Heliyon 10 (2024) e32336

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Anal human papillomavirus (HPV) disagreement by Linear Array compared to SPF10 PCR-DEIA-LiPA25 system in young sexual minority men

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ARTICLE INFO

Keywords:
Human papillomavirus
Discordance
anal cancer
Sexual minority men
Screening test

ABSTRACT

Introduction: Young sexual minority men (SMM) bear the greatest burden of anal human papillomavirus (HPV) infections. We assessed anal HPV genotype discordance between the Linear Array (LA) and SPF10 PCR-DEIA-LiPA25 (LiPA25).
Methods: Discordance was assessed between LA and LiPA25 using self-collected anal swabs from 120 SMM aged 18–29 who were recruited in 2014–2016. Multiple-type infection was explored as a potential confounder of testing agreement, along with clinical and behavioral factors such as HIV status, syphilis status, incarceration history, health insurance coverage, having 3 or more sex partners in the past 6 months, and co-infection with HPV-16.
Results: Significant discordance was found for HPV-6, -11, -16, -31, -42, -54, and -59. Exploratory analyses suggest higher prevalence of genotype discordance in those living with HIV, those with 3 or more sex partners, and those who were positive for 4 or more HPV types.
Conclusions: Our results highlight the importance of HPV detection methods which may inform different interpretations of research assessing anal HPV natural history among SMM at highest risk for HPV.

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https://doi.org/10.1016/j.heliyon.2024.e32336
Received 14 August 2023; Received in revised form 30 May 2024; Accepted 2 June 2024
Available online 3 June 2024

1. Introduction

Though anal cancer is rare, the incidence has continued to increase over the past two decades with recent data suggesting a 1.1 % annual increase in anal cancer incidence among males from 2000 to 2019 [1,2]. Further, a disproportionate increase has been observed in non-Hispanic White men and non-Hispanic Black men with annual anal cancer incidence increases of 2.2 % and 1.7 %, respectively [1]. Additionally, sexual minority men (SMM) bear 32-fold higher risk of anal cancer when compared to the general U.S. population, with a 52-fold higher risk in SMM living with human immunodeficiency virus (HIV) [3,4]. Infection with human papillomavirus (HPV), specifically oncogenic HPV types 16 and 18, is the main driver of increasing anal carcinomas as more than 80 % of anal cancers have been associated with HPV infection [5].

Anal carcinomas are largely preventable, and vaccination against HPV will help in reducing risk [6]. Further, the development of effective screening methods is important in the reduction of anal cancer risk, specifically in SMM. Recent studies have examined anal precancer screening to assess the prevalence of abnormal cytology and anal HPV infection [7], yet there is still no proven biomarker for anal precancers. As oncogenic potential is largely associated with HPV type, standardized HPV genotyping is needed to adequately assess the natural history and epidemiology of each type. Different assays are available for HPV genotyping, with many studies assessing their performance utilizing cervical specimens [8–13] but fewer have assessed performance with anal samples in the most vulnerable population.

Additionally, it is uncertain whether testing accuracy is influenced by concurrent HPV infections, however studies have suggested that multiple-type infections may lead to false-negative genotyping results in some assays due to competition between types during amplification, specifically in people living with HIV [14–17]. Multiple-type HPV infections are common among anal cancer patients [18,19], thus it is imperative that specific assays perform accurately in SMM with multiple-type infections. Individual-level factors that may be associated with higher risk of multiple-genotype infections should be assessed for their influence, if any, on assay performance. Specifically, incarceration history and risk behaviors such as having multiple sex partners may increase the risk of exposure to multiple HPV genotypes. In this case, certain social determinants of health (SDOH) could lead to multiple HPV infections, which may influence assay results.

The Linear Array (LA) HPV genotyping assay (Roche Molecular Diagnostics, Alameda, CA) was considered the gold standard, often being utilized for HPV genotyping in cervical samples, however production of the LA was discontinued in 2019. Alternative assay performance, specifically using anal specimens, has been compared to LA over the years [14,20–26], yet no gold standard exists for anal HPV genotyping. One current alternative to the LA is the SPF10 PCR-DEIA-LiPA25 (LiPA25) system (DDL Diagnostic Laboratory, Rijswijk, The Netherlands). LiPA25 has been shown to have high analytical sensitivity, especially in cervical specimens [27] and oral

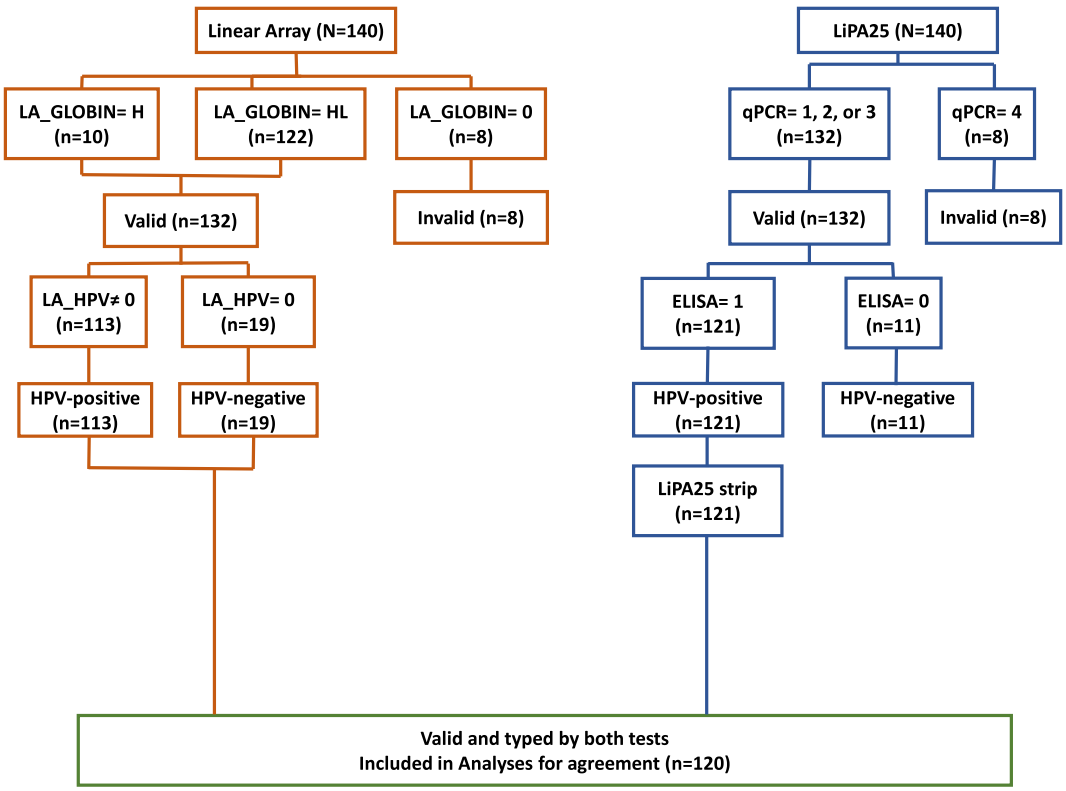


Fig. 1. Flow of inclusion for samples (n = 120).

gargle samples [28], yet the results attained by LiPA25 have not yet been compared to LA using anal specimens. This study aims to assess the percent discordance of anal HPV detected by LA compared to LiPA25 in a sample of young SMM, who tend to bear a high burden of anal HPV infections and exhibit a high prevalence of high-risk genotypes. Additionally, we explore the association between individual-level clinical, behavioral, and SDOH factors and multiple-type infections that may confound the ability to accurately detect distinct HPV types, especially those involving the highest oncogenic potential.

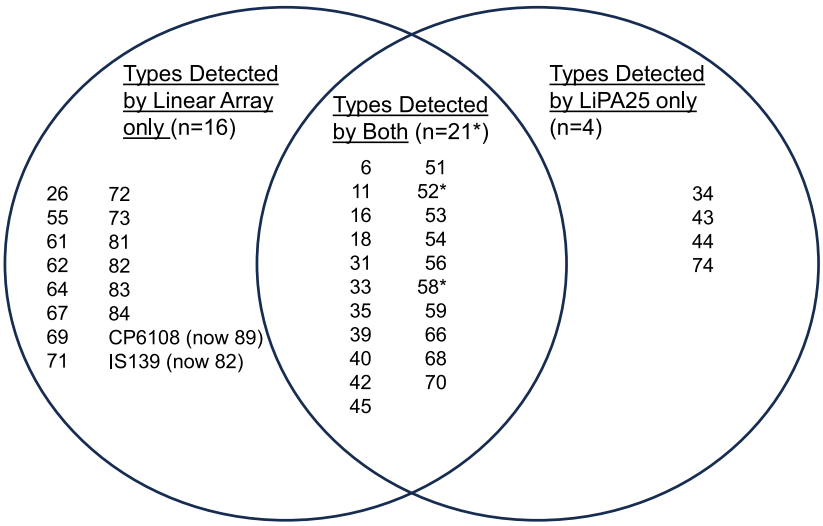
2. Material and methods

2.1. Study sample

We analyzed data from 140 young SMM aged 18–29 years who were a part of the larger Young Men's Affiliation Project (YMAP) wave-1 of data collection in Houston, TX [29]. The details of this study have been reported elsewhere [30]. In short, young SMM aged 18–29 years who were male-identifying and assigned male at birth, and had sexual contact (oral or anal) with another male in the past 12 months were recruited during 2014–2016 in Houston, TX, using respondent driven sampling methods [29]. The participants completed an interviewer-administered behavioral survey, as well as had blood samples taken for HIV and syphilis testing. Additionally, anal specimens were self-collected using a polyethylene terephthalate swab. The swab was inserted into the anal canal and rotated as it was removed. The swab was then stored in a tube and frozen within 6 h of collection [30]. The YMAP study protocol was reviewed and approved by the Committee for the Protection of Human Subjects at the University of Texas Health Science Center at Houston (approval number HSC-SPH-12-0830).

2.2. HPV genotyping

Anal specimens were sent to the Moffitt Cancer Center where HPV genotyping analysis was conducted. Though our original data had 140 samples, some samples were determined invalid for genotyping. Specifically, 2 samples were considered invalid for LA only, 2 samples were considered invalid for LiPA25 only, and 6 samples were considered invalid for both LA and LiPA25. An additional 10 positive samples were not typable for any of the 25 specific genotypes detectable by LiPA25 (included in Table 4). The present analysis focuses on 120 samples that were valid for both LA (conducted in 2016) and LiPA25 (conducted in 2019) genotyping methods. The flow of sample inclusion is available in Fig. 1. Notably, the samples were freshly isolated for each testing assay. Deoxyribonucleic acid (DNA) was extracted from anal canal cell pellets using the automated BioRobot MDx (Qiagen, Inc.) following the manufacturer's instructions. The same DNA extraction was used for both the LA and LiPA25 system. The LA assay requires DNA concentration quantification prior to loading the polymerase chain reaction (PCR), and sample concentration was determined via Nanodrop. Samples with DNA concentrations above the targeted 1 ng/μL were diluted accordingly. PCR was performed and carried out using the manufacturer protocol via thermal cycler. Once completed, the LA assay was performed per manufacturer protocol using 50 μL of PCR product. Results were obtained by comparing each strip to the included Roches' LA HPV genotyping card. Gel electrophoresis was performed to confirm sample results. The LA assay tests for 37 HPV types [6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, CP6108 (now 80), IS139 (now 82)]. Notably, if the LA assay was positive for both types 52 and 58, the 52 result was grouped into type 58, thus we do not present agreement data for these genotypes.



*Of note, n=19 types were included in direct comparison analyses due to the grouping of types 52 and 58 on Linear Array.

Fig. 2. Flow of inclusion and exclusion for direct HPV-typing comparison (n = 19).

The LiPA25 system is a three step process that includes: (a) qPCR that determines sample adequacy; (b) a DNA enzyme immunoassay (DEIA) or enzyme linked immunosorbent assay (ELISA) method that detects 65 HPV types; and (c) a LiPA25 genotyping multiplex PCR that selectively identifies the following 25 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) [28]. The ELISA was performed according to the manufacturer's protocol using 10 μL of PCR and was used to determine HPV positivity, though non-specifically. Samples were concluded positive for HPV when the ELISA optical density value for the sample was greater than or equal to the borderline control optical density value. All positive samples were then genotyped using an automated system with the LiPA25 assay. After processing, each strip was visually inspected and analyzed using the reverse hybridization assay (RHA) HPV LiPA25 interpretation chart included and specific HPV genotypes were determined. All types that were defined as high-risk were analyzed by both assays (type 16, 18, 31, 33, 35, 39, 45, 51, 56, 59, 68). However, not all types defined as low-risk were analyzed by both methods. We defined a total of 24 low-risk HPV types and 8 of the 24 were analyzed by both LA and LiPA25 (type 6, 11, 40, 42, 53, 54, 66, 70). Notably, HPV-55 was reclassified as a subtype of HPV-44 [31] , thus we did not include a direct comparison for type 44 in this study. The flow of HPV-type inclusion for direct comparison (n = 19) is available in Fig. 2.

2.3. Measures

Along with HPV genotype test agreement, we explored seven participant characteristics to assess potential patterns of discordance between LA and LiPA25. These factors included HIV status (seronegative, seropositive), syphilis status (due to common co-infection with HIV among this population [32,33] and high prevalence with HPV 16/18 positivity [34]; seronegative, seropositive), ever being detained/arrested/jailed (yes, no), health insurance coverage (yes, no), having 3 or more sex partners in the 6 months prior to the interview (yes, no), being co-infected with HPV-16 (yes, no), and being infected with 4 or more types of HPV. HIV status was determined by the fourth generation Alere rapid test and confirmed by either multispot or viral load quantitative testing, while syphilis status was determined using fluorescent treponemal antibody (FTA) test (Immunofluorescence Assay FTA-Absorption Test System, Zeus Scientific, New Jersey, USA).

2.4. Statistical analysis

Data analyses were conducted using R version 4.2.1. We employed McNemar's exact test to assess the discordance between LA and LiPA25 for each HPV type that was available. In examining the HPV genotype by test agreement, both the exact- and mid-*p* values are presented. The mid-*p* [35] was ultimately used to determine statistical significance at an alpha level of 0.05. We did not report the Cohen's Kappa statistic as high agreement was expected between the LA and LiPA25; however, the estimated Cohen's Kappa values ranged from 0.00 to 1.00 (median: 0.714) for the nineteen HPV types included in direct comparison (data not shown in tables). In additional exploratory analyses, we presented the frequency and percentage of each participant characteristic due to the limited number of samples with discordant HPV results.

This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.

Table 1
Linear Assay (LA) vs. Line Probe Assay (LiPA25).

HPV genotype	(LA, LiPA25)					
	(-, -)	(+, +)	(-, +)	(+, -)	*Exact-P*	*Mid-P*
High risk types						
16	95	17	1	7	0.0703	0.0391
18	102	14	2	2	1.0000	0.8125
31	99	10	9	2	0.0654	0.0386
33	113	7	0	0	–	–
35	101	16	2	1	1.0000	0.6250
39	103	6	3	8	0.2266	0.1460
45	102	9	3	6	0.5078	0.3438
51	97	18	2	3	1.0000	0.6875
56	108	4	5	3	0.7266	0.5078
59	95	3	0	22	<0.0001	<0.0001
68	95	10	4	11	0.1185	0.0768
Low risk types						
6	90	21	8	1	0.0391	0.0215
11	103	11	6	0	0.0313	0.0156
40	113	4	1	2	1.0000	0.6250
42	107	0	0	13	0.0002	0.0001
53	92	14	6	8	0.7905	0.6072
54	113	0	0	7	0.0156	0.0078
66	99	18	1	2	1.0000	0.6250
70	113	5	1	1	1.0000	0.7500

Abbreviations: HPV, human papillomavirus; LA, Linear Array; LiPA25, Line Probe Assay.

3. Results

The median age for the 120 participants included in the direct comparison is 25.09 years (*IQR* = 22.79–27.39). Table 1 shows the discordance of anal HPV results in LA compared to LiPA25. Among high-risk HPV types, statistically significant discordant results were found for HPV-16 ($p = 0.04$) where in 7 discordant pairs LA was positive while LiPA25 remained negative; HPV-31 ($p = 0.04$) where in 2 discordant pairs LA was positive while LiPA25 remained negative; and HPV-59 ($p < 0.0001$) where in 22 discordant pairs LA was positive while LiPA25 remained negative. Among low-risk types, significant discordant results were found for HPV-6 ($p = 0.02$) where in 8 discordant pairs LA was negative while LiPA25 was positive; HPV-11 ($p = 0.02$) where in 6 discordant pairs LA was negative while LiPA25 was positive; HPV-42 ($p = 0.0001$) where in 13 discordant pairs LA was positive while LiPA25 remained negative; and −54 ($p = 0.008$) where in 7 discordant pairs LA was positive while LiPA25 remained negative.

Table 3 shows the prevalence of selected characteristics by discordant HPV genotyping results. Our overall study sample's HIV prevalence was 55 % (Table 2), whereas among discordant samples, the prevalence appears to be higher than 60 % for most HPV types, with the exceptions of HPV-31, -11, -40, -54, and -70 (Table 3), with 100 % of discordant samples for HPV-16 being HIV-positive ($p = 0.008$). Our overall study sample's syphilis prevalence was 41 % (Table 2), and we did not see any clear pattern for discordant test results and syphilis status. The prevalence among individuals with discordance results ranges from 0 % to 67 % (Table 3). Roughly 56 % of our study sample had been detained, arrested, or jailed at least once in their lifetime (Table 2). Among the individuals with discordant results, there does not appear to be any pattern with ever being jailed, as the ever-jailed prevalence of discordance ranges from 20 % to 88 % (Table 3). Approximately 46 % of our study sample had health insurance coverage (Table 2), however again, the estimated prevalence of health insurance coverage among individuals with discordant results does not show any indication of pattern. Among the samples with discordant test results, health insurance coverage ranges from 0 % to 100 % (Table 3). Roughly 53 % of our overall study sample had 3 or more sex partners in the past 6 months (Table 2), whereas among discordant samples, the prevalence appears to be 50 % or higher for most HPV types, with the exceptions of HPV-16, -35, and -68 (Table 3), with 100 % of discordant samples for HPV-56 having 3 or more sex partners in the past 6 months ($p = 0.014$). Roughly 34 % of our study sample was infected with 4 or more HPV types (Table 2), and among discordant samples, the prevalence is more than 60 % for all HPV types except for two (HPV-6, -53, and -70, with more than 70 % of discordant samples for HPV-16 ($p = 0.025$), HPV-39 ($p = 0.022$), and HPV-59 ($p = 0.005$) having 4 or more HPV infections (Table 3). Lastly, we did not observe any apparent pattern of HPV-16 co-infection with discordance test results; however, 67 % of discordant samples for HPV-35 ($p = 0.015$) and HPV-40 ($p = 0.001$) had HPV-16 co-infection (Table 3).

Table 4 shows the HPV results and typing for the 10 HPV-positive samples that were not typable for any of the 25 specific genotypes detectable by LiPA25. For these 10 samples, all were positive for HPV DNA on LiPA25 but did not yield typing results, while only 6 of these samples were positive for HPV DNA and typed on LA.

4. Discussion

The results of our study indicate statistically significant discordance in anal HPV genotyping between the LA assay and the LiPA25 system. Though there have been no standardized screening methods for anal HPV prevention, the LA was often utilized as the gold standard for HPV genotyping specifically in cervical samples prior to its discontinuation. Genotyping agreement is needed among current assays to ensure the accurate detection of unique HPV genotypes, especially in anal HPV specimens which tend to exhibit multiplicity and diversity in HPV types [36]. The accurate detection of prevalent anal HPV types and those with the highest invasive potential can be utilized to inform vaccine effectiveness, specifically in SMM where adequate vaccine coverage may be the best form of prevention against the development and recurrence of anal lesions [37,38].

We previously observed that approximately three-fourths of the sample population used in the present study was positive for infection with at least one type of high-risk HPV [30]. With almost all anal squamous cell carcinomas being positive for HPV [19], it is of great importance to understand the factors associated with persistent HPV infection which can lead to the development of anal cancers.

Our findings also suggest a pattern between HIV serostatus and genotyping discordance. Specifically, among discordant pairs, there was generally a higher prevalence of HIV seropositivity compared to those with genotype agreement. The reason for this is unknown, however findings from a prior study in people living with HIV suggested that PCR-based genotyping systems may have increased likelihood of discordant results in samples with multiple-type infections [14]. Specifically, the researchers found lower genotype agreement among anal samples and those samples from participants living with HIV [14]. Additionally, our findings show that among

Table 2
Prevalence of selected characteristics among the overall study sample (N = 120).

Variable	% Prevalence
HIV positive	54.6 (65/119)
Syphilis positive	40.9 (47/115)
Ever jailed	55.5 (66/119)
Have health insurance	46.2 (55/119)
Have 3+ sex partners in the past 6 months	52.9 (63/119)
Infected with 4+ types of HPV	34.2 (41/120)

Abbreviations: HIV, human immunodeficiency virus; HPV, human papillomavirus.

Table 3
Prevalence of selected characteristics among individuals who have discordant HPV genotype results.

HPV genotype	Number of discordant samples	Prevalence in %						
		HIV positive % (n/N)	Syphilis positive % (n/N)	Ever jailed % (n/N)	Have health insurance % (n/N)	Had 3+ sex partners in the past 6 months % (n/N)	Co-infected with HPV-16 % (n/N)	Infected with 4+ types of HPV % (n/N)
High risk types								
16	8	**100.0 (8/8) P=0.008**	42.9 (3/7) P = 1.000	87.5 (7/8) P = 0.074	12.5 (1/8) P = 0.067	25.0 (2/8) P = 0.146	–	**87.5 (7/8) P=0.025**
18	4	75.0 (3/4) P = 0.625	0.0 (0/4) P = 0.144	50.0 (2/4) P = 1.000	50.0 (2/4) P = 1.000	75.0 (3/4) P = 0.621	25.0 (1/4) P = 0.216	75.0 (3/4) P = 0.345
31	11	45.5 (5/11) P = 0.543	18.2 (2/11) P = 0.195	54.5 (6/11) P = 1.000	45.5 (5/11) P = 1.000	54.5 (6/11) P = 1.000	9.1 (1/11) P = 0.092	63.6 (7/11) P = 0.342
35	3	66.7 (2/3) P = 1.000	66.7 (2/3) P = 0.566	66.7 (2/3) P = 1.000	66.7 (2/3) P = 0.595	33.3 (1/3) P = 0.601	**66.7 (2/3) P=0.015**	66.7 (2/3) P = 0.609
39	11	63.6 (7/11) P = 0.752	36.4 (4/11) P = 1.000	45.5 (5/11) P = 0.536	63.6 (7/11) P = 0.342	54.5 (6/11) P = 1.000	**36.4 (4/11) P<0.001**	**81.8 (9/11) P=0.022**
45	9	87.5 (7/8) P = 0.070	50.0 (4/8) P = 0.714	75.0 (6/8) P = 0.296	37.5 (3/8) P = 0.724	62.5 (5/8) P = 0.721	**22.2 (2/9) P=0.028**	66.7 (6/9) P = 0.301
51	5	60.0 (3/5) P = 1.000	40.0 (2/5) P = 1.000	20.0 (1/5) P = 0.170	40.0 (2/5) P = 1.000	80.0 (4/5) P = 0.369	0.0 (0/5) P = 1.000	20.0 (1/5) P = 0.373
56	8	85.7 (6/7) P = 0.125	28.6 (2/7) P = 0.699	28.6 (2/7) P = 0.240	57.1 (4/7) P = 0.702	**100.0 (7/7) P=0.014**	12.5 (1/8) P = 0.129	62.5 (5/8) P = 0.475
59	22	72.7 (16/22) P = 0.095	40.0 (8/20) P = 1.000	54.5 (12/22) P = 1.000	59.1 (13/22) P = 0.237	54.5 (12/22) P = 1.000	**31.8 (7/22) P<0.001**	**72.7 (16/22) P=0.005**
68	15	66.7 (10/15) P = 0.410	38.5 (5/13) P = 1.000	46.7 (7/15) P = 0.581	33.3 (5/15) P = 0.407	40.0 (6/15) P = 0.407	**33.3 (5/15) P<0.001**	73.3 (11/15) P = 0.056
Low risk types								
6	9	66.7 (6/9) P = 0.509	37.5 (3/8) P = 1.000	44.4 (4/9) P = 0.509	44.4 (4/9) P = 1.000	55.6 (5/9) P = 1.000	**33.3 (3/9) P=0.009**	55.6 (5/9) P = 0.506
11	6	50.0 (3/6) P = 1.000	50.0 (3/6) P = 0.687	66.7 (4/6) P = 0.691	16.7 (1/6) P = 0.215	50.0 (3/6) P = 1.000	**33.3 (2/6) P=0.020**	66.7 (4/6) P = 0.411
40	3	33.3 (1/3) P = 0.590	33.3 (1/3) P = 1.000	33.3 (1/3) P = 0.585	33.3 (1/3) P = 1.000	66.7 (2/3) P = 1.000	**66.7 (2/3) P=0.001**	100.0 (3/3) P = 0.116
42	13	61.5 (8/13) P = 0.770	50.0 (6/12) P = 0.545	61.5 (8/13) P = 0.771	46.2 (6/13) P = 1.000	61.5 (8/13) P = 0.568	**23.1 (3/13) P=0.001**	61.5 (8/13) P = 0.385
53	14	64.3 (9/14) P = 0.571	53.8 (7/13) P = 0.375	64.3 (9/14) P = 0.574	35.7 (5/14) P = 0.570	50.0 (7/14) P = 1.000	14.3 (2/14) P = 0.066	42.9 (6/14) P = 1.000
54	7	28.6 (2/7) P = 0.243	33.3 (2/6) P = 1.000	28.6 (2/7) P = 0.240	28.6 (2/7) P = 0.449	85.7 (6/7) P = 0.118	0.0 (0/7) P = 1.000	71.4 (5/7) P = 0.261
66	3	66.7 (2/3) P = 1.000	33.3 (1/3) P = 1.000	33.3 (1/3) P = 0.585	100.0 (3/3) P = 0.096	66.7 (2/3) P = 1.000	33.3 (1/3) P = 0.121	66.7 (2/3) P = 0.593
70	2	50.0 (1/2) P = 1.000	50.0 (1/2) P = 1.000	50.0 (1/2) P = 1.000	0.0 (0/2) P = 0.499	50.0 (1/2) P = 1.000	0.0 (0/2) P = 1.000	50.0 (1/2) P = 1.000

Abbreviations: HIV, human immunodeficiency virus; HPV, human papillomavirus.
Fisher's exact test was used. A p-value less than 0.05 indicates a significant difference between the prevalence among discordant samples compared to the prevalence among the agreement samples.

Table 4
Description of Samples not Typable for any of the 25 Specific Genotypes Detectable by LiPA25 (n = 10).

Sample	Linear Array		LiPA25	
	HPV result	HPV type	HPV result	HPV type
A	–	None	+	Untyped
B	–	None	+	Untyped
C	–	None	+	Untyped
D	+	26	+	Untyped
E	+	62, 70	+	Untyped
F	+	45	+	Untyped
G	+	58, 62	+	Untyped
H	+	61, 81, 83	+	Untyped
I	+	45, 81	+	Untyped
J	–	None	+	Untyped
Total (+)	6		10	
Total (−)	4		0	
Total	10		10	

Abbreviations: HPV, human papillomavirus; LiPA25, Line Probe Assay.

statistically significant different cases between discordant and concordant pairs, all eight cases of discordant pairs for high-risk HPV-16 (100 %) were HIV seropositive, and a majority (~75 %) of discordant pairs for high-risk type -18, -45, and -56. The rationale behind this finding is unknown, thus more research is needed to assess the effect of HIV serostatus, as well as multiple-type HPV infection, on assay accuracy.

Notably, we discovered higher discordance in those who were concurrently positive for 4 or more HPV genotypes and those with 3 or more recent sex partners, as most (>70 %) discordant pairs for HPV-16, -39, and -59 were infected with 4 or more distinct HPV types. This may indicate other potential confounders of discordant results perhaps due to limitation in testing methods. Those with a higher number of sex partners are likely at increased risk of concurrent HPV infection with different genotypes which may impact assay accuracy. Additionally, with high diversity in HPV types exhibited in anal HPV specimens, assays must have the ability to accurately detect distinct HPV types, especially those with the highest oncogenic potential. Another important factor and potential complication to consider is that there are different prevalence rates of HPV, and specifically high-risk HPV, based upon race [30]; thus, it would be important to know if there were emerging or other "high-risk" HPV-types that are less common than HPV-16 and -18, but that are within these populations. This could be crucial to understand as a factor related to race differences in HPV prevalence and pathogenesis [39].

Due to our study's limited number of discordant pairs and sample size, our ability to further assess these potential patterns were limited, however future studies with larger sample sizes should assess the association, if any, between HIV seropositivity and anal HPV genotyping, as well as the effect of concurrent HPV infection with multiple types and having a higher number of sex partners. Those living with HIV are at increased risk for anal carcinomas and anal HPV infection [40,41], as well as those with higher number of sex partners thus it is of great importance to understand how HIV seropositivity, sexual behavior, and concurrent HPV infection with 4 or more types may affect anal HPV genotyping in certain assays. Further, it is of particular importance that assays accurately estimate type-specific persistence being that persistence is important in natural history. Misclassifying one as having nonpersistent infection may change our understanding of the strength of the association between persistence and precancers. This could inform the use of certain assays for persons with specific characteristics to attain the most accurate results possible and provide the best information for vaccine effectiveness and future interventions, especially in SMM living with HIV, those with more diverse HPV infectivity, and those with increased sexual activity.

The findings from this paper highlight the importance of anal HPV detection methods and potential confounders of other behavioral and SDOH factors that could be related to multiple HPV genotypes and discordance, which may inform different interpretations of research findings that study anal HPV natural history and vaccine effectiveness among SMM. Future research is needed to develop adequate and standardized screening methods for anal HPV infection in SMM who bear the heaviest burden of HPV-related anal disease, as well as to consider potential behavioral and SDOH factors at risk for multiple HPV infections.

4.1. Limitations

The results of our study should be interpreted in the context of certain limitations. Our sample size of discordant pairs was limited; therefore, we could not perform complex statistical analysis to assess the effect of individual characteristics on HPV genotyping discordance. Additionally, the assays were not performed at the same time (LA was performed in August 2016, and LiPA25 was performed in November 2019), thus the data gathered was not explicitly controlled for an assay comparison. More research controlled for explicit assay comparison is needed, specifically with anal samples, to further investigate the findings from our study. In addition, our study sample was limited to young SMM aged 18–29 years, therefore our results may not be generalizable to older SMM. However, studies have shown that anal HPV infections do not peak with age unlike cervical HPV infections [42,43], therefore, participant age may not have any effect on the results presented. Further, being that our sample was mostly Black, more research is needed to assess these associations in non-Black SMM. Lastly, we do not have HPV vaccination status for this sample, however in our wave-2 data that was collected later, HPV vaccine uptake was estimated at 35 % among those who had heard of HPV [44]. We anticipate this to be similar among the present study population.

5. Conclusions

There is a great need for standardized anal HPV genotype screening methods, specifically among predominantly racial minority young SMM who bear a disproportionately high risk of anal carcinomas. Our study aimed to compare results obtained from the LiPA25 system and LA assay on anal samples collected from this population. The results from our study indicate significant discordance among certain high-risk and low-risk HPV genotype results from LiPA25 compared to LA. Further research is needed to assess the accuracy of LiPA25 on anal specimens, especially in light of the discontinued production of LA. Additionally, research involving larger sample sizes is needed to assess different factors that may affect test agreement in SMM, specifically in those living with HIV, those with multiple HPV infections, and those with increased sexual activity.

Declarations

Ethics statement: The YMAP study protocol was reviewed and approved by the Committee for the Protection of Human Subjects at the University of Texas Health Science Center at Houston (approval number HSC-SPH-12-0830) on February 8, 2013.

Funding

Dr. Amboree is supported by the National Institute on Minority Health and Health Disparities, United States (NIMHD) [grant number 3R01MD013715-04S1]. This research extends from a parent study that was supported by the National Institutes of Health, United States [Recipients: Fujimoto, Schneider – grant number NIH/NIMHD 1R01MH100021]. Additional funds came from the University of Texas Health-MD Anderson Population Health Initiative Collaborative Project Award [Recipients: Fujimoto, Chiao]; Center for Health Promotion and Prevention Research, and Department of Health Promotion & Behavioral Sciences, UTHealth Houston, United States, and the Sally W. Vernon, Ph.D., Distinguished Professorship in Social Determinants of Health [Recipient: Fujimoto]. The content of this publication is solely the responsibility of the authors and does not necessarily represent the official views of the NIH, NIMHD, the University of Texas Health Science Center at Houston, or the University of Texas MD Anderson Cancer Center, United States.

Role of funding source

The funders had no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

Data availability statement

Data are available on reasonable request. Data can be obtained by the corresponding author after the approval of UTHealth Houston Committee for the Protection of Human Subjects.

CRediT authorship contribution statement

Trisha L. Amboree: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Jacky Kuo:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Bradley A. Sirak:** Writing – review & editing, Validation, Investigation. **John A. Schneider:** Writing – review & editing, Funding acquisition, Conceptualization. **Alan G. Nyitray:** Writing – review & editing, Conceptualization. **Lu-Yu Hwang:** Writing – review & editing, Resources. **Elizabeth Y. Chiao:** Writing – review & editing. **Anna R. Giuliano:** Supervision, Resources, Investigation, Conceptualization. **Kayo Fujimoto:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We acknowledge project staff and Dr. Mayumi Imahashi, MD, MPH. A part of these results was presented at the 37th Annual Meeting of the Japanese Society for AIDS Research on December 3, 2023, Kyoto, Japan.

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