecular technologies, such as isothermal amplification platforms and GeneXpert HPV tests, can increase testing accessibility and affordability. Strengthening public-private collaborations is also critical to ensuring a consistent supply of chemicals and proper equipment maintenance. Furthermore, including HPV testing into larger women's health initiatives, such as HIV and reproductive health services, encourages a comprehensive and resource-efficient approach. Collectively, these integrated, cost-effective solutions can help close the gap between scientific competence and population-level implementation, in line with the WHO's cervical cancer elimination road-map **[18]**.

6. Discussion and Perspectives

6.1. Integrating molecular testing with clinical management

Molecular HPV testing has transformed the paradigm of cervical cancer screening, moving from cytology-based detection toward viral DNA and RNA-based assays with higher sensitivity and reproducibility **[38]**. However, the

integration of molecular diagnostics into clinical algorithms for HPV-associated cancers, particularly in low-resource regions, remains uneven.

In North Africa, despite progress in tertiary centers, screening coverage and follow-up linkage to care are still suboptimal due to logistical and socioeconomic barriers **[38]**. The potential of HPV molecular tests extends beyond cervical cytology triage. In oropharyngeal, anal, and penile cancers, HPV DNA and E6/E7 mRNA assays are being increasingly applied for tumor typing, prognosis, and therapeutic stratification **[39]**. These emerging clinical uses highlight the need for harmonized diagnostic pathways across cancer sites.

6.2. Molecular precision and genotype-specific surveillance

From a public health perspective, genotype-resolved molecular surveillance is crucial. HPV16 and HPV18 remain dominant globally, but the regional prevalence of HPV31, 33, 45, and 52 in North Africa underscores the need for locally optimized vaccine coverage and screening panels **[11, 40]**. Commercial assays that detect ≥14 high-risk genotypes (e.g., Cobas 4800, Anyplex II) offer a strategic advantage for molecular epidemiology and post-vaccination monitoring.

Future molecular workflows should integrate next-generation sequencing (NGS) or multiplex PCR genotyping, enabling fine-resolution tracking of variant evolution and co-infections (such as HPV–EBV, as observed in Algerian cohorts). This precision surveillance would inform vaccine impact assessments and guide second-generation vaccine introduction (e.g., nonavalent formulations).

6.3. Cost and sustainability

While cost remains a key obstacle, evidence suggests that centralized testing models—where PCR-based assays are batched regionally—can halve per-sample costs **[41]**. Combining self-sampling and pooled testing strategies could further increase efficiency in North African settings with limited lab networks **[26]**. The long-term sustainability of HPV molecular screening will depend on domestic production of reagents, regional procurement alliances, and integration with vaccination and public health infrastructure **[18]**.

6.4. Emerging technologies and translational opportunities

Several innovations are poised to make molecular HPV testing more accessible and clinically relevant. Isothermal amplification assays, such as loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA), offer field-adaptable solutions suitable for remote or resource-limited screening sites. Portable PCR platforms, including GeneXpert and QIAstat, enable

rapid testing with short turnaround times and minimal laboratory infrastructure. Moreover, mRNA-based oncogene detection methods like Aptima and PreTect Proofer provide higher specificity for identifying clinically relevant infections. Finally, advancements in digital pathology and AI-assisted cytology hold promise for integration with HPV testing, enabling automated triage and improving diagnostic efficiency (2022). Combining these technologies within screen-and-treat algorithms could accelerate early diagnosis and reduce cancer incidence by up to 60% over 20 years, as modeled in other LMIC contexts **[37]**.

6.5. Regional collaboration and policy frameworks

The Maghreb region shares similar epidemiological, cultural, and infrastructural profiles, presenting an opportunity for multinational collaboration in HPV control.

Creating a North African HPV Surveillance Consortium, which would bring together molecular laboratories, epidemiologists, and oncologists, would allow for standardized molecular testing protocols and robust QA/QC systems, pooled procurement to reduce costs, and the generation of cross-border data on genotype shifts and vaccine impact. This collaboration would also establish consistent protocols for screening, follow-up, and referral across the region. Such a project might work closely with the WHO's Global HPV Laboratory Network (GHLI) and the African Regional Cervical Cancer Elimination project (ARCCI). Furthermore, Algeria's ongoing cancer control strategy (Plan Cancer 2021-2030) **[16]** could provide a governmental foundation for incorporating molecular diagnostics into preventive oncology.

6.6. Future perspectives

In the next decade, North African countries could advance from opportunistic to organized screening by leveraging digital health platforms, mobile sample collection, and AI-enhanced diagnostics. Clinical laboratories should adopt HPV genotyping as a standard reflex test for all cervical intraepithelial neoplasia (CIN) and oropharyngeal cancers, ensuring data integration into national registries. Ultimately, linking molecular diagnostics, vaccination, and public education will be key to achieving equitable cancer prevention in the region.

Conclusion

Human papillomavirus (HPV) testing represents a cornerstone of modern cancer prevention. While molecular HPV assays have transformed cervical cancer screening, access and implementation vary considerably across regions. High-income countries increasingly adopt primary HPV testing as a standard, while resource-limited regions often rely on cytology or VIA (visual inspection with

acetic acid). Bridging these disparities requires integrating affordable molecular assays, improving laboratory infrastructure, and strengthening public health policies. Global harmonization of diagnostic standards and investment in next-generation testing technologies, including point-of-care and self-sampling methods, will be essential to achieving the WHO's goal of eliminating cervical cancer as a public health problem.

Conflict of Interest

The authors declare that there are no known competing financial interests or personal relationships that could have influenced the work reported in this article.

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