Performance of human papillomavirus DNA detection in residual specimens taken for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* nucleic acid amplification testing in men who have sex with men

Supplementary material

Word count = 535

Laboratory methods

On arrival to the HPV testing laboratory at PHE, specimens were vortexed to agitate the material from the swab into the transport medium and aliquots of 300 µl were stored at -25°C. Thawed aliquots were lysed with 40µl Qiagen Protease and 265µl Qiagen buffer and nucleic acid was extracted on a BioRobot Universal platform using QIAampDNA Blood BioRobot MDx kit (Qiagen, UK). Ten microlitres of the 100µl elution was used for PCR amplification using an in-house single-round multiplex PCR and type-specific infections were resolved using a genotyping assay based on the Bio-Plex (Luminex xMAP, Bio-Rad Laboratories, UK) platform.(1)

PCR primers and probe targeting glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were optimized for the ABI 7500 Fast PCR machine (Applied Biosystems) using Platinum UDG Supermix (Life Technologies). Estimated numbers of cells per mL of sample were made based upon the amount of cellular extract (10µL) used in the GAPDH qPCR (copies per reaction / 2) and scaling up to the volume (300µL) used for extraction(2)

Sample size

We used a precision-based sample size calculation based on a desired maximum 95% CI width of ±12.5% for the estimated sensitivity of pooled or residual rectal samples to detect HPV relative to the dedicated samples. For the purpose of the sample size calculation, the dedicated specimen was the assumed gold standard for anal HPV detection. As the maximum variance for an estimate of proportion from a sample is for a true value of 0.5, we used this as the 'true proportion' in the sample size calculations. Using these values gave a required number of true-positive cases of 62. Based on an observed prevalence of 52.5% of any of the HPV genotypes detected by the assay,(3) a sample size of 119 was required to achieve 62 true-positive cases of any genotyped HPV.

Comparison of HPV prevalence with prior HPV prevalence study at the same site

Our measures of qHPV, 9vHPV and HR-HPV DNA all exceeded those recorded in a prevalence study carried out amongst MSM at the same clinical site in 2012, 6 years before our study was run.(3, 4) However, our study included a higher proportion of HIV positive participants (24% vs 5%) in whom HPV prevalence is known to be higher. Table S1 shows a comparison of the data collected by King *et al* with prevalence measures from our study results, including an adjustment to correct for the higher proportion of HIV positive participants in our study. Adjusted figures show the estimated prevalence of qHPV, 9v-HP and HR-HPV in our study had the proportion of HIV positive participants been equal to that recruited King *et al*'s study, calculated based on our measured prevalence of HPV in HIV positive and negative participants respectively. The comparison suggests that rates of HPV infection in MSM have either remained stable or increased since 2012, in contrast to the substantial reductions in anogenital wart diagnoses reported amongst women and heterosexual men ascribed to the introduction of the UK's universal HPV vaccination programme in adolescent females.(5)

Concordance

Table S2 shows the frequency of discordant results between specimen pairs by individual HPV genotype. Discordant results occurred for all genotypes with the exception of HPV82 which was of very low prevalence (0.8%) in our study participants.

Table S1: Comparison of anal qHPV, 9vHPV and HR-HPV prevalence with King *et al*, adjusted for HIV status

	King *et al*	Nugent *et al* (unadjusted)	Nugent *et al* (adjusted)	Nugent *et al* (HIV negative)	Nugent *et al* (HIV positive)
N	511	123	123	93	30
qHPV HPV	29.1%	33.3%	29.1%	28.0%	50.0%

9v-HPV	40.1%	51.2%	44.8%	43.0%	76.7%
HR-HPV	40.5%	54.5%	50.6%	49.5%	70.0%

Table S2: Frequency of discordant infections by genotype

		Positive in any specimen	Dedicated (D) vs residual pooled (P) specimens		Dedicated (D) vs residual rectal (R) specimens		Residual pooled (P) vs residual rectal (R) specimens	
			D+ P-	D- P+	D+ R-	D- R+	P+ R-	P- R+
Total pairs available for comparison			123		125		119	
LR qHPV	HPV6	18	2	1	1	4	0	4
	HPV11	19	1	1	0	4	0	3
HR qHPV	HPV16	18	0	3	1	3	3	2
	HPV18	11	2	1	4	1	4	2
HR 9vHPV	HPV31	5	1	0	1	2	0	2
	HPV33	5	2	0	3	1	0	1
	HPV45	14	2	4	3	5	2	2
	HPV52	22	4	2	3	1	4	3
	HPV58	14	1	4	2	4	2	0
Other HR HPV	HPV35	8	2	0	2	1	0	1
	HPV39	11	0	3	0	4	1	1
	HPV51	14	2	3	1	4	1	3
	HPV56	15	4	1	3	3	1	4
	HPV59	6	0	3	0	2	1	0
	HPV68	21	5	1	2	5	0	6
	HPV26	12	4	1	6	0	4	1

Possible HR HPV	HPV53	14	2	4	2	3	2	2
	HPV66	6	0	2	2	1	3	0
	HPV70	13	3	3	2	6	1	5
	HPV73	5	2	2	1	0	2	1
	HPV82	1	0	0	0	0	0	0
	Total		39	39	39	54	31	43

References

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