Direct Comparison of HPV16 Serological Assays Used to Define HPV-Naïve Women in HPV Vaccine Trials

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Abstract

Background: Two HPV serological assays, the competitive Luminex immunoassay (cLIA), and an enzyme-linked immunoassay (ELISA) against HPV16 have been used to define HPV-naïve subcohorts within large HPV vaccination trials. Some of the variation in estimated vaccine efficacies may be due to the differences in these assays used to define the HPV-naïve subgroups. To guide the interpretation of published results, we compared these assays.

Methods: Replicate enrollment sera from a stratified sample of 388 unvaccinated women from the control arm of the Costa Rica HPV 16/18 Vaccine Trial were measured for antibodies against HPV16 using cLIA and ELISA. Agreement between the assays was estimated using standard and alternative assay cutoffs.

Results: Using laboratory-determined seropositivity cutoffs, sampling-adjusted HPV16 seropositivity was 24.8% by ELISA and 7.2% by cLIA. Comparing cLIA and ELISA antibody levels based on the standard cutoffs, overall agreement was 53% (positive-agreement = 49%). The poor agreement was mainly driven by the higher sensitivity of the ELISA than cLIA, resulting in 30% of the ELISA-positive sample that were cLIA-negative (none of the ELISA-negatives were cLIA-positive). Increasing ELISA cutoff to 54 ELISA units (EU)/mL (the level which maximized agreement with cLIA; ELISA standard cutoff is 8 EU/mL) resulted in higher agreement (overall agreement = 91%; positive agreement = 78%).

Conclusions: ELISA and cLIA are different from each other based on the laboratory-determined cutoff. Increasing ELISA cutoff increased agreement with cLIA, which could facilitate comparisons among studies that use different assays.

Impact: Keeping cLIA at the laboratory-determined cutoff but altering ELISA cutoff for seropositivity might facilitate vaccine efficacy comparisons in the naïve cohorts defined by cLIA. *Cancer Epidemiol Biomarkers Prev; 21(9); 1547–54. ©2012 AACR.*

Introduction

Carcinogenic human papillomavirus (HPV) types are the etiologic agents for cervical cancer (1, 2) and a subset of other anogenital and oropharyngeal tumors (3). Two HPV prophylactic vaccines (Gardasil and Cervarix) are now licensed for use in over 120 countries. Because available vaccines are prophylactic and HPV infection typically occurs shortly after sexual debut, vaccination during adolescence (before sexual debut) is recommended.

The large randomized phase III clinical trials that led to licensure of the 2 prophylactic HPV vaccines included young adult women, many of whom were already sexually experienced and exposed to HPV. Within these phase III trials, in an effort to estimate the impact of vaccinating HPV-naïve populations (i.e., adolescent populations), subcohorts of likely naïve women were defined and examined. While there are currently no reliable methods to distinguish HPV-naïve from exposed groups within sexually active populations, a combination of HPV DNA testing of cervical samples and anti-HPV antibody testing of serum were used to define subgroups most likely to represent naïve individuals. Thus, in analyses that restricted to likely HPV-naïve, that is, women who were negative for cervical HPV DNA at enrollment and sero-negative for anti-HPV antibodies (HPV16 and HPV18) in serum, reported vaccine efficacy against CIN2 or worse lesions irrespective of causal HPV, were 42.7% for

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Gardasil (4) and 64.9% for Cervarix (5). However, because different serology assays were used in the 2 different trials to define naïve populations, it is unclear whether a direct comparison of the reported vaccine efficacy estimates is valid.

The assay used to define likely naïve women at enrollment in the phase III trials of Gardasil is a virus-like particle (VLP)-based competitive Luminex immunoassay (cLIA) that was designed to measure presence of HPV-16 and HPV-18 type-specific serum IgM, IgA, and/or IgG antibodies that compete with monoclonal antibodies (V5 epitope) known to have neutralizing ability (6). The assay used to define likely naïve women at enrollment in the phase III trials of Cervarix is a VLP-based direct enzyme–linked immunoassay (ELISA) that measures serum IgG antibodies to both neutralizing and nonneutralizing epitopes that bind to HPV-16 or HPV-18 VLPs (7, 8). In addition to platform and format differences, these assays use different reference standards that report in different units and use serostatus cutoffs that were developed using different methods. Thus far direct comparisons between these assays have been sparse (9–11). The limited understanding of how these assays relate to each other in the context of unvaccinated populations/natural infection makes comparison across studies complex.

To help guide the interpretation of analyses within the vaccine trials restricted to naïve women defined using serology, we conducted a study of unvaccinated women to directly compare the cLIA and ELISA. For completeness, we also compared each of these 2 assays against the third, commonly used assay in HPV Vaccine Trials to define an individual's ability to neutralize HPV virions, secreted alkaline phosphatase neutralization assay (SEAP-NA).

Materials and Methods

Study population

Samples were from the enrollment/prevaccination phase of the publicly funded, community-based randomized phase III HPV16/18 Vaccine Trial in Costa Rica (CVT). The trial has been described in detail elsewhere (12). In brief, a total of 7,466 women consented and were enrolled at which time they were randomized to receive either the HPV vaccine or the hepatitis A vaccine and were followed for 4 years. At enrollment and all follow-up visits, participants provided a serum sample and for sexually experienced women, a pelvic exam was conducted and exfoliated cervical cells were collected for cytology and HPV DNA infection determination. Protocols were approved by the US National Cancer Institute and the INCIENSA, Costa Rica Institutional Review Boards.

For this specific project, we designed a study using prevaccination samples from 388 HPV16 DNA-negative women nested within the control arm of the CVT (Fig. 1). We embedded this methods-evaluation study within our previous study, which evaluated whether naturally
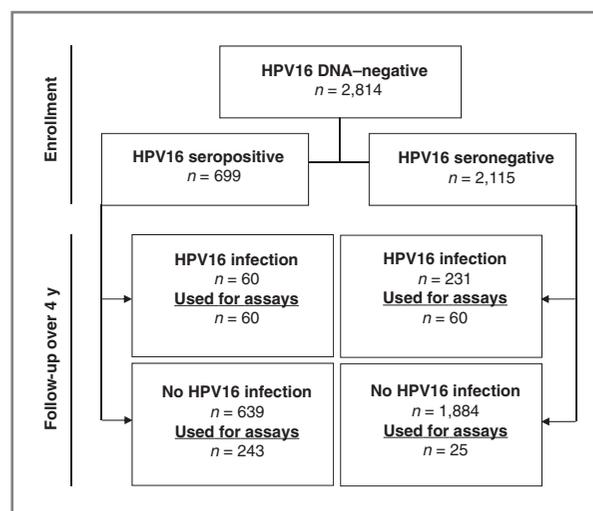

Figure 1. Consort diagram of samples used in the comparison of the cLIA, HPV 16/18 ELISA, and the L1 + L2 pseudovirion SEAP assay. Total number of samples (N) = 388. DNA positive/ELISA positive = 60, DNA negative/ELISA positive = 243, DNA positive/ELISA negative = 60, and DNA negative/ELISA negative = 25.

acquired antibodies among women who were HPV16 cervical DNA-negative at study entry protected against future reinfection (13). Thus, we selected samples on the basis of direct ELISA status at enrollment and incident/newly detected HPV (HPV16 and HPV18) infection as measured by DNA testing (12) through the 4 years of follow-up. Selection was designed to capture women who were (i) HPV16 ELISA–seropositive at enrollment and developed an incident HPV16 infection during follow-up (i.e., infected despite ELISA positivity), (ii) HPV16 ELISA–seropositive at enrollment and did not develop an infection over the follow-up (i.e., immune protected and/or unexposed group), (iii) HPV16 ELISA–seronegative at enrollment and developed an incident infection during follow-up (i.e., infected in the absence of ELISA positivity), and finally (iv) HPV16 ELISA–seronegative at enrollment and did not develop an infection over the follow-up (Fig. 1). This targeted sampling was designed to enable us to compare the HPV16 direct ELISA against each of the other assays; the approach also allowed us to weight back the finding to the full base population. Replicate enrollment and prevaccination serum samples from the 388 participants were tested by the ELISA, cLIA, and SEAP-NA for HPV16 antibodies.

HPV serologic and DNA measurements

ELISA. Serum collected at enrollment was used to determine HPV16 IgG serostatus at GSK Biologicals using a VLP-based direct ELISA developed by GSK that measures serum polyclonal IgG antibodies that bind HPV16 VLPs as described previously (7). 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% w/v skim milk with 0.2% v/v Tween-

20. Serum samples were serially diluted in the blocking solution starting at 1:100 in 2-fold 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 (peroxidase-labeled affinity purified antibody to human IgG (γ), F(ab')2, produced in goat; Kirkegaard and Perry Laboratories) 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 the 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 cutoff points were determined by GSK and calculated from antibody titer values 3 SDs above the geometric mean titers taken from 2 groups of known HPV-negative individuals (7, 14). The variability of this assay in the laboratory we used for testing has been shown to be very low (mean CV = 12.31%; ref. 7).

cLIA. Seropositivity by the multiplex cLIA assay was measured at PPD Vaccines and Biologicals as previously described (6). In brief, VLPs were expressed in yeast, coupled to Luminex microspheres, and pooled into a multiplex system. Type-specific, monoclonal antibodies binding to neutralizing epitopes (H6.B10.5, H11.B2, H16.V5, and H18.J4) were labeled with phycoerythrin (PE) and used at a final concentration of 0.5 μg/mL for H6.B10.5, 1.0 μg/mL for H11.B2, 1.0 μg/mL for H16.V5, and 1.25 μg/mL for H18.J4. Diluted sera (1:4) and PE-tagged antibodies were incubated before VLP-microspheres were added to the well. After incubation overnight at room temperature, plates were washed 3 times with PBS and 1% TX100. Mean fluorescence intensities (MFI) were measured using a Luminex 100 instrument and MFIs were converted to arbitrary milli-Merck unit (mMU/mL) values using standard curves. The serostatus cutoffs were set using a clinical sensitivity/specificity algorithm to maximize the distinction between "likely negative" and "likely positive" samples (15) and for HPV16 was 20 mMU/mL, as described previously. We estimated assay reproducibility using coefficients of variations based on blinded duplicate samples ($n = 25$) randomly placed in each batch to be 9.5% for HPV16.

SEAP-NA. Neutralizing antibody titers were determined using SEAP-NA as previously described at the HPV Immunology Laboratory of the National Cancer Institute (Frederick, MD; ref. 8). Briefly, 293TT cells were expanded and cultured and seeded in 96-well, flat-bottom plates at a cell density of 3×10^4 cells per well and incubated at 37°C and 5% CO2 for 2 or more hours before addition of controls and diluted samples. Twenty-five microliters of serially diluted serum (diluted 1/10 in 4-fold increments up to 1/163,840, and tested in duplicate) were incubated with 100 uL of HPV16 or 18 VLPs at 4°C

for 1 hour. The samples were then incubated with 293TT cells at 37°C for 72 hours. Clarified supernatants were transferred to 96-well plates and frozen at −70°C until further testing. The Great EscAPe SEAP assay kit was used according to the manufacturer's protocol (BD Biosciences-Clontech Laboratories Inc.). Neutralization titers were calculated by linear interpolation and defined as the reciprocal of the dilution that caused 50% reduction in SEAP activity compared with control wells. The reported neutralization titers reflect the mean value of duplicate testing for each sample. Titer values below our lowest dilution (1/10) were arbitrarily given a value of 5 titer units (TU). SEAP-NA titers greater than or equal to 10 TU (1/10 dilution) were defined as positive for the respective HPV types and are presented. The serostatus cutoffs were set by using a cut point 3 SDs over HPV16 DNA and seronegatives (8) and for HPV16 was 25.1 TU. We estimated assay reproducibility using coefficients of variations based on blind duplicate samples ($n = 25$) randomly placed in each batch to be 27.6% for HPV16.

HPV DNA-SPF$_{10}$/DEIA/LiPA$_{25}$. HPV DNA detection and genotyping was conducted at DDL Diagnostic Laboratory as described previously (16, 17). Extracted DNA was used for PCR amplification with the SPF$_{10}$ primer sets. 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), which detects 25 HPV genotypes.

Because the Costa Rica HPV16/18 Vaccine Trial 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. Amplimers from the TS-PCRs were detected by DEIA similar to the method used for SPF$_{10}$ amplimer detection (16–18).

Statistical analysis

Positivity at the developer's suggested cutoff was used to dichotomize results from each assay. Percentage agreement, positive agreement, and κ statistics were calculated for agreement between dichotomized results. Our primary objective was to compare ELISA and cLIA. For completeness, we also compared both the ELISA and cLIA against SEAP-NA.

To account for the sampling scheme used for sample selection, we report the sampling-adjusted estimates such as geometric mean titers (GMT), interquartile range, and prevalence. We calculated sampling fractions as the ratio of the numbers of stratum members in the stratified sample and the cohort. The reciprocal of the sampling fractions for each stratum were then used as the sampling weight for every individual in the stratum, and used to estimate results for the full cohort.

We also compared the raw (continuous) values generated by the assays, assigning values below the laboratory-determined cutoff as negative. In addition, we compared

ELISA and cLIA agreement at alternate ELISA cutoffs. We used 3 approaches to determine the ELISA cutoffs. First, we estimated a cutoff based on a ROC analysis using cLIA as the gold-standard. Using this approach, we determined an ELISA cutoff of 26 EU/mL by maximizing Youden Index (sensitivity+specificity-1). Second, we estimated an ELISA cutoff that maximized positive agreement between cLIA and ELISA. Using this approach, we determined an ELISA cutoff of 54, which maximized positive agreement between cLIA and ELISA. In addition, based on our previously published report that showed that HPV16 DNA–negative women with enrollment ELISA levels above 60 were half as likely to acquire a newly detected subsequent HPV16 infection over 4 years of follow-up compared with HPV16 seronegatives (13), we also used the ELISA cutoff of 60 EU/mL. However, results using this cutoff were similar to the results using the cutoff of 54 and thus are not reported.

Results

Overall positivity

Sampling adjusted HPV16 antibody seropositivity by ELISA was 24.8%, compared with 7.2% by cLIA and 13.6% by SEAP-NA (Fig. 2). Among the seropositives, HPV16 ELISA titers ranged from 8 to 3,202 EU/mL (weighted GMT 33.8 EU/mL), cLIA ranged from 20 to 3,370 mMU/mL (weighted GMT 92.7 mMU/mL), and SEAP-NA ranged from 25.1 to 2,560 TU (weighted GMT 72.6 TU; Table 1).

Comparison of ELISA and cLIA

Comparing the raw values, after weighting back to the entire cohort, based on the standard cutoff (for both ELISA and cLIA) 75.2% of the samples were negative by both assays, 7.2% were positive by both assays. All of the women with discordant results were ELISA-positive, and cLIA-negative (17.7%; Fig. 3A), resulting in an overall agreement of 82.4% (positive agreement = 44.9%).

Using the ELISA cutoff of 26 EU/mL determined from the ROC analysis, (keeping cLIA cutoff at 20 mMU/mL)
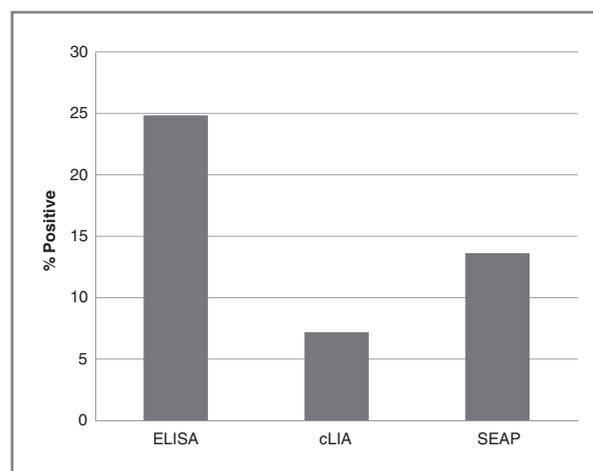
Figure 2. HPV16-specific cLIA, ELISA, and SEAP positivity based on developer suggested seropositivity cutoff (weighted prevalence).

overall agreement increased to 94.6% (positive agreement = 72%). 87.6% of the samples were negative by both assays and 7.0% were positive by both assays. Of the discordant, 0.2% were classified as cLIA-positive samples that were ELISA-negative, however, at this cutoff ELISA-positive/cLIA-negatives decreased from 18% (at the recommended cutoff) 5.2% (Fig. 3B).

At the higher ELISA cutoff of 54 EU/mL which was determined to maximize positive agreement with cLIA, (and keeping cLIA cutoff at 20 mMU/mL), overall agreement increased to 97% (positive agreement = 78%). Ninety-one percent of the samples were negative by both assays and 5.7% were positive by both assays. Of the discordants, 1.4% were classified as cLIA-positive and ELISA-negative, however, at this cutoff, ELISA-positive/cLIA-negatives decreased from 18% (at the recommended cutoff) to 1.8% (Fig. 3C).

Comparison of HPV16 ELISA and cLIA with SEAP-NA

Agreement between ELISA and cLIA with SEAP-NA is presented in Table 2 (based on weighted data). Forty-two

Table 1. Comparison of the experimental data from HPV16 specific cLIA, ELISA, and SEAP-NA

Assay (units and cutoff)	All measured specimens						Positive specimens only					
	Min[b]	Max	GMT[a] (Wtd)	25% (Wtd)	Median (Wtd)	75% (Wtd)	Min	Max	GMT (Wtd)	25% (Wtd)	Median (Wtd.)	75% (Wtd.)
VLP-based direct ELISA (EU/mL; 8+)	<8	3202	6.69	<8	<8	<8	8	3202	33.76	14	25	72
cLIA (mMU/mL; 20+)	<11	3370	6.91	<11	<11	<11	20	3370	92.72	44	76	191
SEAP-NA (TU; 25.1+)	5	2560	11.97	5	12.31	16.55	25.24	2560	72.64	28.27	52.18	128.48

Abbreviations: GMT, geometric mean titer; Wtd, weighted; TU, titer unit.
[a]For the geometric mean calculation, values less than the limit of detection were coded as 1 and missing values omitted.
[b]Values less than the limit of detection are shown as "limit of detection."
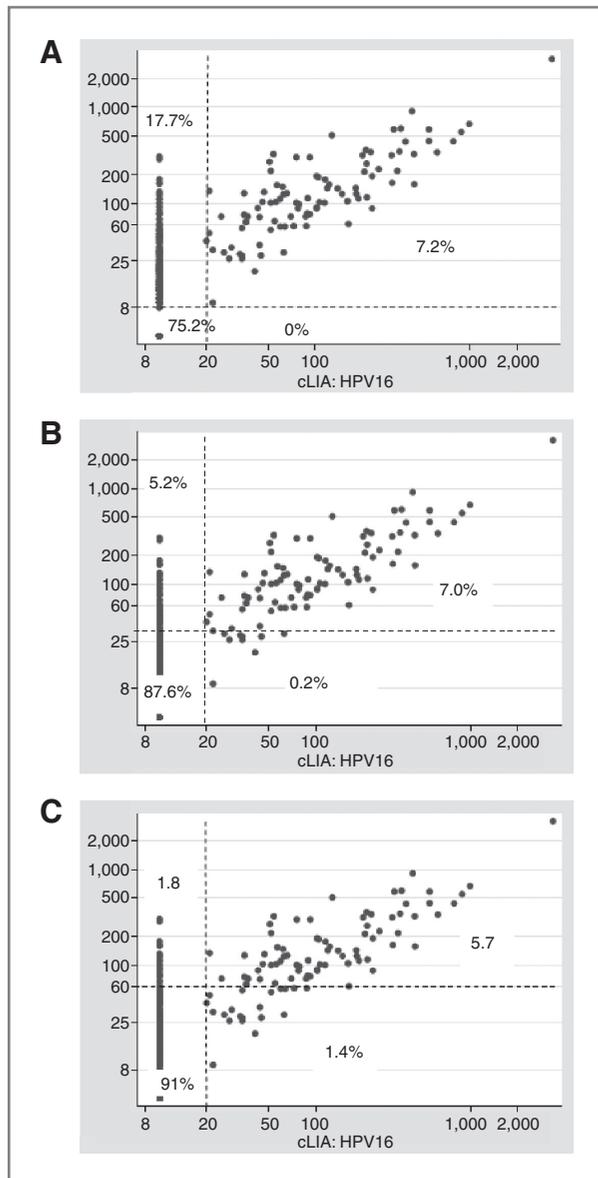Manufacturer-determined seropositivity thresholds.

Figure 3. Comparison of ELISA and cLIA based on the laboratory-determined suggested cutoffs (A, cutoff for ELISA = 8, cLIA = 20); alternate seropositivity cutoff of 26 for ELISA which maximized agreement with cLIA (B, ELISA=26, cLIA=20); alternate seropositivity cutoff of 54 for ELISA, that is, a cutoff determined to maximize positive agreement with cLIA (C, ELISA=54, cLIA=20). Sampling adjusted numbers are shown.

percent (296/699) of the ELISA-positive samples were SEAP-positive, compared with 99% (199/201) of the cLIA-positives that were SEAP-positive. Conversely, 77% (296/383) of the SEAP-positive were ELISA-positive, and 52% (199/383) of the SEAP-positives were cLIA-positive. Examining the ELISA-SEAP discordant samples revealed that more than half of these samples had low ELISA levels. Thus, after weighting back to the entire cohort of the 403 ELISA-positive and SEAP-negative discordant samples, 227 (57%) were in the lowest tertile of

Table 2. Agreement of ELISA and cLIA with SEAP-NA based on weighted values

	L1 + L2 Pseudovirion SEAP (>25.1 TU)	
	Positive	**Negative**
ELISA		
Positive	296	403
Negative	87	2,028
cLIA		
Positive	199	3
Negative	184	2,428

NOTE: SEAP-NA and ELISA: overall agreement = 83%; positive agreement = 55%; κ = 0.45.
SEAP-NA and cLIA: overall agreement = 93%; positive agreement = 68%; κ = 0.65.

ELISA levels (ELISA range 8–18 EU/mL), 137 (34%) were in the middle tertile (ELISA range 19–54 EU/mL), and 38 (9%) were in the highest tertile (ELISA range 57–3204 EU/mL). At the cutoffs suggested by the developers, ELISA had moderate agreement with SEAP-NA (positive-agreement of 55%, κ of 0.46), whereas cLIA had better agreement with SEAP-NA (positive agreement of 68%, κ of 0.65).

In an effort to further characterize these assays, we converted both to International units (IU). Each laboratory established the conversion factor to IU using the WHO 05/134 international reference standard (19). The conversion factor for GSK's ELISA Unit to international unit for HPV-16 direct ELISA is 1IU = 6.1 ELISA Units. Similarly, the conversion factor to translate mMU/mL to international unit for the HPV16 is 1 IU = 13.28 mMU/mL. Table 3 presents the conversions to IU. The seropositivity cutoff for both ELISA and cLIA were similar in terms of IU (1.31 IU and 1.5 IU, respectively), the range of the cLIA was 2-fold narrower than ELISA (maximum levels of 525 and 254 for ELISA and cLIA, respectively).

Conclusions

Explanation of vaccine efficacy differences in type-independent CIN2+ between Gardasil and Cervarix is likely multifactorial, and include (i) differences in cross-protection among the 2 vaccines, (ii) differences in incidence of other carcinogenic HPV types, and finally (iii) differences in serologic exclusion due to use of different assays. To address the role of serologic exclusions, we compared ELISA and cLIA serologic assays in unvaccinated individuals to assess the potential impact of using qualitatively and quantitatively different assays on reported vaccine efficacies in analyses of subsets of women defined as "HPV-naïve" in phase III HPV Vaccine Trials. Our results showed that these assays are very different from each other based on the laboratory-

Table 3. Comparison of conversion of HPV16 ELISA and cLIA values among seropositives to International units

	Minimum	Maximum	Median
ELISA	8 EU = 1.31 IU	3202 EU = 525 IU	29 EU = 4.75 IU
cLIA	20 mMU = 1.5 IU	3370 mMU = 254 IU	88 mMU = 6.63 IU

NOTE: ELISA conversion is 6.1 EU = 1 IU.
cLIA conversion is 13.28 mMU = 1 IU.

determined cutoffs. ELISA positivity was 3-fold higher than cLIA positivity suggesting that the observed differences in vaccine efficacies among the subset of HPV naïve-populations may be due, in part, to the inconsistent cutoffs in the assays used to define the HPV-naïve populations. The fact that cLIA did not classify as many seropositives as the ELISA suggests that the cutoff used in the cLIA assay is higher (thus not sensitive) for detecting exposure; in contrast, previous published work suggests that ELISA assays are a good measure of HPV exposure (20–22).

Comparison of the outcomes in cohorts of women classified as HPV-naïve can be misleading when different serology assays were used to define the cohorts. Understanding the relation of ELISA and cLIA among the unvaccinated can provide a comparison between vaccine efficacies in cohorts defined as HPV-naïve using different assays, such as the vaccine trials. As an example, the prophylactic efficacy in prevention of any HPV related CIN2+ in girls and women ages 16–26, among participants who were defined to be HPV-naïve, i.e., seronegative for HPV16 and 18 at baseline and HPV DNA-negative for the 14 carcinogenic HPV types at baseline was 42.7% [95% confidence interval (CI), 23.7–57.3] for Gardasil (4) in contrast with a reduction of 64.9% (95% CI, 52.7–74.2) for Cervarix (5). These differences could be due to the vaccine itself; however, they could partly be due to the assays used to define the analytic population of HPV-naïve individuals. Merck used cLIA to define their HPV16/18 seronegative, which is designed to measure presence of HPV type–specific serum IgM, IgA, and/or IgG antibodies that compete with monoclonal antibodies (HPV16 V5 epitope) known to have neutralizing ability. Because cLIA is based on the competition of serum antibodies with one specific epitope (V5 for HPV16), it may not detect other antibodies binding to different epitopes. In contrast, as the ELISA assay measures serum IgG antibodies to both neutralizing and nonneutralizing antibodies that bind to the VLPs, it is more sensitive for measuring exposure and thus would have resulted in a "cleaner" HPV-naïve population for the efficacy findings and which could partly explain the observed higher reduction of CIN2/3 or AIS using Cervarix compared with Gardasil.

Use of the most sensitive assays to detect prior infection would maximize ability to better define naïve subcohorts. In the meantime, to make results from existing trials more comparable, it might be possible to consider alternative cutoffs of positivity that make the 2 assays more comparable. To guide such reanalyses, in the present work, we considered alternative cutoffs that increased agreement between cLIA and ELISA. This was possible by increasing ELISA cutoff (i.e., making it more specific); it was not possible to lower cLIA cutoff (i.e., making it more sensitive), as cutoff provided by the laboratory is already at the limit of detection for that assay. By altering the ELISA cutoff, we showed that ELISA compared well with cLIA when a higher cutoff was applied.

While in separate studies, the ELISA and cLIA have been compared with SEAP-NA in vaccinated individuals and have shown good agreement in the initial years after vaccination (7, 8), comparisons using serum samples from unvaccinated individuals are lacking. In the context of unvaccinated populations, we showed that the cLIA results compare better than the ELISA results with the SEAP-NA results, which is in agreement with the fact that the cLIA measures antibodies against a specific neutralization epitope. In contrast, the ELISA results were not a good measure of neutralizing potential when the standard positivity cutoff recommended by the assay developer is used because ELISA captures the total polyclonal IgG response independently of the neutralization capability. A previous study, which compared the ELISA to cLIA among unvaccinated individuals, similarly showed that cLIA assay detected as positive a subset of individuals that ELISA defined as exposed (11).

Conversion of the EU/mL and mMU/mL to the international units could, in theory, facilitate interpretation of assay findings across different studies. We found that while the minimum and median levels were similar in terms of the IU, the range of the cLIA was narrower than the ELISA. Whether this is due to the fact that cLIA measures a subset of the ELISA measured antibodies, or whether the cLIA assay is less sensitive at lower levels and not linear in the higher ranges is unknown. However, because these assays measure different aspects of the antibody response, and have different performances, it may be that a simple conversion to IU will facilitate comparisons of results for the same assay across studies but will not facilitate interassay comparisons.

We used samples from the control arm of the community-based, randomized Costa Rica HPV16/18 HPV Vaccine Trial that included women selected from a population census. We designed a study to follow-up on our

findings that showed women with elevated HPV16 and HPV18 antibody levels (measured with ELISA assay) following natural infection had a significant 50% and 60% reduced risk of subsequent new HPV16 or HPV18 infection compared with seronegatives (13). We were interested to investigate the role of other assays such as cLIA as a measure of immunity. Rather than conducting the different assays on samples from all the eligible women in the control arm ($n \sim 7,500$ assays), we designed that study using knowledge of our prior study and thus selected women on the basis of their enrollment ELISA seropositivity. In addition to the primary objective, the same sample set, allowed us to conduct the current methods study. While this resulted in an efficient sampling, a limitation was that the samples selected were on the basis of seropositivity by ELISA. We believe this does not bias our study, as it measures a broader range of antibodies, and we previously showed this ELISA assay to be a good biomarker of exposure at the suggested cutoff (20, 21).

ELISA and cLIA are different from each other based on the laboratory-determined cutoff. Increasing the ELISA cutoff increased agreement with cLIA, which could facilitate comparisons among studies that use different assays.

Disclosure of Potential Conflicts of Interest

Authors' Contributions

Grant Support

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