Comparison of the Analytical and Clinical Performance of Five Tests for the Detection of Human Papillomavirus Genital Infection

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Abstract

HPV-based screening provides greater protection against cervical cancer (CC) than cytology-based strategies. Currently, several molecular diagnostic assays for the detection of human papillomavirus (HPV) are available. In this study, we analyzed 5 different HPV testing and genotyping techniques (Hybrid Capture 2 [HC2; Qiagen, Hilden, Germany], AnyplexTMII HPV28 [Anyplex; Seegene, Seoul, Korea], Linear Array [Roche, Branchburg, NJ, USA], GP5+/6+ PCR-EIA-RH [Labo Bio-medical Products, Rijswijk, The Netherlands] and CLART2 [Genomica, Madrid, Spain]) in 295 women referred to the hospital Colposcopy Clinic from 2007 to 2008 due to positive HPV test results or an abnormal Pap test. DNA extraction for HPV genotyping was performed in cervical sample specimens after Pap test and HPV detection by HC2. The inclusion criteria were: (1) adequate cervical sampling with sufficient material for the Pap test and HPV detection and genotyping, and (2) colposcopically-directed biopsy and/or endocervical curettage. HC2 showed the highest sensitivity for high-grade squamous intraepithelial lesion and CC (HSIL+) detection (96.1%), but all the HPV genotyping tests showed a higher specificity. (Anyplex 86.8%; Linear Array 86.0%; GP5+/6+ 78.8%; CLART2 76.5%). The agreement between HC2 results and the other techniques was similar: (82.4%, kappa = 0.650 for Anyplex; 83.4%, kappa = 0.670 for Linear Array, 79.93%, kappa = 0.609 for GP5+/6+ and 82.4%, kappa=0.654 for CLART2. HPV 16 and/or 18 infection was a risk factor for underlying HSIL+ in the univariate analysis. Anyplex showed the highest risk of underlying HSIL+ after positive HPV 16 and/or 18 tests (OR 31.1; 95% IC 12.1-80.0).

INTRODUCTION

High-risk human papillomaviruses (hr-HPV) are the causative agents of cervical cancer (CC) and its precursors. (1;2) A consequence of this well-established causal link between hr-HPV infection and CC development (3) is the introduction of hr-HPV DNA testing in CC screening programs, initially implemented as a complement to the Pap test and, in the last few years, as the first line screening test. (4;5) hr-HPV DNA testing has shown a higher sensitivity than cytology in detecting high-grade squamous intraepithelial lesions or CC (HSIL+) (6-8), and there is evidence that HPV-based screening provides better protection against CC than Pap test-based strategies. (9) hr-HPV DNA testing is also the recommended method in the follow-up of patients treated for HSIL+, since it is more accurate than repeated cytology in diagnosing residual disease or relapse. (10;11)

Currently, several molecular diagnostic assays for the detection of HPV are available. Hybrid Capture 2 (HC2, Qiagen, Hilden, Germany) was the first technique approved by the US Food and Drug Administration (FDA) and has become the reference test against which the newly developed HPV assays have to be assessed. (12) The Cervista HPV HR Test (Hologic, Madison, WI, USA) (13), and the Roche Cobas 4800 HPV Test (Roche, Branchburg, NJ, USA) have also received FDA approval for the detection of hr-HPV in CC screening (14;15), and the Abbott RealTime High-Risk HPV test (Abbott Molecular, Des Plaines, IL, USA) has obtained CE Marking. (16) All these tests, designed for screening, simultaneously detect different hr-HPV

genotypes and do not allow specific typing, although some (Roche Cobas 4800 HPV Test, Abbott RealTime High-Risk HPV) provide specific genotyping information for HPV 16 and 18, which are considered the HPV types with the highest carcinogenic risk. (17-20)

A number of commercially available techniques allow specific genotype identification: INNO-LiPA HPV Genotyping Extra kit (Innogenetics, Ghent, Belgium), CLART2 (Genomica, Madrid, Spain), Linear Array assay (Roche, Branchburg, NJ, USA), GP5+/6+ PCR-EIA-RH (GP5+/6+, Labo Bio-medical Products, Rijswijk, The Netherlands), Anyplex TMII HPV28 (Anyplex, Seegene, Seoul, Korea). These techniques have been approved within the European Union (CE Marking) and have shown to be useful in epidemiological studies to improve the triage of HPV-positive women by single type risk stratification, (20;21) and the follow-up of persistent infection. (18;21)

The aim of the present study was to compare the analytical and clinical performance of Anyplex, Linear Array, GP5+/6+ and the CLART2 assay with HC2, which is the reference test routinely used in many laboratories for HPV detection in women referred to colposcopy.

MATERIAL AND METHODS

Study design and patient selection

This transversal study was performed at the Hospital Clinic of Barcelona, Spain. Data from all women referred to the hospital from 2007 to 2008 due to a positive hr-HPV test result or a Pap test result of atypical squamous cells, atypical glandular cells, low-grade squamous intraepithelial lesions (LSIL), HSIL, or CC within the 6 months previous to admission were reviewed.

From all women referred in this period we selected women who fulfilled the following inclusion criteria: (1) adequate cervical sampling with sufficient material for cytology (Pap test) and all the HPV tests (HC2, Anyplex, CLART2, Linear Array assay, and GP5+/6+); and (2) adequate colposcopy examination with at least a colposcopically-directed biopsy and/or endocervical curettage. A total of 295 women met the inclusion criteria and were therefore included in the study.

The study was approved by the institutional Ethical Review Board of the Hospital Clinic. All patients provided written consent for the use of biological specimens for research purposes after the clinical procedures were completed.

Patient Management

Prior to the colposcopy procedure, a cervical sample was collected from all the women using a cytobrush, which was transferred to PreservCyt solution (Hologic, Marlborough, MA, USA). The first part of the sample was used for ThinPrep liquid-based cytology. The residual material was used first for hr-HPV testing by HC2 and second to test the different assays for HPV detection and genotyping.

Colposcopy was performed using an Olympus Evis Exera II CV-180 colposcope (Olympus, Barcelona, Spain) after preparing the cervix with 5% acetic acid. A colposcopically-directed biopsy was taken in all patients on the identificaton of an abnormal area. (22;23) When the transformation zone was not completely visible, endocervical curettage using a Kervokian curette was also performed. A random biopsy from the transformation zone was performed

in all the women with a completely visible transformation zone having no colposcopic abnormalities. (24;25)

Liquid-based cytology and histological diagnosis

Thin-layer cytology slides were prepared using the Thinprep T2000 slide processor (Hologic) and stained using the Papanicolaou method. Cytology slides were evaluated by a cytotechnologist and confirmed by a pathologist using the revised Bethesda nomenclature. (26) Formalin-fixed, paraffin-embedded 4-mm sections were routinely stained with hematoxylin and eosin (H&E). All the histological samples were reviewed by one of the authors (JO) to confirm the presence or absence of cervical lesion and its grade. The histological diagnoses were established using pure morphologic criteria based on the H&E-stained sections, with no knowledge of HPV status or the cytology result. The LAST nomenclature was used for the histological diagnosis. (27)

Routine HPV detection (hybrid capture II)

Detection of hr-HPV was performed in cytological samples. Initially hr-HPV detection was undertaken with the Hybrid Capture 2 (HC2) system (Qiagen, Hilden, Germany) in the samples collected in liquid-based media (PreservCyt). This test detects the following genotypes: 16, 18, 32, 34, 36, 39, 45, 51, 52, 56, 58, 59, and 68. A relative light unit of 1 (1.0 pg/mL) was used as the cut-off to classify a specimen as positive for hr-HPV. (28)

Detection of HPV by genotyping tests

After the initial processing that included the Pap test and HC2 testing the residual material was centrifuged and the pellets stored at -80ºC until processing. For all the other genotyping tests DNA extraction was performed using 250µL of the cervical sample specimen to obtain 100 µL of eluate with the QIAamp MinElute Virus Spin kit (QIAgen Inc., Valencia, CA, USA) according to the manufacturer's protocol. DNA yields were quantified spectrophotometrically using the Nanodrop ND-1000 (NanoDrop Technologies, USA). A negative and a positive internal control were used in each genotyping assay according to the manufacturer's procedure. All genotyping assays were tested twice with each sample. A sample was considered invalid for an specific test when both results were invalid.

Anyplex II HPV28 (Anyplex) was performed according to the manufacturer's instructions with using 5 µl DNA in each of the two 20µl reaction mixtures with primer set A or B and a CFX96 real-time thermocycler (Bio-Rad, Hercules, CA, USA). A-set has 14 hr-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) and B-set covers five HR and nine LR types (26, 53, 69, 73, 82, 6, 11, 40, 42, 43, 44, 54, 61, and 70). Anyplex uses the Tagging Oligonucleotide Cleavage and Extension (TOCE) technology (Seegene, Seoul, Korea) a novel approach that enables the detection of multiple targets in the same fluorescence channel of real-time PCR. The *L1* gene of HPV and human beta-globin was simultaneously co-amplified as an internal control to monitor DNA purification efficiency, PCR inhibition, and cell adequacy. (29) The thermal cycler conditions consisted of an initial incubation at 50ºC for 4 minutes, denaturation at 95ºC for 15 minutes, followed by 50

cycles of denaturation at 95ºC for 30 seconds, annealing at 60ºC for 1 minute, and elongation at 72ºC for 30 seconds.

Linear Array HPV genotyping test (Linear Array). This assay recognizes the following HPV types: hr-HPV types 16, 18, 31, 33, 35, 39, 43, 44, 45, 51, 52, 56, 58, 59, and 68 and lr-HPV types 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 81, 73, 82, 83, 84, and IS39 and CP6108. (30) Amplification, hybridization, and detection steps were performed as recommended by the manufacturer. Briefly, ten µL of extracted DNA was employed in the PCR reaction. PCR was performed in a final reaction volume of 100 µl. The mixture was incubated for 2 minutes at 50°C and for 9 minutes at 95°C, followed by 40 cycles of denaturation at 95ºC for 30 seconds, annealing at 95°C for 30 seconds, and elongation at 72ºC for 1 minute.

GP5+/6+ PCR-EIA-RH (GP5+/6+). Ten µL of isolated DNA were amplified by the GP5+/6+ PCR, and hr-HPV was detected by the EIA (Diassay, Rijswijk, The Netherlands) according to the manufacturer's instructions. (31) GP5+/6+ PCR was performed in a total volumen of 50 µl. The mixture underwent 4 minutes denaturation step at 94°C, followed by 40 cycles of denaturation at 94°C for 20 seconds, annealing at 40 °C for 2 minutes and a chain elongation step at 72°C for 1 minutes. The first cycle was preceded by a 4 min denaturation at 94 °C and the last cycle was extended by a 4 min elongation at 72 °C. (32) Fourteen hr-HPV types can be targeted with the GP5+/6+ test: HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. After GP5+/6+ PCR, EIA was performed. Three times the mean OD of the PCR negative controls (OD ≤0.120) was used as the cut-off value to classify samples as positive for HPV. This assay does not identify HPV genotypes individually.

Thus, next, the EIA-positive GP5+/6+ amplimers were genotyped by Reverse Hybridization using Line Probe Assay, according to the manufacturer's instructions. Briefly, ten µl of the biotinylated products of PCR were mixed in test troughs and incubated at room temperature for 5 minutes after that, 1ml of the prewarmed (37°C) hybridization solution and one strip was added to each trough. Hybridization was performed for 1hour at 50ºC in a closed water bath with back-and-forth shaking. The strips were washed twice with 1ml of wash solution, at room temperature for 20 seconds and once at 50°C for 30 minutes. After the washing step, strips were rinsed twice with 1ml of a standard rinse solution. (33) Strips were incubated on a rotating platform with an alkaline phosphatase-labeled streptavidin conjugate diluted in a standard conjugate solution for 30minutes at 25°C. Strips were then washed twice with 1ml of rinse solution and once with standard substrate buffer, and color development was initiated by addition of 5-bromo-4-chloro- 3-indolylphosphate and nitroblue tetrazolium to 1ml of substrate buffer. (33) After 30minutes of incubation at room temperature, the color reaction was stopped by aspiration of the substrate buffer and addition of distilled water. After drying, the strips were visually interpreted using a grid.

CLART HPV2 Assay (CLART2). This test uses biotinylated MY09/11 consensus primers and is able to detect 35 HPV types, including 20 hr-HPV types (type 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82, 85) and 15 lr-HPVs (6, 11, 40, 42, 43, 44, 54, 61, 62, 71, 72, 81, 83, 84, 89). The test was performed according to the manufacturer's instructions. (34;35) Briefly, five µL of eluted DNA were added to 45 µL of Genomica Master Mix for HPV testing. After an initial denaturation step at 95ºC for 5

minutes, reaction mixtures underwent 40 cycles of denaturation at 94°C for 30 seconds followed by annealing at 55ºC for 60 seconds, and elongation step at 72ºC for 90 seconds, and finally a cycle of 4ºC for 8 minutes. Detection of PCR product was made by a low-density microarray platform, CLART (Clinical Array Technology). Results were automatically analyzed in CLART Human Papillomavirus 2 specific software as well as manually surveyed using the CLART grid (Genomica).

Final Diagnosis

The diagnosis of CC and HSIL was established in all cases after histological confirmation. Diagnosis of LSIL was determined based on either histological confirmation or the LSIL result in the Pap test. Women with a negative biopsy and normal Pap test results were classified as negative for intraepithelial lesion or malignancy.

Data analysis

Data analyses were performed with the SPSS version 18.0 (SPSS Inc, Chicago, IL, USA). The statistical methods used in the study were mostly descriptive. The Student t-test or analysis of variance was used to compare quantitative variables. Qualitative variables were compared with the Chi square test. A p value ≤ 0.05 was considered statistically significant. Sensitivity, specificity and positive (PPV) and negative predictive values (NPV) were determined by comparing the results of the HPV testing assays with the final diagnoses. For these values, 95% confidence intervals (CI) were assessed using either a binomial or normal distribution according to the data. The κ value and its standard deviation (SD) were calculated as a measure of

agreement for positive testing and between the HPV genotypes observed in the different tests.

HPV genotype concordance among the different HPV genotyping tests was analyzed and classified as follows: 1) identical if all genotypes were identified by the different tests; 2) concordant when the analysis showed at least one identical genotype; and 3) different if there were no similarities between the genotypes found.

Univariate logistic regression was performed to identify the risk of a positive HPV test and a positive result for HPV16 and/or 18 for underlying HSIL+, with the odds ratio (OR) reported as an estimate of relative risk.

RESULTS

The mean age of the women included in the study was 37.4 ± 13.1 years (range 15-78). The final diagnosis after the completion of the study was CC in 9 women (3.1%; 6 squamous cell carcinomas, 3 adenocarcinomas), HSIL in 44 women (14.9%), LSIL in 78 women (26.4%), and negative in 164 women (55.6%). Of the 78 women classified as LSIL, 43 (55.1%) had a histological diagnosis, whereas in 35, the diagnosis was established on the basis of LSIL cytology with a negative biopsy.

Table 1 shows the number of valid samples for each assay and the percentage of women with positive HPV results by HC2 and the four HPV genotyping tests according to each final diagnostic category. The agreement between HC2 results and the results obtained with the other techniques (positive vs. negative testing) was similar: 82.4% (95%CI= 77.6-86.4%), kappa = 0.650 ± 0.044 for Anyplex; 83.4% (95% CI= 78.7-87.3%), kappa = 0.670 ±

0.043 for Linear Array, 79.93% (95%CI=75.0-94.1%), kappa = 0.609 ± 0.042 for GP5+/6+ and 82.4% (95%CI 77.1-86.7%), kappa=0.654 ± 0.046 for CLART2.

Table 2 shows the sensitivity, specificity and PPV and NPV for the detection of HSIL+ with all the molecular HPV tests. Among the four genotyping tests Anyplex showed the highest sensitivity for HSIL and CC detection.

Among the positive cases, 42.0% (58 out of 138 positive cases) showed multiple HPV types with Anyplex, 44.0% (62/141) with Linear Array, 20.4% (19/93) with GP5+/6+ and 40.7% (44/108) with the CLART2 test. The differences between the rates of multiple-infected lesions were statistically significant (Supplementary tables 1-6).

The genotype distribution among the Anyplex positive cases identified by each specific test is shown in Table 3. The comparison of the genotype distribution between Anyplex and Linear Array in the 125 cases positive for both techniques showed identical genotypes in 73 (58.4%) samples, concordant genotypes in 47 (37.6%) and different genotypes in 5 (4.0%). Both tests were negative in 133 samples. The comparison between Anyplex and GP5+/6+ in the 86 samples positive for both assays showed identical genotypes in 53 (61.6%), concordant results in 31 (36.1%) and different HPV genotypes in 2 (2.3%). One hundred forty-five samples were negative for both tests. On comparing Anyplex and CLART2, both tests were positive in 103 samples. Identical genotypes were found in 61 (59.2%), concordant genotypes in 39 samples (37.9%) and different genotypes in 3 (2.9%). Both tests were negative in 106 samples.

Table 4 shows the risk of a positive hr-HPV test for underlying HSIL+ lesion according to the HC2 test and the risk of a positive result for HPV non 16 non 18 and positive result for HPV 16 and/or 18 for underlying HSIL+ lesion according to the different assays. Positive HPV testing, especially positive results for HPV 16 or 18 were associated with the risk of an underlying HSIL+ lesion. Anyplex showed the highest risk of underlying HSIL+ results after a positive result for the HPV 16 or 18 test (OR 31.1; 95% CI 12.1-80.0).

DISCUSSION

The present study compared different HPV tests in a routine diagnostic setting. HC2 showed the highest sensitivity for HSIL+ detection while the sensitivity of Anyplex and Linear Array was 90% of that shown by the HC2 test, (36) and they could therefore be considered candidate tests for CC screening according to the international guidelines for HPV test validation. On the other hand, CLART2 and GP5+/6+ showed a lower sensitivity, although the latter showed the highest specificity. Patients under 30 years of age present a high prevalence of HSIL lesions, (15) most of which regress and are not the objective of CC screening strategies. Thus, the lower sensitivity of CLART2 and GP5+/6+ could be helpful in this specific age group. All the HPV tests showed a higher sensitivity than the cited 51% benchmark of cytology sensitivity as a stand-alone test. (9;37) All HPV genotyping tests showed a higher specificity than HC2 for the detection of HSIL+. The similar clinical sensitivity and superior or equal specificity of the four HPV genotyping tests compared to HC2, observed in primary screening, is in agreement with the findings from previous studies on its performance in the triage of women with minor cytological abnormalities. (12;29;36;38)

The agreement in terms of positivity/negativity of the different HPV genotyping tests compared with HC2 was about 80% or higher. The high agreement between the tests is in line with previous comparative reports. (38-43) Similarly, genotype concordance was over 80%. Different HPV types were found in less than 5% of the HPV positive samples. Despite the different HPV types included in each test and the differences in terms of sensitivity and specificity, high concordance has been also reported between genotype distribution in previous studies. (38;39;41-44)

In the present study, the rate of multiple HPV infections significantly varied from 20.4% to 44.0% depending on the test used. This is in line with previous studies comparing different methods for HPV typing and describing considerable differences in the multiple infection rates of the individual tests. These series have shown that 15% to 50% of women with prevalent SIL of all grades have multiple hr-HPV genotypes. (45-47) The clinical significance of multiple HPV infections has been analyzed previously. (48;49) However, only limited conclusions can be drawn from HPV typing in cytology, as it includes all infections present on the cervico-vaginal surface including transforming infections related to HSIL lesions, transient infections, and possibly sexually deposited HPV DNA. (46)

In line with the data of the present study, several previous reports have shown an increased risk of underlying or developing HSIL+ after an HPV 16 and/or 18 infection. (18;20;21;50) Indeed, recent guidelines recommend HPV genotyping for HPV 16 and 18 as a triage strategy for women testing positive for HPV. (5;51) Recently, Cuzick et al. reported that the most common hr-HPV detected in women with HSIL histological lesions was HPV16. (52) This

is in line with previous reports and with the present study, in which HPV 16 was the HPV type most frequently identified with all the techniques. (18;21)

The main strength of our study is that it includes a series of women studied according to a well-defined protocol routine, which included liquid-based cytology, hr-HPV testing, and colposcopy with directed biopsies, with endocervical curettage being performed in the case of a non visible transformation zone. Thus, the results of HPV testing and genotyping of the cytology sample are directly correlated with a colposcopy and a histological sample. The implementation of highly sensitive analytical HPV assays could detect most underlying high-grade disease, but it can also lead to a large proportion of clinically irrelevant positive results, which would result in unnecessary diagnostic procedures and treatments, increased costs and psychological distress in healthy women. Thus, as previously stressed, clinical sensitivity is more relevant than analytical sensitivity in CC screening, and any new technique should be validated in a clinical setting. Another strength of the present study is that all the genotyping tests were performed in all the women included, thereby avoiding bias in the analysis of clinical performance of the tests studied.

This study has some possible limitations. No follow-up data was available; thus the possible relation between specific type of hr-HPV detected by a test and the risk of developing HSIL+ could not be assessed. It has been suggested that HPV 16 and/or 18 can identify women at higher risk of underlying HSIL+ lesions (51;53) but they are also related to the risk of developing high-grade disease in the follow-up. (18;21) Likewise, HPV 31, 33 or 45 have been related to a higher risk of HSIL+. (18;20;21) However, the

current guidelines do not support different follow-up algorithms according to the HPV genotype isolated in a cervical lesion. Another possible limitation is related to the accuracy of colposcopy to guide biopsy sampling, namely when single biopsy from the most worrisome lesion was taken (24) which might miss an underlying HSIL lesion at initial evaluation in a proportion of women. In spite of this possible limitation, colposcopy is currently considered the gold standard to guide biopsy sampling to confirm the diagnosis in these patients. (24)

In conclusion, this study show that most of the HPV tests currently available yield very high concordance and show a similar clinical sensitivity and specificity for HSIL+ detection. (54) Besides accuracy, other assay characteristics should be taken into account when the choice of the screening test is considered. The individual genotyping and the range of targeted genotypes are factors that may play a role in the determination of the preferred HPV assay. Objective tools for quality assurance and monitoring of HPV tests within HPV-based screening programs are warranted.

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Table 1. Absolute numbers and percentages of positivity for high-risk human papillomavirus (hr-HPV) in each diagnostic category. Values are shown in numbers and percentages.

Final diagnosis	**HC2 (n/N)**	**Anyplex (n/N)**	**Linear Array (n/N)**	**GP5+/6+ (n/N)**	**CLART2 (n/N)**
valid HPV samples	295	290	281	294	239
Negative	42/164 (25.6%)	37/161 (23.0%)	38/159 (23.9%)	20/163 (12.3%)	21/120 (17.5%)
LSIL	68/78 (87.2%)	55/76 (72.4%)	60/70 (77.9%)	32/78 (41.0%)	47/67 (70.1%)
HSIL	44/44 (100.0%)	39/44 (88.6%)	37/44 (84.1%)	35/44 (79.5%)	33/43 (76.7%)
CC*	7/9 (77.8%)	7/9 (77.8%)	6/8 (75.0%)	6/9 (66.7%)	7/9 (77.8%)

HC2: Hybrid Capture 2, Anyplex: Anyplex TMII HPV28; GP5+/6+: GP5+/6+ PCR-EIA-RH; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; CC: cervical cancer. * Women with squamous cell carcinoma and adenocarcinoma were included.

Table 2. Sensitivity, specificity, and positive and negative predictive values (PPV and NPV) for high-grade intraepithelial lesion or carcinoma (HSIL+) of high-risk human papillomavirus (hr-HPV) detected with Hybrid Capture 2 (HC2), AnyplexTMII HPV28, Linear Array, GP5+/6+ PCR-EIA-RH and CLART2, assay tests.

	HC2		**Anyplex**		**Linear Array**		**GP5+/6+**		**CLART2**	
	%	**(95% CI)**	**%**	**(95% CI)**	**%**	**(95% CI)**	**%**	**(95% CI)**	**%**	**(95% CI)**
Sensitivity	96.1	(87.0-98.9)	86.8	(74.7-93.3)	86.0	(73.8-93.0)	78.8	(66.0-88.0)	76.5	(63.2-86.0)
Specificity	54.8	(48.5-60.9)	61.4	(55.1-67.4)	61.2	(54.8-67.2)	73.7	(67.8-79.0)	62.9	(55.8-69.5)
PPV	31.4	(24.7-39.0)	33.1	(25.7-41.4)	32.3	(25.0-40.7)	39.4	(30.6-49.0)	36.1	(27.7-45.5)
NPV	98.5	(94.7-99.6)	95.4	(90.8-97.8)	95.3	(90.6-97.7)	94.1	(89.8-96.7)	90.7	(84.4-94.6)

HC2: Hybrid Capture 2, Anyplex: Anyplex TMII HPV28; GP5+/6+: GP5+/6+ PCR-EIA-RH

Table 3. Human papillomavirus (HPV) type-specific results obtained with each test compared to Annyplex TMII HPV28.

Anyplex	n	Linear Array	GP5+/6+	CLART2
HPV6	5	4 (80.0%)	-	3 (60.0%)
HPV 11	1	1 (100.0%)	-	1 (100.0%)
HPV 16	49	46 (93.4%)	46 (93.4%)	44 (89.8%)
HPV 18	5	5 (100.0%)	3 (60.0%)	3 (60.0%)
HPV 31	17	16 (94.1%)	14 (82.4%)	12 (70.6%)
HPV 33	2	1 (50.0%)	2 (100.0%)	2 (100.0%)
HPV 35	5	5 (100.0%)	3 (60.0%)	4 (80.0%)
HPV 39	10	3 (30.0%)	4 (40.0%)	2 (20.0%)
HPV 40	2	0 (0.0%)	-	0 (0.0%)
HPV 42	17	8 (47.1%)	-	0 (0.0%)
HPV 43	5	-	-	0 (0.0%)
HPV 45	3	3 (100.0%)	2 (66.7%)	1 (33.3%)
HPV 51	6	5 (83.3%)	2 (33.3%)	6 (100.0%)
HPV 52	12	11 (91.7%)	2 (16.7%)	10 (83.3%)
HPV 53	17	14 (82.4%)	0 (0.0%)	10 (58.8%)
HPV 54	3	3 (100.0%)	-	1 (33.3%)
HPV 56	12	8 (66.7%)	9 (75.0%)	4 (33.3%)
HPV 58	11	7 (63.6%)	8 (72.7%)	8 (72.7%)
HPV 59	10	7 (70.0%)	4 (40.0%)	6 (60.0%)
HPV 61	8	7 (87.5%)	-	7 (87.5%)
HPV 66	10	6 (60.0%)	7 (70.0%)	8 (80.0%)
HPV 68	8	2 (25.0%)	1 (12.5%)	1 (12.5%)
HPV 69	1	1 (100.0%)	-	-
HPV 70	3	2 (66.7%)	-	3 (100.0%)
HPV 73	7	4 (57.1%)	2 (28.6%)	-
HPV 82	4	1 (25.0%)	-	2 (50.0%)

** Blankets correspond to HPV genotypes not included by each specific tets*

Anyplex: Anyplex TMII HPV28; GP5+/6+: GP5+/6+ PCR-EIA-RH

Table 4. Risk of positive high-risk human papillomavirus (hr-HPV) test and positive result for HPV16 and/or 18 for underlying high-grade intraepithelial lesion or carcinoma (HSIL+). Results are shown with odds ratio (OR) and 95% confidence interval (CI).

HPV test	Result	OR (95%CI)	p
Hybrid capture	Negative	1	
	Positive	30.6 (7.2-128.53)	<0.001
Anyplex II	Negative	1	
	HPV non 16 non 18	4.3 (1.7-10.9)	0.002
	HPV16 and/or 18	31.1 (12.1-80.0)	<0.001
Linear Array	Negative	1	
	HPV non 16 non 18	3.1 (1.3-7.5)	0.012
	HPV16 and/or 18	15.3 (6.6-35.8)	<0.001
GP5+/6+	Negative	1	
	HPV non 16 non 18	5.1 (2.0-12.8)	0.001
	HPV16 and/or 18	23.2 (10.4-53.0)	<0.001
CLART2	Negative	1	
	HPV non 16 non 18	1.8 (0.7-4.5)	0.185
	HPV16 and/or 18	17.5 (7.5-40.6)	<0.001

HC2: Hybrid Capture 2, Anyplex: Anyplex TMII HPV28; GP5+/6+: GP5+/6+ PCR-EIA-RH