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Clinical performance of the novel full-genotyping OncoPredict HPV Quantitative Typing assay using the VALGENT framework

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Abstract

Clinical validation of human papillomavirus (HPV) assays according to international criteria is prerequisite for their implementation in cervical cancer screening. OncoPredict HPV Quantitative Typing (QT) assay (Hiantis Srl, Milan, Italy) is a novel full-genotyping multiplex real-time PCR quantitative assay targeting E6/E7 genes, allowing individual viral load determination of 12 high-risk (HR) HPV types. Quality controls for sample adequacy, efficiency of nucleic acid extraction and PCR inhibition are included in the assay. Clinical performance of OncoPredict HPV QT test was assessed as part of the "Validation of HPV Genotyping Tests" (VALGENT-2) framework, consisting of 1300 cervical liquid-based cytology (LBC) samples of women aged between 20 and 60 years who had originally attended for routine cervical screening in Scotland. The clinical accuracy of the OncoPredict HPV QT (index test) for the detection of CIN2+ was assessed relative to the GP5+/6+ Enzyme Immuno-Assay (GP5+/6+ EIA) (comparator test), using noninferiority criteria. Intra- and interlaboratory reproducibility of the assay was assessed on a subpopulation, comprising 526 samples. The relative sensitivity and specificity for OncoPredict HPV QT vs GP5+/6+-PCR-EIA were 1.01 (95% CI: 0.99-1.03) and 1.03 (95% CI: 1.0-1.06) respectively. The *P*-values for noninferiority were ≤0.001. The intra- and inter-laboratory reproducibility demonstrated a high concordance (>98.7%) with kappas for individual types ranging from 0.66 to 1.00. OncoPredict HPV QT fulfills the international validation criteria for the use of HPV tests in cervical cancer screening.

Abbreviations: BSCC, British Society for Clinical Cytopathology; CCR5, C-C motif chemokine receptor 5; CI, confidence interval; CIN, cervical intraepithelial neoplasia; CIN1, cervical intraepithelial neoplasia grade 1; CIN2, cervical intraepithelial neoplasia grade 2; CIN3, cervical intraepithelial neoplasia grade 3; Ct, cycle threshold; GPEIA, GP5+/6+ Enzyme ImmunoAssay; HC2, Hybrid capture 2; HPV, human papillomavirus; HR, high-risk; HR-HPV, high-risk human papillomavirus; LBC, liquid-based cytology; NHS, National Health Service; PTP, Parco Tecnologico Padano; QC, quality control; QT, quantitative typing; UniMiB, University of Milano-Bicocca; VALGENT, Validation of HPV Genotyping Tests; VIA, visual inspection with acetic acid.

Clementina Elvezia Cocuzza and Sharonjit Kaur Dhillon contributed equally to this work.

KEYWORDS

cervical cancer prevention, clinical performance study, full-genotyping HPV viral load, OncoPredict HPV quantitative typing (QT), VALGENT framework

What's new?

Testing for high-risk HPV infection is an effective screening tool for the prevention of invasive cervical cancer. Many HPV assays are commercially available, but few have been fully validated according to international guidelines. Here, the authors evaluated the clinical accuracy and reproducibility of OncoPredict HPV Quantitative Typing assay, a novel full-genotyping assay targeting the 12 oncogenic HPV types. They showed that OncoPredict HPV QT performed with comparable accuracy to the well-established GP5+/GP6+ Enzyme ImmunoAssay and had good reproducibility within and between labs. Its ability to determine the genotype-specific viral load makes the OncoPredict HPV QT also useful for the risk stratification and follow-up monitoring of HPV-positive women.

1 | INTRODUCTION

Cervical cancer is the second most common cause of death in women of reproductive age worldwide.[1] Persistent infection with high-risk HPV (HR-HPV) is now known to be associated with progression to precancer and ultimately invasive cancer.[2] Several large, randomized trials have shown that HR-HPV testing offers better protection in the prevention of invasive cervical cancer as compared to cytology or visual inspection with acetic acid (VIA), allowing also for longer screening intervals.[3-5] Moreover, some of the HR-HPV tests recently introduced on the market have extended or full genotyping capability, which in clinical practice may be useful in the risk-stratification and triage of HPV-positive women.[6-8] Consequently, many countries worldwide have already, or are now shifting to HR-HPV primary screening, using HPV tests that have undergone clinical validation according to international criteria.[9] In recent years many HPV assays have become commercially available but only a relatively small number have been fully validated according to the international guidelines.[10,11] The European VALidation of HPV GENotyping Tests (VALGENT) collaborative framework was designed to support robust evaluation and validation of HPV tests including those with genotyping capacity. It involves the application of HPV tests to samples derived from a screening population and a disease-enriched population, where clinical outcomes are known.[12] Our study aimed to evaluate the clinical accuracy and intra- and interlaboratory reproducibility of the novel quantitative full-genotyping assay, OncoPredict HPV QT, which allows normalized genotype-specific viral load determination. Evaluation of clinical performance and reproducibility was performed through the VALGENT-2 iteration.

2 | MATERIALS AND METHODS

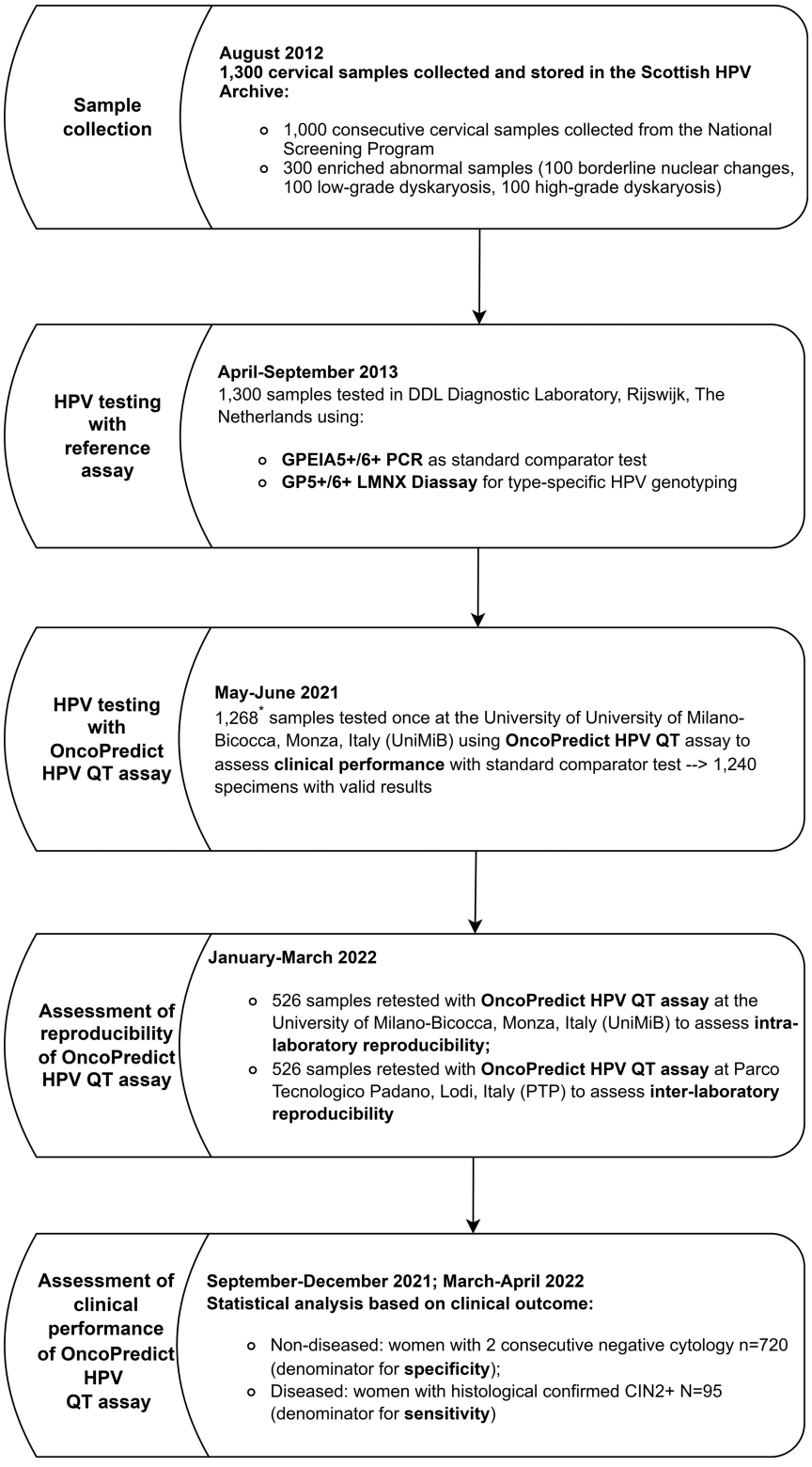
2.1 | Study population and VALGENT-2 panel

The VALGENT-2 framework/collection is based on samples collected from the organized screening program in Scotland in 2012. Dimensions of the sample are described in detail elsewhere.[13] However, in brief, the population included 1300 samples comprising of 1000 consecutive samples collected from the routinely screened population (screening population) enriched with 300 cytologically abnormal samples (disease-enriched population). All liquid-based cytology (LBC) samples resuspended in PreservCyt liquid medium (Hologic, Bedford, MA) were aliquoted and stored at −80°C in the Scottish HPV Archive. A separate aliquot of the original LBC specimen was defrosted only once before its use for the validation of an HPV DNA assay as part of the VALGENT-2 Framework. When considering the total population (screening and disease-enriched) women's median age was 38 years (ranging from 19 to 68 years).

Figure 1 describes the complete flowchart of sample collection and testing procedure used in this validation study.

2.2 | Testing of samples with OncoPredict HPV QT

OncoPredict HPV QT (Hiantis Srl, Milan, Italy) is a CE-marked full-genotyping quantitative assay, based on multiplex real-time PCR technology, targeting specific E6 and E7 DNA sequences for the detection of 12 HR-HPV types: HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59. The E6/E7 amplicons detected by the OncoPredict HPV QT test vary according to the high-risk HPV genotypes detected, ranging from 60 to 128 bp. The assay is composed of five separate reaction wells (as shown in Figure 2): four of them (QT1 to QT4) allow the independent identification of three separate HR-HPV types and of an external amplification control primed to detect an exogenous target (synthetic-custom designed gene/not human DNA); the fifth serves as a quality control (QC) reaction to reflect sample adequacy and end-to-end sample processing. The QC assessment includes an accurate evaluation of: the number of human cells present in the sample, by the quantitative determination of the single-copy human gene *C-C Motif Chemokine Receptor 5* (CCR5); the efficiency of nucleic acid extraction, by the recovery of an exogenous control gene target (synthetic gene—firefly luciferase DNA) added to the sample before preanalytical processing; and the evaluation of potential PCR inhibition, by the amplification of a control target (synthetic-custom designed gene/not human DNA) included in the OncoPredict HPV QT.



* 14 samples not available for HPV testing with OncoPredict HPV QT assay

FIGURE 1 Flowchart of samples tested for the evaluation of clinical accuracy and reproducibility of OncoPredict HPV QT within the VALGENT-2 framework.

Fully automated nucleic acid extraction and real-time PCR plate preparation, using OncoPredict HPV QT assay, were performed at the University of Milano-Bicocca (Monza, Italy) from May to June 2021, by means of a Fluent 480 (Tecan, Switzerland) liquid-handler workstation. DNA extraction was performed using *Quick*-DNA/RNA MagBead kit (Zymo, Irvine, CA), starting from a 400 µL of sample volume; the extracted nucleic acids were eluted in a final volume of 100 µL and transferred into a clean 96-well plate. OncoPredict HPV QT testing was performed according to the manufacturer's protocol. Briefly, the automated liquid handler dispensed the PCR master mix containing 10 µL of QC reagents and 10 µL for each of the four additional mixes (QT1 to QT4) per sample well. Subsequently, a 5 µL volume of DNA template

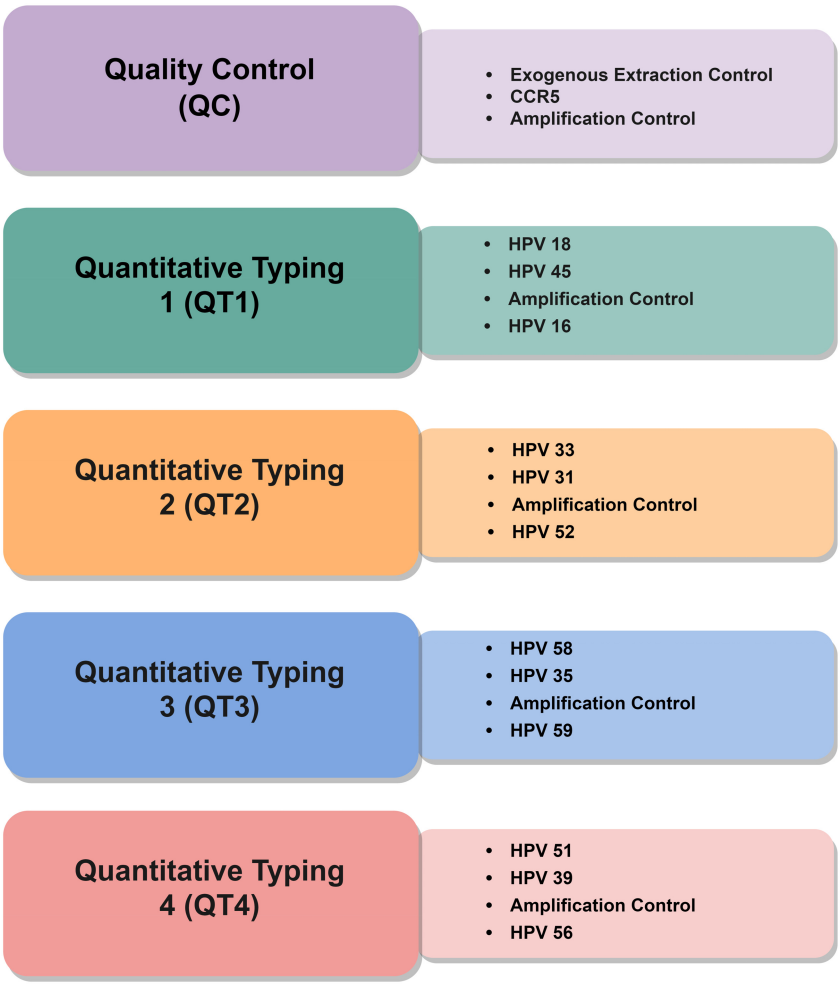


FIGURE 2 Set-up of OncoPredict HPV QT assay composed by quality control (QC) for the evaluation of sample adequacy and 4 Quantitative modules (QT1 to QT4) for the assessment of type-specific viral loads.

was added to each sample well, resulting in a 15 µL total reaction volume. Negative and positive controls, provided by the manufacturer, were included in each PCR run. Standard curves were constructed based on the cycle threshold (Ct) values of quantitative gene targets calibrators, included in the OncoPredict HPV QT kit, allowing for the determination of the normalized viral load of each of the 12 HR-HPV types (viral genomic units/10^4 cells). PCR was carried out using a CFX384 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA). Samples were considered adequate for the HR-HPV analysis if at least 400 cells/reaction were detected, if nucleic acid extraction efficiency was ≥10% and if the amplification control cycle threshold (Ct) was ≤30. Samples were then considered to be HR-HPV positive if a viral load ≥50 copies/10^4 cells was detected. In the case of multiple HR-HPV infections, the HPV type with the highest viral load or predominant HR-HPV type was used for assessing positivity.

2.3 | Testing of samples with standard comparator test

In VALGENT-2, the GP5+/6+ PCR enzyme immunoassay (GPEIA)[14] was used as the standard comparator test to assess noninferior clinical accuracy of HR-HPV testing with OncoPredict HPV QT. LMNX Genotyping Kit GP HR (*LMNX*; *Diassay* BV, Rijswijk, the Netherlands), subsequently referred to as "LMNX Diassay,"[15] was used to evaluate the genotyping capability of OncoPredict HPV QT. The overall HR-HPV prevalence in both the screening and disease-enriched populations according to baseline cytology and age was determined using OncoPredict HPV QT and LMNX Diassay. Type-specific agreement was assessed using the kappa statistics for the 12 HR-HPV types resolved by the OncoPredict HPV QT assay.

Testing of VALGENT-2 samples with the GPEIA and LMNX Diassay was performed at DDL Diagnostic Laboratory, Rijswijk, The Netherlands from April to September 2013[16] (Figure 1).

2.4 | Reproducibility assessment

The intra- and interlaboratory reproducibility of the OncoPredict HPV QT was assessed on a subset of 526 samples, randomly selected from the original VALGENT-2 panel imposing 30% HR-HPV GPEIA positives samples, as indicated by the validation guidelines.[9] The reproducibility panel included 157 HR-HPV positive and 369 HR-HPV negative samples, as indicated in Figure 1.

The remaining extracted DNA following initial testing was frozen and subsequently thawed to perform reproducibility analysis. Intralaboratory reproducibility of the OncoPredict HPV QT testing was assessed by repeated testing at UniMiB, whereas the interlaboratory reproducibility assessment was performed by repeating the testing at Parco Tecnologico Padano (PTP) Laboratory, Lodi, Italy.

2.5 | Clinical outcomes

The British Society for Clinical Cytopathology (BSCC) terminology and the cervical intraepithelial neoplasia (CIN) nomenclature were used for reporting cytology and histology results, respectively.[16,17] Women with abnormal cervical cytology were managed according to the Scottish guideline criteria. In particular, women with abnormal cytological results were referred to colposcopy and colposcopy-directed biopsies were taken when clinically indicated according to local protocols. HPV test results did not influence patients' management.

The clinical sensitivity of OncoPredict HPV QT assay was based on test performance for the detection of histologically confirmed diagnosis of cervical intraepithelial neoplasia (CIN) grade 2 or worse (CIN2+) within 18 months of sample collection. The clinical specificity was determined using samples from women who had two consecutive negative cytology samples across two screening rounds (control group). Clinical performance of the index test was assessed for women irrespective of age as well as separately for women ≥30 years of age.

2.6 | Statistical analyses

The absolute clinical sensitivity for CIN2+ and specificity for ≤CIN1 of the OncoPredict HPV QT were calculated with 95% CI for women irrespective of age and in women aged 30 years and older. Noninferior clinical accuracy of the index test compared to the standard comparator test was assessed as proposed by Tang[18] applying the benchmarks of 0.90 for relative sensitivity and 0.98 for relative specificity.

Differences in accuracy between index and comparator tests were also assessed by 95% confidence intervals (95% CIs) around the relative sensitivity and specificity taking the paired design into account and by McNemar (McN) tests.

The reproducibility was expressed as the overall percentage agreement, which is the proportion of the number of concordant results (positive on both assays + negative on both assays) overall test results. The reproducibility validation criterion was considered as fulfilled when the left 95% CI bound for HR-HPV concordance exceeded 87% and the kappa >0.5.[9]

The analytical concordance between OncoPredict HPV QT and LMNX Diassay was assessed using Kappa statistics[19] for the overall HR-HPV positivity and individually for the 12 HR-HPV types detected by the OncoPredict HPV QT assay. Kappas were categorized as proposed by Landis[20]: ($1.00 \geq K > 0.80$): excellent; ($0.80 \geq K > 0.60$): good; ($0.60 \geq K > 0.40$): moderate; ($0.40 \geq K > 0.20$): fair; ($0.20 \geq K > 0.00$): poor.

The level of statistical significance was set at 0.05. Statistical analyses were performed with STATA version 16 (College Station, TX).

3 | RESULTS

3.1 | Cytopathological findings, adequacy of specimens for HPV testing

The cytopathological findings in the screening population were as follows: 89.8% were cytology negative; 5.4% had borderline nuclear changes; 3.8% low-grade dyskaryosis; 1% moderate to severe (high-grade) dyskaryosis. The disease-enriched population was composed by design[13] of 100 samples with borderline nuclear changes, 100 with low-grade dyskaryosis and 100 with high-grade dyskaryosis.

Out of the total 1300 samples comprising the VALGENT-2 panel, 1286 were available for the purpose of this clinical performance study. Out of the 1286 available samples, a further 46 samples (25 from the screening and 21 from the disease-enriched population) had to be excluded from the analysis due to insufficient starting volume (17 samples' aliquots contained <400 µL volume required for nucleic acid extraction and testing according to OncoPredict HPV QT protocol) or to "invalid result" due to low sample cellularity or reduced nucleic acid extraction efficiency (6 samples with <400 cells/reaction; 23 with nucleic acid recovery <10%). Testing of the VALGENT-2 panel with GPEIA at DDL Diagnostic Laboratory yielded two samples that were previously excluded due to operational issues. The final number of matched samples where valid results were available for both GPEIA and OncoPredict HPV QT were n = 1239.

Overall, there were a total of 95 women with CIN2+ (denominator for clinical sensitivity); of these 50 women had CIN3+ or worse. A total of 720 women had two consecutive cytology negative results (denominator for clinical specificity).

3.2 | HR-HPV prevalence

The prevalence of HR-HPV for OncoPredict HPV QT assay was 14.3% (95% CI: 12.1%-16.6%) (138/968) and 67.5% (95% CI: 61.6%-73.1%) (183/271) in the screening and the disease enriched population respectively. Comparatively, the prevalence of HR-HPV when tested with the comparator assay (GPEIA) was 17.3% (95% CI: 14.9%-19.8%) (167/968) in the screening population and 71.6% (95% CI: 65.8%-76.9%) (194/271) in the diseased enriched population. The prevalence of HR-HPV by cytological findings can be found in Tables S1 (single and multiple HR-HPV infections) and S2 (only single HR-HPV infections).

3.3 | Clinical accuracy of OncoPredict HPV QT

Table 1 provides a summary of the data on clinical sensitivity and specificity for both assays as well as relative sensitivity and specificity for women of all ages and for women >30 years of age.

In the total study population, OncoPredict HPV QT showed a relative sensitivity for CIN2+ of 1.01 (95% CI: 0.99-1.03) and a relative specificity for ≤CIN1 of 1.03 (95% CI: 1.00-1.06) compared to GPEIA. The sensitivity for CIN2+ and specificity for ≤CIN1 of OncoPredict HPV QT was noninferior to GPEIA (P = <0.0001 for both). Similar results were found when restricting the analysis to women aged 30 years and older.

TABLE 1 (a) Absolute and (b) relative sensitivity for CIN2+ and CIN3+ and specificity for ≤CIN1 of OncoPredict HPV QT compared to GPEIA.[a]

(a)	OncoPredict HPV QT				GPEIA			
Age group and outcome	Sensitivity % (95% CI)	n/N	Specificity % (95% CI)	n/N	Sensitivity % (95% CI)	n/N	Specificity % (95% CI)	n/N
Total study population								
CIN2+	94.7 (93.2-96.3)	90/95			93.7 (92.0-95.4)	89/95		
CIN3+	98.0 (97.0-99.0)	49/50			98.0 (97.0-99.0)	49/50		
≤CIN1[b]			93.6 (91.9-95.4)	674/720			90.1 (88.1-92.2)	649/720
Women ≥ 30 years								
CIN2+	92.9 (90.9-94.8)	39/42			92.9 (90.9-94.8)	39/42		
CIN3+	95.5 (93.8-97.1)	21/22			95.5 (93.8-97.1)	21/22		
≤CIN1[b]			94.9 (93.2-96.6)	579/610			92.3 (90.3-94.3)	563/610

(b)		Relative accuracy of OncoPredict HPV QT vs GPEIA (95% CI)			
	Outcome	Relative sensitivity	Relative specificity	McNemar, *P*[c]	Noninferiority *P*[d]
Total study population	CIN2+	1.01 (0.99-1.03)		1.000	<.0001
	CIN3+	1.00 (1.00-1.06)		1.000	.009
	≤CIN1[b]		1.03 (1.00-1.06)	.0001	<.0001
Women ≥30 years	CIN2+	1.00 (0.95-1.05)		1.000	.0187
	CIN3+	1.00 (0.92-1.09)		1.000	.06
	≤CIN1[b]		1.02 (1.01-1.05)	.005	<.0001

[a]Positivity for the detection of 12 HR-HPV types.
[b]Women with two consecutive negative cytology results.
[c]*P* for the McNemar test for a difference between matched proportions; *P* values of >.05 indicate that the sensitivity or specificity of the OncoPredict HPV QT assay is not significantly different from that of GPEIA.
[d]*P* for the test of noninferiority; *P* values of <.05 indicate that the sensitivity or specificity of the OncoPredict HPV QT assay is not significantly lower than that of GPEIA.

TABLE 2 Intra- (a) and inter- (b) laboratory reproducibility of HR-HPV testing with the OncoPredict HPV QT assay evaluated by the laboratories of UniMiB and PTP.

(a)		Second testing at UniMiB		
		Positive	Negative	Total
First testing at UniMiB	Positive	122	2	124
	Negative	2	397	399
	Total	124	399	523

(b)		Second testing at PTP		
		Positive	Negative	Total
First testing at UniMiB	Positive	121	3	124
	Negative	4	395	399
	Total	125	398	523

3.4 | Intra- and interlaboratory reproducibility of OncoPredict HPV QT assay

The intra- and interlaboratory reproducibility results are reported in Table 2. Among the subset of 526 samples, included in the reproducibility panel, 3 samples were excluded because they were invalid on at least of one retesting. Out of the 523 valid samples, 519 were concordant over the two intralaboratory runs, while 516 concordant results were obtained on repeating testing at PTP. The intra- and interlaboratory reproducibility was 99.2% (95% CI: 98.1-99.8; $k = 0.98$) and 98.7% (95% CI: 97.3-99.5; $k = 0.96$), respectively.

Reproducibility was also high at type-specific level with general concordance >99% and kappa values ranging from 0.66 to 1.00, as reported in Table 3.

3.5 | HPV genotyping prevalence of OncoPredict HPV QT assay and GP5+/6+ LMNX

Overall, 321/1239 (25.9%; 95% CI: 23.5-28.4) and 338/1239 (27.2%; 95% CI: 24.8-29.9) women were found to be HR-HPV positive using OncoPredict HPV QT and LMNX Diassay, respectively. HR-HPV type-specific agreement between OncoPredict HPV QT and LMNX Diassay ranged from good to excellent with kappa values ranging from 0.64 for HPV 58 to >0.90 for HPV types 16, 31, 33, 39 and 52. Full details of the analytical concordance for the detection of the 12 HR-HPV types detected by both assays in the total population are illustrated in Table 4.

Values of genotype-specific viral load (copies/10^4 cells) are reported in the Table S3.

TABLE 3 Intra- and interlaboratory reproducibility of HR-HPV and type-specific HPV testing with OncoPredict HPV QT evaluated by the laboratories of UniMiB and PTP.

	Intralaboratory reproducibility		Interlaboratory reproducibility	
HPV type	Concordance, % (95% CI)	*K* value (95% CI)	Concordance, % (95% CI)	*K* value (95% CI)
HR-HPV	99.2 (98.1-99.8)	0.98 (0.96-1.00)	98.7 (97.3-99.5)	0.96 (0.94-0.99)
HPV 16	99.6 (98.6-100.0)	0.95 (0.89-1.00)	99.8 (98.9-100.0)	0.98 (0.93-1.00)
HPV 18	99.8 (98.9-100.0)	0.92 (0.77-1.00)	99.0 (97.8-99.7)	0.66 (0.38-0.94)
HPV 31	99.8 (98.9-100.0)	0.96 (0.90-1.00)	99.8 (98.9-100.0)	0.96 (0.895-1.00)
HPV 33	100.0 (99.3-100.0)	1.00 (1.00-1.00)	100.0 (99.3-100.0)	1.00 (1.00-1.00)
HPV 35	100.0 (99.3-100.0)	1.00 (1.00-1.00)	100.0 (99.3-100.0)	1.00 (1.00-1.00)
HPV 39	100.0 (99.3-100.0)	1.00 (1.00-1.00)	100.0 (99.3-100.0)	1.00 (1.00-1.00)
HPV 45	100.0 (99.3-100.0)	1.00 (1.00-1.00)	100.0 (99.3-100.0)	1.00 (1.00-1.00)
HPV 51	99.6 (98.6-100.0)	0.95 (0.89-1.00)	99.2 (98.1-99.8)	0.91 (0.81-1.00)
HPV 52	99.6 (98.6-100.0)	0.95 (0.87-1.00)	99.4 (98.3-99.9)	0.92 (0.82-1.00)
HPV 56	100.0 (99.3-100.0)	1.00 (1.00-1.00)	100.0 (99.3-100.0)	1.00 (1.00-1.00)
HPV 58	99.6 (98.6-100.0)	0.92 (0.80-1.00)	99.8 (98.9-100.0)	0.96 (0.88-1.00)
HPV 59	100.0 (99.3-100.0)	1.00 (1.00-1.00)	99.8 (98.9-100.0)	0.95 (0.86-1.00)

Note: Color legend (adapted from Landis and Koch)[20] for the concordance: dark green (1.00 ≥ *K* > 0.80): excellent; light green (0.80 ≥ *K* > 0.60): good; yellow (0.60 ≥ *K* > 0.40): moderate; orange (0.40 ≥ *K* > 0.20): fair; red (0.20 ≥ *K* > 0.00): poor.

TABLE 4 Concordance of OncoPredict HPV QT and LMNX Diassay results in the total population for types individually resolved by both assays.

HPV type	Negative in both assays (n)	Positive in both assays (n)	Positive in OncoPredict HPV QT only (n)	Positive in LMNX Diassay assay only (n)	Kappa (95% CI)
HPV 16	1133	89	3	14	0.91 (0.86, 0.95)
HPV 18	1200	20	0	19	0.67 (0.53, 0.81)
HPV 31	1187	45	2	5	0.92 (0.87, 0.98)
HPV 33	1203	30	0	6	0.91 (0.83, 0.98)
HPV 35	1220	15	3	1	0.88 (0.76, 1.00)
HPV 39	1196	36	6	1	0.91 (0.84, 0.98)
HPV 45	1209	16	0	14	0.69 (0.54,0.84)
HPV 51	1191	34	13	1	0.82 (0.73, 0.91)
HPV 52	1200	35	4	0	0.94 (0.89, 1.00)
HPV 56	1192	33	6	8	0.82 (0.73, 0.91)
HPV 58	1201	18	18	2	0.64 (0.49, 0.78)
HPV 59	1204	22	7	6	0.77 (0.64, 0.89)

Note: Color legend (adapted from Landis and Koch)[20] for the concordance: dark green (1.00 ≥ *K* > 0.80): excellent; light green (0.80 ≥ *K* > 0.60): good; yellow (0.60 ≥ *K* > 0.40): moderate; orange (0.40 ≥ *K* > 0.20): fair; red (0.20 ≥ *K* > 0.00): poor.

4 | DISCUSSION

Worldwide cervical cancer screening programs are transitioning from cytology to primary HPV testing resulting in molecular HPV assays becoming increasingly available commercially; however only a relatively small number of them have been assessed according to international criteria designed to validate HPV DNA tests for use in cervical cancer screening.[10,21]

This clinical performance study demonstrated the noninferior accuracy of OncoPredict HPV QT compared to GPEIA for the detection of cervical precancer. The assay also showed excellent intra- and interlaboratory reproducibility of qualitative HPV detection. Furthermore, OncoPredict HPV QT was evaluated as part of the 2019 HPV Labnet international proficiency study, which reported it to be 100% proficient in the detection of the targeted HR-HPV types.[22]

OncoPredict HPV QT assay is a novel quantitative full-genotyping assay targeting E6/E7 viral genes, allowing normalized genotype-specific viral load determination of 12 HR-HPVs defined as 1A carcinogens by the International Agency for Research on Cancer.[23] A key and unique feature of this assay is the extent of quality controls

included to assess sample adequacy, efficiency of nucleic acid extraction and amplification. Sample adequacy is evaluated through the quantitative assessment of a single-copy human gene, CCR5, located on chromosome 3, allowing the number of human cells present in the clinical sample to be determined as well as allowing the accurate normalization of viral loads, as previously described.[24,25] In particular, CCR5 is not a pseudogene, as in the case of several housekeeping genes used as internal controls in nucleic acid amplification tests (NAATs) which have variable copy numbers in the human genome. Moreover, the detection of this single-copy human gene in a reaction-well separate from those dedicated to the amplification of HR-HPV targets prevents "amplification competition," as previously reported in samples with high viral loads,[26] which could compromise the accuracy of viral load determination. The efficiency of nucleic acid recovery, through an external control introduced in the sample before nucleic acid extraction, provides information on the quality of this important preanalytical step. It also supports evaluation, validation and comparison of the assay with different extraction methods/protocols. Finally, an external amplification control is included in each of the five separate real-time PCR reaction wells, part of the analytical step, allowing potential PCR inhibition and/or reagent failures to be discerned.

The OncoPredict HPV QT assay has been CE marked for its use in full automation, combining high-throughput automated DNA extraction and amplification/detection by real-time PCR, as well as for a manual preanalytical and analytical set-up, thus allowing the use of the test both in large scale screening and in smaller molecular diagnostics laboratories.

The additional characteristics of the OncoPredict HPV QT assay, such as the possibility to determine the full-genotype specific normalized viral load (viral copies/no. of human cells), support its application not only in screening programs for cervical cancer prevention but also in the risk-stratification and follow-up of HPV-positive women.[27-30] Studies are ongoing to evaluate the potential value of OncoPredict HPV QT in the molecular triage of HPV-positive women following primary HPV-based screening. Moreover, HR-HPV genotype-specific viral load is expected to be a useful marker in the post-treatment management of women with high-grade dysplasia (test-of-cure). In the present study the genotyping performance of OncoPredict HPV QT assay has been compared to that of GP5+/6+ PCR EIA, according to the current validation guidelines.[9] The latter has been replaced operationally by newer assays in HPV-based screening programmes[21] and as a result guidelines are in the process of being updated to include alternative potential comparators in addition to HC2 and GP5+/6+ PCR EIA. In case of multiple infections, the HPV genotype with the highest viral load was defined as the predominant HPV-type and was used to assess OncoPredict HPV QT positivity, as previously reported for RIATOL qPCR assay.[26,31] Moreover, validation of RIATOL qPCR assay showed similar results in terms of sensitivity and specificity to detect CIN2+ when considering either the cumulative hrHPV viral loads or the genotype with the highest viral load.[31]

Meijer Guidelines[9] suggest that clinical validation of new candidate HPV tests for their use in cervical cancer screening should be performed on a selected panel of CIN2+ cases and <CIN2 controls from women of at least 30 years of age. However, in the current validation study, conducted as part of the VALGENT-2 framework,[13] cervical samples were collected in 2012 in Scotland, when at the time, routine screening was offered to women from 20 to 60 years of age; representing a "real-life" screening population. We also note that samples collected in VALGENT-1 and VALGENT-4 framework came from an HPV-primary screening setting, while those collected during VALGENT-2 and VALGENT-3 from a cytology-based program; however, a multivariate analysis confirmed that primary screening modality did not affect validation outcomes; this aids in extrapolating the findings to contemporary settings.[21]

While the present study focused on clinician-taken samples, there is an increasing global practice and appetite to apply HPV tests to self-taken samples. In the future, there will be a greater onus on demonstrating tests are validated for both clinician- and self-collected specimens.[32] To this end, accuracy of type-specific viral load determination with OncoPredict HPV QT on self-collected as compared to clinician-collected specimens is currently being addressed in the VALHUDES framework.[33]

In conclusion, OncoPredict HPV QT is a novel full-genotyping HR-HPV test with the unique features of allowing genotype-specific normalized viral load determination as well as independent quality controls to assess sample adequacy and preanalytical/analytical processing. The present study demonstrated noninferior accuracy to detect cervical precancer compared to GPEIA in addition to robust intra- and interlaboratory reproducibility. OncoPredict HPV QT, therefore, fulfills all three international validation criteria for HPV tests suitable for primary cervical cancer screening.

AUTHOR CONTRIBUTIONS

Clementina Elvezia Cocuzza: Conceptualization; Methodology, validation and/or data curation; Formal analysis and writing – review & editing. **Sharonjit Kaur Dhillon:** Conceptualization; Formal analysis and writing – review & editing. **Marianna Martinelli:** Methodology, validation and/or data curation. **Chiara Giubbi:** Methodology, validation and/or data curation. **Ruth Chinyere Njoku:** Methodology, validation and/or data curation. **Ramya Bhatia:** Methodology, validation and/or data curation. **Kate Cuschieri:** Conceptualization; Methodology, validation and/or data curation; Formal analysis and writing – review & editing. **Marc Arbyn:** Conceptualization; Formal analysis and writing – review & editing. The work reported in the article has been performed by the authors, unless clearly specified in the text.

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CONFLICT OF INTEREST STATEMENT

CEC received research funding or gratis consumables to support research from the following commercial entities in the last 3 years: BD, Seegene, Copan, Novosanis and Hiantis; CEC is a minority shareholder of Hiantis Srl. SKD and MA's institution has received support from VALGENT, as explained in the VALGENT protocol (Arbyn 2016 J Clin Virol). MA and SKD were supported by the Horizon 2020 Framework Programme for Research and Innovation of the European Commission, through the RISCC Network (Grant No. 847845) and by the European Commission funded SME Instrument Phase 2 Project (Grant agreement ID: 806551). KC's institution has received research funding or gratis consumables to support research from the following commercial entities in the last 3 years: Cepheid, Roche, Vaccitech, Euroimmun, GeneFirst, SelfScreen, Hiantis, Seegene and Hologic. The work was carried out when RB was employed within NHS Lothian and University of Edinburgh, while at the time of publication, RB is employed by QIAGEN as a full-time employee. Marianna Martinelli, Chiara Giubbi and Ruth Chinyere Njoku have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

Data will be provided upon reasonable request to the corresponding author and in line with due process of governance.

ETHICS STATEMENT

The VALGENT-2 samples were obtained from the Scottish HPV Archive which comes under the guardianship and approval of the National Research Scotland Lothian Bioresource (East of Scotland Research Ethics Service Ref 20/ES/0061). Samples were provided for the present study following approval from the Scottish HPV Archive Steering Committee (HPV Archive application Ref-0060).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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