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Research article

Comparison of AmpFire and MY09/11 assays for HPV genotyping in anogenital specimen of Rwandan men who have sex with men

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ABSTRACT

Introduction: The AmpFire HPV genotyping Assay (Atila Biosystems, Mountain View, CA, USA) is a new test for which there are few data regarding its analytic performance and reliability. Using anal and penile swab specimens from a cohort study of men who have sex with men (MSM) in Rwanda, we compared high-risk HPV (hrHPV) detection by AmpFire done at two laboratories, one at University of California San Francisco (UCSF) and the other Rwanda Military Hospital, and well-validated MY09/11-based assay done at UCSF.

Methods: Anal and penile specimens collected from 338 MSM from March 2016 to September 2016 were tested for high-risk HPV genotypes (hrHPV) by MY09/11, AmpFire UCSF and AmpFire RMH. Cohen's kappa coefficient was used to test for reproducibility.

Results: The hrHPV positivity by MY09/11 and AmpFire UCSF was 13% and 20.7% ($k = 0.73$) for anal specimens and was 26.3% and 32.6% ($k = 0.67$) for penile specimens. Specifically, good reproducibility was for types 16 and 18 ($k = 0.69$ and $k = 0.71$) for anal specimens and ($k = 0.50$ and $k = 0.72$) for penile specimens. The hrHPV positivity by AmpFire at UCSF and RMH was 20.7% for both laboratories ($k = 0.87$) for anal specimens and was 34.9% and 31.9% ($k = 0.89$) for penile specimens. Specifically, excellent reproducibility was for types 16 and 18 for anal specimens ($k = 0.80$ and $k = 1.00$) and penile specimens ($k = 0.85$ and $k = 0.91$).

Conclusion: Results show that MY09/11 and AmpFire assays have good reproducibility while the AmpFire UCSF and RMH assays have excellent reproducibility. These results show that AmpFire is a promising HPV genotyping test.

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1. Introduction

Human papillomavirus (HPV) is the most common sexually transmitted infection worldwide and is associated with warts (condylomas) and a variety of cancers in both men and women, anal, penile and oropharyngeal cancers [1]. HPV is the underlying cause of roughly 5% of all cancers worldwide [2].

HPV genotyping tests are useful for the study of the natural history of HPV infection. Among the HPV genotyping tests that are the currently used is the standard MY09/11 assay with dot blot detection of 37 HPV genotypes including 14 high-risk HPV (hrHPV) types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) and 23 low-risk HPV types (6, 11, 26, 32, 40, 42, 53, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 73, 81, 82 subtype (1S39), 83, 84 and 89(CP6108) [3–6]. The MY09/11 assay has been largely used in clinical research [4]. The AmpFire Genotyping High Risk HPV Real Time Fluorescent Detection Assay ("AmpFire Assay"; GHPVF-100, Atila BioSystems, Mountain View, CA, USA), a new technology that detects 15 HPV types in common with the MY09/11 test, including the same 14 hrHPV types and HPV53, is now available [7,8].

In Rwanda, a *low* resource setting, affordable, transferable and accurate molecular tests to detect HPV for research purposes are limited. The AmpFire HPV testing assay can be easily performed with Atila Powergene qPCR 96 well plate reader, a piece of portable equipment. The assay detects HPV from raw samples without needing extraction. It simplifies the process and makes the results available within an hour for 96 specimens. It is well-suited to be a point-of-care HPV test and is perfect for low- and middle-income countries (LMIC) due to its simplicity (no DNA extraction is required), low cost and high throughput [9]. Compared with other HPV testing assays, AmpFire showed similar sensitivity to Cobas, with good specificity [10]. We therefore committed to implementing the technology and evaluating its performance in Rwanda. This included establishing in-country capacity building with the acquisition of lab equipment and materials for HPV genotyping using the AmpFire assay.

The aim of this study was to confirm the successful transfer and validity of HPV genotyping using the AmpFire assay in Rwanda. Baseline anal and penile swab specimens collected from a cohort study of men who have sex with men (MSM) living in Kigali, Rwanda [8] were tested for HPV genotypes. Specimens were tested using the AmpFire assays performed at the Rwanda Military Hospital (RMH). These results were compared to reference results from masked testing of the specimens by MY09/11 and AmpFire assays done at the University of California San Francisco (UCSF).

2. Materials and methods

2.1. Study design and population

This was a cross-sectional analysis done on HPV results of anal and penile specimens collected at study entry as part of a longitudinal cohort study assessing HIV and HPV prevalence among 300 MSM of unknown HIV status and 50 known to be living with HIV in Kigali, Rwanda [8]. These specimens were collected between March 2016 and September 2016. We included self-identified MSM who lived in Kigali, were 18 years or older, reported any sex with a man in the past 6 months, were willing to have HIV testing and, viral load and CD4 cell count taken if found to be living with HIV, were willing to be contacted by the research team for follow-up as part of the study, and consented to participate. Detailed study methods have been previously described [8].

2.2. HPV genotyping

From the 20 ml PreservCyt that contained the specimen, two mL aliquots from each anal and penile specimen were prepared and shipped to the UCSF laboratory to be tested with MY09/11 and AmpFire; an additional one mL aliquot from each specimen was also prepared and used for testing with AmpFire at RMH. Due to resource limitations, only 190 penile samples were tested by MY09/11 at UCSF and were considered for comparison with AmpFire penile hrHPV results.

2.2.1. DNA preparation from PreservCyt and HPV genotyping by MY09/11 assay

The ThinPrep vial was swirled to suspend cells. 750 μL were pipetted into matching labeled microfuge tubes. The vial was swirled again and another 750 μL added to the microfuge tube to make a total of 1.5 mL. Cells were pelleted by centrifugation at 13.2 K RPM for 15 min in an Eppendorf centrifuge. After centrifugation, the tubes were carefully removed from the centrifuge and the ThinPrep solution was decanted. Cell pellets were allowed to dry overnight upside down.

The pellet was suspended in 1xTE (10 mM Tris, 0.1 mM EDTA), and 2 μL 10 mg/mL proteinase K were added and tubes briefly vortexed. Tubes were incubated in a 56 °C water-bath for 1–2 h and then heated in 95 °C water-bath for 15 min to inactivate the proteinase K. Tubes were allowed to cool to room temperature and then spun for 30 s to bring down condensate. Extracted DNAs were stored at −80 °C until PCR was performed.

HPV Genotyping by MY09/11 PCR. Polymerase chain reaction (PCR) was performed using primer pair MY09/11 and it was followed by HPV typing using the dot blot method for 14 hrHPV and 23 LR-HPV. A sample was defined as HPV-positive if it reacted positively to the consensus probe mixture. Hybridization was done using biotin-labeled probes for 14 high-risk HPV genotypes and 23 low-risk HPV genotypes. The hybridization products were then detected using enhanced chemiluminescence (ECL). After amplification and hybridization, the positive result was judged by the intensity of the blue spots on the membrane by the naked eye. The intensity of the dot blot was categorized as 1, 2, 3, 4 and 5 where the intensity of 1 was considered negative and not reported because the signal was too faint on the film, the intensity of 2 considered positive and result reported but considered weak due to the low signal intensity of the dot

on the film and the intensity of 3, 4 and 5 strong positive and reported. Beta globin was used as an internal control and commercial HPV standards were blotted onto each membrane as positive controls for their respective genotypes. This technique has been described and published [6,8].

2.2.2. HPV genotyping with AmpFire HPV genotyping assay

The AmpFire assay is an isothermal nucleic acid amplification based real-time fluorescence detection that detects 14 hrHPV types, 1 low-risk HPV type (HPV 53), and an internal control (IC, human β-globin gene) in 4 reaction tubes. The 23 LR-HPV detected by MY09/11 are not detected by AmpFire. Testing was done according to the manufacturer's protocol. The Thin-Prep solution was mixed by vortexing, 1 mL pipetted into corresponding labeled microfuge tube, centrifuged at 10,000 rpm for 10 min, supernatant removed completely and the pellet lysed by lysis buffer, followed by incubation for 10 min at 95 °C. After incubation, 2 μL of treated sample were mixed with 12 μl of Reaction Mix and 11 μl of each of the four Primer Mixes. The resulting four reaction tubes for every sample were tested using the Powergene 9600 fluorescence real-time PCR system (Atila Biosystems Inc., USA) at 60 °C with fluorescence from FAM/HEX/ROX/CY5 channels measured every minute.

After approximately 1 h of amplification cycling, the results were interpreted according to exponential curves developed during the process. If the negative control showed no exponential curves and the positive control showed an exponential curve, this experiment run was judged to be valid. The next step was to examine the set of four tubes corresponding to a specimen. Multiplex HPV infections could result in multiple exponential curves for a specimen. If no exponential curve other than internal control (Hex channel in PM-3 tube) was present for a sample, this sample called negative for the 15 targeted HPV types. If there was no exponential amplification curve in any of four tubes/any fluorescence channels, the sample failed the test. A failed sample usually indicated not enough DNA in the sample and another aliquot was reprocessed or another specimen was re-taken from the patient. This technique has been previously described [8,11].

2.3. Statistical analysis

Only 15 HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68 detected by both assays were considered for comparison. We calculated the overall, type specific and multiple genotypes concordance between the AmpFire and the MY09/11 assays and between the two AmpFire determinations at UCSF and at RMH. Cohen's kappa coefficient was used to test for reproducibility; $k > 0.75$ was considered excellent, $0.4 \leq k \leq 0.75$ good reproducibility, and $0 \leq k < 0.4$ poor reproducibility [12]. All analyses were performed with SAS statistical software (9.4; SAS Institute, Cary, NC).

3. Ethical consideration

All subjects provided written, informed consent. The study protocol was reviewed and approved by the Rwanda National Ethics Committee and the Institutional Review Boards of Albert Einstein College of Medicine and UCSF.

4. Results

4.1. Socio demographics and sexual behavior of study participants

Three hundred forty five of 350 participants had verified results [8], and of these 345, 338 were considered for this analysis, with the remaining 7 participants having inconclusive results. Sixty-seven (19.8%) tested positive for HIV.

4.2. HPV genotyping with MY09/11 and AmpFire at UCSF

Overall, for high-risk HPV (hrHPV) that could be found by both assays, for anal specimens analyzed at UCSF (Table A. 1.), 44 (13%) were positive and 268 (79.3%) were negative for both MY09/11 and AmpFire. 26 (7.7%) were negative for MY09/11 and positive for AmpFire and none (0%) were positive for MY09/11 and negative for AmpFire with good reproducibility, $k = 0.73$ (95%CI: 0.63–0.83). For type-specific hrHPV, good reproducibility was found for types 16, and 18 respectively with $k = 0.69$ (95%CI: 0.51–0.86) and 0.71 (95%CI: 0.44–0.98) and similarly for types 53, 35, 33, 39, 58, 68, 51 and 52 with $k = 0.49$–0.75. Excellent reproducibility was demonstrated for HPV 31 $k = 0.79$ (95%CI: 0.57–1.00). For multiple genotypes, good reproducibility was found for the types HPV16+others (others = hrHPV except HPV 16 and 18) and HPV others with $k = 0.56$, and $k = 0.67$, respectively.

For penile hrHPV specimens analyzed at UCSF (Table A. 2.), overall, among the 190 tested by MY09/11, 43 (22.6%) were positive and 121 (63.7%) were negative for both MY09/11 and Ampfire. 7 (3.7%) were positive for MY09/11 and negative for AmpFire and 19 (10%) were negative for MY09/11 and positive for AmpFire with good reproducibility, $k = 0.67$ (95%CI: 0.56–0.79). For type-specific hrHPV, good reproducibility was found for types 16 and 18 respectively with $k = 0.50$ (95%CI: 0.22–0.78). and $k = 0.72$ (95%CI: 0.42–1.00). and similarly for types 56, 39, 33, 58 and 66 with $k = 0.50$–0.71. Excellent reproducibility was for types 68, 51, 31 and 53 ($k = 0.76$–0.79). For multiple genotypes, good reproducibility was for types HPV16+others, HPV others and HPV18+others with $k = 0.45$, $k = 0.55$ and $k = 0.66$ respectively.

4.3. HPV genotyping with AmpFire at UCSF and at RMH

Overall, of the 338 anal specimens (Table A. 3.) 63 (18.6%) were positive and 261 (77.2%) were negative for AmpFire performed at both UCSF and at RMH. 7 (2.1%) were positive for AmpFire at UCSF and negative for AmpFire at RMH and 7 (2.1%) were negative for AmpFire at UCSF and positive for AmpFire at RMH with excellent reproducibility, k = 0.87 (95%CI: 0.81–0.94) (Table A. 3.). For type-specific hrHPV, excellent reproducibility was demonstrated for types 16 and 18 with k = 0.80 (95%CI: 0.68–0.93). and k = 1.00 (95% CI: 1.00–1.00). respectively and for types 33, 56, 35, 66, 68, 31 and 45 with k = 0.86–1.00. Good reproducibility was found for types 52, 39, 58, 51 and 53 with k = 0.57–0.75. For multiple genotypes, excellent reproducibility was found for HPV others, HPV 16 + 18 and HPV18+ others k = 0.81–1.00 and good reproducibility was for HPV 16 + others k = 0.66.

For penile hrHPV results (Table A. 4.), overall, 105 (31.1%) were positive and 217 (64.2%) were negative for AmpFire performed at both UCSF and at RMH, 13 (3.8%) were positive for AmpFire UCSF and negative for AmpFire RMH and 3 (0.9%) negatives for AmpFire UCSF and positive for AmpFire RMH with excellent reproducibility, k = 0.89 (95%CI: 0.84–0.94). For type-specific hrHPV, excellent reproducibility was found for types 16 and 18 respectively with k = 0.85 (95%CI: 0.74–0.97) and k = 0.91 (95%CI: 0.80–1.00) and for types 39, 51, 52, 68, 53, 33, 58, 66 and 56 with k = 0.76–0.96 and good reproducibility for types 45, 35, 59 and 31 with k = 0.45–0.70. For all multiple genotypes, excellent reproducibility was found with k = 0.80–0.93. For the sensitivity and specificity, comparing AmpFire assay and MY09/11 for both anal and penile specimens, the sensitivities were 100% and 86% respectively and the specificities were 91% and 86% respectively.

5. Discussion

This is the first study to compare MY09/11 (also known as MY09/11 consensus PCR, dot blot hybridization, or the dot blot method) and AmpFire. For the testing done at UCSF, the reproducibility of results was generally good between MY09/11 and AmpFire assays. The AmpFire assay demonstrated excellent reproducibility in tests performed at both UCSF and RMH. From MY09/11 and AmpFire HPV genotyping assays for the testing performed at UCSF, in HPV genotyping of the 14 hrHPV and HPV53 for anal specimens, reproducibility was good for overall hrHPV with k = 0.73. This result indicates that the two tests have high agreement and that either of the two tests would serve the same purpose. While different studies comparing MY09/11 to other molecular techniques on HPV genotyping for different types of specimens have been performed [13,14], to our knowledge there is only one study that compared AmpFire to another molecular test for HPV genotyping from anal specimens [15]. This study compared AmpFire assay with Roche human papillomavirus (HPV) linear array for genotyping of anal swab specimens [15].

In our study, comparing MY09/11 with AmpFire for type-specific anal hrHPV, types 16 and 18 had good reproducibility (k = 0.69 and k = 0.71) with MY09/11 detecting less HPV type 16 (14 versus 23/338) and 18 (6 versus 8/338) than AmpFire, whereas excellent reproducibility was found for HPV31 (6 versus 9/338) with k = 0.79. This excellent reproducibility between MY09/11 and AmpFire would be most likely due to the fact that both techniques have equal sensitivity for HPV 31. Independently, each of the two tests has been compared to other molecular tests, but there are limited data on AmpFire using anal specimens [6,16,17]. Despite this, similar results were obtained in another study conducted in Harare, Zimbabwe, where HPV31 was among the most frequently detected high-risk HPV genotypes (11%) by MY11/09 from anal specimens [6]. Although our results indicate good reproducibility of HPV16 between MY09/11 and AmpFire, MY09/11 detected less HPV16 (14 versus 23/338) (k = 0.69). In a single available study comparing AmpFire and Roche, HPV16 showed the highest overall agreement at 93.3% (139/149, k = 0.84) [15].

For penile results, MY09/11 and AmpFire showed good reproducibility for overall hrHPV results with k = 0.67. These results indicate that the two tests have a relatively moderate agreement on penile specimens. Despite the lack of comparison data between MY09/11 and AmpFire using penile specimens, previous studies that compared MY09/11 to newer technologies such as next-generation DNA sequencing demonstrated that the MY09/11 primer set for PCR and the dot blot method for HPV genotyping are not able to detect all possible HPV types in the anogenital region [6]. In a study on the prevalence of HPV DNA in penile cancer cases from Brazil, PCR using specific primers detected five HPV infections in MY09/11 negative samples and it was revealed that generally, there is a loss of 10% of samples in MY09/11 PCR when compared with type-specific PCR [18]. Let us point out that from other different studies, the MY09/11 assay has been compared with other molecular tests for HPV genotyping from different samples [19, 20].

In our comparison of type-specific penile hrHPV, types 16 and 18 had good reproducibility (k = 0.50 and k = 0.72) with MY09/11 detecting less HPV types 16 (7 versus 12/190) and 18 (4 versus 7/190) than AmpFire, whereas excellent reproducibility was found for types 68, 51, 31 and 53 (k = 0.76–0.79). Similar results were found in comparisons of MY09/11 with other molecular tests but none of these included AmpFire. Other similar results with excellent reproducibility were found in another study where southern blot (SB) and dot blot (DB) hybridization were compared with PCR for the detection of human papillomavirus DNA in biopsies of the uterine cervix from women with dysplasia and where the agreement between DB and PCR was similar for each of the HPV types and ranged from a low of 89% for type 6/11 with DB hybridization to a high of 97% for type 16 with SB hybridization [21]. In our comparison of MY09/11 and AmpFire, the AmpFire shown high sensitivity (100%) and specificity (91%) for anal specimens and equal sensitivity and specificity (86%) for penile specimens. In another study that compared AmpFire assay with a Cobas assay on cervical specimens, the AmpFire assay showed similar sensitivity to the Cobas assay with good specificity [10].

On interlaboratory comparison tests performed at both USCF and RMH using AmpFire, in HPV genotyping of the 14 hHPV and HPV53 for anal specimens, reproducibility was excellent for overall hrHPV with k = 0.87. We found no previous data comparing AmpFire to AmpFire for anal HPV genotyping in different settings. The results of our study confirm that this technology can be successfully transferred to low income settings.

For type-specific hrHPV in anal specimens, excellent reproducibility was found for the types 16, 18, 33, 56, 35, 66, 68, 31 and 45 with k = 0.80–1.00. Similar results were reported in the study that compared AmpFire with the Roche Cobas and Linear Array assays [11]. In our study, the performance of AmpFire was based on its ability to detect type-specific hrHPVs from anal specimens, not at the place or person performing the test.

For penile specimens, AmpFire showed excellent reproducibility for overall hrHPV with k = 0.89 and for the types 16, 18, 39, 51, 52, 68, 53, 33, 58, 66 and 61 with k = 0.76–0.96. However, there are no previous studies found related to penile HPV genotyping with AmpFire. The excellent reproducibility on penile specimens by AmpFire at UCSF and RMH should most likely be due to the excellent analytic sensitivity and specificity of the assay.

In collaboration with developed countries, technology transfer is possible and the AmpFire assay can be used for hrHPV detection in LMICs.

6. Study limitations

There are few limitations to this study. The first is the small number of studies on HPV genotyping with AmpFire, MY09/11, and comparisons with other molecular tests for HPV genotyping, particularly from anal and penile samples. The second concerns the different settings of the two laboratories. There is a limited number of penile samples tested with MY09/11 at UCSF which was only 190 instead of 350. The last limitation relates to low-risk HPV types tested only with MY09/11 and which were not taken into account in the comparison.

7. Conclusion

The results showed good reproducibility for overall and excellent reproducibility for the specific hrHPV types between the MY09/11 and AmpFire tests, done at UCSF. AmpFire assays done at UCSF and RMH showed excellent inter-laboratory reproducibility for most of the 14 hrHPV types and HPV53. These findings indicate that AmpFire is a real-time test that can be used as a point-of-care test for screening and genotyping of anal, penile, and possibly other anatomical sites of the anogenital region, potentially impacting cancer prevention at these sites. Based on these findings, technology transfer from developed countries to LMICs is a possibility.

Author contribution statement

Faustin Kanyabwisha: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Hae-Young Kim; Qiuhu Shi; Gad Murenzi; Anthere Murangwa; Onesphore Turizigiye; Boniface Nsengiyumva: Analyzed and interpreted the data.

Patrick Tuyisenge; Maria Da Costa; Xin Chen: Performed the experiments.

Gallican Kubwimana; Athanase Munyaneza: Contributed reagents, materials, analysis tools or data;

Leon Mutesa; Kathryn M. Anastos: Conceived and designed the experiments.

Joel M. Palefsky: Conceived and designed the experiments; Analyzed and interpreted the data.

Data availability statement

The authors do not have permission to share data.

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.

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Appendices.

A. 1

Comparison of the MY09/11 results and the AmpFire HPV genotyping Assay results both done at UCSF: anal specimens (n = 338).

Results	MY09/11 UCSF/AmpFire UCSF	Kappa (SE)	95% CI

(*continued on next page*)

A. 1 (*continued*)

Results	MY09/11 UCSF/AmpFire UCSF				Kappa (SE)	95% CI
Anal hrHPV results	+/+	+/−	−/+	−/−		
Overall	44 (13%)	0	26 (7.7%)	268 (79.3%)	0.73	0.63–0.83
HPV genotypes						
16	13	1	10	314	0.69	0.51–0.86
18	5	1	3	329	0.71	0.44–0.98
31	6	0	3	329	0.79	0.57–1.00
33	2	0	2	334	0.66	0.23–1.00
35	4	0	6	328	0.56	0.25–0.88
39	3	0	3	332	0.66	0.31–1.00
45	.	.	3	335	NA	NA
51	6	0	4	328	0.74	0.50–0.99
52	3	0	2	333	0.75	0.41–1.00
53	4	0	8	326	0.49	0.19–0.79
56	1	0	4	333	0.33	−0.16–0.82
58	5	2	3	328	0.66	0.38–0.94
59	1	0	5	332	0.28	−0.15–0.72
66	3	0	13	322	0.30	0.04–0.57
68	5	1	4	328	0.66	0.38–0.94
Multiple genotypes						
HPV16 + 18	1	1	2	334	0.39	−0.15–0.94
HPV16 + Others	4	0	6	328	0.56	0.25–0.88
HPV18 + Others	1	0	5	332	0.28	−0.15–0.72
HPV Others	24	2	18	294	0.67	0.54–0.81

Legend: +/+: HPV positive by both techniques; +/−: HPV positive by the first technique and negative by the second; −/+: HPV negative by the first technique and positive by the second; −/−: HPV Negative by both techniques.

A. 2

Comparison of the MY09/11 results and the AmpFire HPV genotyping Assay results both done at UCSF: penile specimens (n = 190).

Results	MY09/11 UCSF/AmpFire UCSF				Kappa	95% CI
Penile hrHPV results	+/+	+/−	−/+	−/−		
Overall	43 (22.6%)	7 (3.7%)	19 (10%)	121 (63.7%)	0.67	0.56–0.79
HPV genotypes						
16	5	2	7	176	0.50	0.22–0.78
18	4	0	3	183	0.72	0.42–1.00
31	6	1	2	181	0.79	0.56–1.00
33	4	0	5	181	0.60	0.29–0.92
35	1	2	5	182	0.21	−0.16–0.57
39	4	2	5	179	0.52	0.20–0.83
45	1	0	3	186	0.39	−0.15–0.94
51	12	2	4	172	0.78	0.62–0.95
52	0	1	5	184	−0.01	−0.02–0.01
53	4	1	1	184	0.79	0.52–1.00
56	1	0	2	187	0.50	−0.10–1.00
58	4	2	2	182	0.66	0.34–0.97
59	1	0	7	182	0.21	−0.14–0.57
66	8	1	5	176	0.71	0.49–0.93
68	5	3	0	182	0.76	0.50–1.00
Multiple genotypes						
HPV16 + 18	.	.	1	189	NA	NA
HPV16 + Others	3	0	7	180	0.45	0.11–0.78
HPV18 + Others	2	1	1	186	0.66	0.22–1.00
HPV Others	27	12	17	134	0.55	0.41–0.70

Legend: +/+: HPV positive by both techniques; +/−: HPV positive by the first technique and negative by the second; −/+: HPV negative by the first technique and positive by the second; −/−: HPV Negative by both techniques.

A. 3

Comparison of the AmpFire HPV gemotyping Assay Results done at UCSF and at RMH: anal specimens (n = 338).

Results	AmpFire UCSF/AmpFire RMH				Kappa	95% CI
Anal hrHPV results	+/+	+/−	−/+	−/−		
Overall	63 (18.6%)	7 (2.1%)	7 (2.1%)	261 (77.2%)	0.87	0.81–0.94
HPV genotypes						
16	20	3	6	309	0.80	0.68–0.93
18	8	0	0	330	1.00	1.00–1.00
31	9	0	1	328	0.95	0.84–1.00
33	3	1	0	334	0.86	0.58–1.00
35	9	1	1	327	0.90	0.76–1.00

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A. 3 (*continued*)

Results	AmpFire UCSF/AmpFire RMH				Kappa	95% CI
39	5	1	4	328	0.66	0.38–0.94
45	3	0	0	335	1.00	1.00–1.00
51	7	3	2	326	0.73	0.50–0.96
52	2	3	0	333	0.57	0.13–1.00
53	8	4	1	325	0.75	0.55–0.96
56	4	1	0	333	0.89	0.67–1.00
58	5	3	2	328	0.66	0.38–0.94
59	0	0	6	332	NA	NA
66	14	2	1	321	0.90	0.79–1.00
68	8	1	0	329	0.94	0.82–1.00
Multiple genotypes						
HPV16 + 18	3	0	1	334	0.86	0.58–1.00
HPV16 + Others	7	3	4	324	0.66	0.42–0.89
HPV18 + Others	6	0	0	332	1.00	1.00–1.00
HPV Others	34	8	6	290	0.81	0.71–0.90

Legend: +/+: HPV positive by both techniques; +/−: HPV positive by the first technique and negative by the second; −/+: HPV negative by the first technique and positive by the second; −/−: HPV Negative by both techniques.

A. 4

Comparison of the AmpFire HPV genotyping Assay Results done at UCSF and at RMH: penile specimens (n = 338).

Results	AmpFire UCSF/AmpFire RMH				Kappa	95% CI
Penile hrHPV results	+/+	+/−	−/+	−/−		
Overall	105 (31.1%)	13 (3.8%)	3 (0.9%)	217 (64.2%)	0.89	0.84–0.94
HPV genotypes						
16	19	5	1	313	0.85	0.74–0.97
18	11	1	1	325	0.91	0.80–1.00
31	10	3	5	320	0.70	0.51–0.90
33	11	2	0	325	0.91	0.80–1.00
35	7	5	2	324	0.66	0.42–0.89
39	12	6	1	319	0.76	0.60–0.93
45	3	6	1	328	0.45	0.12–0.79
51	19	5	4	310	0.79	0.66–0.93
52	9	4	0	325	0.81	0.63–0.99
53	11	0	3	324	0.88	0.74–1.00
56	13	1	0	324	0.96	0.89–1.00
58	10	1	1	326	0.91	0.78–1.00
59	6	5	1	326	0.66	0.40–0.91
66	16	2	1	319	0.91	0.81–1.00
68	9	1	3	325	0.81	0.63–0.99
Multiple genotypes						
HPV16 + 18	2	0	1	335	0.80	0.41–1.00
HPV16 + Others	13	5	1	319	0.80	0.65–0.96
HPV18 + Others	7	0	1	330	0.93	0.80–1.00
HPV Others	74	10	5	249	0.88	0.82–0.94

Legend: +/+: HPV positive by both techniques; +/−: HPV positive by the first technique and negative by the second; −/+: HPV negative by the first technique and positive by the second; −/−: HPV Negative by both techniques.

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