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Improving Oral Human Papillomavirus Detection Using Toothbrush Sampling in HIV-Positive Men Who Have Sex with Men

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Pre- and postabrasion oral rinse samples (ORS) and a toothbrush sample detected human papillomavirus (HPV) DNA in at least one sample among 45 (26%) of 173 HIV-positive men who have sex with men. There was moderate agreement for HPV genotype detection between the preabrasion and postabrasion ORS ($\kappa = 0.49$; 95% confidence interval [CI], 0.37 to 0.61). There was good agreement between postabrasion ORS and toothbrushes ($\kappa = 0.70$; 95% CI, 0.60 to 0.80). The sensitivities for HPV genotypes detected were 80% (95% CI, 69 to 88) for preabrasion ORS, 65% (95% CI, 54 to 76) for postabrasion ORS, and 75% (95% CI, 63 to 84) for toothbrushes.

Human papillomavirus (HPV) causes some forms of oropharyngeal cancer, most commonly in the lingual and palatine tonsils or in the base of the tongue (1, 2). It is estimated that high-risk (hr) HPV is detected in more than half of the patients with this form of cancer, the most common genotype being HPV-16 (3, 4). The incidence of oropharyngeal cancer has increased in younger individuals, and the proportion of these tumors associated with HPV continues to rise (5–8).

Currently, there is no universally agreed-on method of oral sampling for the detection of HPV DNA. The most common method is using an oral rinse swirl or gargle to obtain an oral rinse sample (ORS) (9–12). Alternatives include using a flocked nylon swab (13), a biopsy specimen (14), a cytobrush (11, 15), or oral mucosal scraping (16). An untested sampling method is obtaining an ORS immediately after brushing one's teeth and gums. A study by our group found the likelihood of detecting oral HPV fell in a linear fashion by about 14% with each additional hour after brushing the teeth, suggesting that abrasion of oral mucosa may improve the collection of infected cells in an oral rinse (13). This finding suggests that current sampling techniques may be improved by prior epithelial abrasion similar to that used for anogenital HPV detection in men (17). The detection of HPV seems to vary widely depending on the sampling method and the detection methods, with prevalence rates ranging from 7% to 45% even within similar populations (18). It remains unclear whether one sampling method is superior to another for the detection of oral HPV.

Men who have sex with men (MSM) attending the Melbourne Sexual Health Centre (MSHC) HIV clinic from December 2012 to August 2013 were recruited. In the single clinic visit, participants provided 3 samples for HPV detection and genotyping. The first sample was a preabrasion ORS that involved swishing and gargling 20 ml of a sterile saline solution in the oral cavity for 20 to 30 s and then spitting it into a sterile specimen cup. The postabrasion ORS was collected directly after each participant brushed his teeth with a new toothbrush (Dentitex, medium-grade bristles); other than the brushing, the process was identical to that for obtaining the preabrasion ORS. The toothbrush was then placed in 10 ml of phosphate-buffered saline (PBS) and rotated.

The resuspended cells from the toothbrush and ORS were centrifuged for 10 min at 14,000 × *g* and subsequently resuspended in 400 μl of PBS. An aliquot of 200 μl was extracted by the automated MagNA Pure 96 isolation and purification system (Roche Molecular Systems) using DNA and the Viral NA Small Volume kit. Following nucleic acid isolation, each sample was initially assessed for DNA adequacy with a quantitative PCR for a 260-bp fragment of the human beta-globin gene using 10 pmol each of beta-globin primers GH20 (5′-GAAGAGCCAAGGACAGGTAC-3′) and PCO4 (5′-CAACTTCATCCACGTTCACC-3′) and 2 pmol of the adapted probe PCO3 (5′-6-FAM-ACACAACTGTGT TCACTAGC-TAMRA-3′; FAM indicates 6-carboxyfluorescein, and TAMRA indicates 6-carboxytetramethyl rhodamine) (19). Samples which were HPV positive by PCR-enzyme-linked immunosorbent assay (ELISA) (20) were subsequently genotyped by the Linear Array (LA) HPV genotyping test (Roche Diagnostics) (21, 22), with modification as described previously (23, 24). Samples which were HPV positive by PCR-ELISA but negative by the LA test were amplified using the more sensitive HPV SPF10-LiPA 25 assay version 1 (25). hr-HPV genotypes were defined as such according to recent International Agency for Research on Cancer nomenclature (26).

The PCR-ELISA utilized the well-established Ll consensus primers PGMY09/PGMY11 (27, 28) combined with sensitive de-

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TABLE 1 Any HPV genotype detected by preabrasion ORS, postabrasion ORS, and toothbrushes[a]

Sample type and result	No. of HPV genotypes detected from:						Positive on any sample[c]		Sensitivity (% [95% CI])[b]
	Preabrasion ORS		Postabrasion ORS		Toothbrush				
	HPV$^-$	HPV$^+$	HPV$^-$	HPV$^+$	HPV$^-$	HPV$^+$	HPV$^-$	HPV$^+$	
Preabrasion ORS HPV$^-$	140	0					128	12	
Preabrasion ORS HPV$^+$	0	33					0	33	73 (58–85)
Postabrasion ORS HPV$^-$	132	9	141	0			128	13	
Postabrasion ORS HPV$^+$	8	24	0	32			0	32	71 (56–84)
Toothbrush HPV$^-$	130	9	135	4	139	0	128	11	
Toothbrush HPV$^+$	10	24	6	28	0	34	0	34	76 (61–87)

[a] The unit of comparison is an individual with detected HPV. Linear Array detects 37 HPV genotypes (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, 82v, 83, 84, and 89), and SPF10-LiPA detects 25 HPV genotypes (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).
[b] Sensitivity was calculated using positivity from any sample as the reference.
[c] Number of men with HPV detected in pre- and/or postabrasion ORS and/or toothbrush sample.

tection using an HPV Ll generic probe and captured on streptavidin-coated plates (Roche Biochemicals) with the bound hybrid detected by an antidigoxigenin peroxidase conjugate by use of the colorimetric substrate 3-ethylbenzthiazoline-6-sulfonate (ABTS) (20). The established sensitivity of this assay in the laboratory is 10 copies per reaction for the mucosal types detected.

Statistical analyses were performed using Stata (version 13.1). The 95% confidence intervals for the sensitivities were calculated using an exact binomial confidence interval. We used kappa values to quantify the agreement of HPV detection between each sampling method. This research was approved by the Alfred Health Human Ethics Committee (project 384/12).

Two hundred ten HIV-positive MSM attending the Melbourne Sexual Health Centre were approached, and 173 (69%) consented to participate. The mean age of the participants was 52 years (range, 23 to 87 years). They had an average duration of HIV of 11.8 years (range, 2 to 31 years) and a mean current CD4 count of 679 cells/μl, and 91% had a suppressed viral load (<20 copies/ml). Ninety-four percent of them were currently on antiretrovirals, and 32% were current smokers.

Overall, beta-globin, a measure of sample adequacy, was positive in 173 (100%) samples for pre- and postabrasion ORS and positive for 170 (98%) of the toothbrush samples. Twenty-three participants had samples collected which were positive in the HPV DNA-ELISA but negative in Linear Array genotyping; these samples were also tested for HPV genotype by the SPF10-LiPA assay. The cell number estimation performed by the comparison of crossing points from the real-time beta-globin PCR with known standards showed no differences between the cell numbers obtained from HPV-positive and -negative samples (see Table S1 in the supplemental material).

Overall, 45/173 (26%; 95% CI, 20 to 33%) men had detectable HPV of any genotype in at least 1 of the 3 samples. Twenty-three participants had samples collected which were positive in the HPV DNA-ELISA but negative in Linear Array genotyping; these samples were also tested for HPV genotype by the SPF10-LiPA assay. Twenty-six of 173 (15%; 95% CI, 10 to 20%) men had detectable hr-HPV DNA, 8 (5%; 95% CI, 2 to 9%) of which were HPV-16. Fifteen of 26 (58%; 95% CI, 37 to 77%) had 1 high-risk genotype, 5 (19%; 95% CI, 7 to 39%) had 2 high-risk genotypes, and 6 (23%; 95% CI, 9 to 44%) had 3 high-risk genotypes.

When an individual with any HPV genotype detected was used as the unit of comparison, each sampling method missed 24 to 29% of detections compared to the 3 sample methods combined (Table 1). When a specific HPV genotype was used as the unit of comparison, each sampling method missed 20 to 35% of detections compared to the 3 sample methods combined (Table 2). We examined the level of agreement for specific HPV genotype detec-

TABLE 2 Any HPV genotype detected by preabrasion ORS, postabrasion ORS, and toothbrushes[a]

Sample type and result	No. of HPV genotypes detected from:						Positive on any sample[c]		Sensitivity (% [95% CI])[b]
	Preabrasion ORS		Postabrasion ORS		Toothbrush				
	HPV$^-$	HPV$^+$	HPV$^-$	HPV$^+$	HPV$^-$	HPV$^+$	HPV$^-$	HPV$^+$	
Preabrasion ORS HPV$^-$	5,303	0					5,288	15	
Preabrasion ORS HPV$^+$	0	60*					0	60	80 (69–88)
Postabrasion ORS HPV$^-$	5,281	33	5,316	0			5,288	26	
Postabrasion ORS HPV$^+$	22	27	0	49*			0	49	65 (54–76)
Toothbrush HPV$^-$	5,280	27	5,297	11	5307	0	5,288	19	
Toothbrush HPV$^+$	26	30	19	36	0	56	0	56	75 (63–84)

[a] The unit of comparison is detection of a specific HPV genotype. Linear Array detects 37 HPV genotypes (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, 82v, 83, 84, and 89), and SPF10-LiPA detects 25 HPV genotypes (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).
[b] Sensitivity was calculated using positivity from any sample as the reference.
[c] Number of HPV genotypes detected in pre- and/or postabrasion ORS and/or toothbrush sample.

tion between the 3 samples. There was moderate agreement observed between the preabrasion and postabrasion ORS ($\kappa = 0.49$; 95% CI, 0.37 to 0.61) and between the preabrasion ORS and toothbrushes ($\kappa = 0.53$; 95% CI, 0.41 to 0.64). There was good agreement for HPV detection between the postabrasion ORS and toothbrushes ($\kappa = 0.70$; 95% CI, 0.60 to 0.80).

This is the first study to compare simultaneous testing of ORS with oral abrasion and its effect on the sensitivity of oral HPV detection. Results from a single ORS can miss a significant number of oral HPV genotypes. For natural history studies that are reliant on using ORS, differences in HPV detection across time points may result in lower-than-expected HPV prevalence or in accurate estimates of its incidence and persistence. The moderate agreement seen between the three sample methodologies may explain the wide variability of HPV prevalence depending on what sample method is used (18).

The limitations of this study include the study being undertaken in a single center and limited generalizability to HIV-negative populations. The study also had limited power to detect differences in the sampling methods because of the relatively low prevalence. We share the same limitation as other studies where the detection of HPV DNA does not necessarily mean that there is active infection. Testing for HPV RNA to detect active infection may address this in a future study. The two genotyping assays utilized have different analytical performance characteristics and were used to reduce false-negative results, and our beta-globin results indicated that the methods we used obtained adequate sample volumes. However, future studies should be performed to establish the best method of detection and genotyping in such samples diagnostically.

Our findings suggest that multiple sampling methods may be needed to maximize oral HPV detection.

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