Clinical Performance of CLART Human Papillomavirus 2 in Comparison with Hybrid Capture 2

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Keywords:	HPV, CLART Human Papillomavirus 2, HC2, screening, genotyping

TABLE I. Characteristics of the studied cervical samples

CYTOLOGICAL RESULTS (N=425)	**N (%)**
Normal	63 (14.8)
ASCUS	111 (26.1)
LSIL	127 (29.9)
HSIL	118 (27.8)
SCC	6 (1.4)
HISTOLOGICAL RESULTS (N=425)	
Normal	95 (22.4)
CIN1	63 (14.8)
CIN2	118 (27.8)
CIN3	98 (23.1)
SCC	8 (1.9)
ND	43 (10.1)
CLINICAL DIAGNOSIS (cytology + biopsy; N=405)	
≤CIN1	178 (44.0)
≥CIN2	227 (56.0)
ND	20
CLART-13 HR (N=425)	
Positive	268 (63.1)
Negative	157 (36.9)
CLART-17 HR (N=425)	
Positive	275 (64.7)
Negative	150 (35.3)
HC2 (N=425)	
Positive	274 (64.5)
Negative	151 (35.5)

ASCUS, atypical squamous cells of undeterminated significance; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; SCC, squamous cell carcinoma; CIN1, cervical intraepithelial neoplasia grade 1; CIN2, cervical intraepithelial neoplasia grade 2; CIN3, cervical intraepithelial neoplasia grade 3; ND, no determined.

TABLE II. Clinical performance for ≥CIN2 based in Pap smear and biopsy diagnosis of 405 samples

		Clinical Diagnosis		Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
		≤CIN1 (n=178)	≥CIN2 (n=227)				
CLART-13 HR	P	47	218	96.0 (92.6-97.9)	73.6 (66.7-79.5)	82.2 (79.4-82.7)	93.6 (93.0-94.2)
	N	131	9				
	Positivity (%)	26.4	96.0				
CLART-17 HR	P	50	220	96.9 (93.8-98.5)	71.9 (64.9-78.0)	81.5 (79.9-83.1)	94.8 (94.3-95.3)
	N	128	7				
	Positivity (%)	28.1	96.9				
HC2	P	51	218	96.0 (92.6-97.9)	71.4 (64.3-77.5)	81.0 (79.4-82.7)	93.4 (92.8-94.0)
	N	127	9				
	Positivity (%)	28.7	96.0				

≤CIN1, cervical intraepithelial neoplasia grade 1 or less; ≥CIN2, cervical intraepithelial neoplasia grade 2 or worse; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval; P, HPV positive; N, HPV negative.

TABLE III. Analytical performance of CLART and HC2 in 425 samples

		HC2		Concordance	*k*	Sensitivity	Specificity	PPV	NPV
		P	N	% ±SD	(95% CI)	(95% CI)	(95% CI)	(95% CI)	(95% CI)
CLART-13 HR	P	268	0	98.6	0.969	97.8	100	100	96.2
	N	6	151	±1.2	(0.941-0.969)	(96.8-97.8)	(98.1-100)	(98.9-100)	(94.4-96.2)
CLART-17 HR	P	272	3	98.8	0.974	99.3	98.0	98.9	98.7
	N	2	148	±1.1	(0.941-0.988)	(98.1-99.8)	(95.9-98.9)	(97.7-99.4)	(96.5-99.6)

PPV, positive predictive value; NPV, negative predictive value; *k*, agreement; CI, confidence interval; SD, standard deviation; P, HPV positive; N, HPV negative.

TABLE IV. Discordant results between CLART and HC2

Case	CLART-13 HR-HPV	CLART-17 HR-HPV	HC2	PapilloCheck	Cytology / Biopsy
1	N	HPV 66	P	HPV 66	CIN1
2	N	N	P	N	CIN1
3	N	N	P	N	Normal
4	N	HPV 82	P	HPV 82	CIN1
5	N	HPV 53	P	HPV 53	ASCUS
6	N	HPV 66	P	HPV 66	ASCUS

P, HPV positive; N, HPV negative; ASCUS, atypical squamous cells of undeterminated significance; CIN1, cervical intraepithelial neoplasia grade 1.

RESEARCH ARTICLE

Clinical Performance of the CLART Human Papillomavirus 2 Assay Compared with the Hybrid Capture 2 Test

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Running Title:

Validation of CLART Human Papillomavirus 2

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ABSTRACT

Persistent infection by high-risk human papillomavirus (HR-HPV) is a cause of cervical cancer. The use of HPV detection in cervical screening programs may improve the ability to identify women at risk of cervical cancer. Therefore, the development of appropriate methods for the detection of HR-HPV is essential. The aim of this study was to evaluate the clinical performance of the CLART Human Papillomavirus 2 assay (CLART) in comparison with the Hybrid Capture 2 test (HC2), using a clinical cut-off of cervical intraepithelial neoplasia grade 2 or worse. Discrepant results were analysed further by the PapilloCheck HPV genotyping system.

In the 425 studied women, HR-HPV positivity rates were similar by both tests (CLART-13 HR-HPV: 63.1%; CLART-17 HR-HPV: 64.7%; HC2: 64.5%). Agreement between CLART-13 HR-HPV (k=0.969; concordance level 98.6%), CLART-17 HR-HPV (k=0.974; concordance level 98.8%) and HC2 were very good. When 13 HR-HPV types were considered, the two tests showed a clinical sensitivity of 96% (95% CI: 92.6-97.9). The clinical specificity of CLART-13 HR-HPV was 73.6% (95% CI: 66.7-79.5) for cervical intraepithelial neoplasia grade 2 or worse, which was comparable to HC2 (71.4%; 95% CI: 64.3-77.5). When all 17 HR-HPV types were considered, CLART showed a clinical sensitivity of 96.9 % (95% CI: 93.8-98.5) and a clinical specificity of 71.9% (95% CI: 64.9-78.0).

In conclusion, the CLART assay is efficient, sensitive, reproducible, and has a similar performance to HC2 for cervical intraepithelial neoplasia grade 2 or worse. Furthermore, this assay has the advantage of detecting and genotyping 35 HPV types by a single test, which can provide additional information on the predictive value of infection with HR-HPV.

Keywords:

HPV; CLART Human Papillomavirus 2; HC2; screening; genotyping

INTRODUCTION

Epidemiological studies have established that certain human papillomavirus (HPV) genotypes are related etiologically to cervical cancer development [Koutsky et al., 1992; Schiffman et al., 1993; Bosch et al., 1995]. More than 120 HPV types have been identified, of which approximately 40 can infect the mucosa of the genital tract. According to the oncogenic potential, these HPV types can be classified into high-risk (HR), associated with premalignant lesions and cervical cancer, and low-risk (LR), found mainly in benign lesions [Munoz et al., 2003; Bernard et al., 2010]. Most infections will clear spontaneously, but persistent infection with HR-HPV is a strong predictor of the development of high-grade cervical intraepithelial neoplasia (CIN) and cancer of the cervix uteri [Nobbenhuis et al., 1999; Kjaer et al., 2002].

In areas where the Pap smear is the primary screening method, the development of premalignant lesions may be attributed to the low reproducibility and sensitivity of the Papanicolau test [Renshaw, 2002]. Several studies [Bosch et al., 2003; Cuzick et al., 2003, 2008; Bulkmans et al., 2007; Szarewski et al., 2008] have shown that the combined use of cytology and HPV DNA testing in women above 30 years old can improve the sensitivity and the negative predictive value (NPV) of screening. HPV testing can also provide the reassurance of extended intervals between screenings, and can be cost-effective for the detection of high-grade lesions in women with equivocal cytological abnormalities. Indeed, recent studies [Castle et al., 2005; Lai et al., 2007] have shown that a single positive result for either HPV 16 or 18 has a high predictive value for CIN grade 2 or worse (≥CIN2). Several HPV assays with various levels of sensitivity and specificity have been made available recently. However, clinical validation is required before these tests can be used as a "stand-alone" method in cervical cancer screening programs. Tests with significantly higher sensitivity for the detection of HPV DNA than the Hybrid Capture 2 test (HC2) (Qiagen, Hilden, Germany) could detect latent infections that are irrelevant clinically, which may lead to overtreatment of women [Snijders et al., 2003; Meijer et al., 2009]. Recently, an assay based on PCR and hybridization, the CLART Human Papillomavirus 2 (CLART) (Genomica, Madrid, Spain) was developed for the detection and genotyping of 35 HPV types (20 HR-HPV and 15 LR-HPV), in single or multiple infections.

The aim of this study was to evaluate the performance of the CLART assay in comparison with the HC2 test in women for whom histological results were available. Further, the clinical performance was evaluated for each histological grade, using a clinical cut-off of CIN grade 2 or worse (≥CIN2).

MATERIALS AND METHODS

Study Design

The study population comprised 425 archived cervical samples from sexually active women, attending at primary Health-Care Clinics of the National Health Service and Gynaecological Outpatient Clinics. Although not a screening population, the advantage was a broad range of outcomes and a high disease rate, which would enable accurate evaluation of sensitivity and specificity in a relative small sample. All cervical samples were collected in ThinPrep PreservCyt medium (Cytyc UK, Crawley, West Sussex, UK) during clinical examination for cytological analyses. The residual liquid-based cytology (LBC) was used for HPV testing. In 405 samples, the final clinical diagnosis was based on histological examination of biopsy samples obtained at colposcopy. As suggested by Wentzensen and colleagues [2009] and according to histology, 178 out of the 405 women (44%) were considered to have CIN grade 1 or less (≤CIN1, regarded as controls), while 227 (56%) were diagnosed as CIN grade 2 or worse (≥CIN2, regarded as cases). No patient was sampled solely for the purpose of this research study.

All samples were tested by both the CLART and the HC2 assays. Discrepant results were analysed further using the PapilloCheck HPV genotyping system (Greiner Bio-One, Frickenhausen, Germany). To assess the reproducibility of the CLART assay, 75 samples were retested randomly twice by two operators.

Each HPV test was carried out independently of each other according to the manufacturer's instructions. DNA was isolated from 1 ml of cellular suspension by using the NucliSENS easyMAG (BioMerieux, Boxtel, The Netherlands) system, as specified in the manufacturer's instructions. Nucleic acids were eluted in a final volume of 100 µl and stored at -20 ºC until use for PCR analysis.

CLART Human Papillomavirus 2

This methodology uses biotinylated primers that amplify a fragment a 450 bp fragment within the HPV L1 region. Co-amplification of an 892 bp region of the CFTR gene and a 1202 bp fragment of a transformed plasmid provides a control to ensure DNA extraction adequacy and PCR efficiency. Amplicons are detected by hybridization in a low-density microarray containing triplicate DNA probes specific to 35 types (HPV 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 68, 70, 71, 72, 73, 81, 82, 83, 84, 85, and 89). Semi-quantitative results can be obtained in an automatic reader.

Hybrid Capture 2

The HC2 test is a sandwich capture molecular hybridization assay that uses a signal amplification detection method based on chemiluminescence. Thirteen HR-HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) can be detected with this test. The resultant DNA:RNA hybrids are captured on a microplate, and the emitted light is measured in a luminometer as relative light units (RLU). Samples were considered as positive if the ratio RLU/cut-off was greater than 1.0 (equivalent to 1.0 pg HPV DNA/ml). All the cut-offs between 1 and 2.5 were retested and all were greater than 1.0 RLU/cut-off (data not shown).

PapilloCheck HPV Genotyping

The PapilloCheck system allows the genotyping of 24 HPV types (HPV 6, 11, 16, 18, 31, 33, 35, 39, 40, 42, 43, 44/55, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73 and 82). This assay uses a multiplex PCR that amplify a 350 bp fragment of the E1 gene of HPV. Co-amplification of the human gene ADAT1 is used as an internal control. The hybridization is performed on a microarray chip, which is automatically scanned and analysed using the CheckScanner™ at both 532 nm and 635 nm and the CheckReport software, respectively.

Statistical Analysis

For comparative purposes with the HC2 test, results of the CLART assay were considered as positive if one of the following 13 HR-HPV types (CLART-13 HR-HPV) was present: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. In addition, the evaluation was performed also considering 17 (probably) HR-HPV types (CLART-17 HR-HPV) (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82) [Bernard et al., 2010].

The sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) were calculated using 2x2 contingency tables with 95% confidence intervals (95% CI). All *P*-values were obtained using the Fisher's Exact Test or McNemar X^2 for comparison of matched-pair samples. *P*-values <0.05 were considered statistically significant. Agreement between assays was assessed by Cohen's kappa (*k*) statistics. All analyses were conducted using the SPSS (*Statistical Package for the Social Sciences;* SPSS, Inc., Chicago, IL) version 16.0 software.

RESULTS

A total of 425 cervical samples from women aged 25-63 years old (mean age: 34.4±10.0 years; median age: 35 years old) were studied. From these, a subset of 405 samples was available for clinical evaluation of the CLART assay (Table I). Results of HPV DNA assays and clinical diagnosis (based on cytology and biopsy) were shown in Table I. Overall, HR-HPV types were detected in 268 cases (63.1%) with CLART-13 HR-HPV, in 275 cases (64.7%) with CLART-17 HR-HPV, and in 274 (64.5%) with the HC2 test (Table I).

Clinical performance of the two tests for CIN grade 2 or worse (≥CIN2) was very good and comparable, with sensitivities of 96% for CLART-13 HR-HPV and HC2, and 96.9% for CLART-17 HR-HPV. The specificity and NPV of all the tests were also comparable (Table II). No significant statistical differences were found among assays, in terms of sensitivity, specificity and NPV (P=0.000).

Overall, agreement between the CLART and the HC2 was very good (k=0.969, 95% CI: 0.941-0.969; concordance: 98.6% ±1.2 for 13 HR-HPV, and k=0.974, 95% CI: 0.941-0.988; concordance: 98.8% ±1.1 for 17 HR-HPV; P=0.000). Compared with the HC2 test, the CLART-13 HR-HPV assay showed a sensitivity, specificity, PPV, and NPV of 97.8% (95% CI: 96.8-97.8), 100% (95% CI: 98.1-100), 100% (95% CI: 98.9-100), and 96.2% (95% CI: 94.4-96.2), respectively (Table III). For 17 HR-HPV the CLART assay showed a sensitivity, specificity, PPV, and NPV of 99.3% (95% CI: 98.1-99.8), 98% (95% CI: 95.9-98.9), 98.9% (95% CI: 97.7-99.4), and 98.7% (95% CI: 96.5-99.6), respectively (Table III).

Discordant results were observed in 6 samples, which were positive by the HC2 test and negative by the CLART assay for the same 13 HR-HPV types. Considering CLART-17 HR-HPV, 2 samples were negative and 4 were positive (for HPV 53, 66, and 82). Further testing by the PapilloCheck revealed a total concordance with the results obtained by CLART-17 HR-HPV (Table IV).

In addition, the CLART assay was evaluated for reproducibility (n=75). The assay showed a concordance of 98.7% (±2.7%) and an agreement of 0.972 (95% CI: 0.919-1) for the samples tested (data not shown).

DISCUSSION

Recent studies [Castle et al., 2005; Lai et al., 2007; Cuzick et al., 2008] have shown that detection of HR-HPV, in particular of HPV 16 and 18, can be an important aspect to consider in cervical cancer screening programs and algorithms. Since most HR-HPV infections can clear spontaneously, a clinically useful HPV test needs to have an optimal clinical sensitivity and specificity in order to effectively detect CIN grade 2 or worse (≥CIN2). Tests with a better sensitivity than that of the HC2 will detect a large number of latent infections that are clinically irrelevant and lead to an overtreatment of women with transient HPV infections [Snijders et al., 2003; Meijer et al., 2009].

In this study, the performance of a new commercially available CE-marked genotyping assay (CLART Human Papillomavirus 2) that allows the detection and genotyping of 35 HPV types was compared with the HC2 test for the same 13 HR-HPV types, as well as for 17 HR-HPV types, which includes HPV 53, 66, 73 and 82. Moreover, the clinical performance was evaluated on a subset of 405 samples with histological results.

Both the CLART-13 HR-HPV and the HC2 assays showed an identical clinical performance, with a clinical sensitivity of 96%. For 17 HR-HPV types, the clinical sensitivity of the CLART assay was 96.9%. Regarding the specificity and the NPV, the results of the CLART and the HC2 were also very similar. In large population-based trials, the HC2 test had a specificity of ≥95% [Meijer et al., 2009]. In this study, all the assays had a positive rate ranging from 26.4 to 28.7% in the control group (CIN grade 1 or less), which can explain the lower clinical specificity observed (71.9% for CLART-17 HR-HPV, 73.6% for CLART-13 HR-HPV, and 71.4% for the HC2), compared to ≥95% specificity in a screening population [Ronco et al., 2008; Meijer et al., 2009]. Similar data were reported by using other tests [Halfon et al., 2010; Schopp et al., 2010].

Analytical performance of the CLART assay showed highly comparable outcomes, with very good values of sensitivity, specificity, PPV, NPV and concordance compared with the HC2 test. In addition, the CLART assay reproducibility was very good (k=0.972).

HPV discrepancies between the CLART assay and the HC2 test were found in 6 cases. Analysis of these cases by the PapilloCheck system indicated a total concordance with the results obtained by the CLART assay.

As explained elsewhere [Snijders et al., 2003], it is necessary to distinguish between clinical and analytical sensitivity. Whether a method with a higher analytical sensitivity would result in a better performance in terms of clinical sensitivity and specificity is still unknown.

In conclusion, the CLART Human Papillomavirus 2 assay showed an excellent performance, very similar to that of the HC2 test, which has a defined clinically cut-off. The CLART assay is efficient, sensitive, and reproducible, Furthermore, this assay has the advantage of detecting and genotyping 35 HPV types, in single or multiple infections, by a single test, which can be important for the individual risk stratification of women found to be infected persistently by HR-HPV types, as well as for the population studies required for vaccination trials and for monitoring the efficacy of HPV vaccines.

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