

# Evaluation of the FTA Carrier Device for Human Papillomavirus Testing in Developing Countries

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**Liquid-based methods for the collection, transportation, and storage of cervical cells are cumbersome and expensive and involve laborious DNA extraction. An FTA cartridge is a solid carrier device, easier to handle and allowing simple DNA elution for human papillomavirus (HPV) testing. HPV-DNA results from cervical specimens collected in PreservCyt medium (Hologic, Inc.) and the indicating FTA elute cartridge were compared in an area where transportation and storage may affect the performance of the test. Cervical cells from 319 young adult women enrolled in the Costa Rica Vaccine Trial were collected by a nurse using a Cervex brush (Roberts), which was placed on the FTA cartridge and subsequently rinsed in 20 ml of PreservCyt medium. Two 0.5-ml PreservCyt aliquots were frozen for HPV-PCR testing; the FTA cartridges were kept at room temperature. HPV-DNA detection and typing was performed using SPF<sub>10</sub> PCR/DEIA (DNA enzyme immunoassay detection of amplimers)/LiPA<sub>25</sub> system. The percent agreement, agreement among positives, and kappas were estimated. Positivity was higher for FTA compared to PreservCyt specimens (54.5% versus 45.8%,  $P < 0.001$ ). For oncogenic types, the overall agreement was 0.92, the agreement between positives was 0.74, and the kappa was 0.79. For individual HPV types, the overall agreement ranged from 0.97 to 1.00. We did not observe reduced cytology adequacy when specimen collection for cytology was preceded by FTA collection for HPV testing. HPV-DNA detection from FTA cartridges is broadly comparable to detection from PC medium. The higher HPV detection observed for FTA-collected specimens should be explored further. FTA cartridges could provide a simpler and more cost-effective method for cervical cell collection, storage, and transportation for HPV-DNA detection in research settings in developing countries.**

Persistent infection with one of approximately 13 carcinogenic human papillomaviruses (HPVs) is a necessary cause for the development of cervical cancer (14, 23). Detection of some or all of these HPV types has been shown to be useful for cervical cancer screening (1, 15, 26–28).

In clinical trials (including evaluation of current and future HPV vaccines) and epidemiological studies, cervical cells for HPV DNA detection are usually collected and preserved in liquid-based transport medium, which in some cases is used also for cytology slide preparation, allowing the collection of only one sample for both tests (7, 12, 13, 24, 25).

However, these medium samples can be flammable and require stable transport and storage temperatures, which are difficult and expensive to provide in developing and tropical countries. If samples are tested in a different country, as is usually the case for large research studies conducted in developing countries, the exportation of such samples must fulfill international regulations regarding the transport of hazardous samples (i.e., according to the 53rd edition of IATA's DGR, specimens collected in PreservCyt [Hologic, Inc.] or SurePath [Becton, Dickinson and Company] best fulfill the requirements of Flammable Liquids Class 3 and Biological Substance Category B [UN 3373]) (31). Moreover, these liquid-based samples require expensive and laborious DNA extraction procedures susceptible to cross-contamination, especially when manual extraction of DNA is performed.

Solid carriers for DNA transportation and storage, which consist of dried fluid spots on filter paper, have been used widely for postnatal screening of certain congenital disorders and diseases; they have also been used in studies to detect other viruses such as

measles virus, hepatitis B virus, and HIV, as well as genetic research. These devices are easy to store and transport since they dry quickly, they are compact and lightweight, and they do not require a controlled temperature (2–4, 6, 8, 11, 16, 19, 21, 22). The FTA cartridge (GEHC-Whatman) is a paper-based system that immobilizes and stabilizes nucleic acids from fresh samples applied. The paper is impregnated with a patented chemical formula that lyses cells and denatures proteins upon contact, which makes samples collected in FTA cartridges neither hazardous nor infectious as the viruses are denatured upon application. The indicating FTA elute cartridge also contains an indicating dye that changes color when a sample is applied showing the location of the sample. Furthermore, it allows DNA elution by a simple method using only water and heat with easy reduction of the risk of cross-contamination between samples to minimal by simply applying the puncher two to three times on clean paper and wiping it off with 95% ethanol between samples (9, 30). Finally, since not all of the material is used, the residual sample can be stored.

Four studies (two in the Netherlands and two in Sweden) have found good agreement for HPV DNA detection between cervical

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cells collected in this FTA cartridge and liquid-based medium or frozen dry samples. Lenselink et al. compared self-collected samples at home placed in both media from 51 women aged 18 to 29 years with an agreement of 100%. These researchers also found 93% agreement between FTA self-collected and liquid-based physician-collected samples among 45 women aged 23 to 51 years attending a gynecology clinic (20). Gustavsson et al. evaluated cervical samples from 50 women visiting a dermatology and venereology clinic, from whom physician-collected samples were obtained using a cytobrush; each sample was first applied to FTA and then frozen dry, and the agreement was 94% (9).

More recently Gustavsson et al. compared self-collected with physician-collected cervical samples both placed in FTA cartridges among 50 women attending a gynecology clinic and found an agreement of 88% (10). De Bie et al. estimated agreements of 91% for PCR testing and 77.3% for Hybrid Capture 2 (HC2; Qiagen) comparing two physician-collected cervical brushes placed on the liquid-based medium and on FTA cartridges among 88 women attending a gynecology clinic (5).

Given that the use of this cartridge is very promising, especially in developing countries where transportation and storage is expensive and complicated and for multicentric studies with a centralized laboratory, we conducted a study within our HPV16 and HPV18 (HPV16/18) vaccine trial in Costa Rica to evaluate the performance of HPV DNA detection by PCR and genotyping on samples collected on the indicating FTA elute cartridge compared to samples collected in PreservCyt transport medium in the context of research studies. To our knowledge, this is the first study of cervical HPV DNA detection on FTA cartridges in a developing country and is the largest comparing FTA and liquid-based medium.

## MATERIALS AND METHODS

**Study population.** Samples included in this analysis were collected from participants in the Costa Rica Vaccine Trial (CVT), who completed a follow-up screening visit or a colposcopy visit from 24 to 27 November 2009 and from 11 January to 21 May 2010. The methods of the CVT are described in detail elsewhere (12). Briefly, it is a double blind, controlled, randomized, phase III study designed to evaluate the efficacy of the HPV16/18 vaccine (Cervarix; GlaxoSmithKline) for the prevention of HPV16/18 persistent infection and associated cervical lesions (CIN2<sup>+</sup>). The study enrolled 7,466 women residing in the province of Guanacaste and nearby areas of Puntarenas, Costa Rica, between 2004 and 2005. The main eligibility requirements for enrollment were as follows: age, 18 to 25 years; planned residence in the area for the 6 months following enrollment; good general health; and neither pregnant nor breast-feeding. The trial was approved by the U.S. National Cancer Institute IRB and the INCIENSA (Instituto Costarricense de Investigación y Enseñanza en Nutrición y Salud) IRB in Costa Rica, and all of the women provided informed consent.

The women were assigned to receive three doses of Cervarix or hepatitis A virus vaccine as a control vaccine. After the vaccination period, the women were monitored once a year for 4 years. Women with mild cervical abnormalities were monitored every 6 months, and those with high-grade cervical abnormalities or persistent low-grade abnormalities were referred for colposcopy.

At each study visit, one of five trained study nurses performed a pelvic exam on sexually experienced women. Clinician-collected exfoliated cells were obtained using the Cervex brush (Roberts) by inserting the central bristles into the endocervical canal until the external bristles bent against the ectocervix; while the pressure was maintained, the brush was rotated five times in a clockwise direction, and then the brush was removed and

rinsed vigorously in a vial with 20 ml of PreservCyt medium and kept at 15 to 25°C.

Our target number of participants for this substudy was 300, and 319 were included: 218 samples were collected from women attending a regular 1-year screening visit (prevalences of 41.6% for any HPV and 19.3% for any oncogenic HPV), and 101 samples were collected from women attending a colposcopy visit (prevalences of 62.4% for any HPV and 38.1% for any oncogenic HPV). For these participants, immediately following cervical cell collection (detailed above), instead of the Cervex brush being placed directly into the PreservCyt vial, it was first applied to the indicating FTA elute cartridge, and then the Cervex brush was vigorously rinsed in the PreservCyt vial, as indicated above. This was done to avoid using two Cervex brushes that could induce bleeding and obscure the colposcopy evaluation and also because, for use in a clinical or research setting, this would be the most plausible scenario. FTA cartridges were kept at room temperature, while the PreservCyt vial was kept in a cooler at 18 to 25°C. At the end of the day, both samples were sent to a local repository.

According to routine standard trial procedures, within 1 week of collection, two 0.5-ml aliquots for HPV PCR testing were prepared from the PreservCyt sample and frozen in liquid nitrogen (−150°C). The remaining PreservCyt sample was used to produce a cytology slide, and the residual volume was used for HPV testing by HC2 for all women attending regular screening visits and for women attending colposcopy visits only if it was considered necessary to define follow-up or treatment. FTA cartridges were stored in a box at room temperature until they were packed in individual bags and sent to the DDL Diagnostic Laboratory (Rijswijk, Netherlands) by courier for PCR HPV testing.

**DNA isolation from PreservCyt specimen.** Total DNA was isolated from 200 µl of one of the 0.5-ml PreservCyt aliquots by using the MagNA Pure LC isolation procedure (Roche Diagnostics GmbH/Roche Applied Science, Mannheim, Germany) and a total nucleic acid isolation kit (Roche Diagnostics GmbH/Roche Molecular Biochemicals, Mannheim Germany), as described by the manufacturer. DNA was eluted in 100 µl. Each DNA extraction run contained positive and negative controls to monitor the DNA isolation procedure.

**DNA isolation from the indicating FTA elute cartridge.** The FTA cartridges were punched four times using a perforator specifically designed for the FTA cartridges (a 3-mm Harris Uni-Core device; Whatman). For each FTA cartridge, a new sterile perforator was used to avoid any cross-contamination. The FTA elute matrix is chemically treated with proprietary reagents that lyse cells upon contact causing the release of nucleic acids. DNA was recovered from the FTA elute matrix through a simplified elution process using heat and water. The four punches were transferred into a 1.5-ml microfuge tube, and 1,500 µl of sterile water was added to the punches and immediately pulse vortexed three times for a total of 5 s. After centrifugation, the water was removed with a sterile fine tip pipette. An 70-µl portion of sterile water was added to the punches, and the tube was transferred to a heating block at 95°C for 30 min. At the end of the incubation period, the sample was removed from the heating block and pulse vortexed ~6 times. It was additionally centrifuged for 30 s, and the eluted DNA was transferred into a new microcentrifuge tube. Inhibitory components, such as hemoglobin, are retained in the FTA elute matrix. The eluted DNA was stored at −20°C. Finally, 10 µl of the eluate was used for PCR.

**HPV testing and genotyping.** Broad-spectrum PCR-based HPV DNA testing of DNA isolated from FTA cartridges and PreservCyt aliquots was performed at the DDL Diagnostic Laboratory by researchers blinded from the results of the comparative sample, the HC2, and the cytology, using the SPF<sub>10</sub> PCR primer system and a DNA enzyme immunoassay detection of amplimers (DEIA), followed by genotyping using the LiPA<sub>25</sub> (line probe assay, version 1 [Labo Biomedical Products, Rijswijk, Netherlands]) system as described previously (17, 18). LiPA<sub>25</sub> detects 25 HPV genotypes, including HPV16 and HPV18, but to ensure that HPV16 and HPV18 infections were not missed, all specimens that were SPF<sub>10</sub> PCR/

TABLE 1 Comparison of HPV detection results in samples collected in liquid-based medium and solid carrier devices among CVT participants<sup>a</sup>

Participant group	Results by collection method				Total	% Positive		Agreement		Kappa (95% CI)	P <sup>c</sup>
	PC <sup>+</sup> /FTA <sup>+</sup>	PC <sup>-</sup> /FTA <sup>+</sup>	PC <sup>+</sup> /FTA <sup>-</sup>	PC <sup>-</sup> /FTA <sup>-</sup>		FTA	PC	Overall	Among positives		
All visits											
Any HPV	141	33	5	140	319	54.5	45.8	0.88	0.79	0.76 (0.69–0.83)	<0.001
Carcinogenic HPV <sup>b</sup>	78	21	6	214	319	31.0	26.3	0.92	0.74	0.79 (0.72–0.87)	0.006
Colposcopy visits											
Any HPV	60	11	3	27	101	70.3	62.4	0.86	0.81	0.69 (0.54–0.84)	0.057
Carcinogenic HPV	37	10	5	49	101	46.5	41.6	0.85	0.71	0.70 (0.56–0.84)	0.302
Regular screening visits											
Any HPV	81	22	2	113	218	47.2	38.1	0.89	0.77	0.78 (0.69–0.86)	<0.001
Carcinogenic HPV	41	11	1	165	218	23.9	19.3	0.94	0.77	0.84 (0.75–0.93)	0.006

<sup>a</sup> PC, PreservCyt, liquid-based medium; FTA, solid carrier device.

<sup>b</sup> That is, HPV16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -66, and -68/73.

<sup>c</sup> Calculated by using the exact McNemar  $\chi^2$  test.

DEIA positive for HPV DNA but negative for HPV16 or HPV18 were also tested for the presence of HPV16 or HPV18 by using type-specific primers by the TS16 and TS18 DEIA system (29).

**Statistical analysis.** The HPV DNA results obtained from samples collected using both media were compared by calculating the percent overall agreement, the percent agreement between positives, the kappa value, and the weighted kappa value when applicable. The differences were evaluated for statistical significance using the exact McNemar  $\chi^2$  test. We compared detection of any HPV, any oncogenic HPV (HPV16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -66, and -68/73) and individual HPV genotypes.

We stratified the analysis by visit type (regular screening versus colposcopy visit) to evaluate the performance of the FTA in higher- and lower-HPV-prevalence settings. To evaluate the clinical significance of discordant results, we also conducted analyses comparing PC and FTA stratified by HC2 results. Given that among the 319 samples analyzed there were only 3 high-grade squamous intraepithelial lesion (HSIL) and 36 low-grade squamous intraepithelial lesion (LSIL) cytology results, we could not make definitive conclusions from the results stratified by cytology diagnosis. We also compared the number of HPV types detected in each sample to evaluate the performance of the FTA cartridge when multiple infections are present.

Finally, each sample was assigned to one of four cervical cancer risk categories according to the HPV types detected by each collection medium and compared. The four risk categories were defined as follows: (i) HPV16/18 positive; (ii) oncogenic HPV (oncogenic HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 66, and 68/73) positive but HPV16/18 negative; (iii) nononcogenic HPV (nononcogenic HPV types 6, 11, 34, 40, 42, 43, 44, 53, 54, 59, 70, 74, and uncharacterized) positive but negative for all oncogenic types, including uncharacterized types; and (iv) HPV negative. We conducted separate analyses including and excluding results from the HPV16/18 type-specific PCR with comparable results (data not shown).

To investigate whether the placement of the Cervex brush on the FTA cartridge before rinsing it in the PreservCyt medium could decrease the amount of cells placed in the PreservCyt medium, thereby affecting the cytology slide adequacy (which would have relevant implications for clinical management of study participants), we compared the percentages of cytologies considered inadequate for evaluation due to low cellularity among the 319 women included in this analysis to the those for the other participants from CVT whose samples were collected in the same time period and by the same group of nurses but not placed on the FTA ( $n = 262$ ).

Analyses were performed by the programming and statistical staff at the trial's data management center (Information Management Services,

Inc., Silver Spring, MD) under contract to the National Cancer Institute (NCI), supervised by the study investigators, and handled according to standard operating procedures.

## RESULTS

**Study population.** Cervical samples were collected in FTA and PC from 319 women 22 to 31 years of age (mean, 25.0 years) and included in the present analysis. A total of 29.5% of these women had completed elementary school, and 11.9% had attended a university. Most of the women were married or lived like married (58.9%), 33.2% were single, and the remaining women were widowed, separated, or divorced. A total of 47.3% had never been pregnant, while 42.0% had been pregnant once, and 10.7% had been pregnant two or more times.

**Any HPV and any oncogenic HPV detection.** Detection of any HPV type and any oncogenic HPV type by the SPF<sub>10</sub> LiPA<sub>25</sub> in the samples collected using the two different transport media among the 319 samples were compared (Table 1). The overall agreements were 0.88 for any HPV and 0.92 for any oncogenic HPV, the agreements between positives were 0.79 and 0.74, respectively, and the kappa values were 0.76 and 0.79. There was more HPV detection of any type in the samples collected in FTA medium than in the samples collected in PreservCyt (54.5% versus 45.8%, McNemar  $P < 0.001$ ); there was also more detection of oncogenic HPV types in FTA samples (31.0% versus 26.3%, McNemar  $P = 0.006$ ).

We stratified the analysis according to the visit type (regular screening visits versus colposcopy visits) and, although more oncogenic HPV was detected in FTA samples from women attending both types of visits, this difference was significant only among regular screening samples (McNemar  $P = 0.006$  for screening and McNemar  $P = 0.302$  for colposcopy visits). (Table 1).

Detection of any oncogenic HPV in samples collected in the two media was stratified by HC2 positivity (Table 2). Among HC2-positive samples, agreement between positives was 0.96 (kappa = 0.93), while among HC2 negative samples agreement between positives was 0.54 (kappa = 0.66). Samples collected in FTA were more likely to test positive for carcinogenic HPV than PreservCyt samples when HC2 was negative (16.2% versus 10.3%, respectively;  $P = 0.004$ ) but not when HC2 was positive (69.2% in both types of samples).

**TABLE 2** Comparison of carcinogenic HPV detection results in samples collected in liquid-based medium and solid carrier devices among CVT participants by HPV HC2 and cytology results<sup>a</sup>

Analysis	No. of samples				Total	% Positive		Agreement			<i>P</i> <sup>b</sup>
	PC <sup>+</sup> /FTA <sup>+</sup>	PC <sup>-</sup> /FTA <sup>+</sup>	PC <sup>+</sup> /FTA <sup>-</sup>	PC <sup>-</sup> /FTA <sup>-</sup>		FTA	PC	Overall	Among positives	Kappa (95% CI)	
HPV result by HC2											
Positive	44	1	1	19	65	69.2	69.2	0.97	0.96	0.93 (0.83–1.0)	1
Negative	19	14	2	169	204	16.2	10.3	0.92	0.54	0.66 (0.51–0.81)	0.004
Cytology result											
HSIL	3	0	0	0	3	100.0	100.0	1.0	1.0	—	—
LSIL	20	1	2	13	36	58.3	61.1	0.92	0.87	0.83 (0.64–1.0)	1
Normal	193	16	4	54	267	26.2	21.7	0.93	0.73	0.80 (0.71–0.88)	0.012

<sup>a</sup> The carcinogenic HPV types included HPV16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -66, and -68/73. PC, PreservCyt, liquid-based medium; FTA, solid carrier device; HC2, HPV Hybrid Capture 2 test.

<sup>b</sup> Calculated by using the exact McNemar  $\chi^2$  test.

Among the three women with HSIL, the concordance of the FTA and the PreservCyt results was 1.0; one woman was positive for HPV16, one was positive for HPV52, and one was positive for HPV58. Among the 36 women with LSIL (including ASC\_US HPV<sup>+</sup>) cytology results, the agreement between positives was 0.87 (kappa = 0.83) compared to 0.73 (kappa = 0.80) among women with normal cytology. FTA samples were more likely to test oncogenic HPV positive than PC samples (26.2 versus 21.7, respectively; *P* = 0.012) only among women with normal cytology results (Table 2).

**Type-specific HPV detection.** The study was powered for overall HPV detection, but we did not observe statistically significant differences between collection methods in the prevalence of detection for any HPV type tested. The prevalence of individual HPV types was higher in FTA samples for all HPV types except for HPV43, HPV58, and HPV68/73, for which the prevalence was identical, and HPV54 and HPV74, for which the prevalence in PreservCyt samples was higher. The prevalence of uncharacterized HPV types (LiPA<sub>25</sub> positives and DEIA negatives) was statistically higher in FTA samples (Table 3).

**TABLE 3** Comparison of results of individual HPV genotype detection in samples collected in liquid-based medium and solid carrier devices among CVT participants<sup>a</sup>

HPV type	No. of samples				% Positive		Agreement		Kappa (95% CI)	<i>P</i> <sup>b</sup>
	PC <sup>+</sup> /FTA <sup>+</sup>	PC <sup>-</sup> /FTA <sup>+</sup>	PC <sup>+</sup> /FTA <sup>-</sup>	PC <sup>-</sup> /FTA <sup>-</sup>	FTA	PC	Overall	Among positives		
6	4	2	0	313	1.9	1.3	0.99	0.67	0.80 (0.52–1.0)	0.500
11	2	1	0	316	0.9	0.6	1.00	0.67	0.80 (0.41–1.0)	1.000
16	17	4	1	297	6.6	5.6	0.98	0.77	0.86 (0.75–0.98)	0.375
18	4	1	1	313	1.6	1.6	0.99	0.67	0.80 (0.52–1.0)	1.000
31	5	4	2	308	2.8	2.2	0.98	0.45	0.62 (0.33–0.90)	0.686
33	0	2	1	316	0.6	0.3	0.99	0.00	0.00 (–0.01–0.00)	1.000
34	1	2	1	315	0.9	0.6	0.99	0.25	0.40 (–0.15–0.94)	1.000
35	6	2	0	311	2.5	1.9	0.99	0.75	0.85 (0.65–1.0)	0.500
39	4	3	1	311	2.2	1.6	0.99	0.50	0.66 (0.35–0.97)	0.625
40	2	2	1	314	1.3	0.9	0.99	0.40	0.57 (0.13–1.0)	1.000
42	1	1	0	317	0.6	0.3	1.00	0.50	0.67 (0.05–1.0)	1.000
43	3	1	1	314	1.3	1.3	0.99	0.60	0.75 (0.41–1.0)	1.000
44	4	4	0	311	2.5	1.3	0.99	0.50	0.66 (0.35–0.97)	0.125
45	1	2	1	315	0.9	0.6	0.99	0.25	0.40 (–0.15–0.94)	1.000
51	12	8	3	296	6.3	4.7	0.97	0.52	0.67 (0.48–0.85)	0.227
52	17	7	4	291	7.5	6.6	0.97	0.61	0.74 (0.59–0.86)	0.549
53	10	2	0	307	3.8	3.1	0.99	0.83	0.91 (0.78–1.0)	0.500
54	3	3	4	309	1.9	2.2	0.98	0.30	0.45 (0.11–0.79)	1.000
56	8	3	0	308	3.4	2.5	0.99	0.73	0.84 (0.66–1.0)	0.250
58	7	1	1	310	2.5	2.5	0.99	0.78	0.87 (0.70–1.0)	1.000
59	5	3	1	310	2.5	1.9	0.99	0.56	0.71 (0.44–0.98)	0.625
66	13	5	2	299	5.6	4.7	0.98	0.65	0.78 (0.62–0.94)	0.453
68/73	7	5	5	302	3.8	3.8	0.97	0.41	0.57 (0.33–0.81)	1.000
70	7	4	0	308	3.4	2.2	0.99	0.64	0.77 (0.56–0.99)	0.125
74	7	1	2	309	2.5	2.8	0.99	0.70	0.82 (0.62–1.0)	1.000
UNC <sup>c</sup>	23	18	7	271	12.9	9.4	0.92	0.48	0.61 (0.46–0.75)	0.043

<sup>a</sup> PC, PreservCyt, liquid-based medium; FTA, solid carrier device.

<sup>b</sup> Calculated by using the exact McNemar  $\chi^2$  test.

<sup>c</sup> UNC, uncharacterized, SPF10 positive but LiPA negative.

**TABLE 4** Comparison of number of oncogenic HPV types detected in samples collected in liquid-based medium and solid carrier devices among CVT participants<sup>a</sup>

No. of oncogenic HPV types detected in PC samples	No. of oncogenic HPV types detected in FTA samples				Total
	0	1	2	3+	
0	214	17	4	0	235
1	4	50	7	1	62
2	1	3	9	2	15
3+	1	0	0	6	7
Total	220	70	20	9	319

<sup>a</sup> The carcinogenic HPV types included HPV16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -66, and -68/73. The overall agreement was 0.87, the agreement between positives in both media was 0.62, and the weighted kappa (95% CI) was 0.75 (0.68 to 0.83).

**Number of oncogenic HPV types detected.** The agreement between positives between the two collection media for the number of oncogenic HPV types detected in each sample was 0.62, with a weighted kappa of 0.75 (95% confidence interval [95% CI] = 0.68 to 0.83) (Table 4). Detection of two or more oncogenic HPV types in one sample was more common in samples collected in FTA than samples collected in PreservCyt (9.1% versus 6.9%).

**HPV detection by risk categories.** We categorized the results according to the risk (i.e., HPV16/18, oncogenic other than HPV16/18, nononcogenic, and HPV negative); the agreement between positives was 0.71, and the weighted kappa was 0.79 (95% CI = 0.73 to 0.85). Samples collected in FTA tended to be categorized in higher-risk categories than samples collected in PreservCyt. Thus, 8.2 and 22.9% of the FTA samples were classified as HPV16/18 and other oncogenic HPVs, respectively, whereas 7.2 and 19.1% of the PreservCyt samples were classified in these categories ( $P < 0.001$  exact-symmetry  $\chi^2$  test) (Table 5). When we examined only samples positive by both collection media, there was no difference in the proportion of samples assigned to the HPV16/18 positive or the other oncogenic HPV categories (16.3 and 44.0%, respectively, for FTA versus 16.3 and 41.8% for liquid-based) ( $P = 0.82$ ) (data not shown in the tables).

We stratified the analysis by type of visit (regular screening visits versus colposcopy visits), and the pattern was similar, while the agreement and the kappa were higher at regular visits (the agreement between positives was 0.73 and the weighted kappa was 0.80 for regular visits versus 0.68 and 0.73, respectively, for colposcopy visits). Although more samples collected in FTA were

classified in the higher-risk categories than samples collected in PreservCyt for both types of visits, the difference was statistically significant only in specimens from regular screening visits ( $P < 0.001$  versus 0.17; data not shown).

**Unsatisfactory cytologies.** We evaluated the proportions of inadequate cytologies by visit type. The difference between the two collection media was higher for colposcopy visits than for regular visits, but none of them was statistically significant (i.e., the percentage of inadequate cytologies at regular visits was 1.8% when FTA collection preceded the liquid-based cytology (LBC) and 1.4% if no FTA collection was performed [ $P = 0.77$ ], and the percentage of inadequate cytologies at colposcopy visits was 8.9% for FTA samples and 3.5 for liquid-based samples [ $P = 0.13$ ]).

## DISCUSSION

We compared HPV detection from clinician-collected cervical cells placed in a new solid carrier device (i.e., the indicating FTA elute cartridge) to that observed in clinician-collected cervical cells placed in a conventional liquid-based medium (PreservCyt). The agreement for HPV detection using FTA and PC was excellent and was observed in both a lower-risk screening population and a higher-risk colposcopy group. Our findings suggest that almost no infections would be missed by using the FTA instead of the conventional liquid-based medium and indicate that the use of this simplified and less-expensive solid-based carrier (very important characteristics for developing countries) for HPV testing might be practical in the future without the need to sacrifice HPV detection sensitivity. An added advantage of using the solid-phase FTA device over existing liquid-based media is the simplified DNA extraction (20, 30).

Our results are consistent with those from smaller studies ( $n < 100$ ) conducted in Nordic countries that observed a high overall agreement for PCR HPV detection and genotyping between cervical cells collected using a liquid-based medium and specimens collected using the FTA cartridges both among self-collected and clinician-collected samples (5, 9, 10, 20).

We observed that specimens placed on FTA were more likely than those placed on PreservCyt to test positive for HPV (i.e., higher overall prevalence) and to be classified as positive for HPV16/18 or other carcinogenic HPV types. The fact that the differences observed were significant only among women attending regular screening visits and among women negative by HC2 but not among higher-risk women attending a colposcopy clinic and those with an HC2-positive result suggests that the FTA collection method might have reduced specificity for the detection of

**TABLE 5** Comparison of HPV test results in samples collected in liquid-based medium and solid carrier devices in CVT participants, categorized by HPV cancer risk

PC	FTA				Total no. (%)
	HPV16/18	Carcinogenic HPV types other than HPV16/18	Noncarcinogenic HPV	HPV <sup>-</sup>	
HPV16/18	21	2	0	0	23 (7.2)
Carcinogenic HPV types <sup>a</sup> other than HPV16/18	1	54	4	2	61 (19.1)
Noncarcinogenic HPV	1	6	52	3	62 (19.4)
HPV <sup>-</sup>	3	11	19	140	173 (54.2)
Total (%) <sup>b</sup>	26 (8.2)	73 (22.9)	75 (23.5)	145 (45.5)	319

<sup>a</sup> The carcinogenic HPV types included HPV16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -66, and -68/73.

<sup>b</sup> Overall agreement was 0.84, the agreement between positives in both media was 0.71, and the weighted kappa (95% CI) was 0.79 (0.73 to 0.85).

clinically relevant underlying disease. This might be due to an increased ability to detect low-viral-load infections using the FTA compared to the liquid-based collection method due to the fact that FTA samples are more concentrated, leading to a greater proportion of the total sample being tested, but this should be further explored.

One theoretical concern with the use of the FTA device is the need to place the cervical cells on the FTA cartridge prior to producing the sample for cytological evaluation (slide for conventional cytology or liquid-based medium); the possibility exists for reduced cellularity and increased inadequacy of the specimen collected for cytological evaluation. However, we did not observe evidence of reduced cytology specimen adequacy when specimen collection for cytology was preceded by FTA collection for HPV testing.

Our analysis is limited by our inability to compare the FTA and liquid-based collection methods within categories of cytological diagnoses and, more specifically, to evaluate the performance of FTA among women with an HSIL diagnosis. Furthermore, since our study was conducted among young adult women who have a high prevalence of HPV infection, it is uncertain whether our findings can be generalized to older populations with reduced rates of HPV infection.

In our study, the sample was applied to the FTA before being placed in the liquid-based medium. Therefore, we were unable to directly evaluate the possibility that the higher prevalence of HPV detected using the FTA method is partially explained by collection order. However, Gustavson et al. followed the same procedure (i.e., FTA first and dry-frozen cytobrush second) and showed that the number of copies of human single copy gene (housekeeping gene-HMBS) was much higher for the cytobrush sample than for the FTA cards (9), and in our study we did not observe evidence for reduced cytology specimen adequacy, which is consistent with there being sufficient material in the liquid-based sample. Therefore, it is unlikely that the order would be the explanation for the increased prevalence observed, but direct quantification of the DNA present in both samples (which was not conducted in the present study) would be needed to confirm this.

Finally, we evaluated use of the FTA specimen for HPV DNA detection using a single HPV typing system (SPF<sub>10</sub>/DEIA/LiPA<sub>25</sub>), although there is no reason to believe that this collection method would not perform equally well for other, well-characterized PCR-based HPV DNA detection systems.

In conclusion, our analysis demonstrates that the FTA cartridge is an acceptable device for the collection, storage, and transport of cervical cells for HPV testing, especially in developing countries. Use of this collection device could be considered for future epidemiological and clinical studies that require HPV DNA testing.

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