Detection of cervical human papillomavirus infection in filter paper samples: a comparative study

Testing for high-risk human papillomavirus (HPV) types has been recommended for primary cervical cancer screening, as well as for management of women with low-grade cervical cytological abnormalities (IARC, 2005). New, inexpensive screening methods for rapid identification of high-risk HPV types are under evaluation (Lorincz, 2006) and may facilitate the use of HPV testing in low-resource countries.

Cervical exfoliated cells for HPV testing are usually collected in PBS, which is cheap, but requires constant refrigeration, or in other liquid-based preservation media (ThinPrep Solution; Cytoc), which can be stored at room temperature, but are expensive and flammable. Filter paper has been widely used for collecting dry blood spots to screen for various infectious agents (Beebe & Briggs, 1990; Gupta *et al.*, 1992; Kain & Lanar, 1991) and metabolic and genetic diseases (Bickel *et al.*, 1981; Garrick *et al.*, 1973; Maeda *et al.*, 1985). Paper samples would greatly facilitate the collection, storage and transport of exfoliated cervical cells in low-resource countries. Kailash *et al.* (2002) reported similar detection rates of HPV16 in paper and PBS samples among 100 women with cervical dysplasia and carcinoma in India. To further evaluate the issue, we compared the detection of 25 HPV types in paper and PBS samples among young women in Uganda.

Between September 2002 and November 2004, we enrolled 1275 young women attending the Naguru Teenage Information and Health Centre, located in a suburb of Kampala, Uganda, as part of a large HPV survey. Study methods are described elsewhere (Banura *et al.*, 2008). Study participants were informed of the study aims and procedures and signed an informed consent form or assent form in the case of minors. The Institutional Review Boards of the Faculty of Medicine of Makerere University, the Uganda National Council of Science and Technology, and the

International Agency for Research on Cancer approved the study.

The present report includes 111 women randomly selected among study participants. The distribution of the women included in the present report by year of recruitment, age (mean age 20 years; range 16–24), lifetime number of sexual partners (75 % reporting $\geq$ two partners) and HIV infection (eight women were HIV-positive) was well representative of the total study population.

From each woman, a sample was taken from the cervix by rotating (360°) a sterile cotton swab thrice in the cervix, and smearing it within a 0.5–1.0 cm diameter on 3MM Whatman filter paper cut to the size of a small glass slide (5 × 2 cm). The cervical material remaining on the cotton swab was then placed into a 15 ml holding tube containing 5 ml PBS (pH 7.2). Paper samples were dried, placed in auto-seal (ziplock) plastic bags and stored at room temperature (25–30 °C), whereas PBS samples were kept at –20 °C until shipment to DDL Diagnostic Laboratory, Voorburg, the Netherlands, for DNA extraction and HPV genotyping.

Part of the filter paper was punched (~4 mm circle) and transferred to a 0.5 ml microcentrifuge tube containing 50 µl distilled water. DNA was released by boiling for 5 min. For PBS samples, DNA was isolated from 200 µl cell suspension by the MagNA Pure LC instrument (Roche Diagnostics), using the Total DNA isolation kit (Roche Diagnostics). DNA was eluted in 100 µl water, and 10 µl was used for each PCR reaction.

Every PCR reaction included positive and negative controls. Strict laboratory precautions and quality assurance/quality control measures were followed to avoid cross-contamination and carry-over PCR assay. The short PCR fragment (SPF)₁₀ PCR primer set was used to amplify a broad spectrum of HPV genotypes, as

described previously (Kleter *et al.*, 1998, 1999). The same SPF₁₀ amplimers were used to identify the HPV type by reverse hybridization on a reverse hybridization line probe assay (LiPA), containing probes for 25 different HPV types, including HPV6, 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 (SPF₁₀ HPV LiPA version 1; Labo Bio-medical Products).

To compare the types of samples, we calculated the kappa statistic for the detection of overall HPV positivity and presence of each individual HPV type and of multiple-type infections in paper and PBS samples from the same woman.

Overall, the prevalence of any HPV type was 82.9 % in PBS compared to 32.4 % in paper samples (kappa statistic=0.18) (Table 1). Twenty-two different HPV types were detected in 225 infections in PBS samples, but only 18 types were found in 68 infections in paper samples. The most frequently detected high-risk HPV types were HPV52 (22.5 % in PBS, 9 % in paper and 7.2 % in both samples), HPV18 (18.9 % in PBS, 2.7 % in paper and 0.9 % in both samples) and HPV16 (16.2 % in PBS, 4.5 % in paper and 2.7 % in both samples). The most frequently detected low-risk type was HPV6 (20.7 % in PBS, 9 % in paper and 7.2 % in both samples). The majority of kappa statistics for the detection of individual HPV types fell between 0.20 and 0.40, and hence suggested relatively poor agreement. Multiple-type infections were detected in 54.1 % of PBS samples compared to 15.3 % of paper samples. Infection with $\geq$ four HPV types was also relatively common (18.0 %) in PBS samples, but not in paper samples (2.7 %) (Table 1).

In our study of young women from Uganda, HPV detection in paper samples was, therefore, much lower than in PBS samples. In particular, over two-thirds of infections with high-risk types HPV52, 16, 18, 33 and 51 would have been missed, and

Table 1. HPV DNA detection by type of sample among 111 young women in Kampala, Uganda, 2002–2004

HPV	HPV-negative (<i>n</i>)	HPV-positive, by type of sample						Kappa statistic
		PBS		Paper		Both		
		<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	
Any type	19	92	82.9	36	32.4	36	32.4	0.18
HPV6	86	23	20.7	10	9.0	8	7.2	0.41
HPV11	95	16	14.4	2	1.8	2	1.8	0.20
HPV16	91	18	16.2	5	4.5	3	2.7	0.20
HPV18	88	21	18.9	3	2.7	1	0.9	0.04
HPV31	105	6	5.4	3	2.7	3	2.7	0.65
HPV33	96	15	13.5	4	3.6	4	3.6	0.39
HPV35	98	12	10.8	3	2.7	2	1.8	0.23
HPV39	102	9	8.1	0	0.0	0	0.0	0.00
HPV43	110	1	0.9	0	0.0	0	0.0	0.00
HPV44	108	3	2.7	0	0.0	0	0.0	0.00
HPV45	104	6	5.4	3	2.7	2	1.8	0.42
HPV51	94	16	14.4	6	5.4	5	4.5	0.41
HPV52	84	25	22.5	10	9.0	8	7.2	0.38
HPV53	104	5	4.5	3	2.7	1	0.9	0.22
HPV54	103	8	7.2	2	1.8	2	1.8	0.38
HPV56	104	7	6.3	1	0.9	1	0.9	0.24
HPV58	109	2	1.8	1	0.9	1	0.9	0.66
HPV59	106	3	2.7	3	2.7	1	0.9	0.31
HPV66	104	6	5.4	3	2.7	2	1.8	0.42
HPV70	105	6	5.4	0	0.0	0	0.0	0.00
HPV74	107	4	3.6	1	0.9	1	0.9	0.39
HPV68/73	102	7	6.3	4	3.6	2	1.8	0.33
HPV X	105	6	5.4	1	0.9	1	0.9	0.27
Multiple infection	48	60	54.1	17	15.3	14	12.6	0.21
Two types	83	24	21.6	8	7.2	4	3.6	0.16
Three types	92	16	14.4	6	5.4	3	2.7	0.21
≥ Four types	90	20	18.0	3	2.7	2	1.8	0.13

multiple-type infections would have also been underestimated by threefold if only paper samples had been used.

Reasons for the disagreement between our findings and those of Kailash *et al.* (2002), which were much more favourable to paper samples, are unclear, but they could include differences in the load of cells and HPV DNA obtained in the two studies. All women in the Indian study had cervical dysplasia or carcinoma, whereas we included young women who were free of cervical lesions at visual inspection with acetic acid and, on account of their age, were unlikely to harbour cervical carcinoma. In addition, Kailash *et al.* (2002) tested for HPV16 only, whereas we tested for 25 HPV types, using a highly sensitive and well-validated PCR-based assay (Kleter *et al.*, 1999). Conversely, DNA extraction was performed in a similar

way in our study and in that of Kailash *et al.* (2002). Smearing the swab on paper, however, is a highly variable step in the procedure, and it is difficult to control spot size. Therefore, it remains unknown how many cells are placed on the paper, and how representative this paper sample is of the entire cervical swab sample.

Although it is conceivable that DNA extraction from paper samples can be improved, our results do not encourage, for the moment, the use of paper samples for HPV detection. To permit the use of paper samples, a standardized procedure should be developed to ensure equivalence with liquid-based samples.

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