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Clinical validation of the Vitro HPV screening assay for its use in primary cervical cancer screening

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Since HPV DNA tests, which are more sensitive than traditional cytology, are increasingly recommended as the primary screening tool, many commercial assays have been launched on the market. Independent clinical validation before its introduction in screening is essential[1]. The guidelines published in 2009 by Meijer et al. describe the appropriate methodology to evaluate

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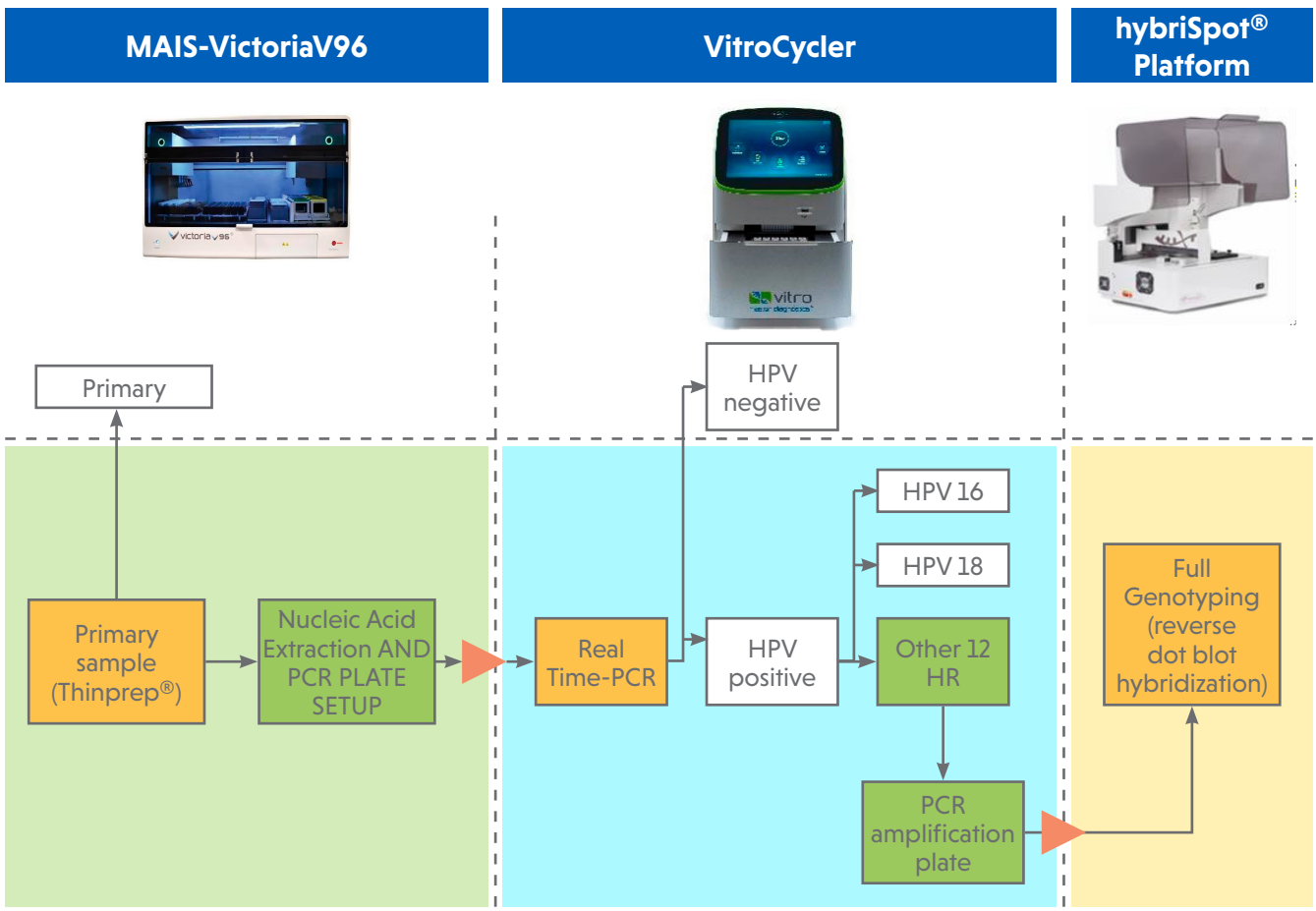
As HPV DNA tests are increasingly recommended as the primary screening tool, many commercial assays have launched on the market, making independent clinical validation essential before their introduction into screening programs.

clinical sensitivity, specificity, and reproducibility for detecting cervical intraepithelial neoplasia grade 2 or worse (CIN2+) of a new HPV test[2].

The Vitro HPV Screening assay (Vitro S.A., Sevilla, Spain) is a fully automated real-time multiplex PCR test targeting the L1 region of the HPV genome. The assay distinguishes HPV16 and HPV18 individually, while the other 12 high-risk (HR) HPV genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) are initially detected as a pool. However, these can be further individually genotyped using the complementary HPV *Direct Flow CHIP* test on the hybriSpot® Platform. The assay utilises a modular, automated, integrated system (MAIS-VictoriaV96) for DNA extraction and PCR setup, ensuring sample traceability and minimising hands-on time. The human beta-globin gene is co-amplified to verify the integrity of the sample. The Vitro HPV Screening assay workflow is shown in Figure 1.

Figure 1
Vitro HPV Screening assay workflow.



The Vitro HPV Screening assay distinguishes HPV16 and HPV18 individually, while the other 12 high-risk HPV genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) are initially detected as a pool, and further individually genotyped using the complementary HPV Direct Flow CHIP test.

Samples were collected from women aged 30 and above in Barcelona, Spain, who were undergoing routine HPV-based cervical cancer screening. The series included 60 cases with histologically confirmed CIN2+ lesions and 844 cases with negative or <CIN2 findings. All samples were collected using ThinPrep® medium (Hologic) and initially tested with the Cobas® 4800 HPV assay (Roche), which served as the comparator[3].

For extended genotyping, the PCR product from positive 12 HR-HPV different from HPV16 and HPV18 samples was hybridised with the HPV Direct Flow CHIP test, based on reverse dot blot hybridisation and colourimetric detection, to identify the 12 HR-HPV genotypes individually. This assay can simultaneously genotype 35 HPV genotypes (both high and low-risk genotypes).

The Vitro HPV assay demonstrated 100% clinical sensitivity and 87.2% specificity for CIN2+, showing non-inferiority to Cobas® 4800.

Intra- and inter-laboratory reproducibility studies were conducted by testing 561 samples in duplicate across two institutions. Consistency in results was assessed via observed agreement and Cohen's Kappa statistical test.

The Vitro HPV assay demonstrated 100% clinical sensitivity and 87.2% specificity for CIN2+, showing non-inferiority to Cobas® 4800 ($p=0.0049$ for sensitivity, $p<0.001$ for specificity). Intra-laboratory agreement was 100% (95% confidence interval [95%CI] 99.2–100) with a Kappa of 1.0, and inter-laboratory agreement was 97.3% (95%CI 95.5–98.4) with a Kappa of 0.95, confirming strong test reliability.

For individual HPV genotypes, the intra-laboratory agreement ranged from 98.2 to 100% with Kappa values between 0.91 and 1.00, except for HPV68 (kappa=0.79). Regarding inter-laboratory reproducibility, the agreement was >96% across all individual genotypes, with kappa values ranging from 0.86 to 0.97.

Among CIN2+ cases, excellent agreement was observed between Vitro and Cobas® for HPV16 and HPV18 (Kappa = 1.0) and the pooled 12 HR-HPV genotypes (Kappa = 0.96 [95%CI: 0.90–1.00]). HPV31, HPV52, and HPV58 were the most frequently detected genotypes.

In <CIN2 cases, excellent agreement (Kappa =1.0) was also found for both HPV16 and HPV18, and 0.99 (95%CI: 0.98–1.00) for the 12 HR-HPV, with HPV66 being the most prevalent genotype.

Extended genotyping showed high concordance in both intra- and inter-laboratory analyses.

Conclusion

The Vitro HPV Screening assay meets all international validation criteria, proving itself a suitable and reliable candidate for cervical cancer screening. Its automation, the availability of results with extended genotyping, and intra- and interlaboratory reproducibility for all HPV-AR genotypes support its potential application in population screening programs. While current guidelines do not mandate individual genotype validation, the data presented supports its clinical relevance for future risk stratification, especially as vaccinated populations age into the screening cohort. ■

CONFLICTS OF INTEREST

Vitro® supplied the plastic products, reagents, and equipment for running all Vitro HPV Screening tests and all HPV Direct Flow CHIP tests in both centers (Hospital del Mar and Catalan Institute of Oncology) for this study, as well as limited funding for coverage of derived costs in both places. Vitro® had no role in the study design, data collection, analysis, or interpretation of the data, manuscript preparation, and the decision to publish present manuscript.

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