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Oral HPV prevalence assessment by Linear Array vs. SPF_{10} PCR-DEIA-$LiPA_{25}$ system in the HPV Infection in Men (HIM) study

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ABSTRACT

Introduction: Oral human papillomavirus (HPV) attributable oropharyngeal cancers are on the rise in many countries. Oral HPV infections among healthy individuals are commonly detected using oral gargle samples. However, the optimal method for HPV genotyping oral gargle specimens in research studies has not been previously evaluated.

Materials and methods: Oral gargle samples from 1455 HPV Infection in Men (HIM) study participants were HPV genotyped using two different methods: Linear Array and the SPF_{10} PCR-DEIA-$LiPA_{25}$. The sensitivity of the two tests for detecting individual HPV types and grouped HPV types, high-risk HPV, low-risk HPV, grouped 4-HPV-vaccine types, and grouped 9-HPV-vaccine-types, and the degree of concordance between the two tests was assessed. We also examined whether socio-demographic-behavioral factors were associated with concordance between the two assays.

Results: The sensitivity of SPF_{10} PCR-DEIA-$LiPA_{25}$ was higher than Linear Array, with the exception of HPV 70, for the detection of oral HPV. The prevalence ratio of SPF_{10} PCR-DEIA-$LiPA_{25}$ to Linear Array varied between 1.0 and 9.0 for individual HPV genotypes, excluding HPV 70, and between 3.8 and 4.4 for grouped 4-valent and 9-valent HPV vaccine types, respectively. There was no association between socio-demographic-behavioral factors and discordance in results between the two tests for oral HPV 16 detection.

Discussion: SPF_{10} PCR-DEIA-$LiPA_{25}$ was more sensitive than Linear Array for detecting HPV in oral gargle samples. Given the growing importance of detecting oral HPV infection for research studies of oral HPV natural history and vaccine effectiveness evaluation, we recommend using methods with higher sensitivity such as SPF_{10} PCR-DEIA-$LiPA_{25}$ for detecting HPV in oral gargle samples.

1. Introduction

Oral human papillomavirus (HPV) is a cause of a sub-set of oropharyngeal cancers (OPCs). The incidence of OPCs attributable to HPV is on the rise among many countries [1,2]. To inform effective preventive and early screening measures as well as to assess HPV prevention vaccine effectiveness it is essential to study the natural history of oral HPV infections and associated risk factors. Many cross-sectional and prospective studies have been conducted to estimate the prevalence and incidence of oral HPV infection in healthy populations such as the Human Papillomavirus Infection in Men (HIM) study [3], the NHANES study [4] and smaller, localized cohorts such as those at the Ohio State University [5] as well as among high risk populations [6].

Most studies of oral gargle HPV utilized the Linear Array method (Roche Molecular Diagnostics, Alameda, CA) for genotyping specimens [3–6]. This test is based on co-amplification of a 450 bp region of the

Abbreviations: HPV, Human Papillomavirus; LiPA, Line Probe Assay

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HPV *L1* gene using biotinylated primer sets PGM09/PGMY11 [7]. Other HPV DNA genotyping tests utilized in studying oral HPV include the SPF_{10} PCR-DEIA-$LiPA_{25}$ system (DDL Diagnostic Laboratory, Rijswijk, the Netherlands), which utilizes amplification of a 65 bp region of the HPV *L1* gene using biotinylated SPF10 primers followed by hybridization of the resulting amplicons with 17–28 HPV-specific oligonucleotide probes immobilized on a nitrocellulose strip [7].

Several studies have compared SPF_{10} based tests and Linear Array for HPV genotyping of formalin fixed paraffin embedded (FFPE) tumor tissue [8–13] and exfoliated cervical cells [14,15]; however, to our knowledge, no study has compared these two tests for HPV genotyping of oral gargle samples, a specimen that is quite different. Unlike FFPE tumor and exfoliated cervical specimens where the majority of the sample is the target tissue that may harbor HPV, oral gargle specimens are comprised of a large diversity of cells, most of which do not harbor HPV; hence, HPV viral load is significantly lower. Given the increasing use of oral gargle samples in oral HPV epidemiological studies and HPV vaccine efficacy and effectiveness studies [16], it is important to compare and assess the suitability of different HPV genotyping methods to prevent spurious HPV infection estimates.

In this study, we utilized data from the oral sub-cohort in the HIM study, one of the earliest and largest prospective studies with a study population of more than 3000 individuals from three different countries (Mexico, US, Brazil). We compared the sensitivity of the Linear Array and SPF_{10} PCR-DEIA-$LiPA_{25}$ system for HPV genotyping of oral gargle samples among HIM study participants at the first oral gargle specimen collection. Oral HPV prevalence is reported using these two methods in samples derived from the same individuals. The level of concordance in the results from these two methods for individual and grouped HPV genotypes was assessed as well as sociodemographic and behavioral characteristics that may be associated with concordance.

2. Materials and methods

The study population was nested within the oral sub-cohort of the HIM study which has been previously described [17]. Men were recruited from São Paulo, Brazil; Cuernavaca, Mexico; Tampa, Florida, and its surrounding areas from March 2005 to December 2009. Baseline oral specimens were collected between November 27, 2007 and September 29, 2009 in the US, between April 7, 2008 and March 23, 2012 in Mexico, and between December 13, 2007 and November 25, 2009 in Brazil. Approval of study procedures before the commencement of the HIM study was obtained from the Centro de Referência e Treinamento de Doenças Sexualmente Transmissíveis e AIDS, Brazil, National Institute of Public Health of Mexico and the Human Subject Committees of the University of South Florida. All participants gave written consent.

2.1. Study population

Men included in the HIM study met the following eligibility criteria: (a) Ages 18–70 years; (b) Residents of one of the three recruitment sites in Brazil, Mexico or the US; (c) Did not report previous diagnoses of anal or penile cancer; (d) Were never diagnosed with genital or anal warts prior to the study; (e) No current reports of a sexually transmitted infection or treatment of a sexually transmitted infection; (f) Not participating in a HPV vaccine study; (g) No history of HIV or AIDS; (h) No history of imprisonment, homelessness, or drug treatment during the past 6 months; (i) Willingness to comply with 10 scheduled visits every 6 months for 4 years with no plans to relocate within the next 4 years. Further details regarding the study population are available elsewhere [18]. Initially, oral specimens of first 1626 individuals were analyzed using the Linear Array method. In 2017 and 2018, these same oral specimens were analyzed using the SPF_{10} PCR-DEIA-$LiPA_{25}$ system. Individuals for whom at least two oral specimens were collected and first oral gargle samples were genotyped for HPV using both Linear Array and SPF_{10} PCR-DEIA-$LiPA_{25}$ system were included in the analyses (N = 1473).

2.2. Sample collection, DNA extraction, and HPV testing

The oral gargle sample was collected in 15 ml of Scope brand mouthwash and processed within 24 h of collection. Participants were asked to perform energetic washing of the oral cavity including the throat by swishing the mouthwash in their mouth vigorously for approximately 15 s and were instructed to cover all surfaces of their mouth. The participant was then instructed to tip their head back and gargle in the throat for another 15 s and spit the mouthwash back into the collection tube which was placed at 20 °C until processing. Within 24 h of collection the gargle specimen was centrifuged at $2000 \times g$ for 15 min at 4 °C. The supernatant was decanted and the resulting cell pellet resuspended in 20 ml cold PBS (4 °C). Centrifugation and pellet washing was repeated twice with the final cell pellet resuspended in 1.2 ml of PBS and maintained at −80 °C until DNA extraction when 300 µl was used for extraction.

For the Linear Array assay, the DNA concentration was determined via Nanodrop and concentrations above 1 ng/µl were diluted accordingly. Samples with concentrations below the targeted 1 ng/µl were not diluted or concentrated prior to use in the assay. Samples were not initially diluted for the SPF_{10} LiPA assay as sample concentration and adequacy were determined during the qPCR portion of the assay. If a sample was deemed inadequate via qPCR, input DNA concentration was adjusted to a more acceptable range, typically 10 ng/µl to 100 ng/µl, and the qPCR portion of the assay was repeated.

The same purification method was utilized in preparation of samples for the two HPV genotyping assays. DNA was extracted from oral gargle cell pellets using the automated BioRobot MDx (Qiagen, Inc.) following the manufacturer's instructions. After specimens thawed, they were placed on the universal extraction machine and the extraction protocol was run. A membrane based procedure was executed by the machine, utilizing optimized buffers to lyse and stabilize DNA, ethanol and wash buffers to wash away impurities and water to elute DNA for use in downstream assays. DNA was eluted into a 96 well plate for easy transfer during PCR procedures. The unused portion of the original samples and any unused extracted DNA was stored in a −80 °C freezer.

Qualitative identification of HPV DNA was performed to identify HPV genotype(s) via two methods: amplification of HPV DNA with the PGMY09/11 L1 consensus primer and HPV genotyping by Linear Array method (Roche Molecular Diagnostics, Alameda, CA), and a SPF_{10} PCR-DEIA-$LiPA_{25}$ system (DDL Diagnostic Laboratory, Rijswijk, the Netherlands). Per protocol, for the Linear Array assay, 50 µl (1 ng DNA/µl) of the processed sample was added to the master PCR mix, and 10 µl (median DNA concentration: 8 ng/µl) of the processed sample was utilized for the SPF_{10} PCR-DEIA-$LiPA_{25}$ system.

Details regarding the protocol for the Linear Array method has been made available elsewhere [17]. The SPF_{10} PCR-DEIA-$LiPA_{25}$ system is an *in vitro* reverse hybridization assay (RHA) [19]. The LiPA25 targets a 65 base pair fragment of the *L1* region of the HPV genome. This assay requires a three step process: (a) qPCR that determines sample adequacy; (b) a DNA enzyme immunoassay (DEIA) or ELISA method that detects 65 HPV types; and (c) a $LiPA_{25}$ genotyping multiplex PCR that selectively identifies the following HPV types by reverse hybridization: 6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68/73, 70, and 74. All samples that were considered as adequate in step (a) were further analyzed via steps (b) and (c).

2.3. Statistical analyses

We included 1455 individuals in our analyses whose oral gargle samples were HPV genotyped using both methods, and whose demographic data were available. Distribution of sociodemographic and behavioral characteristics among the participants were assessed (Table 1). We compared the prevalence of individual and grouped 4

Table 1
Sociodemographic characteristics of the HIM study population for whom oral HPV genotyping was conducted through both Linear Assay and SPF_{10} PCR-DEIA-$LiPA_{25}$ System.

Characteristic	n (%)
Country (N = 1455)	
Brazil	436 (30.0)
Mexico	513 (35.3)
USA	506 (34.8)
Age: Mean (SD)	34.0 (11.7)
Age: Median (Interquartile Range)	32.0 (24.0, 41.5)
Age Category (N = 1455)	
18–30 years	664 (45.6)
31–40 years	393 (27.0)
41–50 years	254 (17.5)
51–73 years	144 (9.9)
Race (N = 1434)	
White	670 (46.7)
Black	32 (2.2)
Asian/Pacific Islander	201 (14.0)
Mexican	467 (32.6)
Other	64 (4.41)
Ethnicity (N = 1446)	
Hispanic	683 (47.2)
Non-Hispanic	763 (52.8)
Education (N = 1448)	
Completed 12 Years or Less	640 (44.2)
13–15 Years	408 (28.2)
Completed at least 16 Years	400 (27.6)
Marital Status (N = 1450)	
Single	617 (42.6)
Married/Cohabiting	123 (8.5)
Divorced/Separated/Widowed	710 (49.0)
Smoking Status (N = 1450)	
Never	868 (59.9)
Former	310 (21.4)
Current	272 (18.8)
Use of Chewing Tobacco or Snuff (N = 1434)	
Never	1362 (95.0)
Sometimes	20 (1.4)
Daily	52 (3.6)
Number of Alcoholic Drinks Consumed in the Past Month (N = 1430)	
None	362 (25.3)
1–30 drinks	656 (45.9)
More than 30 drinks	412 (28.8)
Sexual Orientation (N = 1376)	
MSW[a]	1246 (90.6)
MSM[b]	48 (3.5)
MSMW[c]	82 (6.0)

[a] MSW: Men who have sex with women only.
[b] MSM: Men who have sex with men only.
[c] MSMW: Men who have sex with women and men.

HPV vaccine types (6/11/16/18), grouped 9 HPV vaccine types (6/11/16/18/31/33/45/52/58), any high risk HPV type (16/18/31/33/35/39/45/51/52/56/58/59/68), any low risk type (6/11/40/42/53/54/66/70) and any HPV type (presence of one of the high or low risk HPV types). Overall, twenty one genotypes that were detected by both Linear Array and the SPF_{10} PCR-DEIA-$LiPA_{25}$ system were included in our study.

We used Cohen's Kappa test to assess the agreement between Linear Array and SPF_{10} PCR-DEIA-$LiPA_{25}$ system for each individual and grouped HPV genotype(s) (Table 2). We also visually compared the percentage of samples that tested positive for individual HPV types 6, 11, 16 and 18 and grouped 4 vaccine genotypes (6/11/16/18), grouped 9 vaccine types (6/11/16/18/31/33/45/52/58) and any HPV genotype using grouped bar graphs with standard error bars (Fig. 1).

Going further, we examined the degree of concordance between the results of the two methods for individual and grouped HPV genotype(s) (Table 3) using Cohen's Kappa statistic, prevalence adjusted, bias adjusted Kappa statistic (PABAK) [20] and exact McNemar's test. The Kappa statistic is affected by both bias between the two tests as well as the overall prevalence [20]. Hence, the PABAK statistic, a version of Kappa that adjusts for bias and takes into account the low prevalence of the outcome measure was included alongside the Kappa statistic. McNemar's test is used to examine systematic differences between the results of two tests [21]; hence, it was also included in the analyses. Finally, we examined whether self-reported sociodemographic characteristics such as country of residence, age, race, ethnicity, education, marital status, cigarette smoking, alcohol consumption, use of chewing tobacco and/or snuff and sexual orientation were associated with concordance between the two tests in the detection of HPV 16, the most commonly found oral HPV type, using simple and multi-variable logistic regression models (Table 4). We assessed collinearity between covariates, and excluded covariates with high collinearity in the multivariable logistic regression model. We used naïve Bayesian logistic regression models with weakly informative priors in R using 'arm' package [22] when infinitely wide confidence intervals were encountered in the logistic regression models.

Table 2
Prevalence of different HPV Genotypes with Linear Assay and SPF_{10} PCR-DEIA-$LiPA_{25}$ System within the Oral HIM Sub-cohort that Underwent Both Tests.

HPV Genotype[a]	Linear Array N (%)	SPF_{10} PCR-DEIA-$LiPA_{25}$ System N (%)	SPF_{10} PCR-DEIA-$LiPA_{25}$ System/ Linear Array Prevalence Ratio
6	3 (0.21)	13 (0.90)	4.3
11	2 (0.14)	3 (0.21)	1.5
16	7 (0.48)	26 (1.82)	3.7
18	0 (0.00)	4 (0.28)	N/A
31	0 (0.00)	4 (0.28)	N/A
33	0 (0.00)	1 (0.07)	N/A
35	1 (0.07)	4 (0.28)	4.0
39	1 (0.07)	7 (0.48)	7.0
40	0 (0.00)	0 (0.00)	N/A
42	0 (0.00)	0 (0.00)	N/A
45	0 (0.00)	2 (0.14)	N/A
51	3 (0.21)	13 (0.90)	4.3
52	1 (0.07)	7 (0.48)	7.0
53	1 (0.07)	9 (0.62)	9.0
54	0 (0.00)	2 (0.14)	N/A
56	0 (0.00)	8 (0.55)	N/A
58	1 (0.07)	3 (0.21)	3.0
59	2 (0.14)	2 (0.14)	1.0
66	4 (0.27)	12 (0.83)	3.0
68	0 (0.00)	1 (0.07)	N/A
70	2 (0.14)	1 (0.07)	0.5
Any HPV genotype	28 (1.92)	126 (8.66)	4.5
Any High-Risk HPV	16 (1.1)	76 (5.22)	4.8
Any Low-Risk HPV	12 (0.82)	53 (3.64)	4.4
HPV type 6 and/or 11 and/or 16 and/or 18	12 (0.82)	46 (3.16)	3.8
HPV type 6 and/or 11 and/or 16 and/or 18 and/or 31 and/or 33 and/or 45 and/or 52 and/or 58	14 (0.96)	61 (4.19)	4.4

[a] Total sample size for all HPV types: 1455.

3. Results

Overall, 1455 participants of the total 3098 participants in the oral sub-cohort of the HIM study were included in the analyses. Approximately 30% of participants were from Brazil and around 35% of the participants were from Mexico and the US each. The mean and median age of the participants was 34 and 32 years respectively. Almost half (45.6%) of the participants were aged between 18 and 30 years while less than 10% of the participants were older than 50 years. Most participants reported either White (46.7%) or Mexican (32.6%) races. There was an almost equal distribution between Hispanic (47.2%) and non-Hispanic (52.8%) ethnicities. The majority of participants completed 12 years or less of education (44.2%), were single (42.6%), had

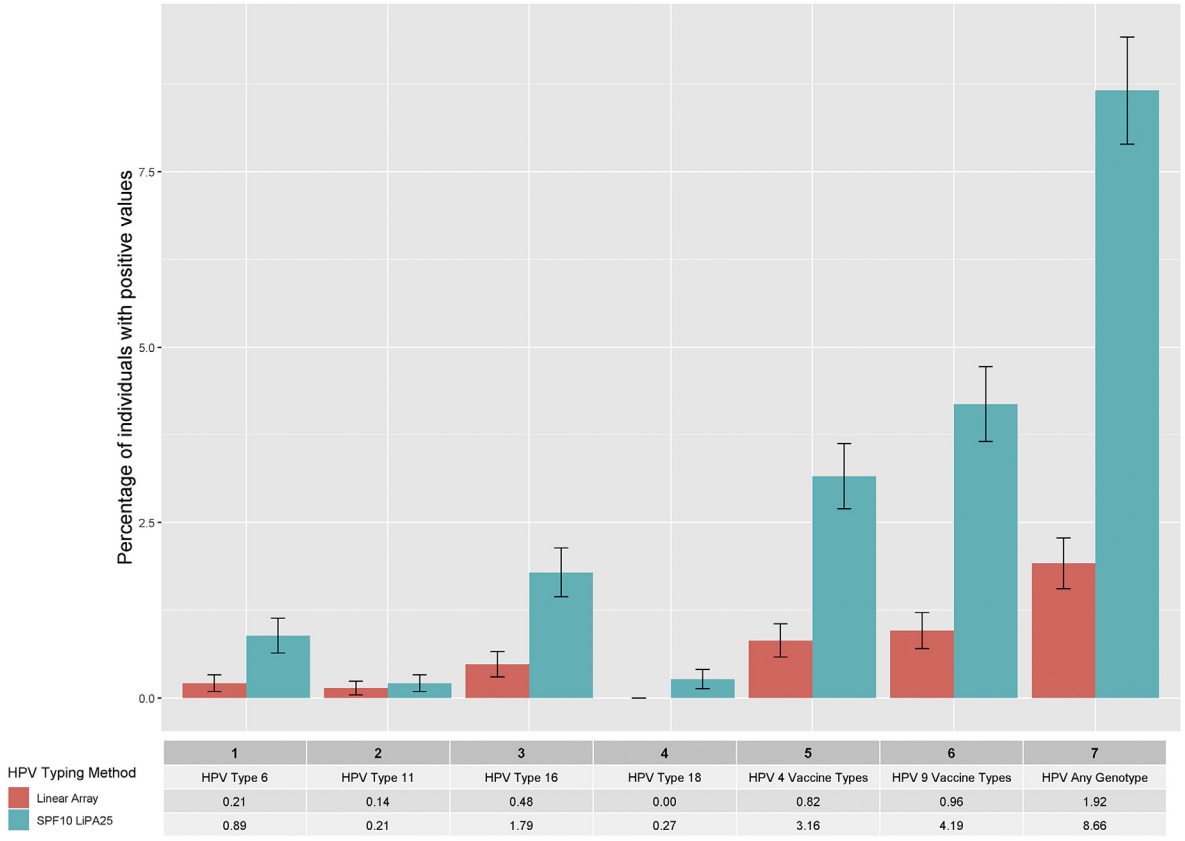

Fig. 1. Oral HPV Prevalence (with standard error bars) among Individual and Grouped HPV Genotypes in the HIM Study at Baseline.

Table 3
Degree of oral HPV detection concordance between linear array and SPF_{10} PCR-DEIA-$LiPA_{25}$ system within the oral HIM sub-cohort that underwent both tests.

HPV Genotype	[a]SPF_{10}-/LA-	[a]SPF_{10}+/LA+	[a]SPF_{10}-/LA+	[a]SPF_{10}+/LA-	Kappa Statistic[b]	PABAK[d]	McNemar test[e] p-value
6	1440	1	2	12	0.12	**0.98**	**0.01**
11	1452	2	0	1	**0.80**	**1.00**	1.00
16	1426	4	3	22	0.24	**0.97**	< **0.01**
18	1451	0	0	4	0	**0.99**	0.12
31	1451	0	0	4	0	**0.99**	0.12
33	1454	0	0	1	0	**1.00**	1.00
35	1450	0	1	4	−0.001	**0.99**	0.37
39	1447	0	1	7	−0.001	**0.99**	0.07
40	1455	0	0	0	N/A[c]	**1.00**	1.00
42	1455	0	0	0	N/A[c]	**1.00**	1.00
45	1453	0	0	2	0	**1.00**	0.50
51	1440	1	2	12	0.12	**0.98**	**0.01**
52	1447	0	1	7	−0.001	**0.99**	0.07
53	1445	0	1	9	−0.001	**0.99**	**0.02**
54	1453	0	0	2	0	**1.00**	0.50
56	1447	0	0	8	0	**0.99**	**0.01**
58	1452	1	0	2	**0.50**	**1.00**	0.50
59	1452	1	1	1	**0.50**	**1.00**	1.00
66	1441	2	2	10	0.25	**0.98**	**0.04**
68	1454	0	0	1	0	**1.00**	1.00
70	1453	1	1	0	**0.67**	**1.00**	1.00
Any HPV	1316	15	13	111	0.17	**0.83**	< **0.01**
Any high risk HPV type	1372	9	7	67	0.18	**0.90**	< **0.01**
Any low risk HPV type	1396	6	6	47	0.17	**0.93**	< **0.01**
HPV 4 vaccine types	1404	7	5	39	0.23	**0.94**	< **0.01**
HPV 9 vaccine types	1388	8	6	53	0.2	**0.92**	< **0.01**

[a] SPF_{10}: SPF_{10} PCR-DEIA-$LiPA_{25}$ system; LA: Linear Array method.
[b] Was obtained using Cohen's Kappa test, a test of agreement, between Linear Array and SPF_{10} PCR-DEIA-$LiPA_{25}$ System.
[c] Kappa statistic was not available when both tests had zero detection for an oral HPV genotype.
[d] PABAK: Prevalence adjusted, bias adjusted Kappa estimate.
[e] Exact McNemar's test was used.

Table 4
Univariate and multivariate adjusted association between sociodemographic characteristics and concordance in oral HPV type 16 detection by linear assay and SPF_{10} PCR-DEIA-$LiPA_{25}$ system.

Characteristic	OR[a] (95% CI[b])	aOR[c] (95% CI[d])
Country		
Brazil	1.96 (0.72, 5.79)	2.05 (0.68, 6.23)
Mexico	1.49 (0.53, 4.47)	1.58 (0.48, 5.21)
USA	Ref	Ref
Age Category		
18–30 years	Ref	Ref
31–40 years	0.77 (0.24, 2.12)	0.59 (0.20, 1.79)
41–50 years	1.44 (0.49, 3.82)	0.99 (0.33, 2.97)
51–73 years	1.26 (0.28, 4.11)	0.66 (0.15, 2.92)
Race[e]		
White	Ref	–
Black	0.50 (0.13, 1.90)	
Asian/Pacific Islander	1.34 (0.21, 8.50)	
Mexican	0.70 (0.29, 1.67)	
Other	0.21 (0.01, 3.62)	
Ethnicity		
Hispanic	1.35 (0.61, 3.12)	–
Non-Hispanic	Ref	
Education		
Completed 12 Years or Less	Ref	Ref
13–15 Years	0.78 (0.27, 2.03)	0.87 (0.28, 2.72)
Completed at least 16 Years	0.93 (0.34, 2.34)	0.98 (0.38, 2.56)
Marital Status		
Single	Ref	Ref
Married/Cohabiting	1.31 (0.54, 3.36)	1.38 (0.48, 4.01)
Divorced/Separated/Widowed	3.23 (0.96, 9.84)	3.39 (0.93, 12.30)
Smoking Status		
Never	Ref	Ref
Former	0.56 (0.13, 1.68)	0.63 (0.19, 2.01)
Current	0.82 (0.27, 2.10)	0.76 (0.27, 2.14)
Use of Chewing Tobacco or Snuff[e]		
Never	Ref	Ref
Sometimes	0.28 (0.01, 5.10)	0.3 (0.02, 5.93)
Daily	2.29 (0.32, 16.44)	1.93 (0.26, 14.30)
Number of Alcoholic Drinks Consumed in the Past Month		
None		Ref
1–30 drinks	1.11 (0.39, 3.57)	1.27 (0.43, 3.74)
More than 30 drinks	1.78 (0.63, 5.75)	2.08 (0.68, 6.36)
Sexual Orientation		
MSW[f]	Ref	Ref
MSM[g]	1.24 (0.07, 6.13)	1.17 (0.18, 7.46)
MSMW[h]	0.72 (0.04, 3.51)	0.72 (0.13, 4.10)

None of the variables were significantly associated with HPV 16 discordance at $\alpha = 0.05$ level.

[a] Unadjusted odds ratio calculated using simple logistic regression.

[b] 95% confidence interval.

[c] Adjusted odds ratio; was calculated using multivariable logistic regression with weakly informative default prior distribution for the regression coefficients. Model was adjusted for country of residence, age, education, marital status, cigarette smoking status, using chewing tobacco or snuff, alcohol consumption and sexual orientation.

[d] 95% Bayesian credible interval; an interval within which an observed parameter value falls with 95% probability.

[e] Weakly informative prior distribution was applied via naïve Bayesian logistic regression model since simple logistic regression yielded infinitely wide confidence intervals.

[f] MSW: Men Having Sex with Women.

[g] MSM: Men Having Sex with Men.

[h] MSMW: Men having Sex with Men and Women.

never smoked cigarettes during their life (59.9%), never chewed tobacco or used snuff (95.0%), had consumed between 1 and 30 drinks during past month (45.9%) and were heterosexual (90.6%) (Table 1).

Among the 21 individual HPV genotypes, the sensitivity of the SPF_{10} PCR-DEIA-$LiPA_{25}$ method was higher than the Linear Array method except for HPV 70. Linear Array did not detect HPV types 18, 31, 33, 54, 56 and 68 whereas the SPF_{10} PCR-DEIA-$LiPA_{25}$ system was able to detect 4, 4, 1, 2, 8, and 1 cases with these HPV types, respectively. Linear Array detected 2 cases of HPV 70 while SPF_{10} PCR-DEIA-$LiPA_{25}$ was able to detect one case. HPV types 40, 42 and 45 were not detected by either method. Excluding types 40, 42, 45 and 70, the prevalence ratio comparing oral HPV prevalence of SPF_{10} PCR-DEIA-$LiPA_{25}$ to Linear Array in our study population varied between 1.0 and 9.0, including 3.8 for HPV 16. Among the grouped HPV categories, the prevalence ratio varied between 3.8 and 4.8 (Table 2). Overall, the prevalence of oral HPV for types 6, 11, 16, and 18, grouped 4 and 9 HPV vaccine genotypes, and any HPV genotype was higher, often substantially, for the SPF_{10} PCR-DEIA-$LiPA_{25}$ system compared to the Linear Array method (Fig. 1). For grouped 4 HPV vaccine types, 9 HPV vaccine types, and all HPV genotypes, the prevalence was 3.2%, 4.2% and 8.7% respectively by the SPF_{10} PCR-DEIA-$LiPA_{25}$ system compared to Linear Array's 0.8%, 1.0%, and 4.3% respectively. Oral HPV prevalence was 3.9 times, 4.4 times and 2.0 times higher for grouped 4 HPV vaccine types, 9 HPV vaccine types, and all HPV genotypes respectively when the SPF_{10} PCR-DEIA-$LiPA_{25}$ system was used compared to the Linear Array method.

In Table 3, we assessed the number of positive cases detected by SPF_{10} PCR-DEIA-$LiPA_{25}$ system but left out by the Linear Array method and vice versa for individual and grouped HPV genotypes. Overall, detection by the SPF_{10} PCR-DEIA-$LiPA_{25}$ system and missed by the Linear Array method occurred much more frequently than vice versa, with the exception of HPV 70. For example, for HPV 16, 22 cases were picked up by the SPF_{10} PCR-DEIA-$LiPA_{25}$ system but not Linear Array, whereas 3 cases were picked up by Linear Array but not the SPF_{10} PCR-DEIA-$LiPA_{25}$ system. Similarly, for grouped any high risk HPV, the SPF_{10} PCR-DEIA-$LiPA_{25}$ system detected 67 cases that Linear Array did not, whereas the Linear Array detected 7 cases that the SPF_{10} PCR-DEIA-$LiPA_{25}$ system did not. While the traditional Cohen's Kappa method found moderate to substantial agreement for only HPV types 11, 58, 59 and 70, the PABAK method found substantial agreement (more than 80%) for all individual and grouped HPV types. The results from the two tests were significantly different for HPV genotypes 6, 16, 51, 53, 56, 66 and all categories of grouped HPV genotypes; for all of these individual and grouped categories, oral HPV prevalence by the SPF_{10} PCR-DEIA-$LiPA_{25}$ system was substantially higher than that of the Linear Array method.

We examined factors that may be associated with concordance of oral HPV 16 detection between the two HPV genotyping assays (Table 4). We did not include race and ethnicity in our adjusted model because of the high degree of collinearity between country of residence and the covariates race and ethnicity: more than 91% and 99% of the Mexican participants reported race of 'Mexican' and ethnicity of 'Hispanic' respectively. None of the unadjusted or adjusted associations achieved statistical significance.

4. Discussion

The SPF_{10} PCR-DEIA-$LiPA_{25}$ system was substantially more sensitive than the Linear Array method for detecting HPV in oral gargle samples; detection of HPV 16, 4 HPV vaccine types, 9 HPV vaccine types, and any HPV genotype was 4 times higher with the SPF_{10} PCR-DEIA-$LiPA_{25}$ system compared to the Linear Array method. This difference is much higher than previous, FFPE tumor tissue based studies [11], where the increase in detection was not more than 2 fold. Any discordance found between the results from the two methods were not systematic i.e. they were not associated with the sociodemographic or behavioral risk factors for oral HPV infection.

The limit of detection for HPV 16, HPV 18, HPV 31, and HPV 45 has been reported to be 6, 41, 96, and 49 HPV DNA copies/PCR reaction respectively for the Linear Array assay [23] and 2, 1, 3, and 9 HPV DNA copies/PCR reaction respectively for the SPF_{10} PCR-DEIA-$LiPA_{25}$ system (personal communication with Dr. Maurits de Koning of DDL Diagnostic Laboratory). The large discrepancy of level of detection between the Linear Array and SPF_{10} PCR-DEIA-$LiPA_{25}$ systems can likely be

explained by the fragment of DNA being amplified buy each assay (~450 bp vs 65 bp, respectively), therefore making the SPF_{10} assay more robust in processes that degrade DNA. In our study, although the SPF_{10} PCR-DEIA-$LiPA_{25}$ system was in general more sensitive than the Linear Array method, the Linear Array method was able to detect a few cases which were not picked up by the SPF_{10} PCR-DEIA-$LiPA_{25}$ system. One potential explanation may be the extremely low HPV DNA load in oral gargle samples leading to variation in HPV DNA composition when different oral gargle aliquots are used, though they have been aliquoted from the same original sample. A similar phenomenon has been observed in a study comparing carcinogenic HPV detection by the SPF_{10} PCR-DEIA-$LiPA_{25}$ system and Hybrid Capture 2 assay in cervical specimens from 5683 women enrolled in the Costa Rican HPV 16/18 Vaccine Trial [24]. In that study, HC2 positive but SPF_{10} PCR-DEIA-$LiPA_{25}$ system negative samples had lower HPV viral load, and the authors hypothesized that sampling error due to low HPV viral load was a potential reason.

The Cohen's Kappa test showed moderate to substantial agreement for only a few HPV genotypes. The finding is different in this study of oral HPV from a previous study [14] where the kappa value was 0.82 when these two tests were compared for any HPV genotyping of cervical specimens. This may be due to difference in HPV prevalence between the two studies. The low Kappa statistic estimates in our study may be due to low HPV viral load in oral gargle specimens compared to cervical specimens. Given that more than 90% (and often more than 99%, depending on HPV type) of the oral specimens are negative for oral HPV, Cohen's Kappa may not be an ideal method for measuring inter-test agreement [25]. Hence, though we presented Kappa test results in this study to increase comparability of results to previous studies, we believe that these statistics should not be considered as an absolute indicator of inter-test agreement between the SPF_{10} PCR-DEIA-$LiPA_{25}$ system and the Linear Array method in oral gargle samples, and other methods such as prevalence adjusted, bias adjusted Kappa estimate (PABAK) may provide better estimates of agreement between the two methods. In our study, good concordance was shown with PABAK despite the poor concordance shown with Kappa test.

None of the unadjusted and adjusted associations between sociodemographic - behavioral risk factors and discordance in HPV 16 results reached statistical significance at $\alpha = 0.05$. These tests were conducted to examine whether discordance in oral HPV genotyping was random or systematic in nature. The discordance in oral HPV genotyping appears to be unbiased by sociodemographic and behavioral risk factors in our data.

Overall, the SPF_{10} PCR-DEIA-$LiPA_{25}$ system had substantially higher sensitivity than the Linear Array method for genotyping HPV in oral gargle samples. Like all studies, this study has a few limitations that should be noted. One of the limitations is the temporal distance between the two methods for testing the oral gargle samples. Since the SPF_{10} PCR-DEIA-$LiPA_{25}$ system was used almost a decade after the Linear Array method, there is a possibility of degradation of DNA within the oral samples with storage. However, it has to be noted that degradation would have led to a lower detection, not higher oral HPV detection as observed in this study. The strengths of this study include use of a large number of oral gargle samples originating from a pool of diverse participants residing in three different countries and belonging to a wide range of age groups. It should also be noted that the assays utilized in this study are assays suitable for research, such as studies of oral HPV natural history, vaccine trials with oral HPV as the endpoint, and vaccine effectiveness, but may not be suitable in clinical practice. At the time of this writing, there is no HPV DNA test that we are aware of that has been validated for clinical evaluation of oral HPV.

In summary, these data indicate the need to utilize highly sensitive HPV genotyping assays with smaller primer sizes for oral gargle specimens to assess oral HPV prevalence. Given the difficulty of localizing asymptomatic oral HPV infections, sampling methods such as oral gargle are used to estimate oral HPV infection but tend to be diluted in comparison with methods such as direct sampling of genital and anal epithelia. Therefore, there is a higher chance of underestimation of oral HPV incidence and persistence compared to that of anogenital and cervical mucosa when methods such as Linear Array, or assays with similar sensitivity, are used. In the light of increasing importance of detection of oral HPV, we recommend using methods with higher sensitivity and smaller primer sizes such as the SPF_{10} PCR-DEIA-$LiPA_{25}$ system, a test that has already been widely used in HPV vaccine trials.

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CRediT authorship contribution statement

Deepti Bettampadi: Conceptualization, Methodology, Formal analysis, Writing - original draft. **Bradley A. Sirak:** Methodology, Investigation, Writing - review & editing. **William J. Fulp:** Methodology, Writing - review & editing. **Martha Abrahamsen:** Investigation, Writing - review & editing, Project administration. **Luisa L. Villa:** Investigation, Writing - review & editing. **Eduardo Lazcano-Ponce:** Investigation, Writing - review & editing. **Jorge Salmeron:** Investigation, Writing - review & editing. **Kimberly A. Isaacs-Soriano:** Investigation, Writing - review & editing. **Maria L. Baggio:** Investigation, Writing - review & editing. **Manuel Quiterio Trenado:** Investigation, Writing - review & editing. **Anna R. Giuliano:** Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Anna R Giuliano is a member of the Merck Advisory Board and her institution has received funding for research through a Merck Investigator-initiated studies program.

Luisa L Villa is a consultant to Merck for the HPV prophylactic vaccine and provides occasional consultancy to BD, Roche and Qiagen concerning HPV tests.

Appendix A. Supplementary data

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References

[1] A.K. Chaturvedi, E.A. Engels, R.M. Pfeiffer, B.Y. Hernandez, W. Xiao, E. Kim, B. Jiang, M.T. Goodman, M. Sibug-Saber, W. Cozen, Human papillomavirus and rising oropharyngeal cancer incidence in the United States, J. Clin. Oncol. 29 (2011) 4294.

[2] A. Näsman, P. Attner, L. Hammarstedt, J. Du, M. Eriksson, G. Giraud, S. Ährlund-Richter, L. Marklund, M. Romanitan, D. Lindquist, Incidence of human papillomavirus (HPV) positive tonsillar carcinoma in Stockholm, Sweden: an epidemic of viral-induced carcinoma? Int. J. Canc. 125 (2009) 362–366.

[3] A.R. Kreimer, A. Villa, A.G. Nyitray, M. Abrahamsen, M. Papenfuss, D. Smith, A. Hildesheim, L.L. Villa, E. Lazcano-Ponce, A.R. Giuliano, The epidemiology of oral HPV infection among a multinational sample of healthy men, Canc. Epidemiol. Prevent. Biomarkers 20 (2011) 172–182.

[4] M.L. Gillison, T. Broutian, R.K. Pickard, Z-y Tong, W. Xiao, L. Kahle, B.I. Graubard, A.K. Chaturvedi, Prevalence of oral HPV infection in the United States, 2009-2010, Jama 307 (2012) 693–703.

[5] R.K. Pickard, W. Xiao, T.R. Broutian, X. He, M.L. Gillison, The prevalence and incidence of oral human papillomavirus infection among young men and women, aged 18–30 years, Sex. Transm. Dis. 39 (2012) 559–566.

[6] D.C. Beachler, K.M. Weber, J.B. Margolick, H.D. Strickler, R.D. Cranston, R.D. Burk, D.J. Wiley, H. Minkoff, S. Reddy, E.E. Stammer, Risk factors for oral HPV infection among a high prevalence population of HIV-positive and at-risk HIV-negative

adults, Canc. Epidemiol. Prevent. Biomarkers 21 (2012) 122–133.
[7] M. Poljak, B.J. Kocjan, Commercially available assays for multiplex detection of alpha human papillomaviruses, Expert Rev. Anti Infect. Ther. 8 (2010) 1139–1162.
[8] D. van Hamont, M.A. van Ham, J.M. Bakkers, L.F. Massuger, W.J. Melchers, Evaluation of the SPF10-INNO LiPA human papillomavirus (HPV) genotyping test and the roche linear array HPV genotyping test, J. Clin. Microbiol. 44 (2006) 3122–3129.
[9] F. Galan-Sanchez, M.A. Rodriguez-Iglesias, Comparison of human papillomavirus genotyping using commercial assays based on PCR and reverse hybridization methods, Apmis 117 (2009) 708–715.
[10] S.E. Tan, S.M. Garland, A.R. Rumbold, S.N. Tabrizi, Human papillomavirus genotyping using archival vulval dysplastic or neoplastic biopsy tissues: comparison between the INNO-LiPA and linear array assays, J. Clin. Microbiol. 48 (2010) 1458–1460.
[11] R. Shanesmith, R.A. Allen, W.E. Moore, D.W. Kingma, S.K. Caughron, E.M. Gillies, S.T. Dunn, Comparison of 2 line blot assays for defining HPV genotypes in oral and oropharyngeal squamous cell carcinomas, Diagn. Microbiol. Infect. Dis. 70 (2011) 240–245.
[12] B.J. Kocjan, L. Hošnjak, M. Poljak, Detection of alpha human papillomaviruses in archival formalin-fixed, paraffin-embedded (FFPE) tissue specimens, J. Clin. Virol. 76 (2016) S88–S97.
[13] F.A. Castro, J. Koshiol, W. Quint, C.M. Wheeler, M.L. Gillison, L.M. Vaughan, B. Kleter, L.-J. van Doorn, A.K. Chaturvedi, A. Hildesheim, Detection of HPV DNA in paraffin-embedded cervical samples: a comparison of four genotyping methods, BMC Infect. Dis. 15 (2015) 544.
[14] P.E. Castle, C. Porras, W.G. Quint, A.C. Rodriguez, M. Schiffman, P.E. Gravitt, P. Gonzalez, H.A. Katki, S. Silva, E. Freer, et al., Comparison of two PCR-based human papillomavirus genotyping methods, J. Clin. Microbiol. 46 (2008) 3437–3445.
[15] S.J. Klug, A. Molijn, B. Schopp, B. Holz, A. Iftner, W. Quint, P. Jf Snijders, K.U. Petry, S.K. Kjær, C. Munk, Comparison of the performance of different HPV genotyping methods for detecting genital HPV types, J. Med. Virol. 80 (2008) 1264–1274.
[16] G. Lawton, S. Thomas, J. Schonrock, F. Monsour, I. Frazer, Human papillomaviruses in normal oral mucosa: a comparison of methods for sample collection, J. Oral Pathol. Med. 21 (1992) 265–269.
[17] A.R. Kreimer, A. Villa, A.G. Nyitray, M. Abrahamsen, M. Papenfuss, D. Smith, A. Hildesheim, L.L. Villa, E. Lazcano-Ponce, A.R. Giuliano, The epidemiology of oral HPV infection among a multinational sample of healthy men, Cancer Epidemiol. Biomarkers Prev. 20 (2011) 172–182.
[18] A.R. Giuliano, E. Lazcano-Ponce, L.L. Villa, R. Flores, J. Salmeron, J.H. Lee, M.R. Papenfuss, M. Abrahamsen, E. Jolles, C.M. Nielson, et al., The human papillomavirus infection in men study: human papillomavirus prevalence and type distribution among men residing in Brazil, Mexico, and the United States, Cancer Epidemiol. Biomarkers Prev. 17 (2008) 2036–2043.
[19] B. Kleter, L.J. van Doorn, J. ter Schegget, L. Schrauwen, K. van Krimpen, M. Burger, B. ter Harmsel, W. Quint, Novel short-fragment PCR assay for highly sensitive broad-spectrum detection of anogenital human papillomaviruses, Am. J. Pathol. 153 (1998) 1731–1739.
[20] T. Byrt, J. Bishop, J.B. Carlin, Bias, prevalence and kappa, J. Clin. Epidemiol. 46 (1993) 423–429.
[21] P. Watson, A. Petrie, Method agreement analysis: a review of correct methodology, Theriogenology 73 (2010) 1167–1179.
[22] A. Gelman, Y.-S. Su, M. Yajima, J. Hill, M.G. Pittau, J. Kerman, T. Zheng, V. Dorie, M.Y.-S. Su, Package 'arm', (2018).
[23] LINEAR ARRAY HPV Genotyping Test. Alameda, CA: Roche Diagnostics.
[24] M. Safaeian, R. Herrero, A. Hildesheim, W. Quint, E. Freer, L.-J. Van Doorn, C. Porras, S. Silva, P. González, M.C. Bratti, Comparison of the SPF10-LiPA system to the Hybrid Capture 2 Assay for detection of carcinogenic human papillomavirus genotypes among 5,683 young women in Guanacaste, Costa Rica, J. Clin. Microbiol. 45 (2007) 1447–1454.
[25] K.L. Gwet, Computing inter-rater reliability and its variance in the presence of high agreement, Br. J. Math. Stat. Psychol. 61 (2008) 29–48.