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Clinical and Analytical Performance of the Onclarity HPV Assay Using the VALGENT Framework

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As the demand for human papillomavirus (HPV)-related cervical screening increases, emerging HPV tests must be evaluated robustly using well-annotated samples, such as those generated in the Validation of HPV Genotyping Tests (VALGENT) framework. Through VALGENT, we assessed the performance of the BD Onclarity HPV assay, which detects 14 high-risk (HR) types and resolves six individual types and three groups of types. Consecutive samples from a screening population ($n = 1{,}000$), enriched with cytologically abnormal samples ($n = 300$), that had been tested previously with the GP5+/6+ PCR enzyme immunoassay (EIA) and the GP5+/6+ PCR LMNX assay (Diassay) were tested with the Onclarity assay. Type-specific HPV prevalences were analyzed according to age and cytological result. The accuracy of the Onclarity assay for the detection of cervical intraepithelial neoplasia grade 2+ (CIN2+) and CIN3+ was assessed relative to the GP5+/6+ EIA results by using noninferiority criteria. Overall agreement and type-specific agreement between the Onclarity assay and the GP5+/6+ LMNX assay were assessed. The prevalence of HPV types 16, 18, 31, and 45 increased with the severity of cytological results (P for trend, <0.05). For the detection of CIN2+, the Onclarity assay had a relative sensitivity of 1.02 (95% confidence interval [CI], 0.99 to 1.05; $P < 0.001$ for noninferiority) and a relative specificity of 0.99 (95% CI, 0.97 to 1.00; $P = 0.186$ for noninferiority). The kappa for agreement between the Onclarity assay and the GP5+/6+ LMNX assay for HR-HPV was 0.92 (95% CI, 0.89 to 0.94), and values for the six individual types ranged from 0.78 (95% CI, 0.68 to 0.87) for HPV-52 to 0.96 (95% CI, 0.93 to 0.99) for HPV-16. These data suggest that the Onclarity assay offers applications for clinical workstreams while providing genotyping information that may be useful for risk stratification beyond types 16 and 18.

The choice of tests for human papillomavirus (HPV) has expanded significantly in the past 5 years, consistent with increased evidence for the use of HPV testing for cervical disease management and epidemiological surveillance (1, 2). Comprehensive reviews have listed over 150 commercially available HPV tests (3). In general, HPV tests may be described as consensus assays, consensus assays with limited typing (often for HPV-16 and HPV-18), and extended genotyping tests (which offer typing beyond HPV-16/HPV-18). The first two types of tests tend to be more automated and thus more geared to high-throughput screening than extended genotyping tests, which provide the detailed data required for epidemiological work (4). Given the increased variety of HPV tests, it is important that their performance is assessed robustly, particularly if the results are to be used in the patient pathway.

The Onclarity HPV assay provides consensus results via a high-throughput automated platform and also offers genotyping beyond HPV-16/HPV-18, with individual resolution of types 16, 18, 31, 45, 51, and 52 and identification of three groups of types: 33/58, 56/59/66, and 35/39/68. Initial work showed the test to be reproducible and clinically applicable in a primary cervical cancer screening setting (5). However, more data on performance, particularly at a type-specific level, is required with regard to clinical applications (6–8).

Using a sample panel collated by the Validation of HPV Genotyping Tests (VALGENT) framework (9–12), the primary objective of this work was to determine the clinical performance of the Onclarity assay for primary cervical cancer screening. This was performed using international validation criteria based on the equivalency criteria described by Meijer et al. (13), with the GP5+/6+ enzyme immunoassay (EIA) being used as the clinically validated comparator test. In addition, information on type-specific prevalence stratified by age and disease status was obtained, as were data on concordance between the Onclarity assay and another comparator test, the LMNX genotyping kit HPV GP HR (GP5+/6+ LMNX assay; Diassay), which offers full genotyping for high-risk (HR) HPV (14 types) and for the six high-risk types individually resolved by the Onclarity assay.

MATERIALS AND METHODS

VALGENT projects and sample collection. The VALGENT projects represent an international collaboration designed to assess, using internationally recognized validation criteria, the comparative performance of HPV tests that have at least limited genotyping capacity, in primary screening contexts. The projects are iterative, with the respective VALGENT sample panels being collated in different countries. The sam-

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ple panel used for the current iteration of VALGENT-2 was collated in Scotland. Scotland has a nationally organized cervical screening program aimed at women between 20 and 60 years of age. Of the women who were eligible for cervical screening in Scotland in 2013, 73% had a cervical screening sample taken within the previous 3.5 years. The VALGENT-2 panel contained consecutive samples (1,000 samples) collected from the routinely screened population (the screening set), as well as a disease-enriched component of 300 abnormal samples (the enrichment set). A total of 419 samples were from women <30 years of age and 881 were from women ≥30 years of age. All samples were collected in PreservCyt solution (Hologic, Bedford, MA), between August 2012 and October 2012. Six aliquots of all of the samples were prepared and sent to participating laboratories for HPV testing. The Onclarity assay was performed at the Scottish HPV Reference Laboratory (Edinburgh, United Kingdom), whereas the GP5+/6+ PCR EIA and the LMNX assay were performed at DDL Diagnostic Laboratory (Rijswijk, the Netherlands).

Ethical approval. Favorable ethical opinion for the project was provided by West of Scotland Research Ethics Committee 4 (reference no. 11/WS/0038).

HPV tests and testing strategy. The Onclarity assay is a qualitative target-amplification test that utilizes real-time PCR and fluorescent probe technology to detect E6 and E7 DNA regions of the HPV genome. In terms of readout, a consensus result is provided (presence or absence of high-risk HPV, i.e., positive for at least one of the following types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, or 68), as well as delineation of six individual types, i.e., types 16, 18, 31, 45, 51, and 52, and three groups of types, i.e., types 33/58, 56/59/66, and 35/39/68. The assay also contains a cellular β-globin control for detection of sample inadequacy or inhibition. All samples were tested, according to the manufacturer's instructions, on the BD Viper platform, which requires 500 μl of sample input. For the purposes of the present evaluation, the comparator assay for clinical performance measurements was the EIA kit HPV GP HR (GP5+/6+ EIA; Diassay, Rijswijk, the Netherlands) (considered the standard comparator assay) (14). For purposes of type-specific comparisons, the Onclarity assay was compared to the LMNX genotyping kit HPV GP HR (GP5+/6+ LMNX; Diassay), which utilizes the amplicon generated from the GP5+/6+ PCR for molecular genotyping, including individual resolution of HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68, and includes an internal chromosomal control. The assay also resolves types 26, 53, 73, and 82, although these were not included in comparisons with the Onclarity assay. The performance of the GP5+/6+ EIA and that of HPV genotyping using the GP5+/6+ LMNX assay with this sample set were presented in detail by Geraets et al. (11). The time frames for the GP5+/6+ EIA, GP5+/6+ LMNX, and Onclarity testing were 24 April to 29 May 2013, 24 April to 24 September 2013, and 9 September to 11 December 2013, respectively.

Type-specific prevalence of HPV according to age and underlying pathology. Overall HR-HPV prevalence according to the Onclarity assay was assessed, according to age, in the overall screening set. The type-specific prevalence of HPV according to cytological results was also collated for the aforementioned types and was compared to the type-specific prevalence detected by the GP5+/6+ LMNX assay. A trend test was used to assess whether HPV test positivity increased with the severity grade of the cytological results for the Onclarity and GP5+/6+ LMNX assays (15).

Clinical outcomes and performance measurements. The British Society for Clinical Cytopathology (BSCC) reporting guidelines and the cervical intraepithelial neoplasia (CIN) nomenclature were used to classify cytological findings and histological outcomes, respectively (16–18). Management for women with abnormal cytological results was performed according to guidelines and algorithms associated with the United Kingdom NHS Cervical Screening Programme, modified for use in the Scottish context (16). Colposcopically directed biopsies were performed as routinely indicated, and HPV results did not influence case management. High-grade disease was indicated when women had a diagnosis of CIN grade 2+ (CIN2+) within 18 months of sample collection. No or low-grade disease was assumed when women had two consecutive cytologically negative samples across two screening rounds, which is prescribed as 3 years according to national recall guidelines and was an average of 3.9 years in the study population.

The sensitivity and specificity of the Onclarity assay, relative to detection of CIN2+ or CIN3+, were calculated. Clinical performance was assessed in the total population and also separately for women ≥30 years of age. The performance of the Onclarity assay was compared with that of the GP5+/6+ EIA by using a noninferiority test (13, 19). The sensitivity and specificity of the candidate test needed to be at least 90% and 98%, respectively, compared with the comparator HPV test (12). The McNemar χ^2 test was applied to assess differences between matched proportions. A McNemar test P of >0.05 indicates that the sensitivity or specificity of the Onclarity assay is not significantly different from that of the GP5+/6+ EIA. A noninferiority test P of <0.05 means that the sensitivity or specificity of the Onclarity assay is not significantly lower than that of the GP5+/6+ EIA.

Type-specific agreement between the Onclarity and GP5+/6+ LMNX assays. With the overall sample set, type-specific agreement was assessed using the kappa statistic for the six types individually resolved by the Onclarity assay. The exact McNemar test was applied to determine whether significant differences in the distribution of discordant results existed.

RESULTS

Demographic and pathological characteristics of the study population. When the total population (i.e., screening and enriched sets together) was considered, the average age was 38 years (range, 18 to 68 years). Cytological stratification of the screening population was as follows: negative cytological result, 89.8%; borderline nuclear change, 5.4%; low-grade dyskaryosis, 3.8%; high-grade dyskaryosis (moderate) or worse, 1%. The enrichment population contained, by design, 100 samples with borderline nuclear changes, 100 with low-grade dyskaryosis, and 100 with high-grade dyskaryosis (moderate) or worse. A total of 102 women had CIN2+, with 55 cases being CIN3 or worse. The majority of CIN2+ cases ($n = 89$) were derived from the enrichment population. A total of 744 women had two consecutive cytologically negative smears, and results were used to calculate clinical specificity according to the primary outcome.

HR-HPV prevalence in the screening population by the Onclarity assay. Of the 1,000 consecutive samples taken from the screening set, only two were ineligible/invalid for HPV testing, as evidenced by the lack of amplification of the internal control. Two additional samples were excluded for operational reasons. Of the remaining samples, 18% (95% confidence interval [CI], 16 to 21%) were positive for HR-HPV by the Onclarity assay. HR-HPV prevalences decreased with age and were 42% (95% CI, 33 to 51%) among women 20 to 24 years of age, 33% (95% CI, 25 to 41%) among women 25 to 29 years of age, 13% (95% CI, 10 to 16%) among women 30 to 54 years of age, and 7.6% (95% CI, 4.4 to 10.8%) among women ≥55 years of age (Fig. 1). Prevalences of HPV types 16, 18, 31, 45, 51, and 52 were 3.0%, 1.8%, 1.9%, 0.9%, 1.9%, and 2.9%, respectively. The type-specific prevalences showed similar patterns according to age, with discrete peaks in the age group of 55 to 64 years for HPV types 16, 18, 45, and 52; these graphs can be viewed in Fig. S1 in the supplemental material.

Type-specific prevalence by the Onclarity and GP5+/6+ LMNX assays according to cytological results. When HR-HPV prevalences in abnormal (i.e., not negative) cytological result categories were assessed with both the screening and enrichment sets,

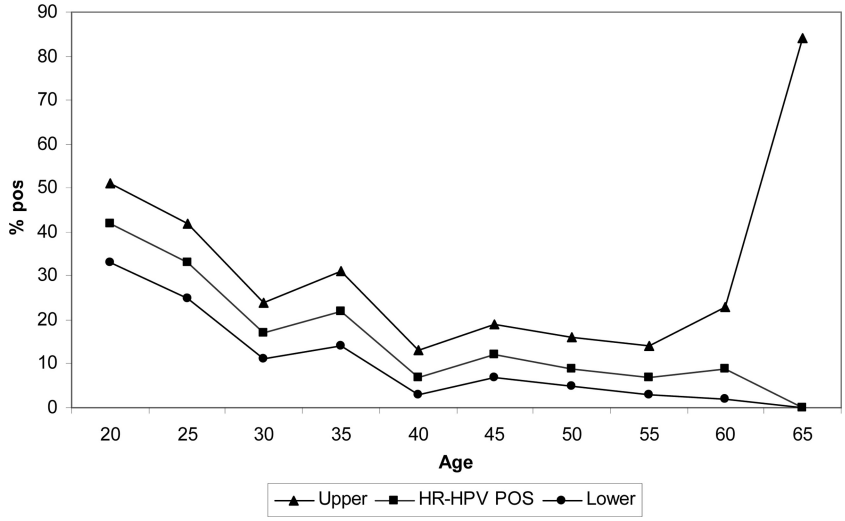

FIG 1 HR-HPV positivity (POS) by 5-year age group, as assessed by the Onclarity assay, in 996 consecutive samples from the Scottish cervical screening population, with upper and lower confidence limits for the proportions.

no significant difference was found; consequently, the data were aggregated (Table 1). HR-HPV positivity by the Onclarity assay increased with the severity of the cytological reporting category. HPV types 16, 18, 31, and 45 increased with the severity of the cytological results (*P* for trend, <0.05). HPV types 51 and 52 were actually less common (*P* for trend, <0.05) in samples with high-grade dyskaryosis (moderate) or worse, compared to low-grade dyskaryosis. The prevalences of HR-HPV (overall) and HPV types 16, 18, 31, and 45, as assessed with the GP5+/6+ LMNX assay, also increased with the severity of the cytological results (*P* for trend, <0.05).

Type-specific agreement between the Onclarity and GP5+/6+ LMNX assays. Agreement for the types individually resolved by both assays varied (according to type), from a kappa of 0.775 (95% CI, 0.683 to 0.868) for HPV-52 to a kappa of 0.956 (95% CI, 0.927 to 0.985) for HPV-16. When only single infections were considered, agreement was slightly lower and ranged from 0.720 (95% CI, 0.597 to 0.844) for HPV-52 to 0.940 (95% CI, 0.900 to 0.979) for HPV-16. Full details are presented in Table 2. Application of the McNemar test indicated statistically significant differences associated with HPV-52 ($P < 0.0001$) and HPV-31 ($P = 0.0156$).

Clinical performance of the Onclarity assay. Table 3 presents cross tabulations of the GP5+/6+ EIA and Onclarity assay results for the total sample set, according to disease grade. Data are presented for all ages and for women >30 years of age. Assay agreement rates were 96.5% for all ages and 97.1% for women >30 years of age; both assays detected 54/55 cases of CIN3+, with one

TABLE 1 HR-HPV and type-specific prevalences in the screening and enrichment populations, using the Onclarity and GP5+/6+ LMNX assays

Assay and HPV type	No. (%) with cytological result of:			
	Negative ($n = 894$)[a]	Borderline ($n = 154$)	Low grade ($n = 138$)	High grade ($n = 110$)
Onclarity				
HR-HPV[b]	110 (12.3)	88 (57.1)	115 (83.3)	101 (91.8)
HPV-16	15 (1.7)	15 (9.7)	34 (24.6)	48 (43.6)
HPV-18	11 (1.2)	7 (4.5)	8 (5.8)	11 (10.0)
HPV-31	8 (0.9)	14 (9.1)	16 (11.6)	23 (20.9)
HPV-45	6 (0.7)	7 (4.5)	4 (2.9)	12 (10.9)
HPV-51	6 (0.7)	12 (7.8)	16 (11.6)	6 (5.5)
HPV-52	16 (1.8)	10 (6.5)	19 (13.8)	14 (12.7)
GP5+/6+ LMNX				
HR-HPV[b]	100 (11.2)	84 (54.5)	108 (78.3)	98 (89.1)
HPV-16	18 (2.0)	15 (9.7)	31 (22.5)	47 (42.7)
HPV-18	13 (1.5)	7 (4.5)	8 (5.8)	12 (11.0)
HPV-31	6 (0.7)	13 (8.4)	13 (9.4)	22 (20)
HPV-45	7 (0.8)	8 (5.2)	3 (2.2)	12 (10.9)
HPV-51	10 (1.1)	8 (5.2)	16 (11.6)	4 (3.6)
HPV-52	10 (1.1)	8 (5.2)	10 (7.2)	10 (9.1)

[a] Cytologically negative samples arose only from the screening population, and two samples were not assessed with the GP5+/6+ LMNX assay (cytologically negative) for operational/technical reasons.
[b] Positive for at least one of 14 HR-HPV types with the Onclarity assay and the GP5+/6+ LMNX assay.

TABLE 2 Concordance of GP5+/6+ LMNX and Onclarity assay results in the total population for types individually resolved by both assays

Infection type and HPV type	No.				κ (95% CI)	McNemar *P*
	Negative by both assays	Positive by both assays	Positive by Onclarity assay only	Positive by GP5+/6+ LMNX assay only		
Single or multiple infection						
HPV-16	1,180	107	5	4	0.956 (0.927–0.985)	1.0000
HPV-18	1,255	36	1	4	0.933 (0.875–0.992)	0.375
HPV-31	1,235	54	7	0	0.936 (0.889–0.983)	0.0156
HPV-45	1,264	27	2	3	0.913 (0.838–0.989)	1
HPV-51	1,252	34	6	4	0.868 (0.787–0.949)	0.7539
HPV-52	1,237	38	21	0	0.775 (0.683–0.868)	<0.0001
Single infection						
HPV-16	1,163	75	5	4	0.940 (0.900–0.979)	1.000
HPV-18	1,216	26	1	4	0.910 (0.832–0.988)	0.375
HPV-31	1,280	33	6	0	0.914 (0.846–0.982)	0.031
HPV-45	1,232	11	2	2	0.845 (0.694–0.995)	1.000
HPV-51	1,214	24	5	4	0.838 (0.734–0.942)	1.000
HPV-52	1,205	24	18	0	0.720 (0.597–0.844)	<0.0001

case (in a woman >30 years of age) testing negative by both assays. A total of 96/102 cases of CIN2+ were positive by both assays, with the Onclarity assay detecting two more cases of CIN2+ than the GP5+/6+ EIA.

These findings translate into high clinical sensitivities of the Onclarity assay for detection of CIN2+ and CIN3+ cases, i.e., 96.1% (95% CI, 94.8 to 97.4%) and 98.2% (95% CI, 97.3 to 99.1%), respectively. When the analysis was confined to women >30 years of age, sensitivities were 95.5% (95% CI, 93.9 to 97.0%) and 95.8% (95% CI, 94.3 to 97.4%) for CIN2+ and CIN3+ cases, respectively. The specificity of the Onclarity assay for CIN1 or less was 89.1% (95% CI, 87.0 to 91.2%) in the total set and was slightly higher when analysis was confined to women >30 years of age, being 91.3% (95% CI, 89.2 to 93.4%).

The performance of the GP5+/6+ EIA in this sample set was described comprehensively by Geraets et al. (11); briefly, the sensitivities of the comparator assay for CIN2+ and CIN3+ were 94.1% (95% CI, 92.5 to 95.7%) and 98.2% (95% CI, 97.3 to

TABLE 3 Cross tabulations of Onclarity assay and GP5+/6+ EIA results for identification of HR-HPV in women with CIN2+ or CIN3+ and in women with two consecutive negative cytological results[a]

Underlying pathology and GP5+/6+ EIA result	No.					
	All ages			Age of ≥30 yr		
	Positive Onclarity assay result	Negative Onclarity assay result	Total	Positive Onclarity assay result	Negative Onclarity assay result	Total
Overall (irrespective of confirmed outcome)						
Positive	378	9	387	154	7	161
Negative	36	873	909	18	698	716
Total	414	882	1,296	172	705	877
CIN2+						
Positive	96	0	96	41	0	41
Negative	2	4	6	1	2	3
Total	98	4	102	42	2	44
CIN3+						
Positive	54	0	54	23	0	23
Negative	0	1	1	0	1	1
Total	54	1	55	23	1	24
CIN1 or less						
Positive	65	7	72	42	6	48
Negative	16	656	672	13	570	583
Total	81	663	744	55	576	631

[a] Women with two consecutive negative cytological results were assumed to have CIN1 or less. The second, third, and fourth sets of rows display data where the defined outcomes were available. The first set of rows incorporates data from the other three sets of rows in addition to women for whom outcomes were not available.

TABLE 4 Absolute sensitivities for CIN2+ and CIN3+ and absolute specificities for CIN1 or less of the Onclarity assay and the GP 5+/6+ EIA[a]

Age group and outcome	Onclarity assay		GP5+/6+ EIA	
	Sensitivity (95% CI) (%)	Specificity (95% CI) (%)	Sensitivity (95% CI) (%)	Specificity (95% CI) (%)
All ages				
CIN2+	96.1 (94.8–97.4)		94.1 (92.5–95.7)	
CIN3+	98.2 (97.3–99.1)		98.2 (97.3–99.1)	
CIN1 or less		89.1 (87.0–91.2)		90.3 (88.3–92.3)
Age of ≥30 yr				
CIN2+	95.5 (93.9–97.0)		93.2 (91.3–95.1)	
CIN3+	95.8 (94.3–97.4)		95.8 (94.3–97.4)	
CIN1 or less		91.3 (89.2–93.4)		92.4 (90.4–94.4)

[a] Positivity is based on the detection of 14 HR-HPV types.

99.1%), respectively, with specificities of 90.3% (95% CI, 88.3 to 92.3%) for the total population and 92.4% (95% CI, 90.4 to 94.4%) for women >30 years of age. Table 4 provides a summary of sensitivity and specificity values for both assays.

The performance of the Onclarity assay relative to the GP5+/6+ EIA is presented in Table 5. The Onclarity assay had a relative sensitivity of 1.02 (95% CI, 0.99 to 1.05), with a McNemar test *P* of 0.157 and noninferiority *P* of <0.001, and thus was considered noninferior to GP5+/6+ EIA for the detection of CIN2+. The relative specificity of the Onclarity assay for CIN1 or less was 0.99 (95% CI, 0.97 to 1.00), with a McNemar test *P* of 0.061, and thus the Onclarity assay was similar to the comparator assay. However, the Onclarity assay did not reach noninferiority (*P* = 0.186 for noninferiority). These observations did not differ when the analysis was confined to women >30 years of age, with the relative sensitivity for CIN2+ being 1.02 (95% CI, 0.98 to 1.07), with a McNemar test *P* of 0.317 and noninferiority *P* of 0.0083 (noninferior to GP5+/6+ EIA), and the relative specificity for CIN1 or less being 0.99 (95% CI, 0.97 to 1.00), with a McNemar test *P* of 0.108 and noninferiority *P* of 0.153 (inferiority to GP5+/6+ EIA cannot be rejected).

DISCUSSION

The Onclarity assay is a clinically sensitive test that has performance similar to that of the GP5+/6+ EIA and offers extended typing capability. The Onclarity assay has the capacity to resolve individually five of the seven HPV genotypes most commonly associated with cervical cancer, i.e., types 16, 18, 45, 33, 31, 52, and 58 (20). As diagnostic investigation was triggered only by a positive cytological result, the observation of additional CIN2+ cases over time will provide further insight into the longitudinal performance of the assay. Consequently, follow-up monitoring of the Scottish VALGENT cohort over subsequent screening rounds is planned

In the present analysis, the Onclarity assay did not demonstrate noninferiority for specificity. This is consistent with the finding that, overall, the Onclarity assay detected more infections than the GP5+/6+ EIA, while not detecting significantly more cases of CIN2+. In the recent work by Ejegod et al. (5), the authors found the Onclarity assay to be noninferior to a different clinically validated reference test (Hybrid Capture 2 [HC2] assay; Qiagen Ltd., Manchester, United Kingdom) for women >30 years of age, with a sensitivity for CIN2+ of 92.9% (95% CI, 87.7 to 96.4%) versus 94.2% (95% CI, 89.3 to 97.3%) for the comparator assay and a specificity of 87.7% (95% CI, 86.8 to 88.7%) versus 88.8% (95% CI, 87.9 to 89.7%) for the comparator assay. There are a number of possible explanations for these findings; absolute accuracy measures are strongly influenced by the specific populations and diagnostic procedures used to determine them, although these factors are somewhat ameliorated when two different HPV assays are applied to the same sample (12, 21). The samples collected were from a different region of the United Kingdom (St. Mary's Hospital, London, United Kingdom), whereas the samples for the present analysis were derived exclusively from Scotland. Previous epidemiological and clinical studies have shown that the prevalence of HR-HPV infection is particularly high in Scotland, higher

TABLE 5 Relative sensitivities for CIN2+ and CIN3+ and relative specificities for CIN1 or less of the Onclarity assay versus the GP 5+/6+ EIA

Age group and outcome	Relative performance (Onclarity assay vs GP5+/6+ EIA)		McNemar *P*[a]	Noninferiority *P*[b]
	Relative sensitivity (95% CI)	Relative specificity (95% CI)		
All ages				
CIN2+	1.02 (0.99–1.05)		0.5000	0.0002
CIN3+	1.00 (0.96–1.03)		1.0000	0.0107
CIN1 or less		0.99 (0.973–1.002)	0.0931	0.1865
Age of ≥30 yr				
CIN2+	1.02 (0.98–1.07)		1.0000	0.0086
CIN3+	1.00 (0.92–1.09)		1.0000	0.0550
CIN1 or less		0.99 (0.974–1.003)	0.1671	0.1551

[a] *P* for the McNemar test for a difference between matched proportions; *P* values of >0.05 indicate that the sensitivity or specificity of the Onclarity assay is not significantly different from that of the GP5+/6+ EIA.

[b] *P* for the test for noninferiority; *P* values of <0.05 indicate that the sensitivity or specificity of the Onclarity assay is not significantly lower than that of the GP5+/6+ EIA.

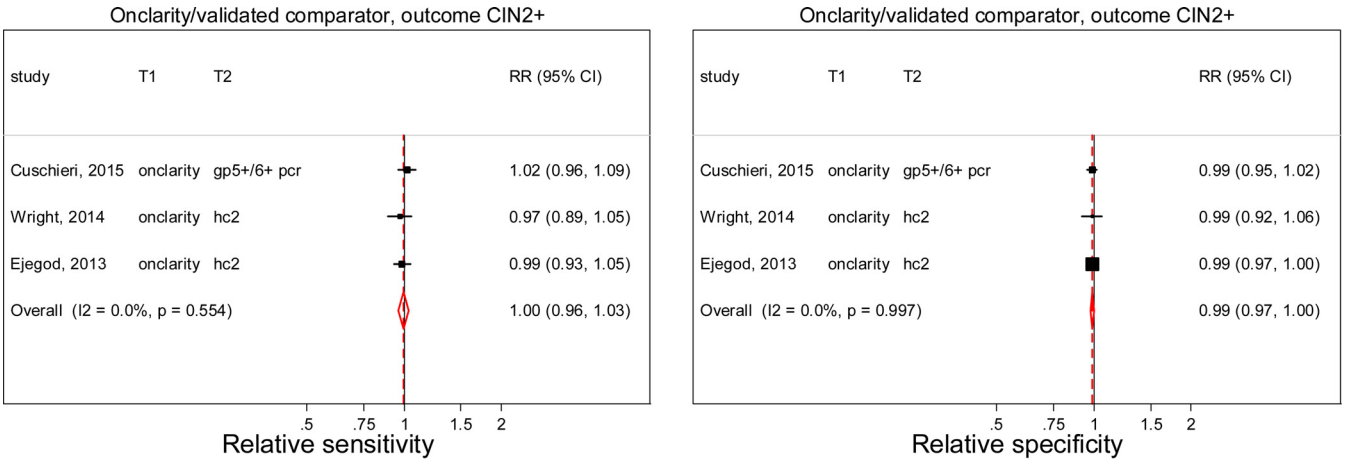

FIG 2 Relative sensitivity and specificity to detect CIN2+ of the Onclarity assay (test 1 [T1]), compared to HPV assays that have been validated for primary HPV screening (test 2 [T2]). RR, relative risk.

than in England (22). It is possible that the Scottish context may present a particular challenge for the specificity of HPV assays (23). In addition, our population included women <30 years of age, whereas the analysis by Ejegod et al. (5) focused on women ≥30 years of age. However, a subanalysis of the presented data that confined performance measurements to women >30 years of age did not lead to a change in the overall conclusions; we note that this reduces the overall numbers available for evaluation. Furthermore, in general terms, the total number of samples assessed, particularly to compute specificity, influences the power of noninferiority testing. When we performed simulations of the data to reflect a larger sample size for the group with no disease, the *P* value for noninferiority became significant (data not shown).

In a multicenter U.S. study of the Onclarity assay using 541 archived samples, Wright and colleagues (24) reported the sensitivity and specificity of the assay for CIN2+ as 90.4% (95% CI, 83 to 95%) and 76.9% (95% CI, 73 to 81%), respectively, which are lower than the values reported in the present study, i.e., 96.1% (95% CI, 94.8 to 97.4%) and 89.1% (95% CI, 87.0 to 91.2%), respectively, and also lower than those reported by Ejegod et al. (5). Again, the different populations must be considered; the U.S. study involved a younger population and histological "adjudication" by a review panel of pathologists, whereas, in the study by Ejegod et al. (5) and the present study, the histological results were those derived from routine practice and were not reviewed. Furthermore, in the U.S. study, the cytological samples were in SurePath preservative fluid, which might have had a bearing on the results (25). In future VALGENT iterations, we aim to include formal histological review and also sample sets collected in SurePath fluid, which will address these potential confounders directly.

Figure 2 shows pooled relative sensitivity and specificity values for the Onclarity assay for CIN2+, compared to the two HPV assays that were initially validated for primary screening, i.e., the HC2 assay and the GP5+/6+ EIA (12). The pooled relative sensitivity and specificity estimates were 0.995 (95% CI, 0.958 to 1.033; $I^2 = 0.0\%$; $P = 0.554$ for heterogeneity) and 0.988 (95% CI, 0.975 to 1.001; $I^2 = 0.0\%$; $P = 0.997$ for heterogeneity), respectively. No heterogeneity was induced by the use of two different comparator tests, i.e., the HC2 assay in the studies by Wright et al. (24) and Ejegod et al. (5) and the GP5+/6+ EIA in the present study.

Although we are limited in our ability to comment on the predictive value of types, given the relatively small number of CIN2+ cases in the screening population and the artificial nature of the enrichment population, it was of interest to note that the two most common HPV types associated with CIN2+ (by both assays) were HPV-16 and HPV-31 (data not shown). According to the GP5+/6+ LMNX HPV test, HPV types 33, 45, 52, and 18 were present in equal numbers after these, consistent with the observation that the type 33/58 channel of the Onclarity assay was the third most positive, after types 16 and 31. Using the Onclarity assay, Wright et al. suggested that HPV types 31 and 33/58 (combined) "may warrant monitoring in future studies," based on their relative risks for CIN2+ and CIN3+, which were comparable to those of HPV-18 (24). HPV-58 is the seventh most common type in cancer globally, with a greater preponderance in CIN2+ cases in Asia; therefore, individual resolution of HPV-58 may be warranted.

When the GP5+/6+ LMNX assay was compared with the Onclarity assay at the type-specific level, the most significant difference was observed for HPV-52, with 21 infections detected by the Onclarity assay only. The other significant difference was with HPV-31, with the Onclarity assay again detecting significantly more infections. While our present analysis indicates that these observations have little bearing on clinical sensitivity, given that HPV-31 and HPV-52 are the fifth and sixth most common types, respectively, associated with cancer, further investigation of discrepant samples is worthwhile. Similar to findings in the present study for the Onclarity assay, Castle et al. showed increased detection of HPV-31 and HPV-52 with the use of SPF10 versus GP5+/6+ primers, which indicates that the latter primers may be less sensitive for these types (26). As discussed, our aim is to continue to monitor this VALGENT-2 cohort, so that longitudinal outcomes can be assessed and related to type-specific results. A potential confounder of these observations (and indeed for any of the between-assay comparisons) is that, while the time frames for testing with the GP5+/6+ LMNX and Onclarity assays overlapped, testing was not simultaneous. However, data indicate that the stability of HPV DNA in liquid-based cytology (LBC) samples collected in PreservCyt solution is high (27). In addition, to investigate type-specific discordance more fully, we

plan a deeper investigation of all discrepant results identified by all assays employed in the VALGENT-2 projects, including sequencing. The influence of viral loads on discordance will also be assessed; an initial analysis with the GP5+/6+ LMNX assay indicated that type-specific mean fluorescence intensity (MFI) values were higher for types 31 and 45 within multiple infections and that type-specific concordance was greater in samples with higher MFI values (data not shown). Finally, we plan to assess the influence of nucleic acid extraction on assay concordance.

In conclusion, the Onclarity assay is a rapid test that offers applications for clinical workstreams, offering genotyping beyond HPV-16/HPV-18 that may be useful for risk stratification for HR-HPV-positive women. The assay has high sensitivity for CIN2+ and specificity similar to that of the GP5+/6+ EIA. Further data on the clinical performance of the Onclarity assay, at the consensus and type-specific levels and including triage and posttreatment contexts, would be welcome.

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K.C. was the local (NHS Lothian) principal investigator for the study and created the manuscript drafts, D.T.G. and W.Q. were responsible for delivery and analysis of the sample panel with the comparator assay, C.M. organized and delivered laboratory testing associated with the Onclarity platform, E.D. coordinated the collation of pathology data, and M.A. is the chief investigator of the VALGENT projects and performed statistical analysis. All authors approved the final article.

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