Laboratory Performance in Molecular Detection of High-Risk HPV: Commercial Kits Outperform Laboratory-Developed Tests

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BACKGROUND

High-risk HPV causes cervical cancer, a global health challenge. Molecular DNA testing enables early detection, allowing timely treatment, improving screening, stratifying risks, and optimizing follow-ups. Reliable results require standardized laboratory performance. External Quality Assessment Programs (EQAP) ensure accuracy (AC) and harmonization in HPV diagnostics.

AIM

To evaluate the performance of laboratories in an EQAP for high-risk HPV detection, organized by a provider accredited to ABNT NBR ISO/IEC 17043:2011.

METHODS

The EQAP consisted of liquid samples from cervical brushing or cell lysate. Participating laboratories received two samples quarterly to evaluate the accuracy (AC), sensitivity (SE), and specificity (SP), which were thoroughly described. Accuracy trends over time were analyzed using the Mann-Kendall test. Sub-group comparisons included in-vitro diagnostic (IVD) kits versus laboratory-developed tests (LDTs), WHO-recommended kits versus others (non-WHO), and qPCR versus non-qPCR methods. Chi-square tests and odds ratios were employed for statistical comparisons (Table 1).

Table 1: Study Design

Component	Details
Sample Type	Cervical brushing or cell lysate (liquid format)
Distribution	Two samples per quarter sent to participating labs
Evaluated Parameters	Accuracy, sensitivity, and specificity
Temporal Trend	Mann-Kendall test applied to accuracy over time
Subgroup Comparisons	IVD vs LDTs; WHO-recommended vs non-WHO; qPCR vs non-qPCR
Inferential Statistics	Chi-square test and Odds Ratio (OR) with 95% CI

RESULTS

Between 2020 and 2024, 69 labs participated in multiple rounds of the program, with a median of 18 laboratories/round (range: 5–51), generating a total of 692 datasets (Table 2). The laboratories achieved 97.98% (678/692) AC, 97.79% (620/634) SE, and 100% (58/58) SP (Table 3). No significant trends in AC were observed over time (τ=-0.046, p=0.795). IVD kits outperformed LDTs, achieving 98.6% (612/621) AC compared to 93.0% (66/71) (OR=5.15, 95% CI 1.68–15.83, p=0.009). WHO-recommended kits and non-WHO kits demonstrated comparable AC rates of 98.4% (185/188) and 98.6% (427/433), respectively (OR=0.87, 95% CI 0.21–3.50, p=1.0). Similarly, qPCR methods showed similar performance to non-qPCR methods, with AC rates of 98.4% (541/550) versus 96.5% (136/141) (OR=2.21, 95% CI 0.73–6.70, p=0.176) (Table 4).

Table 2: Participant Overview:

Participation metric	Results
Total Datasets Generated	692 samples (2020–2024)
Laboratories Enrolled	69 labs; median of 18 per round (range: 5–51)

Table 3: Performance over time:

Performance		
AC: 97.98%	SE: 97.79%	SP: 100%

Table 4: Subgroup comparisons:

IVD kits	LDTs	Statistics
AC: 98.6%	AC: 93%	OR=5.15, 95% CI 1.68-15.83, p=0.009
WHO-recommended kits	**Non-WHO kits**	**-**
AC: 98.4%	AC:98.6%	OR=0.87, 95% CI 0.21-3.50, p=1,0
qPCR methods	**Non-qPCR methods**	-
AC: 98.4%	AC: 96.5%	OR=2.21, 95% CI 0.73-6.70, p=0.176

Legend: Odds ratio (OR); Confidence Interval (CI)

CONCLUSIONS

The High-risk HPV EQAP demonstrated robust diagnostic performance, with IVD kits significantly outperforming LDTs. Both WHO-recommended and non-WHO kits, as well as qPCR and non-qPCR methods, exhibited comparable AC. These findings highlight the quality of HPV diagnostics in participating laboratories and support preferring IVD tests to address the global burden of HPV-related diseases.

DISCLOSURE

The authors confirm that they don't have any conflict of interest to declare.

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