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Evaluation of the polyclonal ELISA HPV serology assay as a biomarker for HPV exposure

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Abstract

Background—Seropositivity to HPV16 and 18 antibodies is used as a measure of cumulative HPV exposure and as a stratifier of HPV exposure for vaccine efficacy analyses. Overall performance of these assays, as a measure of HPV exposure, has not been evaluated.

Methods—Using data from the enrollment phase of the HPV16/18 vaccine trial in Costa Rica, we evaluated the performance of the polyclonal ELISA HPV16 and 18 serological assays as a measure of HPV exposure. Biological (for eg. HPV infection at the cervix) and behavioral characteristics (for eg. lifetime number of sexual partners) with known associations with current and past HPV infection were used to define cases and controls (HPV exposed vs. not exposed). Pre-vaccination serum was measured for antibodies against HPV16 and HPV18 by ELISA; cervical samples were tested for HPV DNA using PCR SPF10/LiPA25. ELISA results were analyzed using receiver-operator-characteristic curves (ROC); performance was evaluated at the manufacturer set cutpoint (HPV16 =8, HPV18 =7) and at cutpoints chosen to optimize sensitivity and specificity (HPV16 =34, HPV18 =60).

Results—Defining cases as type-specific HPV DNA positive with high-grade abnormal cytolzogy (i.e. combined molecular and microscopic markers of infection), HPV16-ELISA gave sensitivity that was lower at the optimal cutpoint than the manufacturer cutpoint (62.2 compared with 75.7, respectively; p=0.44). However, specificity was higher (85.3 compared with 70.4, respectively; p<0.0001). Similarly, HPV18-ELISA gave sensitivity that was lower at the optimal

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Wim Quint and Leen-Jan van Doorn are employees of DDL Diagnostic Laboratory; Catherine Bougelet is an employee of GSK Biologicals. None of the authors have any potential conflicts of interest to report.

cutpoint than the manufacturer cutpoint (34.5 compared with 51.7, respectively; p=0.40), with higher specificities (94.9 compared with 72.6, respectively; p<0.0001).

Conclusions—Modifying cutpoints did not improve the low sensitivity. The low sensitivity of this assay does not support its use for risk stratification or clinical settings.

Keywords

Human Papillomavirus; serology; polyclonal ELISA

INTRODUCTION

Human papillomavirus (HPV) is a common sexually transmitted infection (STI), often acquired shortly after sexual initiation (1, 2). In epidemiologic research, current HPV DNA infections are detected using sensitive polymerase chain reaction (PCR) assays on cervical exfoliated samples (3). HPV DNA based assays are highly sensitive and specific for measuring current infections. However, they are limited as they provide information on current infection only and HPV clearance (i.e. loss of DNA detectability) occurs within months to a few years in the majority of infections (4). Thus, DNA based assays do not provide information on lifetime, cumulative HPV exposure.

Several serological assays with different properties are currently available for research purposes; they measure a wide range of anti-HPV16 and 18 antibodies. Seropositivity to HPV16 and 18 antibodies is being used as an epidemiologic measure of cumulative HPV exposure, a marker of immunity or protection from subsequent infections (5), and in conjunction with DNA-based measures, to define or stratify subgroups of potentially HPV-naïve women for vaccine efficacy analyses. Despite the use of HPV serological assays in many studies, the overall performance of the assays as a measure of HPV exposure have not been evaluated.

The aim of this analysis was to evaluate the performance of the polyclonal ELISA HPV assay as a measure of HPV exposure and to determine whether alternative cutpoints would improve the sensitivity/specificity of the ELISA HPV serology assay as a biomarker of HPV exposure. We also compared the correlates of HPV16 and HPV18 seropositivity (separately) at the manufacturer cutoff and the cutpoint determined to maximize sensitivity and specificity.

MATERIALS AND METHODS

Study population

Data are from the enrollment/pre-vaccination phase of the on-going publicly funded, community-based randomized phase III HPV16/18 vaccine trial in Costa Rica (CVT). The study has been described elsewhere (6). Briefly, the main objectives of the trial are to evaluate the efficacy of a prophylactic HPV16/18 vaccine manufactured by GlaxoSmithKline for prevention of HPV16/18 infection and related precancerous lesions compared to women receiving a control hepatitis A vaccine. A total of 7,466 women provided written, informed consent at enrollment.

At enrollment participants were asked detailed questions regarding demographics, sexual practices, contraceptive use, reproductive and menstrual history, and smoking. For sexually experienced women, a pelvic exam was performed and exfoliated cervical cells were collected for cytology, HPV DNA, *Chlamydia trachomatis* (Ct) DNA, and *Neisseria*

gonorrhoeae (GC) DNA testing. ThinPrep slides were prepared to obtain a Pap stain for cervical cytology interpretation.

All testing was done masked to the results of randomization arm and other test results. Protocols were approved by the US National Cancer Institute and a Costa Rican institutional review board.

HPV serological measurements

Serum collected at enrollment was used to determine HPV16 and -18 IgG serostatus at GSK Biologicals (Rixensart, Belgium) using a VLP-based direct enzyme linked immunoabsorbent assay (ELISA) developed by GSK that measures polyclonal antibodies as described previously (7, 8). All research and development of the assay and testing of the samples was conducted at GSK. Briefly, ELISA microtiter plates were separately coated with 2.7 μg/mL of either HPV16 or HPV18 VLPs that were produced in a baculovirus expression system. The plates were blocked with PBS containing 4% skim milk with 0.2% Tween-20. Serum samples from participants were serially diluted in the blocking solution starting at 1:100 in twofold increments. Serial dilutions of samples, standard, and quality control specimens were added to the microtiter plates. After incubation and washing steps, a peroxidase-conjugated anti-human polyclonal antibody was added. Following incubation and washing, enzyme substrate and chromogen were added to allow color development. Reactions were stopped, and optical density (OD) read at 450 and 620 nm, with background measured at 620 nm and subtracted from the OD reading at 450 nm. Antibody levels, expressed as ELISA units (EU)/mL, were calculated by interpolation of OD values from the standard curve by averaging the calculated concentrations from all dilutions that fell within the working range of the reference curve. The seropositivity cutpoints were determined by GSK and calculated from antibody titer values three standard deviations above the geometric mean titers taken from two groups of known HPV-negative individuals. These groups included: 1) human serum samples previously incubated with corresponding VLP to remove specific antibodies, and 2) human serum taken at day 0 before vaccination from women who did not show an increased immune response after 7 days following the first vaccine (8). Cutpoints were set at OD≥8 EU/ml for anti-HPV16 and OD≥7 EU/ml for anti-HPV18 (8).

HPV DNA- SPF_{10}/DEIA/$LiPA_{25}$

HPV DNA detection and genotyping was performed at DDL Diagnostic Laboratory (Voorburg, Netherlands), as described previously (9, 10). Extracted DNA was used for PCR amplification with the SPF_{10} primer sets (9, 10). The samples were run through an HPV DNA enzyme immunoassay (DEIA) to obtain an OD reading, and categorized as HPV DNA negative, positive, or borderline. The same SPF_{10} amplimers were used on SPF_{10}-DEIA-positive samples to identify HPV genotype by reverse hybridization on a line probe assay (LiPA) (SPF10-DEIA/HPVLiPA25,version 1; Labo Bio-Medical Products, Rijswijk, Netherlands), which detects 25 HPV genotypes.

Since CVT uses the bivalent HPV16/18 vaccine, to ensure detection for these types, HPV16 and 18 type-specific PCR (TS-PCR) primer sets were used to selectively amplify HPV16 and HPV18 from specimens tested SPF_{10} DEIA-positive, but $LiPA_{25}$ HPV16 and/or HPV18 negative (9). Amplimers from the TS-PCRs were detected by DEIA similar to the method used for SPF_{10} amplimer detection (9–11).

Statistical analysis

All analyses were conducted separately for HPV16 and HPV18. We note that the results from the HPV16 and HPV18 models cannot be directly compared to one another because

the HPV16 and 18 ELISAs are not identical. We excluded virginal women (n=1,592) from this analysis because they did not have a pelvic examination and thus we could not assess their HPV DNA or cytology status. However, for completeness, we also performed the analyses including virginal women and assumed they were HPV DNA and cytology negative. As expected specificity increased; however, the main conclusions of the analysis were not altered by restricting to sexually active women (data not shown). We also excluded three women who were outside the trial age range.

We used nonparametric empirical receiver operating characteristic (ROC) analysis to calculate the area under the curve (AUC) (12, 13). We sought to evaluate HPV serology as a biomarker of exposure to HPV. Because HPV DNA is not sensitive for detecting prior infections, we used both biological and behavioral characteristics known to be associated with current and past HPV infection to define cases and controls. Specifically, in separate analyses, cases were defined as either: (1) current type-specific HPV DNA positive at cervix (HPV16 DNA positive for the HPV16 analysis; HPV18 DNA positive for HPV18 analysis); (2) abnormal cytology/histology, defined as ≥HSIL/CIN2+ or ASC-H; (3) Lifetime number of sexual partners >4 (that is the 90th percentile among non-virginal women); (4) Years since sexual debut >4 (median among non-virginal women); and, (5) combined current DNA infection with abnormal cytology/histology (≥HSIL/CIN2+/ASC-H). Given that we cannot attribute specific HPV type to a cytological lesion, for definition #5, we conditioned first on HPV16 DNA positivity (or 18 DNA positivity for the HPV18 analysis) to increase the likelihood of type-specificity. We chose the 90th percentile of number of lifetime sexual partners and median years since sexual debut to increase the probability of accurately classifying women as HPV exposed. The main conclusions were not altered by dichotomizing at different points (data not shown). *A priori*, we considered the last definition to provide the strongest evidence of past exposure because it incorporated both molecular and microscopic evidence of infection. For each of the above five case-definitions, controls were accordingly defined as women not having the specific case-definition.

We estimated how well continuous levels of HPV16 serology (and HPV18 serology for the HPV18 analysis) collected at enrollment discriminates between cases and controls. The y-axis of the ROC graph represents sensitivity, or the true positive rate, i.e. the proportion correctly discriminated or predicted as such by serology among cases. The x-axis represents the complement of specificity, or the false positive rate, which is the proportion incorrectly discriminated by serology amongst non-cases. An area under the ROC curve (AUC) is a commonly used summary measure. An AUC of 1.0 represents a perfect test, while an AUC of 0.5 represents random classification (12). Using the continuous measures of HPV16 and HPV18 serology, we determined the cutpoint that maximized the sum of sensitivity and specificity. This method implicitly weights sensitivity and specificity equally.

In addition, we compared the sensitivity and specificity at the current standard cutpoints (8 EU/mL and 7 EU/mL, for HPV16 and 18 respectively) to three *a priori* specified cutpoints (the 25th, 50th and 75th percentiles among non-virginal seropositive women) and the cutpoint that maximizes the sum of sensitivity and specificity to show the trade-offs in loss and gain of sensitivity and specificity at different cutpoints.

Finally, to investigate whether different serology cutpoints would alter our previous estimates of enrollment correlates of HPV16/18 seropositivity (14), we compared multivariate logistic regression models using the current cutpoints for HPV16 and 18 (8 EU/mL and 7 EU/mL, respectively), to the cutpoint that maximized the sum of sensitivity and specificity.

RESULTS

Of the 7,466 women enrolled in the trial, 5,871 (78.7%) sexually experienced subjects had a pelvic examination, constituting the population for this analysis. The median age of the women included in the analysis was 21 years (interquartile range [IQR], 19 to 23 years); the median time since sexual debut was 4 years (IQR, 3 to 7 years); and the median number of lifetime sexual partners was 2 (IQR, 1 to 3). HPV16 DNA prevalence was 8.3% (n=488/5,868) and HPV18 was 3.2% (n=188/5,868).

Figure 1 shows the ROC graphs and corresponding AUCs comparing how this polyclonal ELISA discriminated cases and controls based on the five different case definitions. For each analysis, the biomarker discriminated significantly better than a completely uninformative model (P <0.001 for each test of the null hypothesis of AUC=0.5). The AUC for the HPV16 DNA only model was 0.70; adding cytology increased AUC to 0.77. For HPV18, the AUC for the HPV18 DNA only model was 0.69, while for combined DNA and cytology was 0.65.

Focusing on the most stringent case definition ‘current DNA infection and abnormal cytology’, using the continuous serology measure, and acknowledging that sensitivity and specificity are weighted equally, for HPV16 a cutpoint of 34 EU/mL yielded the best combination of sensitivity and specificity (sensitivity: 62.2%; specificity: 85.3%); for HPV18, a cutpoint of 60 EU/ mL yielded the best sum of sensitivity and specificity (sensitivity: 34.5%; specificity: 94.9%).

Using the same case definition of ‘current DNA infection and abnormal cytology’, from the lowest to highest cutpoint, sensitivity ranged from 75.7%–37.8%; specificity ranged from 70.4%–92.8% (Table 1A). Comparing 34 EU/mL to 8 EU/mL, there was not a statistically significant difference in sensitivity (P 0.44), but a statistically significant difference in specificity (P <0.0001). Similarly for HPV18 (Table 1B), sensitivity ranged from 51.7%–34.5%; specificity ranged from 72.6%–94.9%. Comparing 60 EU/mL to 7 EU/mL, there was a statistically significant difference in specificity (P <0.0001), but a non-statistically significant difference in sensitivity (P 0.40).

Finally, to investigate whether these new cutpoints would alter our previous estimates of correlates of HPV16/18 seropositivity (14), we compared models using the current cutpoints of 8 EU/mL and 7 EU/mL to the cutpoints that maximized combined sensitivity and specificity, for HPV16 and 18, respectively (Table 2). Although the adjusted odds ratios of the correlates of HPV16 seropositivity we reported previously (14) did not change substantially using the higher cutpoint, some correlates lost statistical significance, possibly due to loss of power. Similar results were seen for HPV18 seropositivity (Table 2). As expected, HPV16 and HPV18 seroprevalence among virgins decreased using the higher cutpoints; however, virginal seroprevalence did not reach 0% (data not shown).

DISCUSSION

HPV serology (mainly against HPV16 and 18, the two most carcinogenic HPV types and the main targets of the prophylactic vaccines) is being used in research settings to identify current and past HPV-exposed individuals. However, the utility and performance of the different serological assays to discriminate HPV exposure is currently unknown. Using data from a large study of unvaccinated, sexually active, young adult women at peak exposure to HPV, we evaluated the sensitivity and specificity of the polyclonal HPV16 and HPV18 ELISA assay developed by GSK over a wide range of antibody levels and at various pre-specified case definitions. Our results showed that the ELISA assays provided moderate discriminative ability to detect cases with a current HPV DNA infection (AUC= 0.7 for both

HPV16 and 18), and for HPV16, it improved slightly with the addition of abnormal cytology (AUC=0.77). Interestingly, addition of cytology to HPV18 DNA decreased the AUC relative to HPV18 DNA alone. While reasons for these discrepancies are unclear, it could be as a result of the differences in the HPV18 serology assay and also detection of HPV18 DNA or cytology. It is not possible to directly compare the two assays, however it is important to note that the HPV18 VLPs in general are more difficult to make and reproduce. It is also noteworthy that HPV18 related lesions are harder to detect with cytology (15–18) compared with HPV16 related lesions. Finally, using a case definition of HPV DNA-positive and abnormal cytology, serology at cutpoints identified by the ROC analysis (34 EU/mL and 60 EU/mL, for HPV16 and HPV18 respectively) had lower sensitivity and statistically significantly higher specificity than manufacturer set cutpoints (8 and 7 EU/mL, for HPV16 and HPV18 respectively).

Accurate classification of both HPV DNA and serostatus are important considerations for epidemiologic studies of HPV infection and vaccine efficacy. For HPV16, using a case definition of HPV16 DNA positive and abnormal cytology, a cutpoint of 8 EU/mL set by the manufacturer had the highest sensitivity (75.7%), resulting in a 24.3% false negative rate. Similarly for HPV18, the set cutpoint of 7 EU/mL (51.7% sensitivity), results in a 48.3% false negative rate. Therefore, even at the highest sensitivity, there is misclassification if HPV serology is used as a biomarker of HPV exposure. This could have implications for studies investigating co-factors influencing progression of HPV-infected cells to precancer and cancer, whereby associations found could be due to residual confounding by HPV positivity. As an example, some studies that have found an association between *Chlamydia trachomatis* (Ct) and cervical cancer, in the absence of HPV DNA exposure, have used HPV serology to account or adjust for HPV status (19–21). Based on our results, given that they used an assay with similar misclassification of HPV exposure as the one we evaluated in this report, 25% of HPV16 and 48% of HPV18 exposed women would be classified as HPV-negative. Thus, an observed positive association could be due to HPV-positive women who were misclassified as negative by the serology assay. In addition, it is premature to use this assay clinically to distinguish HPV exposed from un-exposed.

Our correlates of HPV16 and 18 seropositivity found in a previous analysis did not change substantially at the higher cutpoints, which indicates that the cutpoint set by the manufacturer is specific for epidemiologic studies of correlates of seropositivity. While some correlates lost statistical significance at the higher cutpoints possibly due to decreased power, risk estimates remained similar. Additionally, we found virginal seroprevalences never reached 0%, which is not unexpected as virginal seroprevalence can reflect the inherent biases in self-report of virginity, a technical artifact of non-specific binding, possible transmission through other non-penetrative sexual contact, or perinatal transmission, which we are unable to assess in this analysis.

There are some limitations to be considered. Currently there is no gold standard to classify HPV exposure, and we know from natural history studies that the majority of HPV infections clear (4). Additionally, natural history studies of HPV prevalence and incidence consistently show that the strongest correlates/predictors of HPV infection are those associated with increased exposure and persistence of the virus, such as higher lifetime sexual partners, increased years since sexual debut, abnormal cytology, and a current infection. Taken together, because DNA-positivity does not reflect cumulative infection, we used the aforementioned predictors of past exposure when defining our cases. In addition, not all HPV infected women develop antibody response to natural infection; some seroconvert at very low antibody levels, often below the assays' limit of detection; and, it may take time and repeated exposures to develop such a detectable response (22). Therefore, it is unknown whether the moderate AUCs we obtained are due to poor assay, poor "gold

standard", a combination of the two, or due to biology of HPV infection. Thus, restricting HPV exposed women to biological and behavioral characteristics could have resulted in misclassification of our outcome.

Our analysis is also limited by the cross-sectional nature of our data. Only longitudinal studies can evaluate the serological response to HPV and its relation to HPV exposure, infection, persistence, and immunity. It is also important to note that the results from this study are not applicable to studies using other HPV serology assays. Finally, this study was performed in a young, healthy population; whether the assay performance may be different in older women is unknown.

In summary, this polyclonal ELISA assay provided moderate discriminative ability to detect HPV exposure. In addition, for HPV16 and HPV18 respectively, serology at cutpoints of 34 EU/mL and 60 EU/mL yielded the best combination of sensitivity and specificity, and had lower sensitivity and statistically higher specificity than the current cutpoints (8 and 7 EU/mL) to discriminate HPV exposed women. It is also important to note that even at the highest sensitivity (current cutpoints), a substantial proportion of women are still misclassified. The application of the polyclonal ELISA HPV assays should be considered in the context of the outcome of interest and balance between sensitivity and specificity. If the intent is measuring exposure, the goal may be to maximize sensitivity; thus, the current cutpoints are appropriate as the higher cutpoints yielded higher specificity, but at a detriment to sensitivity. However, if the objective is to measure immunity, it may be more appropriate to maximize specificity and positive predictive value; thus, a higher cutpoint may be more appropriate.

Present evidence does not support use of the ELISA serological assays in risk stratification or clinical setting. Future research on this topic should consider application of the ELISA assays and the important balance between sensitivity and specificity in the specific research setting.

Summary
The low sensitivity of the polyclonal ELISA HPV16 and 18 serological assays to detect HPV exposure highlights the limitations of using this assay for risk stratification or clinical settings.

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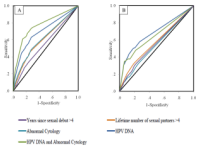

Case Definitions	N	HPV16 AUC	N	HPV18 AUC
Current HPV infection at the cervix*	446	0.70	166	0.69
Cytology positive (+LSIL/CIN2+)	267	0.61	267	0.53
Lifetime number of sexual partners > 4	333	0.61	333	0.60
Years since sexual debut > 4 years	2,903	0.56	2,903	0.53
Current concordant type-specific DNA positive and cytology positive (+LSIL/CIN2+)	75	0.77	29	0.65

Figure 1.

Receiver Operating Characteristics (ROCs) for the Detection of HPV16 (A) and HPV18 (B) According to the Five Different Case Definitions by Serology; HPV16 and HPV18 Area Under the Curve (AUC) for Each Case Definition

NOTE: AUC, Area under the curve; N, number of women positive for outcome

*HPV16 DNA-positive at the cervix for the HPV16 analysis (HPV16 AUC); HPV18-positive at the cervix for the HPV18 analysis (HPV18 AUC).

Table 1

Ability of HPV16 Serology to Detect Women With a Current HPV16 DNA infection and Women With a Current HPV16 DNA Infection and an Abnormal Cytology (≥HSIL/CIN2+) (A); Ability of HPV18 Serology to Detect Women With a Current HPV18 DNA infection and Women With a Current HPV18 DNA Infection and an Abnormal Cytology (≥HSIL/CIN2+) (B)

(A) HPV16				
	HPV16 DNA Positive (n=488)		HPV16 DNA Positive AND Abnormal Cytology[1] (n=75)	
Cut-Point	Sensitivity[2]	Specificity[3]	Sensitivity[2]	Specificity[3]
8[4]	62.5	72.6	75.7	70.4
15	53.1	79.8	68.9	77.8
34[5]	44.3	87.3	62.2	85.3
93	25.4	94.0	37.8	92.8
(B) HPV18				
	HPV18 DNA Positive (n=181)		HPV18 DNA Positive AND Abnormal Cytology[1](n=29)	
Cut-Point	Sensitivity[2]	Specificity[3]	Sensitivity[2]	Specificity[3]
7[4]	58.0	72.8	51.7	72.6
10	51.1	78.9	48.2	77.9
18	43.6	87.2	37.9	86.3
43	31.4	93.9	34.5	93.2
60[5]	26.7	95.4	34.5	94.9

[1] Abnormal cytology defined as: ASC-H, HSIL-CIN2, HSIL-CIN3

[2] Sensitivity defined as: True Positives/ (True Positives + False Negatives)

[3] Specificity defined as: True Negatives/ (True Negatives + False Positives)

[4] Current ELISA-based HPV serological cutpoint as determined by antibody titer values three standard deviations above the geometric mean titers taken from groups of HPV-negative individuals (n=278) and established by GSK.

[5] Cutpoint chosen from the continuous serology measure that optimized combined sensitivity and specificity; for HPV16, also one of the three a priori specified cutpoints

Table 2

Adjusted Odds Ratios (aOR) and 95% Confidence Intervals (95% CI) for the Multivariate Model of the Correlates of HPV16 and HPV18 Seropositivity at Two HPV16 and Two HPV18 Serology Cutpoints:

	HPV16 Seropositivity				HPV18 Seropositivity			
Variable	**8 EU/mL[1] aOR**	**8 EU/mL[1] 95% CI**	**34[2] EU/mL aOR**	**34[2] EU/mL 95% CI**	**7 EU/mL[1] aOR**	**7 EU/mL[1] 95% CI**	**60[2] EU/mL aOR**	**60[2] EU/mL 95% CI**
Years Since Sexual Debut	1.06	1.03, 1.09	1.07	1.04, 1.11	1.07	1.04, 1.10	1.07	1.00, 1.15
Lifetime Sexual Partners	1.00	----	1.00	----	1.00	-----	1.00	-----
1	1.59	1.38, 1.83	1.67	1.39, 2.01	1.38	1.20, 1.59	1.76	1.19, 2.58
2–3	2.30	1.91, 2.76	2.45	1.95, 3.07	1.60	1.33, 1.93	2.60	1.65, 4.10
4+								
Number of Pregnancies	1.00	----	1.00	----	1.00	-----	1.00	-----
0	1.16	1.00, 1.37	1.21	0.98, 1.48	1.10	0.93, 1.30	1.41	0.95, 2.10
1	1.26	1.02, 1.55	1.27	0.98, 1.65	1.19	0.96, 1.30	1.41	0.85, 2.32
2+								
Hormonal Contraceptives	1.00	----	1.00	----	1.00	-----	1.00	-----
Neither	1.11	0.91, 1.34	1.03	0.81, 1.32	1.04	0.86, 1.26	1.39	0.84, 2.28
Ever Only OC[3]	1.45	1.08, 1.94	1.02	0.70, 1.48	1.10	0.82, 1.48	1.18	0.56, 2.50
Ever Only Inj[3]	1.20	0.97, 1.49	1.06	0.81, 1.38	1.12	0.90, 1.38	1.04	0.60, 1.80
Ever Both OC and Inj[3]								
Condom use	1.00	----	1.00	----	1.00	-----	1.00	-----
Never	0.86	0.76, 0.98	0.87	0.74, 1.02	0.98	0.86, 1.12	0.89	0.66, 1.21
Ever								
Smoking	1.00	----	1.00	----	1.00	-----	1.00	-----
Never	1.20	0.94, 1.50	1.02	0.77, 1.36	1.10	0.87, 1.39	0.61	0.32, 1.19
Former	1.26	1.04, 1.53	1.19	0.95, 1.51	1.15	0.94, 1.41	1.28	0.84, 1.94
Current								
Current and/or Past STI[4]	1.00	----	1.00	----	1.00	-----	1.00	-----
No	1.48	1.27, 1.72	1.35	1.13, 1.61	1.40	1.21, 1.63	1.29	0.92, 1.81
Yes								
HC2/Cytology	1.00	----	1.00	----	1.00	-----	1.00	-----
HC2 Neg/Normal	1.00	0.66, 1.50	1.14	0.67, 1.94	1.33	0.90, 1.97	2.60	1.21, 5.55
HC2 Neg/ASCUS or LSIL	1.55	0.82, 2.92	1.33	0.58, 3.06	0.94	0.47, 1.89	0.85	0.11, 6.28
HC2 Neg/ASC-H or HSIL-CIN2 or HSIL-CIN3 or AGC NOS	1.84	1.59, 2.13	1.93	1.61, 2.31	1.48	1.27, 1.72	1.80	1.26, 2.58
HC2 Pos/Normal	2.02	1.66, 2.47	2.36	1.87, 2.98	1.83	1.50, 2.23	2.46	1.60, 3.77
HC2 Pos/ASCUS or LSIL	3.07	2.29, 4.11	3.59	2.63, 4.89	1.62	1.20, 2.18	2.57	1.44, 4.59
HC2 Pos/ASC-H or HSIL-CIN2 or HSIL-CIN3 or AGC NOS								

NOTE: HPV16 and HPV18 multivariate models were simultaneously adjusted for: years since sexual debut, lifetime number of sexual partners, number of pregnancies, hormonal contraceptive use, condom use, smoking history, current and/or past STIs, and HC2/cytology result.

[1]Current cutpoint as defined by GSK

[2]Cutpoint maximizing the sum of sensitivity and specificity as determined using the continuous enrollment HPV16 and HPV18 serology and the most stringent outcome definition of 'HPV DNA positive and abnormal cytology'

[3]Ever only OC = Ever only used oral contraceptives; Ever only Inj = Ever only used injectable contraceptives; Ever OC and Inj = Ever used oral and injectable contraceptives

[4]Current and/or Past STI = Reported ever being diagnosed with warts, herpes, syphilis, Gonorrhea, or other; and/or currently diagnosed with Chlamydia and/or Gonorrhea infection