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Assessment of the Roche Linear Array HPV Genotyping Test within the VALGENT framework

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ABSTRACT

Background: Cervical cancer screening programs are switching from cytology-based screening to high-risk (hr) HPV testing. Only clinically validated tests should be used in clinical practice.
Objectives: To assess the clinical performance of the Roche Linear Array HPV genotyping test (Linear Array) within the VALGENT-3 framework.
Study design: The VALGENT framework is designed for comprehensive comparison and clinical validation of HPV tests that have limited to extended genotyping capacity. The Linear Array enables type-specific detection of 37 HPV types. For the purpose of this study, Linear Array results were designated as positive only if one of the 13 hrHPV types also included in the Hybrid Capture 2 (HC2) was detected. The VALGENT-3 framework comprised 1600 samples obtained from Slovenian women (1300 sequential cases from routine cervical cancer screening enriched with 300 cytological abnormal samples). Sensitivity for cervical intraepithelial neoplasia of grade 2 or worse (CIN2 +) (n = 127) and specificity for < CIN2 (n = 1216) were assessed for Linear Array and for HC2 and non-inferiority of Linear Array relative to HC2 was checked. In addition, the prevalence of separate hrHPV types in the screening population, as well as the concordance for presence of HPV16, HPV18 and other hrHPV types between Linear Array and the Abbott RealTime High Risk HPV test (RealTime) were assessed.
Results: The clinical sensitivity and specificity for CIN2+ of the Linear Array in the total study population was 97.6% (95% CI, 93.3–99.5%) and 91.7% (95% CI, 90.0–93.2%), respectively. The relative sensitivity and specificity of Linear Array vs HC2 was 1.02 [95% CI, 0.98–1.05, ($p < 0.001$)] and 1.02 [95% CI, 1.01–1.03, ($p < 0.001$)], respectively The overall prevalence of hrHPV using the Linear Array in the screening population was 10.5% (95% CI, 8.9–12.3%) with HPV16 and HPV18 detected in 2.3% and 0.9% of the samples, respectively. Excellent agreement for presence or absence of HPV16, HPV18 and other hrHPV between Linear Array and RealTime was observed.
Conclusions: Linear Array showed similar sensitivity with higher specificity to detect CIN2+ compared to HC2. Detection of 13 hrHPV types with Linear Array fulfils the clinical accuracy requirements for primary cervical cancer screening.

1. Background and objectives

In 2012, the International Agency for Research on Cancer (IARC) concluded that at least 12 high-risk human papillomavirus (hrHPV) types (HPV16, HPV18, HPV31, HPV33, HPV35, HPV3, HPV45, HPV51, HPV52, HPV56, HPV58 and HPV59), were carcinogenic to humans for the development of cervical cancer (IARC-2009 hrHPV types)[1]. Furthermore, it has been demonstrated through several randomized controlled trials that hrHPV DNA testing is more effective than cervical cytology in primary screening of women aged 30 years or older [2,3]. Thus, several countries are currently in the process of introducing primary hrHPV based screening for cervical cancer. The first two hrHPV DNA assays that had demonstrated high-quality evidence on efficacy with respect to prevention of cervical cancer in large randomized trials with longitudinal follow-up [3,4] were the Hybrid Capture 2 assay (HC2; Qiagen, Hilden, Germany) [2,5–7] and GP5+/6+ PCR-based

Abbreviations: AGC, atypical glandular cells; ASC-H, atypical squamous cells, cannot exclude high-grade lesion (ASC-H); ASC-US, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; GP5+/6+-EIA, GP5+/6+ PCR-based enzyme immunoassay; HC2, Qiagen Hybrid Capture 2; HSIL, high-grade squamous intraepithelial lesion; hrHPV, high-risk human papillomavirus; Linear Array, Roche Linear Array HPV genotyping test; LSIL, low-grade squamous intraepithelial lesions; NILM, negative for intraepithelial lesion or malignancy; RealTime, Abbott RealTime High Risk HPV test; VALGENT, VALidation of HPV GENotyping Tests

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enzyme immunoassay (GP5+/6+-EIA; Diassay, Rijwijk, the Netherlands) [8–10]. Hence, they represent standard comparator assays in the clinical evaluations of alternative HPV DNA tests [11].

The number of commercially available HPV assays has increased significantly over the last decade with prominent differences in their technology, targeted viral genes, HPV types detected, and level of automation. Unfortunately, many of HPV tests currently on the market lack clinical performance evaluation and are without a single peer-reviewed publication [12–14]. In 2009, international criteria were developed for alternative hrHPV DNA assays, which must be fulfilled in order to be accepted by HPV academic community as clinically validated for the use in primary cervical cancer screening settings [4]. Thus, alternative hrHPV DNA assay should demonstrate good reproducibility together with non-inferior sensitivity and specificity for detecting cervical intraepithelial neoplasia of grade 2 or worse (CIN2+) compared to the HC2 or GP5+/6+-EIA [4]. A list of hrHPV DNA assays that fully or partially fulfil international validation criteria for use in primary cervical cancer screening was published recently [14], most of them targets 12 IARC-2009 hrHPV types plus HPV66 and/or HPV68 [15].

In this study, we assessed the performance of the Linear Array HPV Genotyping Test (Linear Array; Roche Molecular Diagnostics, Branchburg, NJ, USA) through the VALidation of HPV GENotyping Tests (VALGENT) framework. VALGENT is an international study framework aimed at comprehensive comparison and validation of hrHPV DNA tests in primary cervical cancer screening settings. HPV DNA assays evaluated through VALGENT have limited, extended or full genotyping capacity. VALGENT is iterative, using panels collated in different countries. Thus far, two VALGENT panels have been completed [16], using samples collected from Belgium [17–19] and Scotland) [20–23]. VALGENT-3 is using specimens obtained from women participating in the Slovenian national cervical cancer screening programme [7].

Using the VALGENT-3 sample collection, we have evaluated the clinical performance of the Linear Array in comparison to the standard comparator test (HC2) and verified whether the Linear Array fulfils minimal clinical requirements for use in cervical screening. Additionally, we compared the analytical performance of the Linear Array for partial HPV partial genotyping (i.e., using only 14 hrHPV genotypes) with that of Abbott RealTime High Risk HPV test (RealTime; Abbott, Wiesbaden, Germany), another clinically validated hrHPV DNA assay [14].

2. Study design

2.1. Sample collection

The collation of specimens used for the present iteration of VALGENT-3 project was performed in Slovenia, as previously described [7,24]. Briefly, throughout December 2009 and August 2010, a total of 1300 consecutive cervical samples were obtained from women aged 25–64 years who participated in the national cervical cancer screening programme (screening population). Additionally, according to the VALGENT protocol [16], 300 cytological abnormal samples were collected between January 2014 and May 2015, which included 100 women with atypical squamous cervical cells of undetermined significance (ASC-US), 100 women with low-grade squamous intraepithelial lesion (LSIL) and 100 women with high-grade squamous intraepithelial lesion (HSIL) (enrichment population). Ethical approval for the study was obtained from the Medical Ethics Committee of the Republic of Slovenia (consent numbers: 83/11/09 and 109/08/12).

Conventional cytology smears were obtained in compliance with the standard routine gynaecological practice in Slovenia and categorised according to the 2001 Bethesda System [25]. In order to perform HPV DNA testing, a second sample was obtained and placed into ThinPrep vial (ThinPrep PreservCyt solution, Hologic, Marlborough, MA, USA). Coded ThinPrep vials were transported each week to the Laboratory for Molecular Microbiology of the Faculty of Medicine, University of Ljubljana. The 1600 ThinPrep specimens were labelled with anonymous study number and split into several aliquots immediately upon arrival at the laboratory. Two of the aliquots were used for testing with HC2 (4 mL) and RealTime (500 uL). Testing of the screening and the enrichment population was performed in 2010 and 2014, respectively. The remaining aliquots were stored at −70 °C and were used for other HPV DNA tests included in the VALGENT-3 framework [7]. In 2016, the Linear Array testing was performed using 50 uL of DNA extracted from 1 mL of the original ThinPrep aliquot.

2.2. HPV testing

The Linear Array is a HPV genotyping test, which enables identification of 37 high- and low-risk HPV types (HPV6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 44, 45, 51, 52, 53, 54, 56,58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, 89, and IS39) [12,13]. Linear Array is qualitative test which uses biotinylated primers sets PGM09/PGMY11 and PC04/GH20 for simultaneous amplification of a 450 bp and 268 bp fragments of the HPV L1 gene and human beta-globin gene, respectively. Following PCR amplification, genotyping is performed using a single nylon strip coated with HPV type-specific and human beta-globin-specific oligonucleotide probes [13]. Testing was performed in accordance with the manufacturer's instructions.

HC2 detects 13 hrHPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and was used as the standard comparator test for the assessment of the clinical performance of the Linear Array. For the purpose of this study, hrHPV positivity for Linear Array was defined as the presence of one or more of the 13 HPV types also detected by HC2 unless otherwise specified.

The RealTime test is an automated multiplex real-time PCR-based assay, which enables concurrent individual detection of HPV16 and HPV18 and pooled detection of 12 other hrHPV genotypes (HPV31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).

2.3. Clinical outcomes and performance assessment

The algorithm of cytological assessment and referral for colposcopy has been described in detail previously [7]. Briefly, all cervical specimens were evaluated by certified cytologists who were blinded to HPV results. Women with atypical squamous cells, cannot exclude high-grade lesion (ASC-H) or worse were referred to immediate colposcopy according to the Slovenian national screening guidelines [26]. Women who were HPV16 and/or HPV18 positive were referred to colposcopy, regardless of their cytology results. Colposcopy-directed punch biopsies were obtained from areas that were suspicious for high-grade lesions, and pathologists, who were unaware of the HPV results, performed histopathological assessment.

We considered histologically confirmed CIN2+ as the clinical disease outcome. Because women negative for intraepithelial lesion or malignancy (NILM) were not referred to colposcopic verification, we considered them as subjects without disease if they had two or more consecutive NILM cytological results (at enrolment and ≥ 1 year within 36–48 months of follow-up). This group was used to compute the clinical specificity for ≤CIN1. The clinical sensitivity and specificity of the Linear Array for CIN2+ or CIN3+ were calculated. Clinical performance was assessed separately for the total study population and for women > 30 years. Using a non-inferiority statistics with a relative sensitivity threshold of 90% and a relative specificity threshold of 98%, we compared the clinical performance of the Linear Array to that of the HC2 [4,27]. The McNemar test was used in order to compare differences between matched proportions [28]. The level of statistical significance for the non-inferiority test and McNemar was set at a value of 0.05. All analyses were performed using STATA version 14 (College Station, TX, USA).