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Accuracy of genotyping for HPV16 and 18 to triage women with low-grade squamous intraepithelial lesions: a pooled analysis of VALGENT studies

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Background:

Genotyping for the most carcinogenic HPV types (HPV16/HPV18) can identify high risk of underlying cervical precancer and guide further management.

Research design and methods:

A pooled analysis was performed of the clinical accuracy of high-risk HPV testing and HPV16/18 genotyping in triage of women with low grade squamous intraepithelial lesions (LSIL). Data regarding 24 assays evaluated in four VALGENT validation panels were used.

Results:

In women with LSIL, hrHPV had a pooled sensitivity for CIN2+ of 95.5% (95% CI: 91.0-97.8%) and a specificity of 25.3% (95% CI: 22.2-28.6%). HPV16/18 genotyping had a sensitivity and specificity for CIN2+ of 52.9% (95% CI: 48.4-57.4%) and 83.5% (95% CI: 79.9-86.5%), respectively. The average risk of CIN2+ was 46.1% when HPV16/18-positive, 15.5% in women who were HPV16/18-negative but positive for other hrHPV types and 4.3% for hrHPV-negative women.

Conclusions:

Triage of women with LSIL with HPV16/18 genotyping increases the positive predictive value compared to hrHPV testing but at the expense of lower sensitivity. Arguably, women testing positive for HPV16/18 need further clinical work-up. Whether colposcopy referral or further surveillance is recommended for women with other hrHPV types may depend on the post-test risk of precancer and the local risk-based decision thresholds.

Keywords: Cervical cancer screening, human papillomavirus, HPV genotyping, triage, low-grade squamous intraepithelial lesions, diagnostic test accuracy

1. Introduction

In women with minor cytological abnormalities, atypical squamous cells of undetermined significance (ASC-US), triage with high-risk human papillomavirus (hrHPV) testing is recommended in many cervical cancer screening protocols worldwide [1,2]. For moderate cytological abnormalities, low-grade squamous intraepithelial lesions (LSIL), triage with hrHPV testing is less informative due to the high prevalence of HPV [2,3], and the management of LSIL positive women is divergent across settings [2,3]. To avoid immediate referral of all LSIL patients to colposcopy and the adverse effects of overtreatment, triage tools are needed to identify the minority of women with LSIL with underlying or incipient high-grade lesions. Partial genotyping for HPV16 and HPV18 has been proposed as a candidate triage marker. A previous systematic review and meta-analysis [4], published in 2017, indicated that although genotyping for HPV16 and HPV18 has poor sensitivity, it may

be useful as an additional triage tool in LSIL hrHPV positive women in a two-step triage scenario. Here, a risk-based decision-making tool based on post-test risk is presented [4].

The number of commercially available HPV assays enabling HPV16 and HPV18 typing capabilities has increased substantially in the past five years as clinical focus is changing from simple detection of hrHPV towards extended or full genotyping [1,5-8]. Current evidence on the usefulness of HPV16 and HPV18 typing as a triage for the management of women with LSIL is here updated using new accuracy data obtained from the international VALidation of HPV GENotyping Tests (VALGENT) framework. VALGENT aims for the comparison and validation of HPV genotyping tests for clinically relevant outcomes using sample-populations relevant for primary cervical cancer screening [5]. An important objective of VALGENT is to document the accuracy of genotyping for the triage of women with minor cytological abnormalities and the contribution of more than twenty new accuracy datasets on the triage of women with LSIL will complete the current evidence base [4].

In the current pooled analysis of four individual VALGENT studies, we assess the accuracy of genotyping for HPV16/18 in triage of women with LSIL cytology to identify women with underlying cervical intraepithelial neoplasia of grade 2 or worse (CIN2+). The pre-test and post-test risks of CIN2+ were computed to suggest management decisions based on agreed threshold levels [4].

2. Materials and Methods

2.1 VALGENT framework and sample collection

The VALGENT framework contains several iterative sample panels collated in different countries. Up to now, three VALGENT panels have been completed with a fourth ongoing. VALGENT-1 were provided by the AML laboratory using SurePath collected samples (Antwerp, Belgium) [9-13], VALGENT-2 by the Scottish HPV Reference Laboratory using ThinPrep collected samples (Edinburgh, Scotland) [14-16], VALGENT-3 were performed using ThinPrep collected samples from the Laboratory for Molecular Microbiology of University of Ljubljana (Ljubljana, Slovenia) [17-21], and VALGENT-4 using fresh SurePath collected samples by the Molecular Pathology Laboratory of Copenhagen University Hospital (Copenhagen, Denmark) [22]. In each VALGENT panel, the study population comprised a continuous series of 1,000 or 1,300 cervical specimen (archived or fresh) from women participating in the local cervical cancer screening programme supplemented with 300 abnormal pathological samples (100 ASC-US, 100 LSIL and 100 high-grade squamous intraepithelial lesions [HSIL]) [5]. Detailed information about each panel collection, processing and manipulation can be found in previously published VALGENT reports [9-22].

2.2 Clinical outcomes and performance measurement

According to the VALGENT protocol [5], we considered the presence of histologically confirmed CIN2+ as main disease outcome identified through follow-up and management according to national guidelines. Colposcopy was triggered by abnormal cytology in VALGENT-2 [14,15], and by abnormal cytology and/or positive hrHPV testing in VALGENT-1, -3 and -4 [5,22,23]. For the purpose of current pooled analysis of triage

accuracy, we use the number of women found with LSIL cytology and subsequently detected CIN2+ as the denominator for the computation of clinical sensitivity. Specificity was evaluated on women with LSIL index cytology and with normal colposcopy/histology findings and/or a negative cytology outcome through the follow-up period.

2.3 Evaluated tests in VALGENT studies

The full name, abbreviated name and the type specific remit of the evaluated assays, delivered in the VALGENT testing laboratories are summarised in Table 1. Throughout the rest of the paper, assays will be labelled by their shortened name. In total, twenty-four different HPV assays were evaluated in this pooled-analysis. Five assays have limited HPV genotyping capacity, which can identify HPV16 and HPV18 separately (Cobas, Abbott, HPVRisk, HybribioHR and Harmonia); two has extended genotyping capacity (Onclarity and Xpert) and seventeen were full genotyping assays identifying at least 14 hrHPV types (HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58,HPV59, HPV66 and HPV68) separately (Riatol qPCR, BSGP5 +/6+ MPG, GP5+/6+ LMNX, TS-E7-MPG, PapilloCheck, Linear Array, AnyplexHR, Anyplex HPV28, Innolipa, Euroarray, Papiloplex, Genoarray, LifeRiver Venus, CLART, Massarray, Modified GP5+/6+ and BGI).

The evaluated index tests were HPV assays identifying HPV16 alone or HPV16 and HPV18 jointly (HPV16/18). A positive HPV16/18 test was considered positive if HPV16 and/or HPV18 were present and negative when both types are absent. The comparator tests were hrHPV testing with GP5+/6+ PCR EIA (VALGENT-2 and VALGENT-4) and HC2 (VALGENT-3). These two tests are accepted as the standard comparator tests in the validation of new HPV assays with potential application for primary cervical cancer screening [24]. In VALGENT-1, the aggregate of 14 hrHPV types identified with the RIATOL qPCR was used as comparator test since no results generated by HC2 or GP5+/6+ PCR-EIA were available. For Euroarray, the optimised cut-off proposed in the previously published VALGENT report was used for current analysis [19]. The cut-off set by the individual assay manufacturer was used without modification for all the rest evaluated assays.

To triage women with LSIL in order to detect CIN2+, the following questions on test accuracy of the different HPV genotyping assays were addressed: 1) what is the absolute clinical accuracy (sensitivity and specificity) of genotyping for HPV16 alone or for HPV16 and HPV18 combined (HPV16/18); 2) what is the relative clinical accuracy of genotyping for HPV16/18 compared to general hrHPV testing; 3) what is the relative accuracy of combined HPV16/18 genotyping compared to HPV16 genotyping alone; and 4) what is the clinical accuracy of HPV16/18 genotyping when considered as a second triage step in the management of hrHPV-positive LSIL women.

2.4 Statistical analysis

A bivariate normal model for the logit transformations of sensitivity and specificity were used to compute the pooled absolute sensitivity and specificity [25,26]. The relative sensitivity and specificity of the evaluated index tests versus the comparator test were computed by including the test as a covariate in the bivariate normal model [27,28].

Summary receiver-operating characteristics (sROC) plots were used to illustrate the joint overall and study-specific sensitivity and specificity of genotyping for HPV16 and HPV16/18 and hrHPV testing for triage of women with LSIL. Statistical analyses were conducted with STATA 14 (Stata Corp., College Station, Tex) using metandi[25] and a user-written procedure allowing multivariate diagnostic test accuracy meta-regression.

3   Results

3.1 *Characteristics of study populations in different VALGENT panels*
In VALGENT-1, 122 women with LSIL at enrolment(mean age: 33, range: 15-65) had follow-up outcomes with 15 CIN2+ cases identified. The total number of LSIL patients at enrolment with valid clinical outcomes in VALGENT-2 and -3 were 98 (mean age: 30.5, range: 19-62) and 47 (mean age: 35.4, range: 20-65]), respectively. According to the VALGENT protocol and the routinely indicated follow-up and management procedure in Scotland and Slovenia, in total, 20 and 22 confirmed CIN2+ cases were identified respectively. The VALGENT-4 study is on-going, with follow-up data until March 2018; from this 62 LSIL patients (mean age: 39.0, range: 30-57) and 14 CIN2+ were identified.

3.2 Absolute accuracy of genotyping for HPV16 and HPV16/18 compared to hrHPV testing
 HPV16/18 genotyping identified, on average, 52.9% (95% CI: 48.4-57.4%) of CIN2+ while the pooled specificity to exclude CIN2+ was 83.5% (95% CI: 79.9-86.5%) (Figure 1). The pooled absolute accuracy measures for the genotyping of HPV16 and HPV16/18 and the hrHPV testing in triage of women with LSIL to detect underlying CIN2+ with 95% confidence intervals (CIs) are reported in Table 2. The sROC plot displays the sensitivity and specificity of the hrHPV testing (red), of genotyping for HPV16 (blue) and of HPV16/18 (green) in the triage of women with LSIL to detect CIN2+ (Figure 2).

3.3 Relative accuracy of HPV16 and HPV16/18 compared to each other and compared to hrHPV testing
Genotyping of HPV16 and HPV16/18 demonstrated substantially higher specificity for the detection of CIN2+, compared with testing for hrHPV types. The pooled specificity ratios were 3.40 (95% CI: 2.97-3.89; p<0.0001) and 3.20 (95% CI: 2.81-3.64; p<0.0001), respectively (Table 4). The sensitivity of the two partial genotyping methods was lower compared with the hrHPV testing for detecting CIN2+. The pooled ratios were 0.50 (95% CI: 0.45-0.57; p<0.0001) for HPV16 genotyping and 0.57 (95% CI: 0.51-0.63; p<0.0001) for HPV16/18 genotyping. HPV16/18 genotyping detected on average 13% more CIN2+ than HPV16 genotyping, although this was not statistically significant (p=0.06), whereas the specificity to exclude CIN2+ was on average 6% lower which reached significance (95% CI: 3-9%; p<0.0001).

3.4 Pre-test and post-test risk of cervical precancer
In Table 4, the pre- and post-test probabilities of CIN2+ of two triage strategies are presented. The average risk of CIN2+ before triaging test (pre-test risk) was derived from a previously published meta-analysis [4].The post-test probabilities can be computed with the pre-test risk and the sensitivity and specificity derived from the current pooled analysis (Table 2). Women

with LSIL had on average a risk of CIN2+ of 21% before triaging test, triage with a hrHPV test stratifies this risk to25.4% if hrHPV-positive and 4.3% if hrHPV-negative. Triage testing with HPV16/18 positive increased the risk of CIN2+ to 46.1%. Women testing HPV16/18 negative contain two subgroups: a) women who are hrHPV negative and b) those who are positive for other hrHPV types. They have a risk of CIN2+ of 13.0%.

A new pooled-analysis was performed to evaluate the accuracy of triage with HPV16/18 genotyping for CIN2+ restricted to hrHPV positive women (Figure 3). The pooled sensitivity and specificity were 58.1% (95% CI: 53.1-62.5%) and 76.5% (95% CI: 72.5-80.0%), respectively. The post-test risk of CIN2+ is shown in the last row in Table 4; here the pre-test risk of CIN2+ corresponds with the post-test risk for triage with hrHPV testing (25.4%) with risks of 45.2% (if HPV16/18 positive) and 15.5% (if HPV16/18-negative, but other hrHPV-positive).

4 Discussion

This pooled analysis demonstrates the utility and the limitations of genotyping for HPV16 and HPV16/18 in triage of women with LSIL cytology. We found that HPV16 genotyping detects around half of women with LSIL cytology and underlying CIN2+. The addition of HPV18 to HPV16 only genotyping increased the sensitivity for CIN2+ with 13% but decreased the specificity with 6%. Due to the substantially larger number of <=CIN1 cases than CIN2+ cases, differences were significant for the specificity but not statistically significant for sensitivity. The pooled specificity of HPV16/18 genotyping to exclude CIN2+ is 84%, which was, as expected, substantially more specific but less sensitive than testing for all hrHPV types.

The underlying risk of cervical precancer should determine management [29]. A good triage test to manage LSIL patients should have good discriminatory power to indicate colposcopy referral when the triage test is positive and a return to either routine screening or re-test at a defined interval when the triage test is negative. Based on results of test accuracy obtained from current pooled analysis and the knowledge of CIN2+ prevalence in women with LSIL from the previously published meta-analysis [4], the post-test probabilities of CIN2+ of different triage strategies could be computed and translated into patient management algorithms.

A one-step HPV16/18 triage strategy clearly is not clinically acceptable since it does not allow to distinguish two groups (hrHPV negative and other than HPV16/18 positive) with clearly different risks. When HPV16/18 genotyping is applied in a two-step triage scenario to hrHPV-positive, its sensitivity is slightly higher, and its specificity is slightly lower compared to HPV16/18 when applied as a single triage.

The average risk of underlying CIN2+, pooled from the VALGENT studies, was 46% if HPV16/18-positive, 16% in women who were positive for other hrHPV types but negative for HPV16/18 and 4% for LSIL hrHPV-negative women. In European settings, a risk of CIN2+ (or positive predictive value) of >20% has been proposed as a threshold to indicate colposcopy [30-32]; an interval for surveillance testing 6-12 months later could be proposed

if the risk of CIN2+ is between 2-20%, and an interval for routine testing three years later is proposed if the risk for CIN2+ is <2%. In contrast, in the USA, if the risk of CIN2+ exceeds 10.2%, colposcopy referral is proposed [33]. Summarizing, use of HPV16 and/or 18 positive outcomes in women with LSIL could be considered useful in a European setting, effectively selecting those women who would have a colposcopy referral versus those with other hrHPV types who can be referred for re-testing at a defined interval.

With genotyping capability being an increasing feature of HPV testing platforms, our findings of the current study may help inform patient management pathways for women with LSIL through description of the underlying risks associated with HPV16/18 positivity.

In total, we included 24 HPV tests, which performed overall similarly with respect to clinical sensitivity and specificity in the different triage scenarios. However, limitations should be noted. First, in VALGENT, cross-sectional and short-term longitudinal endpoints are defined in agreement with the local follow-up guidelines within a single screening round. The follow-up periods for screen-positive women varied between 0 and 20 months. Short-term outcomes do not provide insights on the risk of developing high-grade disease over time. How the sensitivity and specificity of HPV16/18 genotyping change between cross-sectional or longitudinal endpoint has been assessed in a previously published meta-analysis [4]. The absolute longitudinal sensitivity of genotyping for HPV16/18 (>=2 years after the index finding of LSIL) was 12-15 percent lower whereas the longitudinal specificity was 1-2 percent higher than the respective cross-sectional accuracy measures. Another limitation was the small number of LSIL women who had a final diagnosis of CIN3+ in each of the VALGENT panels (always <10). Although it would be better to use histologically confirmed CIN3+ as main disease outcome, the low number of CIN3+ would make the sensitivity estimations unstable and imprecise. Therefore, we had to restrict our analysis to the endpoint of CIN2+.

The pooled results are comparable with the evidence in the previously published meta-analysis of genotyping with HPV16/18 in women with low grade cervical lesions [4]. It suggests that partial genotyping tests can be used to risk stratify precancer in hrHPV-positive women and to inform about need for immediate colposcopy or re-test within a defined interval. However, the clinical utility of HPV16/18 genotyping in LSIL patients is moderate, since negative triage results do not bring down the risk to a sufficiently low level allowing for a safe relieve to routine screening. Therefore research for more performant triage markers should be continued. Since LSIL reflects a prevalent HPV infection, finding appropriate triage markers might be a relevant setting to discover triage tests that are also useful for the management of hrHPV-positive women in a context of primary HPV-based screening.

5   Conclusion
In Conclusion, triage of women with LSIL with partial genotyping of HPV16/18 increases the positive predictive value compared to detection of all hrHPV types but at the expense of loss in sensitivity. Women testing positive for HPV16/18 need further diagnostic and/or therapeutic work-up. Women testing HPV16/18-negative but positive for other hrHPV types may also be referred to colposcopy or kept under further surveillance depending on local

decision thresholds. HrHPV-negative LSIL patients may be kept under surveillance or released to routine screening also depending on local decision thresholds. Further development and optimization of triage markers is needed to manage women with LSIL beyond limited genotyping.

6   Five-year view

Triage of minor cytology will stay important in several countries as long as cytology remains the primary cervical cancer screening test. Additional triage options beyond genotyping for HPV16 and 18 which allow better management are still needed. However, it is expected that within 5 years, many countries will have switched to HPV-based cervical cancer screening. In the future, triage of HPV-positive women will become a major topic for new meta-analytical work. However, defining evidence-based algorithms for management will continue to be driven by the assessment of risks of significant disease and how these risks change by screening and triage tests.

Key issues

- Triage of women with LSIL by HPV16/18 genotyping in a one-step triage strategy is poorly sensitive and not clinically acceptable.
- Genotyping for HPV16/18 to triage women with LSIL may be useful as a second triage test for women testing hrHPV-positive.
- Women with LSIL testing positive for HPV16/18 can be referred to colposcopy directly.
- Women with LSIL testing positive for other hrHPV types may also be referred to colposcopy or maintained under surveillance depending on local decision thresholds.

Declaration of interest

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- IB is a clinical pathologist working in the private laboratory AML . AML has received research support in the form of free kits, reduced prices or funding from Abbott, Hologic, Cepheid, Roche, Becton Dickinson, Seegene, Biomérieux, Rover Medical devices, Aprovix and My Sample. IB has also received travel grants to attend symposia, conferences and meetings from Hologic and Abbott. IB, nor AML, has received any financial advantage from test manufacturers nor any payment for work and tests conducted in the framework of VALGENT.
- KC has no conflicts of interest to declare, KC's institution has received research funding and or associated gratis consumable from the following in the last 3 years:

Author's contributions

Conception and design of the study: MA and LX

Panel testing and reporting: IB, KC, MP and JB

Statistical analysis: LX

Writing of the manuscript: LX and MA

Revising of the paper critically: all

Decision to submit: all

Figure 1. Pooled analysis of the sensitivity (left) and specificity (right) of genotyping for HPV16/18 to detect CIN2+ in women with LSIL. CIN: cervical intra-epithelial neoplasia; HPV: human papillomavirus; LSIL: low-grade squamous intraepithelial lesion.
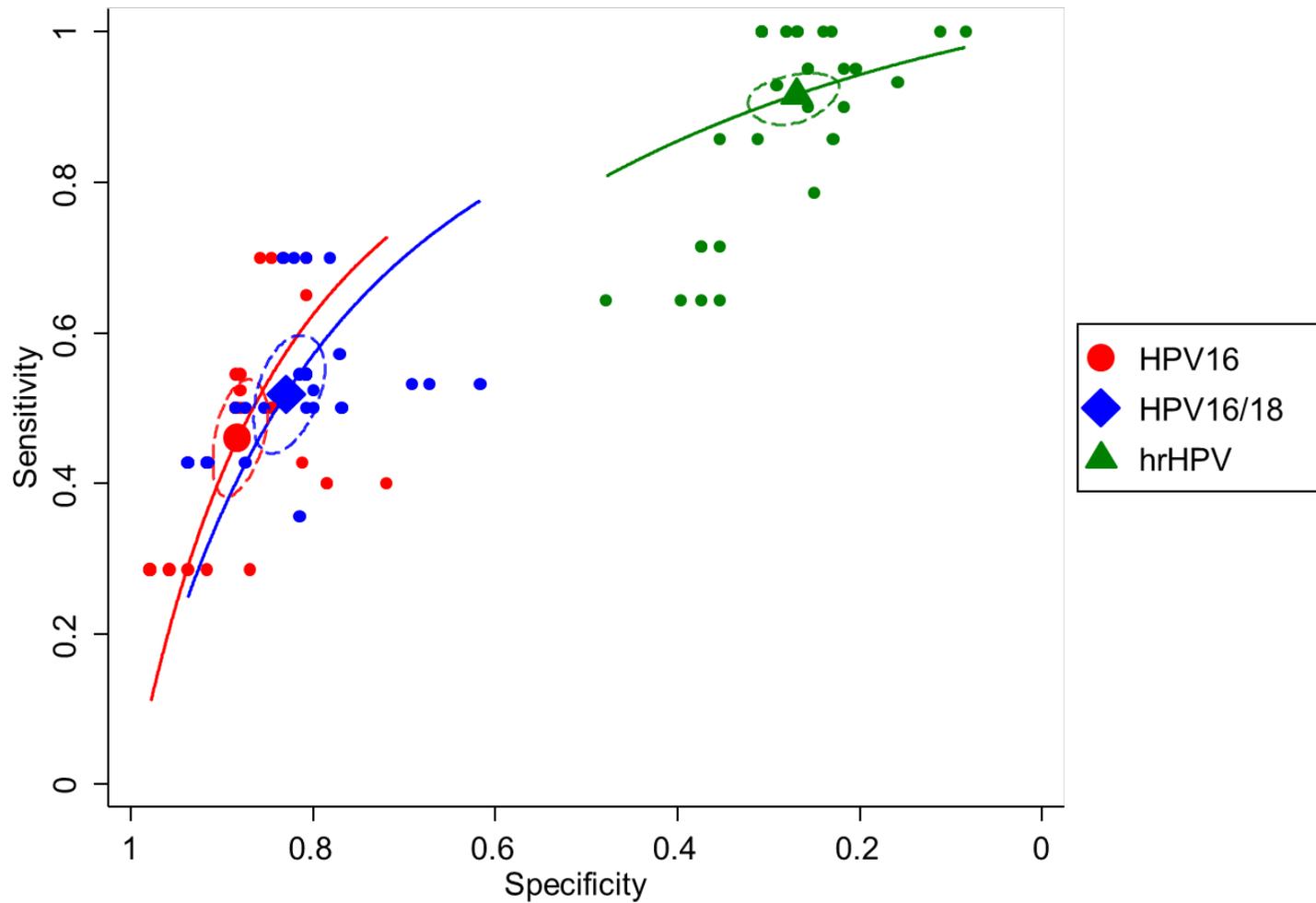
Figure 2. Summary receiver operation characteristic plot of the sensitivity as a function of the specificity of hrHPV and genotyping for HPV16/18 and HPV16 alone to detect CIN2+ in women with LSIL.
CIN: cervical intra-epithelial neoplasia; HPV: human papillomavirus; hr: high-risk; LSIL: low-grade squamous intraepithelial lesion.
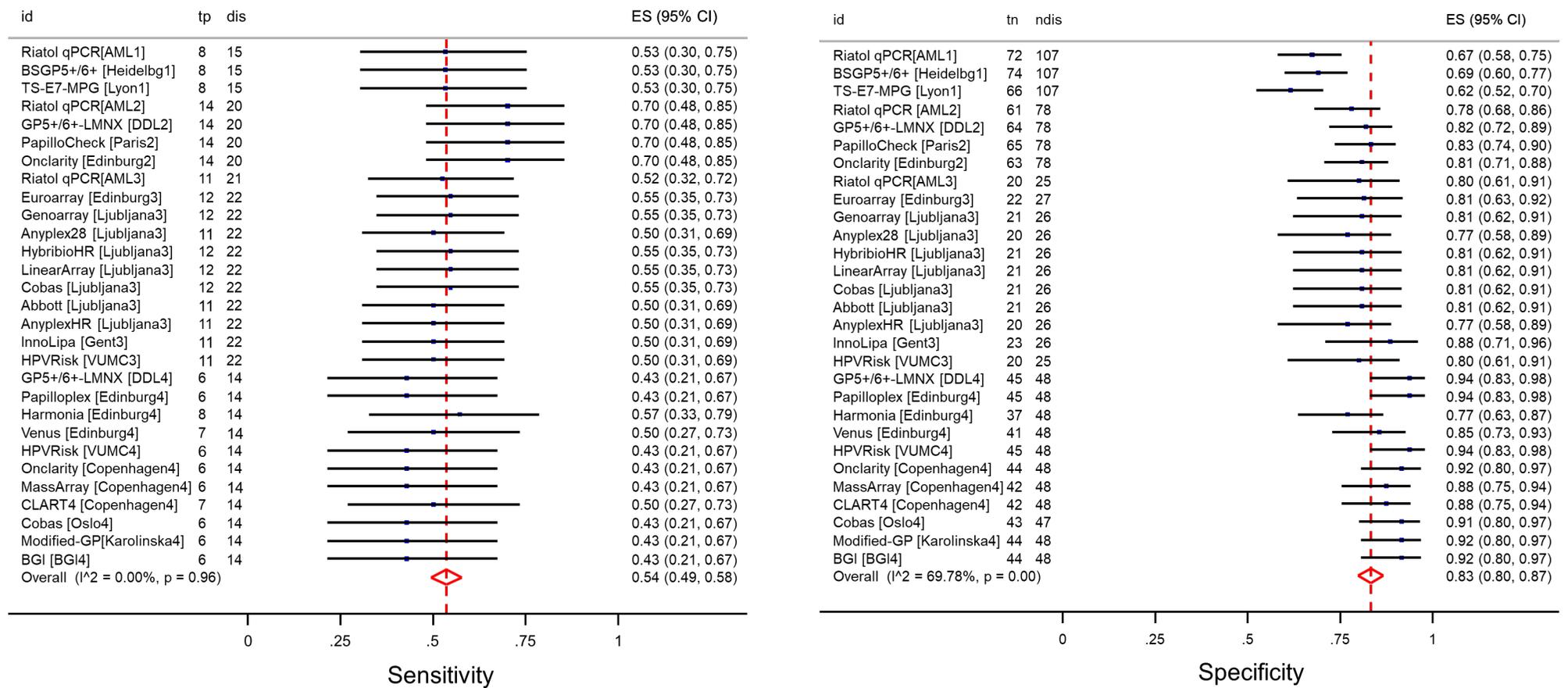
Figure 3. Pooled analysis of the sensitivity (left) and specificity (right) of genotyping for HPV16/18 to detect CIN2+ in women with LSIL who were hrHPV-positive. CIN: cervical intraepithelial lesion. HPV: human papillomavirus; hr: high-risk; LSIL: low-grade squamous intraepithelial lesion.
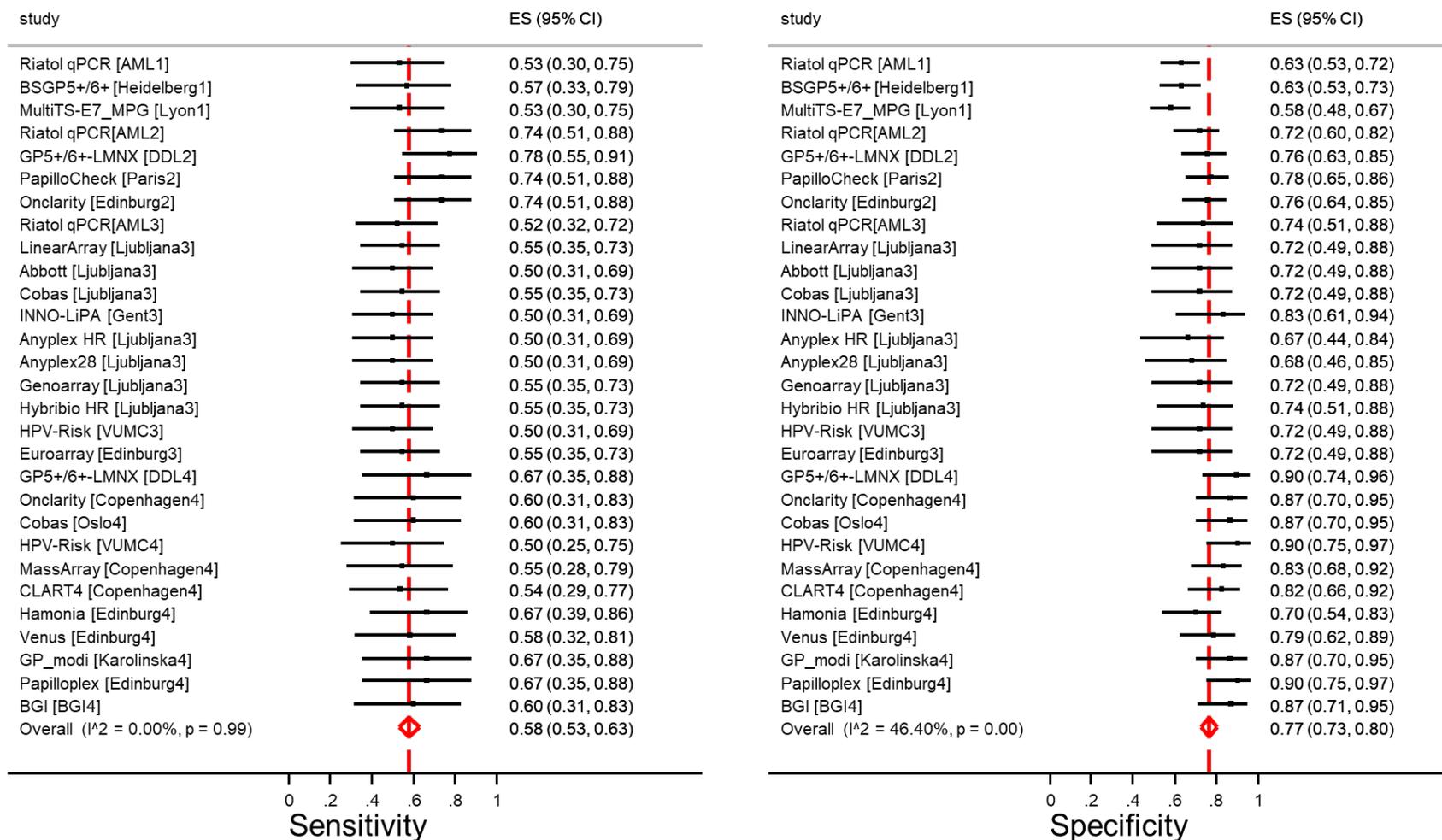
Table 1. Characteristics of the different HPV tests evaluated in VALGENT framework

	Assay (**abbreviated name**; manufacturer)	Performed by	Evaluated in Valgent No:	Test genotyping capacity
Standard comparator tests				
*	Hybrid Capture 2 High-Risk HPV DNA Test (**HC2**; Qiagen, Gaithersburg, MD, USA)	Laboratory for Molecular Microbiology, Faculty of Medicine, University of Ljubljana, Slovenia	3	No separate genotyping. Detects 13 hrHPV types in aggregate: 16,18,31,33,35,39,45,51,52,56,58,59 and 68.
*	GP5+/6+ PCR- EIA (**GP5+/6+ EIA**; Diassay B.V., Rijkswijk, the Netherlands)	DDL Diagnostic Laboratory, Rijswijk, The Nederland	1,2,4	No separate genotyping. Detections in bulk 14 hrHPV types in aggregate: same as HC2 plus 66.
Evaluated tests				
1	Riatol qPCR assay (**Riatol qPCR**; lab in-house assay)	AML laboratory, Antwerp, Belgium	1,2,3	Individual detection of: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68; Detects also phr type 53 and lr types 6 & 11.
2	BSGP 5+/6+-PCR/MPG assay (**BSGP5 +/6+ MPG**; lab in-house assay)	Department of Genome Modifications and Carcinogenesis, DKFZ, Heidelberg, Germany	1	Individual detection of: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68b;pHr types 26, 53, 67, 70, 73, 82, 6, 11, 30, 42, 43, 44, 7, 13, 32, 34, 40, 54, 61, 62, 71, 72, 74, 81, 83, 84, 85, 86, 87, 89, 90, 91, 97, 102, 106, 55, 64 and 68a.
3	GP5+/6+ PCR Luminex genotyping kit (**GP5+/6+ LMNX**; Diassay B.V., Rijkswijk, the Netherlands)	DDL Diagnostic Laboratory, Rijswijk, The Nederland	1,2,4	Individual detection of 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68; Detects also phr types: 26,53,73,82.
4	TS-E7-MPG assay (**TS-E7-MPG**; in-house assay)	IARC, Lyon, France	1	Individual detection of 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, and 82.
5	BD Onclarity HPV assay (**Onclarity**; BD Diagnostics, Sparks, MD, USA)	SHRL, Edinburg, Scotland & Hvidovre Hospital, Dept. Pathology, Hvidovre, Denmark	2, 4	Individual detection of 16, 18, 31, 45, 51, 52, 33/58, 56/59/66 and 35/39/68.
6	Xpert HPV (**Xpert**; Cepheid, Sunnyvale, CA, USA)	SHRL, Edinburg, Scotland	2	16, 18/45 and 11 other hrHPV types (See GP5+/6+ PCR- EIA).
7	PapilloCheck HPV-screening (**PapilloCheck**; Greiner Bio-One, Frickenhausen, Germany)	French HPV Reference Laboratory, Institut Pasteur, Paris, France.	2	Individual detection of 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82, 6, 11, 40, 42, 43, 44.
8	Linear Array HPV genotyping (**Linear Array**; Roche	Laboratory for Molecular Microbiology, Faculty of Medicine, University of Ljubljana, Slovenia	3	Individual detection of 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82, 6, 11, 40, 42, 54, 61, 70, 72, 81, CP6108, 55, 62, 64, 67, 69, 71, 83, 84, and IS39.

	Molecular Diagnostics, Branchburg, NJ, USA)		
9	Cobas 4800 HPV test (**Cobas**; Roche Molecular System, Pleasanton, CF, USA)	Laboratory for Molecular Microbiology, Faculty of Medicine, University of Ljubljana, Slovenia	3	16,18 and 12 other hr types (See GP5+/6+ PCR- EIA)
10	Abbott RealTime High Risk HPV test (**Abbott**; Abbott, Wiesbaden, Germany)	Laboratory for Molecular Microbiology, Faculty of Medicine, University of Ljubljana, Slovenia	3	16,18 and 12 other hr types (See GP5+/6+ PCR- EIA)
11	Anyplex II HPV HR assay (**AnyplexHR**; Seegene, Seoul, South Korea)	Laboratory for Molecular Microbiology, Faculty of Medicine, University of Ljubljana, Slovenia	3	Individual detection of 14 hrHPV types: 16,18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 66, 68
12	Anyplex HPV28 detection assay (**Anyplex28**; Seegene, Seoul, South Korea)	Laboratory for Molecular Microbiology, Faculty of Medicine, University of Ljubljana, Slovenia	3	Separate identification of 28 types: 14 hr types (16,18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 66, 68); 6 phr types (26, 53, 69, 70, 73, 82); 8 lr types (6, 11, 40, 42, 43, 44, 54, 61).
13	INNO-LiPA Extra II HPV Genotyping assay (**Innolipa**; Fujirebio Europe, Ghent, Belgium)	Ghent University Hospital, Ghent, Belgium	3,4	Separate identification of 28 different HPV types, 14 hr types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68); 5 phr types (26,53,73, 70,82); 6 lr types (6, 11, 40, 43, 44, 54); 3 types with undetermined risk (69, 71, 74).
14	EUROArray HPV (**Euroarray**; EUROIMMUN; Lübeck, Germany)	SHRL, Edinburg, Scotland	3	Separate identification of 30 types: 14 hr types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68); 4phr types (26, 53, 73, 82); 12 lr or ur types (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, 89).
15	HPV-risk array (**HPVRisk**; Self-screen BV, Amsterdam, The Netherlands)	Cancer Center Amsterdam, VU University Medical Center, Amsterdam, The Netherlands	3,4	HPV16, 18 and 13 other hr types ((See GP5+/6+ PCR- EIA plus 67)
16	Hybribio 21 HPV Genoarray diagnostic kit (**Genoassay**; Hybribio, HongKong, China)	Laboratory for Molecular Microbiology, Faculty of Medicine, University of Ljubljana, Slovenia	3	Separate identification of 21 types: 14 hr types (16,18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 66, 68); 1 phr types (53); 6 lr types (6, 11, 42, 43, 44, 81).
17	Hybribio 14 High-risk HPV with 16/18 Genotyping Real-time PCR kit (**HybribioHR**; ; Hybribio, HongKong, China)	Laboratory for Molecular Microbiology, Faculty of Medicine, University of Ljubljana, Slovenia	3	16,18 & 12 other hr types (See GP5+/6+ PCR- EIA)
18	Papilloplex High Risk HPV (**Papiloplex;** GeneFirst, Oxfordshire, UK)	SHRL, Edinburg, Scotland	4	Individual detection of 14 hr types (See GP5+/6+ PCR- EIA)
19	Liferiver Harmonia HPV assay (**Harmonia**; Liferiver, Shanghai, China)	SHRL, Edinburg, Scotland	4	16,18 & 12 other hr types (See GP5+/6+ PCR- EIA)

20	Liferiver Venus HPV assay (**LiferiverVenus**; Liferiver, Shanghai, China)	SHRL, Edinburg, Scotland	4	Separate identification of 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 & 82
21	CLART HPV4 assay (**CLART**; Genomica, Madrid, Spain)	Hvidovre Hospital, Dept. Pathology, Hvidovre, Denmark	4	Individual detection of : 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
22	Agena HPV MassArray assay (**Massarray**; Agena Bioscience, Hamburg, Germany)	Hvidovre Hospital, Dept. Pathology, Hvidovre, Denmark	4	Individual detection of: 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 73
23	Modified GP5+/6+ PCR (**Modified GP5+/6+**; Karolinska University Hospital, Stockholm, Sweden)	International HPV Reference Center, Karolinska University Hospital, Stockholm, Sweden	4	Individual detection of 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68; Detects also phr types: 26,53,73,82.
24	BGI SENTIS HPV test (**BGI**; Shenzhen, China)	BGI Institute, HongKong, China	4	Individual detection of 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68; Detects also lr types: 6 & 11.

hrHPV: high-risk HPV types; phr types: probably/possibly carcinogenic HPV types; lr types: low-risk HPV types.

Table 2. Pooled absolute sensitivity and specificity of genotyping for HPV16 and HPV1618 and hrHPV testing in triage of women with LSIL to detect underlying CIN2+

Genotyping	Outcome	No of studies/tests	Pooled value, in %	
			Sensitivity (95% CI)	Specificity (95% CI)
HPV16	CIN2+	30	46.9 (41.6-52.3)	89.2 (86.0-91.8)
HPV16/18	CIN2+	29	52.9 (48.4-57.4)	83.5 (79.9-86.5)
HrHPV	CIN2+	32	95.5 (91.0-97.8)	25.3 (22.2-28.6)

Table 3. Pooled analysis of the relative sensitivity and relative specificity of A) genotyping for HPV16 compared to hrHPV testing; B) genotyping for HPV16/18 compared to hrHPV testing and C) genotyping for HPV16/18 versus genotyping for HPV16 only, in triage of women with LSIL to detect CIN2+.

Outcome	Number of comparisons	Relative sensitivity	P	Relative specificity	p
A) Comparison HPV16 vs hrHPV					
CIN2+	30	0.50 (0.45-0.57)	<0.0001	3.40 (2.97-3.89)	<0.0001
B) Comparison HPV16/18 vs hrHPV					
CIN2+	29	0.57(0.51-0.63)	<0.0001	3.20 (2.81 –3.64)	<0.0001
C) Comparison HPV16/18 vs HPV16					
CIN2+	32	1.13 (0.99-1.28)	0.06	0.94 (0.91-0.97)	<0.0001

Table 4. Pre-test and post-test probabilities of CIN2+ of triage with hrHPV testing or HPV16/18 genotyping among women with LSIL. Data for triage with HPV16/18 genotyping among women hrHPV+ LSIL is shown in the grey row (two-step triage).

Triage Group	Test	Pre-test risk*	Post-test risk	
			if test+	if test-
			PPV	cNPV
LSIL	hrHPV	21%*	25.4%	4.3%
	HPV16/18	21%	46.1%	13.0%
LSIL&hrHPV+	HPV16/18	25%	45.2%	15.5%

*Pre-test risk based on pooled prevalence from previously published meta-analysis (AIM2016). For triage of hrHPV-positive LSIL patient, the pre-test risk corresponds with the post-test risk after hrHPV testing.

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