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Technical Advance

The Indicating FTA Elute Cartridge

A Solid Sample Carrier to Detect High-Risk HPV and High-Grade Cervical Lesions

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The clinically validated high-risk human papillomavirus (hrHPV) Hybrid Capture 2 (HC2) and GP5+/6+-PCR assays were analyzed on an Indicating FTA Elute cartridge (FTA cartridge). The FTA cartridge is a solid dry carrier that allows safe transport of cervical samples. FTA cartridge samples were compared with liquid-based samples for hrHPV and high-grade cervical intraepithelial neoplasia (CIN) detection. One cervical sample was collected in a liquid-based medium, and one was applied to the FTA cartridge. DNA was eluted directly from the FTA cartridge by a simple elution step. HC2 and GP5+/6+-PCR assays were performed on both the liquid-based and the FTA-eluted DNA of 88 women. Overall agreement between FTA and liquid-based samples for the presence of hrHPV was 90.9% with GP5+/6+-PCR and 77.3% with HC2. The sensitivity for high-grade CIN of hrHPV testing on the FTA cartridges was 84.6% with GP5+/6+-PCR and only 53.8% with HC2. By comparison, these sensitivities on liquid-based samples were 92.3% and 100% for GP5+/6+-PCR and HC2, respectively. Therefore, the FTA cartridge shows reasonably good overall agreement for hrHPV detection with liquid-based media when using GP5+/6+-PCR but not HC2 testing. Even with GP5+/6+-PCR, the FTA cartridge is not yet capable of detecting all high-grade CIN lesions. ***(J Mol Diagn 2011, 13:371–376; DOI: 10.1016/j.jmoldx.2011.02.003)***

Infection with human papillomavirus (HPV) is indicated as the causal role in cervical cancer development. Primary high-risk (hr) HPV screening appeared to be more sensitive than cytological features in detecting cervical intraepithelial neoplasia grade 2 or worse (CIN2+) and, therefore, displayed superior protection against cervical precancer and cancer.[1,2] Interestingly, material from cervicovaginal lavages or cervicovaginal brushes proved to be highly representative of cervical hrHPV status.[3–8] Moreover, analysis of cervicovaginal self-samples appears to be as reliable as physician-obtained samples for detecting cervical (pre)malignant disease after hrHPV analysis.[9,10]

Screening via self-samples obtained in the privacy of women's own homes is likely to result in better attendance than screening via samples obtained by physicians or other health care providers.[11,12] Therefore, cervicovaginal self-sampling is an attractive alternative for physician-obtained cervicovaginal material.[5,13]

Most previous studies[3,11,12,14–16] used cervical samples with liquid-based collection systems. In principle, the use of liquid-based self-samples has the impractical consequence that fluids may leak; in addition, special precautions have to be taken for transport. In case alcohol-containing preservation fluids are used, problems such as inflammability and harm to eyes and skin may occur. These problems may be circumvented when applying self-sampled specimens to a solid dry carrier, the Whatman Indicating FTA Elute cartridge (FTA cartridge). FTA cartridges are biohazard free because the sample is denaturized on application. These properties solve storage and transport problems often seen with liquid samples. More important, the FTA cartridge indicates dye changes from purple to white when a sample is applied, thereby confirming that women performed the procedure properly. This solid dry carrier might

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Disclosures: Rovers Medical Devices B.V. supplied the Rovers Cervex-Brush, and GE Healthcare provided the Whatman Indicating FTA Elute cartridges.

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be beneficial for specimens collected by nonphysicians in remote areas, which would need transportation to the laboratories. A proof-of-principle study was previously performed to assess the potential of HPV detection directly on eluted DNA from the FTA cartridge. The SPF_{10} Line Blot 25 assay was used, and 98% agreement with physician-obtained samples was found.[17] However, the SPF_{10} Line Blot 25 assay is sensitive in HPV detection, and it is unknown how clinically validated hrHPV assays with a lower analytical sensitivity would perform on FTA cartridge samples. In the current study, we evaluated the clinically validated Hybrid Capture 2 (HC2) and GP5+/6+-PCR[18] methods on physician-obtained cervical samples applied to the FTA cartridge for the detection of hrHPV and cervical premalignancies in women visiting a gynecological outpatient clinic.

Materials and Methods

Study Subjects

Between May 25 and December 18, 2009, 94 women were recruited at the Department of Obstetrics and Gynecology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands. The cohort consisted of women with different risk factors for HPV infection and CIN. All women visited the gynecologist at the outpatient department, and two cervical samples for liquid-based and cartridge collection were obtained, as specified later. The volume of liquid-based samples of six women was not sufficient to perform the two different hrHPV tests, in addition to liquid-based cytological testing. Therefore, these women were excluded. The remaining 88 patients constituted the study population.

Sample Collection

Two Cervex-Brushes (Rovers Medical Devices B.V., Oss, the Netherlands) were used to obtain cervical samples. The first brush was rinsed in a Thinprep vial (Cytyc Corp, Boxborough, MA) on which regular liquid-based cytological examination and HPV testing by HC2 and GP5+/6+-PCR were performed. The second brush was applied to the FTA cartridge (the Indicating FTA Elute micro card; Whatman/GE Healthcare, Kent, UK), as previously described.[17] Again, HC2 and GP5+/6+-PCR HPV testing was performed on the DNA eluted from these FTA cartridges.[17]

To assess the samples anonymously, all FTA cartridge and cervical liquid-based samples were labeled with a unique patient code before sending them to the laboratory. Histological results were retrieved from the medical records in case a biopsy specimen was obtained from the cervix during colposcopy or in case of surgery. Histological results were considered superior to cytological results.

Liquid-Based Samples

All Thinprep vials were used for regular cytological examination. Papanicolaou smear abnormalities were interpreted and classified by using the Bethesda system.

For the HC2 assay, 5 mL of liquid-based homogenized medium was used according to the manufacturer's instructions (Qiagen, Gaithersburg, MD).

For the hrHPV GP5+/6+-PCR, DNA was isolated from 500 μL of medium of the liquid-based cervicovaginal samples, using the MagNAPure LC Isolation station (Roche Diagnostics GmbH, Roche Applied Science, Mannheim, Germany). Nucleic acids were resuspended in a final volume of 50 μL; 10 μL was used for the GP5+/6+ assay, as previously described.[19,20]

FTA Cartridges

The Indicating FTA Elute matrix contains an indicating dye that changes from purple to white on application of a colorless sample, such as a cervicovaginal swab. The white parts on the FTA cartridges were punched using a sterilized perforator specifically designed for the FTA cartridges (3-mm Harris Uni-core device, Whatman). DNA was recovered from the FTA Elute matrix through a simplified elution process using heat and water. Inhibitory components, such as hemoglobin, are retained on the FTA Elute matrix.

For elution, four 3-mm punches from the matrix were transferred into a 2.0-mL Microfuge tube; and 2.0 mL of sterile H_2O was added to the punches and immediately pulse vortexed three times, for 5 seconds each. The H_2O was removed with a sterile fine-tipped pipette. Sterile H_2O, 75 μL, was added to the punches; and the tube was transferred to a heating block at 95°C for 30 minutes. At the end of the incubation period, the sample was removed from the heating block and pulse vortexed approximately 60 times. Subsequently, the tube was centrifuged for 30 seconds and the supernatant with eluted DNA was transferred to a new microcentrifuge tube. The eluted DNA was stored at -80°C for further use. Finally, according to protocol, 10 μL of the eluate was used for the GP5+/6+-PCR and 50 μL was used for the HC2.

HPV Detection by HC2 and GP5+/6+-PCR

Liquid-based homogenized medium, 5 mL, and a separate 50 μL of eluted DNA from the cartridges were used for the HC2, according to the manufacturer's instructions. The HC2 assay included a mixture of probes for the following HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. RNA/DNA hybrids were captured with antibodies, and a secondary signaling antibody generated a chemiluminescent signal that was ultimately expressed as relative light units per cutoff value (RLUs/CO), representing the ratio between the emission from a sample to the average of three positive controls (ie, 1 pg/mL of cloned HPV 16 DNA). Samples were considered HC2 positive in case of an RLU/CO value $\geq$1.0 (equivalent to a signal of 1 pg/mL HPV 16 DNA). Repeat testing is recommended for RLU/CO ratios between 1.0 and 2.5. Because priority was given to clinical testing, in the context of this study, material was limited. Therefore, the primary test result was considered definitive.

Separately, 10 μL of isolated DNA from the liquid-based specimens and 10 μL of DNA eluted from the FTA cartridges were used for HPV testing by the GP5+/6+-PCR assay. The GP5+/6+-PCR was performed using the

enzyme immunoassay readout system with a probe cocktail of 14 hrHPV types (ie, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), as previously described.[19,20] The standard CO of three times the mean OD value of the PCR-negative controls was used.[20,21]

Statistics

All data were analyzed using SPSS version 16.0 for Windows (SPSS Inc., Chicago, IL). Agreement was measured by absolute agreement and Cohen's κ statistics, a measure of the agreement between two methods that is in excess of that due to chance.

Results

The study group consisted of 88 women (median age, 37 years; SD, 10 years; range, 24 to 72 years). In 18 of the 88 cases, histological specimens of the cervix were obtained. A histological feature was indicated by a liquid-based cytological result of at least a low-grade squamous intraepithelial lesion (LSIL). However, in 10 LSIL cases, no biopsy specimens were obtained because of nonsuspicious colposcopy results. Of the 18 histological specimens, four showed normal tissue, one showed a low-grade CIN lesion (CIN1), and 13 showed a high-grade CIN lesion (CIN2-3). In total, the cervical samples were within normal limits in 46 cases (52.3%) and atypical

Table 1. Liquid-Based and FTA Cartridge Samples Analyzed with the Two Different hrHPV Assays (HC2 and GP5+/6+-PCR), according to Cytological/Histological Features

Sample no.	Liquid-based samples				FTA cartridge samples	
	Cytological feature	Histological feature	HC2 (RLU/CO)	GP5+/6+-PCR	HC2 (RLU/CO)	GP5+/6+-PCR
1	WNL	—	**1.23**	Neg	Neg	Neg
2	WNL	—	**1.23**	Pos	Neg	Pos
3	WNL	—	**2.91**	Neg	Neg	Neg
4	WNL	—	**2.93**	Pos	Neg	Neg
5	HSIL	Normal	**4.50**	Neg	Neg	Neg
6	WNL	—	**8.98**	Pos	Neg	Neg
7	WNL	—	**76.00**	Neg	Neg	Pos
8	WNL	—	**213.00**	Pos	**16.24**	Pos
9	LSIL	Normal	**783.58**	Pos	**2.61**	Pos
10	ASC-US	—	**1.09**	Neg	Neg	Pos
11	ASC-US	—	**1.45**	Neg	Neg	Pos
12	ASC-US	—	**2.99**	Pos	Neg	Pos
13	ASC-US	—	**4.36**	Neg	Neg	Neg
14	ASC-US	—	**95.00**	Neg	Neg	Neg
15	ASC-US	—	**460.00**	Pos	**19.41**	Pos
16	ASC-US	—	**543.00**	Pos	**218.43**	Pos
17	ASC-US	—	**635.00**	Pos	**34.83**	Pos
18	ASC-US	—	**737.00**	Pos	**140.70**	Pos
19	LSIL	—	Neg	Pos	Neg	Neg
20	LSIL	—	**2.24**	Pos	Neg	Neg
21	LSIL	—	**2.73**	Pos	**1.47**	Pos
22	LSIL	—	**13.59**	Neg	Neg	Neg
23	LSIL	—	**35.92**	Pos	**4.49**	Pos
24	LSIL	—	**196.00**	Pos	**6.38**	Pos
25	LSIL	—	**238.94**	Pos	**2.61**	Pos
26	LSIL	—	**318.00**	Pos	**185.67**	Pos
27	LSIL	—	**552.60**	Pos	**10.34**	Pos
28	LSIL	—	**771.27**	Pos	**1.37**	Pos
29	ASC-US	CIN1	**4.74**	Pos	**4.94**	Pos
30	LSIL	CIN2	**11.15**	Neg	Neg	Neg
31	LSIL/HSIL	CIN2	**13.40**	Pos	**1.73**	Pos
32	LSIL/HSIL	CIN2	**73.19**	Pos	**1.43**	Pos
33	ASC-US	CIN2	**1085.49**	Pos	Neg	Pos
34	HSIL	CIN3	**1.34**	Pos	Neg	Pos
35	HSIL	CIN3	**1.90**	Pos	Neg	Neg
36	HSIL	CIN3	**27.07**	Pos	**18.28**	Pos
37	HSIL	CIN3	**100.75**	Pos	**4.20**	Pos
38	ASC-US	CIN3	**108.72**	Pos	**3.04**	Pos
39	HSIL	CIN3	**294.65**	Pos	**8.12**	Pos
40	HSIL	CIN3	**422.95**	Pos	Neg	Pos
41	HSIL	CIN3	**467.28**	Pos	**8.85**	Pos
42	HSIL	CIN3	**794.08**	Pos	Neg	Pos
43–79	WNL	*	Neg	Neg	Neg	Neg
80–88	ASC-US		Neg	Neg	Neg	Neg

Bold text indicates positive result.
*Unspecified histological feature.
—, no histological specimen taken; HSIL, high-grade squamous intraepithelial lesion; Neg, negative result; Pos, positive result; WNL, within normal limits.

Table 2. Concordant and Discordant Results for Lesion Type, Sample Type, and Assay Used

Cytological/histological features	FTA versus LB (HC2)		FTA versus LB (GP5+/6+)		GP5+/6+ versus HC2 (LB)		GP5+/6+ versus HC2 (FTA)	
	c	d	c	d	c	d	c	d
Normal*	39	7	43	3	42	4	44	2
ASC-US*	13	5	16	2	14	4	15	3
LSIL*	8	2	8	2	8	2	10	0
CIN1†	1	0	1	0	1	0	1	0
CIN2†	2	2	4	0	3	1	3	1
CIN3†	5	4	8	1	9	0	6	3
Total	68	20	80	8	77	11	79	9
Concordance (%)	77.3		90.9		87.5		89.8	
κ (95% CI)	0.53 (0.37–0.69)		0.80 (0.67–0.93)		0.75 (0.61–0.88)		0.76 (0.61–0.90)	

*Cytological features, without subsequent histological features.
†Histological features after abnormal cytological features.
c, concordant; d, discordant; LB, liquid-based sample.

squamous cells of undetermined significance (ASC-US) in 18 cases (20.5%); in 24 cases (27.3%), the smear appeared to be LSIL or more severe.

GP5+/6+-PCR

Table 1 shows that in 32 (36.4%) of the 88 liquid-based samples, hrHPV was detected using the GP5+/6+-PCR. By comparison, 30 (34.1%) of the 88 FTA cartridge–eluted DNA samples were hrHPV positive with GP5+/6+-PCR. Of the 32 GP5+/6+-PCR–positive liquid-based samples, 27 (84.4%) showed concordant hrHPV-positive results on the eluted DNA from the corresponding FTA cartridge samples. Five women had GP5+/6+-PCR–positive liquid-based samples but negative FTA cartridge samples. Two of these five women had normal cytological results, two had LSIL cytological results, and one had a histological CIN3 lesion. In three women, hrHPV was detected on the eluted DNA from the cartridge but was negative on the liquid samples with GP5+/6+-PCR. One of these women had a normal cytological result, and the other two women had ASC-US. In the total group of 88 samples, 80 FTA cartridge samples showed concordant hrHPV test results with the liquid-based samples [concordance, 90.9%; κ, 0.80; 95% confidence interval (CI), 0.67 to 0.93] when the GP5+/6+-PCR was used for detection (Table 2).

There were 13 histologically confirmed high-grade CIN lesions. The liquid-based samples of 12 of these 13 lesions were hrHPV positive with GP5+/6+-PCR (sensitivity, 92.3%). The FTA cartridges showed 11 hrHPV-positive samples with GP5+/6+-PCR (sensitivity, 84.6%).

HC2 Assay

By using the HC2 assay for the detection of hrHPV, 41 (46.6%) of 88 liquid-based samples were positive (Table 1). These included all samples with high-grade CIN (sensitivity, 100%). Of the positive samples, only 21 were also positive with HC2 using the eluted DNA from the FTA cartridge samples. Of the 67 women with a negative FTA cartridge sample, 54 had a normal or ASC-US cytological result and four had a normal histological result. However, of 13 HC2 hrHPV liquid-based–positive women with histologically confirmed high-grade CIN lesions, six were missed using the HC2 on eluted DNA (sensitivity, 53.8%). The RLU of the FTA cartridge samples detected with four punches was low (mean, 33.1; SD, 63.9) compared with the corresponding RLU of the liquid-based samples (mean, 225.3; SD, 296.7). Indeed, 13 of the 20 hrHPV-positive liquid-based samples with a negative result on the FTA cartridge samples had a low RLU (<10).

In 68 of the total 88 samples, concordant hrHPV HC2 results were found between the liquid-based samples and the FTA cartridge samples (concordance, 77.3%; κ, 0.53; 95% CI, 0.37 to 0.69) (Table 2).

GP5+/6+-PCR versus HC2

Only one liquid-based sample was negative by HC2 but positive by GP5+/6+-PCR. This was an LSIL sample. Ten samples were positive with HC2 but negative with the GP5+/6+-PCR. Most (8 of 10) of these HC2-positive but GP5+/6+-PCR–negative liquid-based samples were within the normal cytological or histological group ($n = 4$) or ASC-US ($n = 4$). One high-grade CIN lesion (ie, CIN2), positive by HC2 on liquid, was negative by GP5+/6+-PCR on liquid. Absolute agreement between the two hrHPV test results was 87.5% (κ, 0.75; 95% CI, 0.61 to 0.88) for the liquid-based samples (Table 2).

All FTA cartridge samples that were positive with HC2 were also positive with the GP5+/6+-PCR. Nine FTA cartridge samples were negative with HC2 but positive with GP5+/6+-PCR. These comprised samples that were cytologically classified as normal ($n = 2$) or ASC-US ($n = 3$) or histologically classified as CIN2 ($n = 1$) or CIN3 ($n = 3$). Absolute agreement between the two hrHPV test results was 89.8% (κ, 0.76; 95% CI, 0.61 to 0.90) for the FTA cartridge samples (Table 2).

Tables 1 and 2 show a summary of the hrHPV results of the HC2 and GP5+/6+-PCR tests of both the liquid-based and the FTA cartridge samples, as well as the concordances.

Discussion

We aimed to test whether the clinically validated HC2 and GP5+/6+-PCR systems could be performed on the FTA cartridge material to detect high-grade CIN lesions. We found a reasonably good concordance in hrHPV detection between liquid-based and FTA cartridge systems when using the GP5+/6+-PCR. This concordance was much lower using HC2. Moreover, the sensitivity of hrHPV detection on the FTA cartridge for high-grade CIN lesions was 84.6% with GP5+/6+-PCR but only 53.8% with HC2.

Although more liquid-based samples were hrHPV positive with HC2 than with GP5+/6+-PCR (41 versus 32), only 21 of the 41 FTA cartridge samples were hrHPV HC2 positive. A possible explanation for this is that the amount of DNA eluted from the punches is insufficient to allow reliable HC2 testing, a method that does not use target amplification. Optimization of the processing steps (eg, by increasing the number of punches) may improve the HC2 outcome. However, increasing the number of punches is labor intensive and the H_2O volume used needs to be increased equivalently. In addition, we used the accepted RLU/CO value of 1.0 for signifying a positive HPV HC2 test result. This is arbitrary because these values were developed and validated in conjunction with liquid-based tests. It might be possible that values <1.0 RLU/CO represent positive test results when other media and different amounts of DNA are used. Moreover, results with RLU/CO values between 1.0 and 2.5 are not repeated within this study, as recommended according to protocol. Because priority was given to clinical tests, there was limited availability of sampled material. These samples could have been negative with repeat testing.

Based on the results so far, HC2 might not be the preferred method for hrHPV detection using FTA cartridges. To obtain sufficient (clinical) sensitivity, amplification-based methods might be more suitable. Results for the FTA cartridge with the sun protection factor 10 line probe assay PCR were previously reported.[17] With an overall agreement for hrHPV between the FTA cartridges and the liquid-based samples of 98% (κ, 0.94), the sun protection factor 10 line probe assay proved to be a highly reliable method for hrHPV testing on the FTA cartridges. In addition, Gustavsson et al[22] reported an agreement in hrHPV positivity between the Cytobrush and FTA samples of 94% (κ, 0.88; 95% CI, 0.748 to 1), again by using a real-time PCR-based assay. Although the results of the clinically validated GP5+/6+-PCR in this study are promising, they do not completely confirm previous findings on PCR-based hrHPV testing on FTA cartridges.

Because the sensitivity of detecting hrHPV and high-grade cervical lesions on the FTA cartridge is still not sufficient, there are aspects that need further consideration. Transferring a sample collected with a sampling device onto the surface of a solid sample carrier, such as the FTA cartridge, might cause the same problems that plagued the conventional Papanicolaou test (ie, not all cells collected are transferred, but they remain on the sampling device). This may result in a nonrepresentative sample. Moreover, in case of high-grade CIN, the HPV copy number per cell tends to be lower as the HPV tends to be integrated. Especially then, showing that there is DNA in the sample does not necessarily mean that the HPV-infected cells are transferred to the FTA cartridge. Previous studies have shown that cells from dysplastic cervical lesions are more likely to show aberrant expression of adhesion molecules and might fail to exfoliate. Similarly, such cells might be relatively less likely to be transferred to a solid substrate, like the FTA cartridge.

Given the cross-sectional nature of this study, only 18 women had a histological specimen. Women who do not have colposcopically detectable lesions, regardless of whether they are hrHPV positive, are not usually subjected to blind biopsies. The biopsy specimens would be highly unlikely to show significant pathological features.

Furthermore, only physician-obtained cervical samples were used in this study to enable an optimal comparison between liquid-based and FTA cartridge samples. The aim of this study was to compare the use of two collection methods (ie, FTA cartridge versus liquid) and to avoid any influence from sampling different sides. Therefore, only physician-obtained cervical samples were analyzed. For self-sampling, vaginal, rather than cervical, samples are brushed. Consequently, no direct applicability of this study to self-sampling is legitimate. In addition, the fact that the two Cervex-Brushes were obtained consecutively because of diagnostic reasons might have induced a bias against the FTA cartridge method. The second brushes were used for the FTA cartridges. Most of the relevant material might already have been sampled by the first brush.

This study concerns a population of women who visited the gynecological outpatient department and consequently involved an hrHPV group. Therefore, to consider the use of the FTA cartridge in a screening population, a thorough analysis of the FTA cartridge in such a population is necessary.

Nevertheless, our data have shown that, in the context of GP5+/6+-PCR, but not HC2 testing, the FTA cartridge holds promise as a collector of cervical specimens for screening. However, an ultrahighly or a highly sensitive method for HPV detection, such as PCR-based HPV DNA testing, is required. The clinical implication of those tests must be further assessed. The sensitivity is still not equal to that of hrHPV testing by HC2 on Thinprep vials; therefore, further optimization is recommended.

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