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Comparative performance of three HPV DNA detection assays for cervical cancer screening in Algeria

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Nabila Masdoua[1,2], Lamia Boublenza[2], Ilyes Zatla[3], Lamia Lablack[1], Nabila Sadouki[4] and Michel Segondy[5]*

Higher School of Biological Sciences of Oran, Oran, 31000, Algeria[1]; Laboratory of Microbiology applied to the Food industry, Biomedical and the Environment, Faculty of Natural and Life Sciences, Earth and Universe Sciences. Department of Biology. University of Tlemcen, Tlemcen, 13000, Algeria[2]; Independent Researcher, Tlemcen, Algeria[3]; Virus and Cancer Laboratory, Pasteur Institut, Algiers, 1600, Algeria[4]; CHU Montpellier, Saint-Eloi Hospital – University of Montpellier, UMR 1058 INSERM, MIVEGEC, PCCEI, Montpellier, France[5]

***For Correspondence**: Email*: ilyes.zatla@aol.com*; Phone: +213-540-315422

Abstract

Persistent infection with high-risk human papillomavirus (HR-HPV) is the principal etiological driver of cervical cancer, making accurate HPV detection central to contemporary screening strategies. This study compared three molecular assays—INNO-LiPA HPV Genotyping Extra, Cobas 4800 HPV, and Amplicor HPV—using overlapping cervical-sample subsets from Algerian women at increased risk of cervical disease. Among the 94 specimens tested by both INNO-LiPA and Cobas, HR-HPV was detected in 54.3% and 29.8%, respectively; INNO-LiPA also identified HPV16 and HPV18 more frequently. In the 18-specimen subset tested by all three assays, HR-HPV positivity was 27.8% with INNO-LiPA, 33.3% with Cobas, and 50.0% with Amplicor. Pairwise agreement ranged from weak to substantial (κ=0.116–0.667), and discordance was significantly associated with multiple HPV infections (OR=5.88, 95% CI 1.29–26.85; p=0.012). HR-HPV positivity was more frequent in specimens with abnormal cytology, with the strongest association observed for INNO-LiPA (OR=4.9, 95% CI 1.5–15.9; p=0.005). Using the study-defined composite reference classification, INNO-LiPA showed higher sensitivity (93%) but lower specificity (62%) than Cobas (71% and 88%, respectively). These findings demonstrate substantial assay-dependent variation in HR-HPV detection and highlight the value of detailed genotyping for epidemiological investigation, while supporting the use of clinically validated HR-HPV assays for screening applications. *(Afr J Reprod Health 2026; 30 [21]: 123-134).*

Keywords: Human papillomavirus; HR-HPV; INNO-LiPA; Cobas 4800; Amplicor; cervical cancer screening; molecular diagnostics

Résumé

L'infection persistante par le papillomavirus humain à haut risque (HPV-HR) constitue le principal facteur étiologique du cancer du col de l'utérus, ce qui fait de la détection précise du HPV un élément central des stratégies contemporaines de dépistage. Cette étude a comparé trois tests moléculaires — INNO-LiPA HPV Genotyping Extra, Cobas 4800 HPV et Amplicor HPV — à partir de sous-ensembles de prélèvements cervicaux provenant de femmes algériennes présentant un risque accru de lésions cervicales. Parmi les 94 échantillons analysés à la fois par INNO-LiPA et Cobas, un HPV-HR a été détecté dans 54,3 % et 29,8% des cas, respectivement ; INNO-LiPA a également identifié les génotypes HPV16 et HPV18 plus fréquemment. Dans le sous-ensemble de 18 échantillons analysés par les trois tests, la positivité pour le HPV-HR était de 27,8 % avec INNO-LiPA, de 33,3 % avec Cobas et de 50,0% avec Amplicor. L'accord entre les méthodes, évalué deux à deux, variait de faible à substantiel (κ = 0,116–0,667), tandis que les discordances étaient significativement associées aux infections multiples par le HPV (OR = 5,88 ; IC à 95% : 1,29–26,85 ; p = 0,012). La positivité pour le HPV-HR était plus fréquente dans les échantillons présentant une cytologie anormale, l'association la plus forte ayant été observée avec INNO-LiPA (OR = 4,9 ; IC à 95 % : 1,5–15,9 ; p = 0,005). Selon la classification de référence composite définie dans le cadre de cette étude, INNO-LiPA présentait une sensibilité plus élevée (93%), mais une spécificité plus faible (62 %) que Cobas (71% et 88%, respectivement). Ces résultats mettent en évidence une variation substantielle de la détection du HPV-HR selon le test utilisé et soulignent l'intérêt du génotypage détaillé dans les études épidémiologiques. Ils confortent également l'utilisation de tests de détection du HPV-HR ayant fait l'objet d'une validation clinique pour les applications de dépistage. *(Afr J Reprod Health 2026; 30 [21]: 123-134).*

Keywords: Papillomavirus humain; HPV-HR ; INNO-LiPA ; Cobas 4800 ; Amplicor ; dépistage du cancer du col de l'utérus ; diagnostics moléculaires

Introduction

Human papillomaviruses (HPVs) are epitheliotropic DNA viruses responsible for a broad spectrum of cutaneous and mucosal lesions and are among the most prevalent sexually transmitted infections worldwide.[1–4] Most HPV infections are transient and resolve spontaneously; however, persistent infection with oncogenic genotypes is the critical event underlying cervical carcinogenesis.[5]

Among the more than 100 HPV genotypes historically described, a subset is classified as high risk because of its strong association with cervical and other anogenital precancers and cancers.[6] Persistent HR-HPV infection is considered a necessary cause of cervical cancer, and HR-HPV DNA is detected in the great majority of invasive cervical cancers.[7–9] Cervical cancer remains a major global health burden, with a disproportionate impact in settings where organized screening and preventive services are limited.[10,11] The burden of persistent HPV infection and HPV-related disease is particularly important among women living with human immunodeficiency virus (HIV), who experience greater persistence of oncogenic HPV and an increased risk of cervical neoplasia.[12] Because progression from persistent infection to high-grade precancer and invasive disease generally occurs over several years, cervical cancer is highly amenable to screening and prevention.

Organized cervical-screening programs have substantially reduced cervical cancer incidence in many high-income settings. Cytology has traditionally been the cornerstone of screening, but molecular detection of oncogenic HPV has become increasingly important because it targets the causal agent and offers high sensitivity for clinically relevant cervical disease. In Algeria, cervical cancer prevention has historically relied predominantly on cytological screening, while molecular HPV testing and HPV vaccination have had limited integration into routine services. Epidemiological information on HPV prevalence, genotype distribution, and assay performance in Algerian populations—particularly among women with HIV or gynecological pathology—also remains limited.

The present study therefore evaluated HPV infection in Algerian women at increased risk of cervical abnormalities, including women living with HIV and women presenting with gynecological conditions. The primary objective was to compare the detection performance and agreement of three HPV assays and to examine their relationship with cervical cytology. The assays assessed were the INNO-LiPA HPV Genotyping Extra assay, which provides broad genotype-level characterization, and the Cobas 4800 HPV and Amplicor HPV assays, which were developed for high-risk HPV detection in clinical testing contexts.

Methods

Sampling

Cervicovaginal specimens for HPV testing were collected with a cytobrush and preserved in ThinPrep liquid medium (Hologic, Villepinte, France) after collection of a conventional cervical smear for cytological examination. Sampling was conducted in two phases.

First collection: 98 cervicovaginal specimens were obtained from women attending private gynecology and pathology practices in Tlemcen between December 2010 and October 2011. Amplicor testing was performed at the Pasteur Institute of Algeria laboratory in October 2011. Eighteen of these specimens were subsequently tested with INNO-LiPA HPV Genotyping Extra at INSERM U1058, Montpellier, in January 2012 and with Cobas 4800 HPV at the Ibn Sina laboratory, Constantine, in June 2012.

Second collection: 88 cervicovaginal specimens comprised 56 samples collected from the same Tlemcen facilities and 32 samples from women living with HIV attending the STI/HIV/AIDS reference center for Western Algeria at the University Hospital of Oran. These specimens were collected from October 2011 to January 2012 and tested with INNO-LiPA in Montpellier in January 2012. Seventy-six were also tested with Cobas 4800 HPV at the Ibn Sina laboratory in June 2012.

Overall, 18 specimens from the first collection had valid results from all three assays (Amplicor, INNO-LiPA, and Cobas), whereas 76 specimens from the second collection were available for paired INNO-LiPA/Cobas comparison.

Inclusion and exclusion criteria

Exclusion criteria

For the paired INNO-LiPA/Cobas analysis, specimens were excluded if either assay was not

performed or yielded an invalid result, or if a corresponding conventional cervical-smear result was unavailable.
For the three-assay comparison, the same criteria were applied and specimens without a valid Amplicor result were additionally excluded.

Inclusion criteria

The paired INNO-LiPA/Cobas analysis included 94 specimens (18 from the first collection and 76 from the second) with valid results from both assays and an available cytological interpretation.
The three-assay comparison included the 18 specimens with valid Amplicor, INNO-LiPA, and Cobas results and an available cytological interpretation.

Description of assays

The three molecular platforms differed in analytical principle, genotype coverage, and amplicon length (Table 1). As described above, Amplicor, INNO-LiPA, and Cobas testing were performed at their respective study laboratories. Cobas 4800 HPV is a multiplex real-time PCR assay that individually detects HPV16 and HPV18 while concurrently detecting a pool of 12 other high-risk genotypes (HPV31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68).
The Cobas workflow comprised two principal steps: Automated sample preparation on the Cobas x480 instrument, including nucleic-acid extraction and purification using magnetic glass particles under chaotropic conditions.
Real-time PCR amplification and fluorescence detection targeting an approximately 200-nucleotide region of L1, with a human β-globin target serving as an internal control. Separate fluorescence channels identify β-globin, HPV16, HPV18, and the pooled group of 12 other HR-HPV genotypes. The Cobas 4800 HPV assay received U.S. FDA approval in 2011 and has been used in cervical cancer screening and triage applications.

Study design

For the paired INNO-LiPA/Cobas analysis, only the 94 specimens with valid results from both assays were considered. To harmonize genotype coverage, INNO-LiPA was classified as HR-HPV positive only when it detected one of the 14 genotypes targeted by Cobas; low-risk, probable high-risk, and undetermined-risk types were treated as negative for this specific comparison. Results were also compared according to oncogenic category (HPV16, HPV18, other HR-HPV, or negative).
For analyses involving Amplicor, only the 18 specimens tested by all three assays were included, and INNO-LiPA positivity was restricted to the 13 HR-HPV genotypes targeted by Amplicor.
Assay results were subsequently examined in relation to the corresponding cytological findings.
For the study-defined sensitivity and specificity analysis, a composite reference classification was constructed as follows:
A specimen was classified as reference-positive when INNO-LiPA and Cobas were both positive, or when a discordant specimen was supported as positive by Amplicor and/or abnormal cytology.
A specimen was classified as reference-negative when INNO-LiPA and Cobas were both negative, or when a discordant specimen had both a negative Amplicor result and normal cytology. Because histological confirmation was unavailable, these estimates should be interpreted as study-defined comparative performance rather than definitive clinical sensitivity and specificity for CIN2+ or CIN3+ disease.

Table 1: Analytical characteristics of the three HPV assays

Test	INNO-LiPA	Cobas	Amplicor
HPV types detected	28 genotypes	14 high-risk genotypes	13 high-risk genotypes
Identified types	6 ; 11 ; 16 ; 18 ; 26 ; 31 ; 33 ; 35 ; 39 ; 40 ; 43 ; 44 ; 45 ; 51 ; 52 ; 53 ; 54 ; 56 ; 58 ; 59 ; 66 ; 68 ; 69 ; 71 ; 70 ; 73 ; 74 ; 82.	16, 18 (individually)	Not specified
Principle	DNA amplification + reverse hybridization + chromogenic detection	Real-time PCR + fluorescent detection	DNA amplification + hybridization + absorbance reading
Amplicon size	65 bp	200 bp	165 bp
Target region	L1	L1	L1
Manufacturer	Innogenetics®	Roche Diagnostics®	Roche Diagnostics®
Approval	CE-marked for EU use	FDA approved	CE-marked for EU use

Statistical analysis

Statistical analyses were performed in the Epidemiology Department of CHU Tlemcen using SPSS version 21.
Inter-assay agreement was quantified using Cohen's kappa (κ), interpreted as follows:
0.00–0.20: very weak
0.21–0.40: weak
0.41–0.60: moderate
0.61–0.80: strong
0.81–1.00: almost perfect
McNemar's test was used to assess asymmetry in paired assay results.
Associations were evaluated using chi-square tests and odds ratios (ORs) with 95% confidence intervals (CIs).
Microsoft Excel 2007 was used for data processing and supplementary calculations. A two-sided p value <0.05 was considered statistically significant.

Ethical considerations

Ethical requirements relating to informed consent, anonymity, and confidentiality were addressed during the study. Ethical clearance was obtained from the Scientific Research Ethics Committee of the University of Tlemcen, Algeria.

Results

HR-HPV detection across all assay-specific samples

Across all specimens tested by each platform, HR-HPV was detected in 17.1% of the 76 specimens analyzed with Amplicor, 52.0% of the 123 specimens analyzed with INNO-LiPA, and 29.8% of the 94 specimens analyzed with Cobas. These percentages arise from different assay-specific sample sets and therefore should not be interpreted as a direct head-to-head comparison.

HPV detection in directly comparable sample subsets

Among the 94 specimens tested by both INNO-LiPA and Cobas, HR-HPV positivity was 54.3% and 29.8%, respectively. INNO-LiPA detected HPV16 in 24.5% and HPV18 in 14.9% of specimens, compared with 8.5% and 2.1%, respectively, with Cobas. When all genotypes detectable by INNO-LiPA were considered, irrespective of Cobas genotype coverage, overall HPV positivity was 60.6%.
Within the 18-specimen subset tested by all three assays, HR-HPV positivity was 50.0% with Amplicor, 33.3% with Cobas, and 27.8% with INNO-LiPA (Table 2).

Table 2: HPV detection rates according to assay and genotype category

Test	N	Genotypes Detected	HPV Rate (%)
Cobas	94	14 HR-HPV[1]	28 (29.8)
		HPV 16	8 (8.5)
		HPV 18	2 (2.1)
		Other HR	23 (24.5)
INNO-LiPA	94	28 HPV	57 (60.6)
		13 HR-HPV[2]	49 (52.1)
		14 HR-HPV[1]	51 (54.3)
		HPV 16	23 (24.5)
		HPV 18	14 (14.9)
		Other HR	28 (29.8)
		Low-Risk HPV (LR)	11 (11.7)
Amplicor	18	13 HR-HPV[2]	9 (50.0)

[1] Among the 14 genotypes detectable by Cobas
[2] Among the 13 genotypes detectable by Amplicor

Inter-assay agreement for HR-HPV detection

Pairwise concordance

Among the 18 specimens tested by all three assays, 14 (77.8%) yielded concordant results across the relevant pairwise comparisons. Among the 94 specimens tested by INNO-LiPA and Cobas, 51 (54.2%) were concordant. Pairwise agreement was weakest between INNO-LiPA and Cobas (κ=0.116), intermediate between INNO-LiPA and Amplicor (κ=0.556), and highest between Cobas and Amplicor (κ=0.667) (Table 3).

Direction of inter-assay discordance

Among the 94 paired INNO-LiPA/Cobas specimens, 43 (45.7%) were discordant for binary HR-HPV positivity: 33 (35.1% of all paired specimens) were INNO-LiPA positive/Cobas negative and 10 (10.6%) were Cobas positive/INNO-LiPA negative.
Of these 43 binary discordances, 76.7% (33/43) were INNO-LiPA positive/Cobas negative.

Table 3: Pairwise concordance and agreement among HPV assays

Comparison	N	Pos/Pos (%)	Pos/Neg (%)	Neg/Pos (%)	Neg/Neg (%)	*P-value	% Agreement	Kappa (κ)
INNO-LiPA vs Cobas	94	18 (19.1)	33 (35.1)	10 (10.6)	33 (35.1)	0.001	54.2	0.116
INNO-LiPA vs Amplicor	18	5 (27.8)	0	4 (22.2)	9 (50.0)	0.125	77.8	0.556
Cobas vs Amplicor	18	6 (33.3)	0	3 (16.7)	9 (50.0)	0.250	83.3	0.667

*P-value from McNemar's test

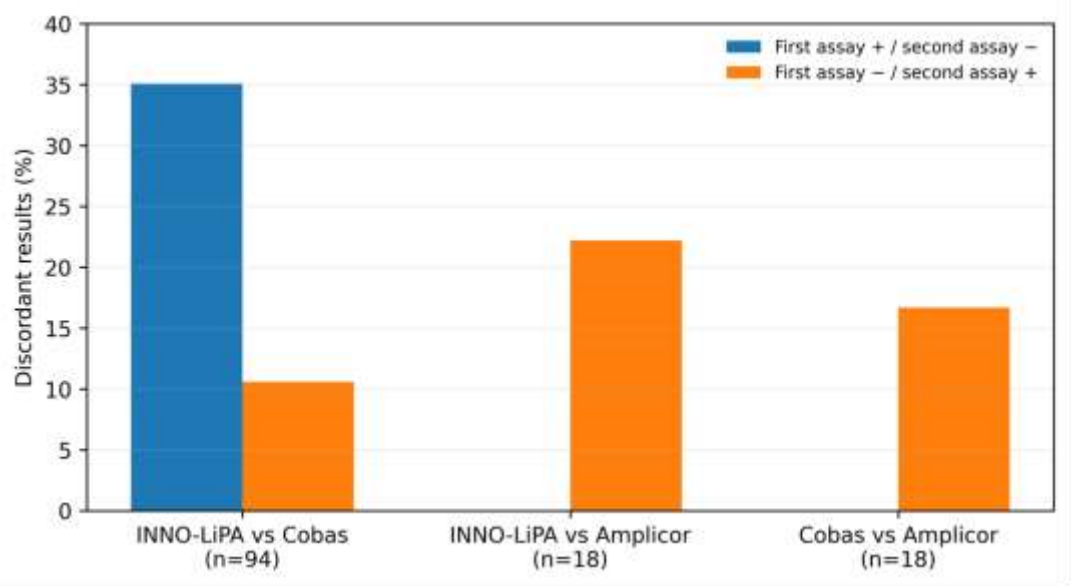


Figure 1: Direction and magnitude of discordant HR-HPV results between assay pairs. Percentages are calculated within each pairwise comparison. Positive/negative indicates that the first assay was positive and the second negative; negative/positive indicates the reverse.

Among the 10 Cobas-positive/INNO-LiPA-negative specimens, four (40%) contained low-risk or probable high-risk genotypes detectable by INNO-LiPA (HPV11, HPV54, HPV70, or HPV53). Among the 18 specimens tested by all three assays, four (22.2%) were Amplicor positive/INNO-LiPA negative; three of these were also Cobas negative (Figure 1).

Comparison of INNO-LiPA and Cobas by oncogenic category

Concordance by oncogenic category

When results were classified as HPV16, HPV18, other HR-HPV, or negative, agreement between INNO-LiPA and Cobas remained low (κ=0.183; Table 4).

Discordance by oncogenic category

When genotype category was considered, 47 of 94 specimens (50.0%) showed discordant classifications. INNO-LiPA identified HPV16 (24.5% vs 8.5%) and HPV18 (7.4% vs 1.1%) more frequently than Cobas within this cross-classified analysis. The 47 discordant classifications comprised:

INNO-LiPA HPV16/Cobas negative: 18/47 (38.3%).

INNO-LiPA HPV18/Cobas non-HPV18: 6/47 (12.8%).

INNO-LiPA other HR-HPV/Cobas discordant category: 9/47 (19.2%).

Cobas HPV16/INNO-LiPA non-HPV16: 3/47 (6.4%).

Cobas other HR-HPV/INNO-LiPA negative: 7/47 (14.9%).

Table 4: Cross-classification of INNO-LiPA and Cobas results by oncogenic category

Cobas Result	INNO-LiPA HPV 16	HPV 18	Other HR	Negative	Total (%)
HPV 16	3	0	2	3	8 (8.5)
HPV 18	0	1	0	0	1 (1.1)
Other HR	2	0	10	7	19 (20.2)
Negative	18	6	9	33	66 (70.2)
Total (%)	23 (24.5)	7 (7.4)	21 (22.3)	43 (45.7)	94 (100)

κ=0.183; agreement=50.0%; McNemar p=0.002

Table 5: HPV detection by assay and cytological category

Test	N	Genotypes Detected	Normal Cytology (n=73) (%)	Abnormal Cytology (n=21) (%)	ASCUS	LSIL	HSIL
Cobas	94	14 HR-HPV	18 (24.7)	10 (47.6)	2	5	3
		HPV 16	4 (5.5)	4 (19.1)	0	1	3
		HPV 18	1 (1.4)	1 (4.8)	1	0	0
		Other HR	17 (23.3)	6 (28.6)	1	4	1
INNO-LiPA	94	28 HPV	39 (53.4)	18 (85.7)	4	11	3
		13 HR-HPV	32 (43.8)	17 (81)	4	11	2
		14 HR-HPV	34 (46.6)	17 (81)	4	11	2
		HPV 16	18 (24.7)	5 (23.8)	0	3	2
		HPV 18	9 (12.3)	5 (23.8)	2	2	1
		Other HR	18 (26)	10 (47.6)	3	7	0
		LR HPV	8 (11)	3 (14.3)	0	2	1
Amplicor	18	13 HR-HPV	5 (66.7)	4 (80.0)	0	2	2

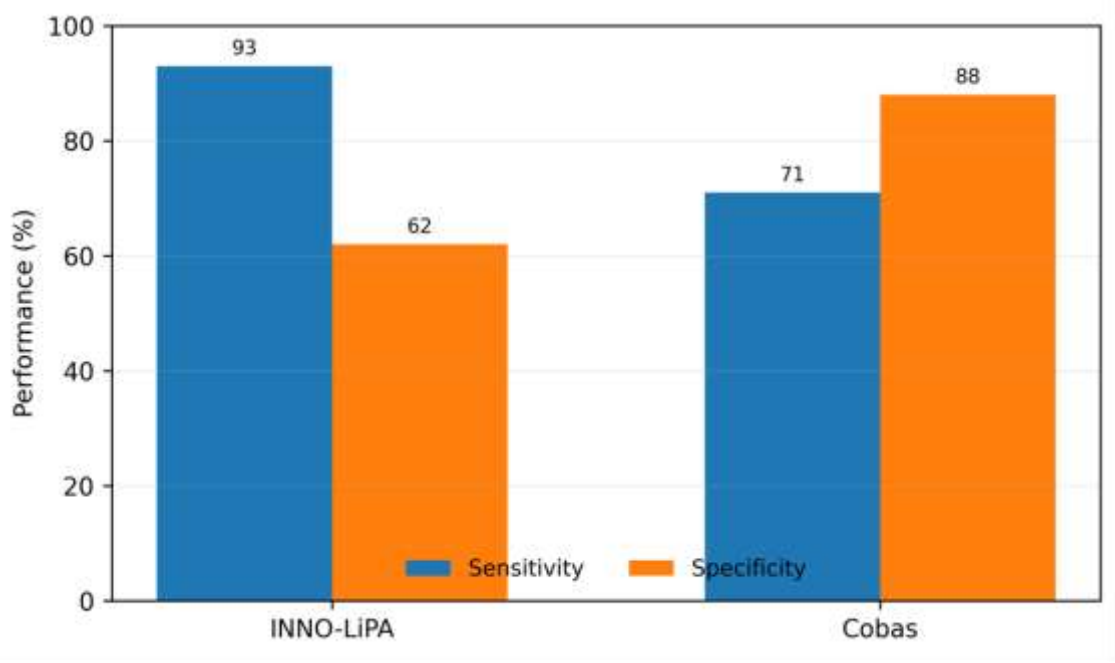


Figure 2: Study-defined sensitivity and specificity of INNO-LiPA and Cobas for HR-HPV detection, based on the composite reference classification described in Methods

Four of 47 discordant classifications (8.5%) occurred when both assays were positive but assigned different oncogenic categories.

Association between multiple HPV infection and assay discordance

Multiple HPV infection was associated with discordant assay results (χ^2=6.285, p=0.012; OR=5.877, 95% CI 1.286–26.853), supporting the possibility that genotype multiplicity contributes to inter-assay disagreement.

Assay results in relation to cervical cytology

Among the 94 specimens with paired assay and cytology data, 73 (77.7%) had normal cytology and 21 (22.3%) had abnormal cytology, including ASC-US (6.4%), LSIL (12.8%), and HSIL (3.2%). HR-HPV positivity was more frequent in specimens with abnormal cytology across assays (Table 5).

Inter-assay concordance by cytology status

Inter-assay concordance was generally higher among specimens with abnormal cytology; in the small three-assay subset, Cobas and Amplicor showed complete agreement among abnormal-cytology specimens (κ=1.0). This subgroup result should be interpreted cautiously because of the limited sample size.

Association between HPV detection and abnormal cytology

INNO-LiPA showed the strongest association between HR-HPV positivity and abnormal cytology (χ^2=7.765, p=0.005; OR=4.9, 95% CI 1.5–15.9) (Table 5).

Study-defined comparative sensitivity and specificity

Using the composite reference classification defined in Methods, INNO-LiPA showed higher sensitivity (93%) but lower specificity (62%) than Cobas (71% sensitivity and 88% specificity) (Figure 2). These estimates describe performance against the study-defined reference and not histologically verified CIN2+/CIN3+ endpoints.

Discussion

The observed HPV positivity varied substantially by assay and by the sample subset analyzed. Across all assay-specific specimens, positivity was 52.0% with INNO-LiPA, 29.8% with Cobas, and 17.1% with Amplicor; however, these overall proportions were derived from different denominators and should not be compared as if they arose from the same cohort.[13–17] In the directly comparable subsets, the same general pattern of assay-dependent variability remained evident, particularly between INNO-LiPA and Cobas. The relatively high HPV burden and frequency of cytological abnormalities in this study are consistent with the enriched-risk composition of the cohort, which included women living with HIV and/or women with gynecological pathology.

HPV detection is strongly influenced by assay design, analytical sensitivity, genotype coverage, and the viral burden of the specimen.[18] Among the three methods, the highest pairwise agreement was observed between Cobas and Amplicor (κ=0.667), followed by INNO-LiPA and Amplicor (κ=0.556), whereas agreement between INNO-LiPA and Cobas was weak (κ=0.116). The Cobas–Amplicor estimate is directionally consistent with previous comparative work, although direct numerical comparison is limited by differences in study populations and reference methods.[19]

Direct head-to-head evidence involving these exact three platforms is limited. The present findings can therefore be interpreted in the context of studies using Hybrid Capture 2 (HC2), a historically important clinically validated HR-HPV assay targeting 13 high-risk genotypes.[20,21] Comparisons of INNO-LiPA, Amplicor, Cobas, and HC2 across prior studies have shown that assay agreement can vary substantially according to analytical thresholds, genotype spectrum, specimen type, and clinical context.[22–35]

The moderate agreement between Amplicor and INNO-LiPA is broadly compatible with prior studies comparing SPF10-LiPA-based genotyping with clinically oriented HR-HPV assays.[22–30] Nevertheless, the present comparison involved only 18 specimens, so the corresponding κ estimates are imprecise and should not be overinterpreted. The particularly low INNO-LiPA/Cobas agreement

likely reflects differences in analytical sensitivity and genotype-level resolution rather than a simple difference in overall assay quality. INNO-LiPA can identify a broader range of HPV genotypes, whereas Cobas is optimized for clinically relevant HR-HPV detection with separate reporting of HPV16 and HPV18.[31–35]

INNO-LiPA uses highly sensitive short-fragment PCR amplification followed by reverse hybridization for genotype identification.[16] The short amplicon can facilitate amplification when viral DNA is present at low concentration or partially degraded, and primer design influences both analytical sensitivity and genotype representation.[36–39] These characteristics may contribute to the higher positivity observed with INNO-LiPA.

Inter-assay concordance may also be influenced by pre-analytical factors such as sampling, transport medium, storage duration and temperature, participant characteristics, and whether assays are performed on the same aliquot or on sequential aliquots from a common specimen.[33,40,41] These factors are especially relevant when discrepant results occur near assay detection thresholds.

ThinPrep was used throughout the present study and is an established liquid-based cytology medium; nevertheless, assay performance can differ across media and storage conditions.[35,42,43] The interval between testing sessions, storage at −20°C, and differences in aliquot handling may have affected DNA yield or the distribution of low-copy HPV DNA between aliquots. These factors offer plausible explanations for some of the observed discordance.

In the small three-assay subset, three specimens (16.7%) were Amplicor positive but negative by both INNO-LiPA and Cobas. Because Amplicor was performed on earlier aliquots and the other assays on later aliquots, stochastic distribution of low-copy DNA, storage effects, or platform-specific detection thresholds are more cautious explanations than assuming that one platform necessarily generated false results.[35,44]

Four of the 10 Cobas-positive/INNO-LiPA-negative specimens contained low-risk or probable high-risk genotypes detectable by INNO-LiPA. Cross-reactivity has been described for some HPV assays, but the present data alone cannot establish cross-reactivity as the mechanism; alternative explanations include coinfection, genotype competition, low-copy HR-HPV, or sampling variability.[35,40,43,45]

Multiple infection was significantly associated with discordance (OR=5.877, p=0.012). In mixed infections, genotype abundance and primer competition can affect amplification efficiency, potentially leading different assays to identify different components of the same infection.[44,46] This finding supports genotype multiplicity as one contributor to assay disagreement.

The higher concordance observed in specimens with abnormal cytology is biologically plausible because clinically significant lesions may contain higher concentrations of oncogenic HPV DNA, increasing the probability of detection across platforms.[32,37,40]

Against the study-defined composite reference, INNO-LiPA showed higher sensitivity and lower specificity than Cobas. This pattern is consistent with the general trade-off between analytical sensitivity and clinical specificity: detecting very low viral loads can increase positivity without necessarily improving identification of clinically significant high-grade lesions.[22,23,29,38,47] Because histological endpoints were unavailable, the present sensitivity and specificity estimates should not be interpreted as validated performance for CIN2+ or CIN3+ detection.

Each platform offers different practical strengths. Amplicor targets 13 HR-HPV genotypes but does not provide genotype-specific results. Cobas detects a pool of other HR-HPV types while individually reporting HPV16 and HPV18, an approach that supports genotype-based risk stratification.[34,35,48,49] INNO-LiPA provides broader genotype resolution and is particularly informative for epidemiological studies, mixed infections, and investigations requiring type-specific characterization.

Accordingly, assay selection should be driven by the intended application. Broad genotyping platforms are valuable for research and surveillance, whereas primary screening requires clinically validated assays with demonstrated performance against high-grade cervical disease endpoints.

Study strengths and limitations

This study provides a direct comparison of three HPV molecular assays within overlapping specimens from the same clinical population. The use of paired samples reduced inter-individual variability, cytological data allowed clinically relevant stratification, and analysis of discordant and multiple infections provided insight into factors that may influence assay agreement.

Several limitations should be considered. The cohort was enriched for women living with HIV and/or women with gynecological pathology and was therefore not representative of a population-based screening cohort. Only 18 specimens were available for the three-assay comparison, limiting precision of agreement estimates involving Amplicor. Assays were performed at different times and, in some cases, on sequential aliquots, so storage and aliquot effects may have contributed to discordance. ThinPrep was the only collection medium evaluated. Most importantly, histological confirmation of CIN2+ or CIN3+ was unavailable; therefore, the reported sensitivity and specificity values are based on a study-defined composite reference and cannot substitute for clinical validation against disease endpoints.

Despite these limitations, the study offers useful comparative evidence on assay agreement, genotype detection, and cytological associations in an Algerian high-risk clinical context.

Conclusion

The three HPV assays showed meaningful differences in positivity, agreement, and genotype resolution. INNO-LiPA detected the broadest range of HPV infections and was particularly informative for genotype-level characterization, whereas Cobas provided clinically oriented HR-HPV detection with separate identification of HPV16 and HPV18. Amplicor showed the highest positivity in the small three-assay subset, but conclusions involving this platform are limited by the sample size. Multiple infections were associated with discordance, and agreement improved in specimens with abnormal cytology. These findings reinforce that HPV assay results are not interchangeable and that assay selection should match the clinical or research objective.

For cervical screening, analytical sensitivity alone is insufficient; clinically validated performance against high-grade disease endpoints remains essential. In research and surveillance, broader genotyping can provide complementary information on genotype diversity and coinfection patterns. Future Algerian studies should include larger population-based cohorts, standardized contemporaneous testing, and histological endpoints to define clinically relevant assay performance more precisely.

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Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon request.

Author contributions

All authors contributed to the work, reviewed the results, approved the final manuscript, and accept responsibility for its content.

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