RESEARCH ARTICLE

Clinical Evaluation of Human Papillomavirus Detection by careHPV™ Test on Physician-Samples and Self-Samples using The Indicating FTA Elute® Card

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Abstract

Objective: To make the clinical evaluation of a solid-state human papillomavirus (HPV) sampling medium in combination with an economical HPV testing method (careHPV™) for cervical cancer screening. Methods: 396 women aged 25-65 years were enrolled for cervical cancer screening, and four samples were collected. Two samples were collected by woman themselves, among which one was stored in DCM preservative solution (called "liquid sample") and the other was applied on the Whatman Indicating FTA Elute® card (FTA card). Another two samples were collected by physician and stored in DCM preservative solution and FTA card, respectively. All the samples were detected by careHPV™ test. All the women were administered a colposcopy examination, and biopsies were taken for pathological confirmation if necessary. Results: FTA card demonstrated a comparable sensitivity of detecting high grade Cervical Intraepithelial Neoplasia (CIN) with the liquid sample carrier for self and physician-sampling, but showed a higher specificity than that of liquid sample carrier for self-sampling (FTA vs Liquid: 79.0% vs 71.6%, *p*=0.02). Generally, the FTA card had a comparable accuracy with that of Liquid-based medium by different sampling operators, with an area under the curve of 0.807 for physician &FTA, 0.781 for physician &Liquid, 0.728 for self & FTA, and 0.733 for self &Liquid (*p*>0.05). Conclusions: FTA card is a promising sample carrier for cervical cancer screening. With appropriate education programmes and further optimization of the experimental workflow, FTA card based self-collection in combination with centralized careHPV™ testing can help expand the coverage of cervical cancer screening in low-resource areas.

Keywords: Whatman Indicating FTA Elute® card - cervical cancer screening - self-sampling - careHPV™ test

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Introduction

Cervical cancer, the most widely screened cancer, has a long natural history with slow progressing precancerous lesions such as cervical intraepithelial neoplasia grade 2 and 3 (CIN 2 and CIN 3) and adenocarcinoma in-situ caused by persistent infection with the oncogenic types of human papillomaviruses (HPV). The precursor lesions may progress to invasive cervical cancer over a period of 5-15 years. Screening tests can identify women with cervical intraepithelial neoplasia as well as early invasive cancer, if provided with quality assurance and by well-trained providers.

It has been shown that Pap smear screening at the population level every three to five years can reduce cervical cancer incidence up to 80% in several developed countries (IARC, 2005). The challenges and resources required in introducing cytology screening and its sub-optimal performance in low- and middle-income countries have prompted evaluation of alternative screening methods such as visual inspection with acetic acid (VIA) and HPV testing which have been found to be effective in preventing cervical cancer (Sankaranarayanan et al., 2007; Arbyn et al., 2012; Ronco et al., 2014). In December of 2013, WHO released the latest guidelines for screening and treatment of precancerous lesions for cervical cancer, and recommended using HPV testing as the primary screening method for cervical cancer in areas with enough resources.

China, a developing country without enough qualified gynecologists and cytologists, the objective HPV testing is considered to be the most expeditious and effective way to expand the coverage of cervical cancer screening. However, most of the current HPV testing methods utilize a liquid-based sample carrier and expensive, which have limitations of large sample volume, risk of leakage, and restriction of storage and transportation, especially in remote rural areas.

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This study sought to make a clinical evaluation of a solid-state HPV sampling medium (Whatman Indicating FTA® Elute card) in combination with an economical HPV testing method (*care*HPV™) for cervical cancer screening. The effect and consistency of physician- and self-collected samples by liquid and solid sample carriers were explored in this project as well.

Materials and Methods

Study Population

This was a population-based study conducted in JiangXi of China. The sample pool was from the enrolled women of a large project called "LCMCCSS, " which was collaboration between the Cancer Institute, Chinese Academy of Medical Sciences (CICAMS) and the Program for Appropriate Technology in Health (PATH). Eligible women were ages 25-65 years, not pregnant and did not have a history of diagnosed CIN, cervical cancer, or hysterectomy. All participants were screened with liquid-based (DCM preservative solution, Qiagen Inc., Gaithersburg, MD, USA) Hybrid capture 2 (HC2, Qiagen Inc., Gaithersburg, MD, USA) and *care*HPV™ test (Qiagen Inc., Gaithersburg, MD, USA). VIA was also utilized in all participants. The "called back population" was instructed to return for colposcopy examination after one week of the primary screening and required to meet one of the following criteria: (a) women with any positive results on HC2, *care*HPV™, or VIA detection; (b) women randomly selected with negative results for all the tests.

Sample Collection

From May to June of 2012, the study was conducted among 396 women who were referral to colposcopy examination in TongGu Women and Children's hospital. Informed consent was obtained from each subject, and then four samples were collected. Specifically, two samples were collected by women themselves using Conical Cervical Sampler (Qiagen Inc., Gaithersburg, MD, USA), among which one was stored in DCM preservative solution (called "liquid sample") and the other was applied on the Whatman Indicating FTA Elute® card (GE Healthcare, Kent, UK, called FTA card and "solid sample" in this study). Secondly, another two samples were collected by physician and stored in DCM preservative solution and FTA card, respectively. The FTA card contains an indicating dye that changes from purple to white upon application of the cervicovaginal sample. All the samples were stored at room temperature and analyzed in the Central Lab of CICAMS. All the women were administered a colposcopy examination, and biopsies were taken if visible lesions were identified. The pathologist in CICAMS was responsible for result reports and high grade CIN case confirmation (see Figure 1). Ethics approval was obtained from the Institutional Review Board of the CICAMS.

Sample Detection

For the liquid sample, 50 microliters of DCM preservative solution was used according to the manufacturer's instructions (Qiagen). The procedures of FTA card-based sample detection by *care*HPV™ test were originally explored in the central Lab of CICAMS after several pilot studies. Firstly, the FTA cards were punched for six disks using a specifically designed sterilized perforator (3-mm Harris Uni-Core device; Whatman). Afterwards, the punched disks were 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. Inhibitory components, such as hemoglobin, were retained on the FTA elute matrix.

The six disks were transferred into 1.5-ml microfuge Tube. Five hundred microliters of sterile water was subsequently added to the tube, and immediately pulse vortexed for 15 seconds, and the water was removed with a sterile fine-tip pipette. This process was performed twice. Sixty microliters of DCM preservative solution and ninety microliters of DEPC water were added to the tube. Then the tube was additionally centrifuged for 30 seconds and transferred to a heating block at 95°C for 30 minutes. During the incubation period, each tube was centrifuged for 30 seconds every 10 minutes to minimize condensation. At the end of heating process, the tube was pulse vortexed approximately 30 seconds and centrifuged for 30 seconds. The eluted DNAs were placed into new microcentrifuge tubes and stored at -80°C until analysis. Finally, 50 microliters of the eluate were used for *care*HPV™ according to the manufacturer's instructions.

Definition of positive result: *care*HPV™ test is signal amplification assay that combine antibody capture of HPV DNA and RNA probe hybrids and chemiluminescent signal detection which provide the ratio of relative light units to standard positive control (RLU/PC) as the semiquantitative measurement of viral load. The cutoff point of 1.0 RLU/PC (approximately equal to 1.0 pg of DNA/ml) was used for the positive definition and colposcopy referrals.

Statistical Analysis

Demographic information such as age, marriage, educational level, occupation, and sexual-related status, was quantified. The accuracy of four combinations of different sampling mediums and operators were presented as sensitivity, specificity, positive and negative predictive values, and Receiver Operating Characteristic (ROC) curves. Proportion between combinations was compared using the Chi-square test. SAS 9.2 was used to analyze data. Statistical significance was assessed by two-tailed tests with an α level of 0.05.

Results

Demographic information

A total of 396 subjects aged 25-65 years were recruited with a mean age of 43.7±8.9 years. All of the subjects were married and the average ages of initiation of sexual activity and menarche were 20.2±2.2 years and 15.5±1.7 yesrs, respectively. Fifty-seven percent of the women (56.5%) reported primary school as their highest educational level, 33% reported middle school, and only 10.5% reported high school or college education. The majority were farmers

Table 1. Demographic Information of the Participants

Variable	Value
Age (Mean±SD[a])	43.7±8.9
Age of Sexual Debut (Mean±SD[a])	20.2±2.2
Age of Menarche (Mean±SD[a])	15.5±1.7
Marriage (YES, %)	100
Highest Education Level (%)	
Primary School	56.5
Middle School	33
High School and Above	10.5
Occupation, (%)	
Farmer	39.8
Housewife	33.7
Other	26.5
Smoking (NO, %)	99.3
Drinking (NO, %)	86.4
Oral Contraceptive (YES, %)	1.9
Sexual Partner (n≥3, %)	3.1
No. of Pregnancy (%)	
≤3	62.7
>3	37.3
No. of Live Birth (%)	
≤3	85.4
>3	14.6

[a]SD, standard deviation and standard error of the mean

or housewives (73.5%), non-smokers (99.3%) and non-drinkers (86.4%). The majority (96.9%) of the women reported to have three or less sexual partners, and 62.7% had three or less pregnancies, 85.4% had three or less live births (Table 1).

Pathological confirmation and valid cases

Among 396 women, 39 were diagnosed as CIN Grade 1 (CIN1), 10 as CIN2, 2 as CIN3, and one case as High Grade Cervical Glandular Intraepithelial Neoplasia (HCGIN). Additionally, 17 samples were used for the pilot studies and one women didn't perform self-sampling during the study, resulting in a valid sample size of 379 and 378 for physician-sampling and self-sampling analysis, respectively (Figure 1).

Accuracy of different screening methods

VIA showed a high specificity (95.8%) for detecting CIN2 or worse (CIN2+) lesions, but a low sensitivity (7.7%). Only one of the thirteen CIN2+ cases was identified by VIA. *care*HPV™ test demonstrated a high sensitivity (66.7%-83.3%) and specificity (71.6%-79.0%) for detecting CIN2+ lesions. Specifically, the accuracies of detecting CIN2+ lesions for four combinations of two sampling mediums with different operators are shown in Table 2. The FTA card demonstrated a comparable sensitivity with the DCM preservative solution both for physician-sampling (FTA vs Liquid: 83.3.0% vs 83.3%, $p>0.05$) and self-sampling (FTA vs Liquid: 66.7% vs 75.0%, $p>0.05$). Two sampling mediums demonstrated comparable specificity (FTA vs Liquid: 77.9% vs 73.0%, $p>0.05$) for the physician sampling, but the FTA card gained a higher specificity than the liquid sample carrier for self-sampling (FTA vs Liquid: 79.0% vs 71.6%, $p=0.02$).

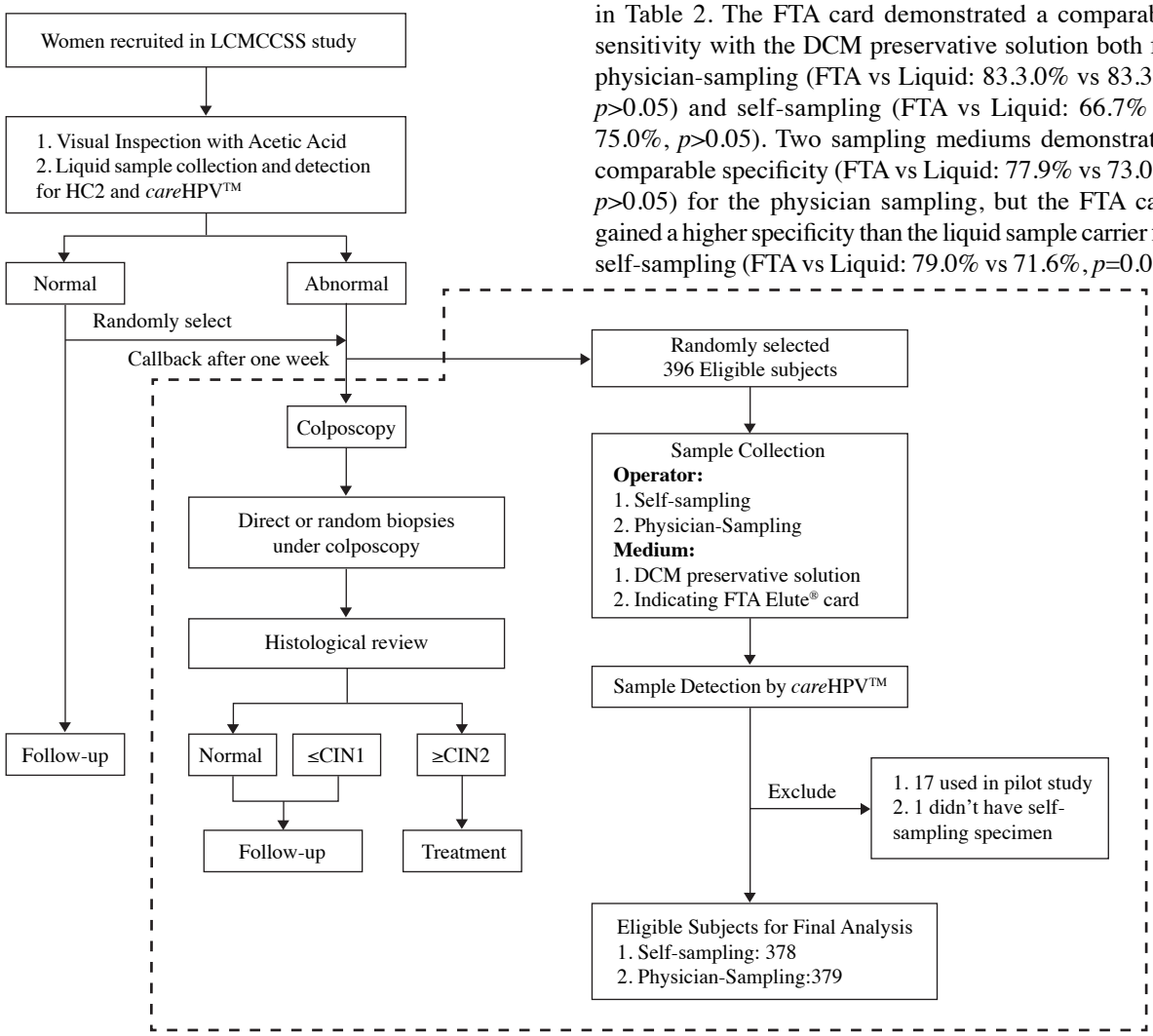

Figure 1. Study Flow Chart; CIN1, cervical intraepithelial neoplasia grade 1; CIN2, cervical intraepithelial neoplasia grade 2; HC2, Hybrid capture 2

Table 2. Accuracy of Detecting CIN2+ by *care*HPV™ Test for different Sampling Medium and Operators

Operator	Sampling Medium	Sample Size	Accuracy							
			Sensitivity		Specificity		Positive Predictive Value		Negative Predictive Value	
			% (n/N)	95% CI[a]	% (n/N)	95% CI[a]	% (n/N)	95% CI[a]	% (n/N)	95% CI[a]
Physician sampling	FTA[b]	379	83.3 (10/12)	62.2-100.0	77.9 (286/367)	73.7-82.1	11.0 (10/91)	4.6-17.4	99.3 (286/288)	98.3-100.0
	Liquid	379	83.3 (10/12)	62.2-100.0	73.0 (268/367)	68.5-77.5	9.2 (10/109)	3.8-14.6	99.3 (268/270)	98.3-100.0
Self sampling	FTA[b]	378	66.7 (8/12)	40.0-93.4	79.0 (289/366)	74.8-83.2	9.4 (8/85)	3.2-15.6	98.6 (289/293)	97.3-99.9
	Liquid	378	75.0 (9/12)	50.5-99.5	71.6 (262/366)	67.0-76.2	8.0 (9/113)	3.0-13.0	98.9 (262/265)	97.6-100.0

[a]95%CI, 95% confidence interval; [b]FTA, Whatman Indicating FTA Elute® card

Table 3. Comparison of Detective Results by *care*HPV™ Test for 13 CIN2+ Cases for Four Combinations of different Sampling Medium and Operators

ID No.	Pathological Result	*care*HPV™ Test Result (Rlu/PC)			
		Physician & liquid	Self & liquid	Physician & FTA	Self & FTA
447	CIN2	0.4*	28.1	17.1	15.4
445	CIN2	51.3	4.4	28.5	0.4*
444	CIN3	170	13.4	173.3	106.6
442	CIN2	117.9	24.7	140.9	56.4
448	CIN2	104.5	9.9	34	68.7
441	CIN2	150.8	126.2	37	72.2
446	CIN2	1.4	0.3*	0.6*	0.7*
443	CIN2	80.8	5	89.5	31.1
450	CIN2	100.7	12.6	61.3	6.6
240	HCGIN	0.3*	0.3*	0.8*	0.4*
265	CIN2	48.4	NA[a]	0.3*	0.3*
288	CIN3	20.7	1.1	3.7	0.6*
344	CIN2	2.7	0.5*	29.7	11.6

[a]without result, sample used in pilot study; *: CIN2+ case failed to be detected; Abbreviation: CIN2, cervical intraepithelial neoplasia grade 2; CIN3, cervical intraepithelial neoplasia grade 3; HCGIN, high grade cervical glandular intraepithelial neoplasia; FTA, Whatman Indicating FTA Elute® card; RLU/PC, the ratio of relative light units to standard positive control

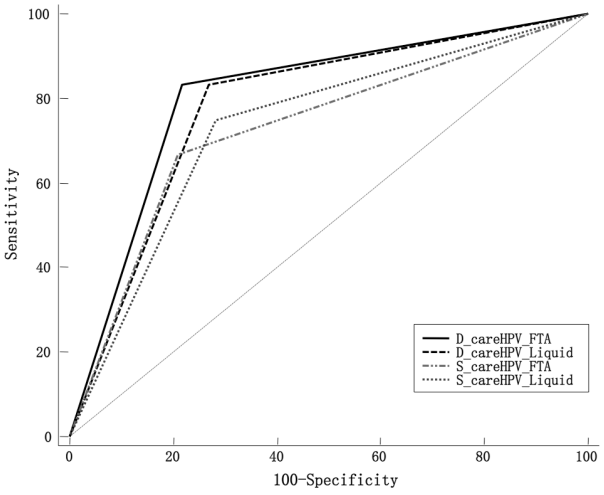

Figure 2. Receiver Operating Characteristic Curves of the Accuracy of Detecting CIN2+ Lesions by Four Different Combinations of *care*HPV™ test; AUC, area under the curve; CIN2+, cervical intraepithelial neoplasia grade 2 or worse; D_careHPV_FTA, physician-sampling & FTA sample; D_careHPV_Liquid, physician-sampling & liquid sample; FTA, Whatman Indicating FTA Elute® card; S_careHPV_FTA, self-sampling & FTA sample; S_careHPV_Liquid, self-sampling & liquid sample

Moreover, the FTA card showed comparable positive predictive value (PPV) and negative predictive value (NPV) both for physician and self-sampling. Detection results of individual analyses for 13 CIN2+ cases are shown in Table 3. The results showed that *care*HPV™ test failed to identify more CIN2+ cases by self-sampling than that of physician-sampling whether using FTA card (Self vs Physician: 5 vs 3) or DCM preservative solution (Self vs Physician: 3 vs 2) as the sample carrier.

We also calculated the overall percent agreement of four different combinations. Good overall percent agreement was found between FTA card and DCM preservative solution either for physician collected samples (83.6%) or women themselves collected samples (84.1%). The results also showed good overall percent agreement of 81.3% and 84.9% between different sampling operators for sample carrier of DCM preservative solution and FTA card, respectively.

ROC curve

To compare the validity of detecting CIN2+ lesions for four different combinations of sampling medium and operators, ROC curves were generated. According to area under the curve (AUC), the FTA card based sampling medium had a comparable accuracy with that of Liquid based medium. Specifically, the AUC as shown in Figure 2 for four different combinations were: physician &FTA, (AUC=0.81, 95% CI: 0.76-0.85), physician & Liquid, (AUC=0.78, 95% CI: 0.74-0.82), self & FTA, (AUC=0.73, 95% CI: 0.68-0.77), self & Liquid, (AUC=0.73, 95% CI: 0.69-0.78) No significance of AUC was found between different combinations (p>0.05).

Discussion

This study was the first to explore the clinical significance of FTA card-based sample carrier in combination with the *care*HPV™ test for precancerous cervical lesion detection. Recently, the FTA card has been actively explored in cervical cancer screening as an alternative for the current liquid-based sample carrier (Gustavsson et al., 2009; Lenselink et al., 2009; Gustavsson et al., 2011). It was promising that good agreement between liquid-based sample carrier and the solid FTA card were found for several PCR-based HPV testing methods, such as GP5+/6+ and SPF (10) PCR/DEIA/LiPA (25) (Gonzalez et al., 2012; Guan et al., 2013). However, PCR-based HPV detective methods are mostly expensive and without clinical significance for detecting precancerous cervical lesions. No study has explored the feasibility and accuracy of using FTA card as sample carrier in combination with a low-cost HPV testing method with clinical significance in area of cervical cancer screening.

*care*HPV™ is a newly-developed and promising

screening system targeting low- and middle-income countries, which can detect for a pool of 14 high-risk HPV genotypes. Previous studies of *care*HPV™ have shown sensitivity and specificity that approach that of HC2, a U.S. Food and Drug Administration-approved test (Qiao et al., 2008; Zhao et al., 2010). Moreover, the workflow of *care*HPV™ is similar but simpler than the HC2 test with a lower cost and simpler administration, and can be run by secondary school graduates without laboratory experience, using a training of the trainer model (Gage et al., 2012). In Dec of 2013, the latest WHO guidelines recommended using HPV testing as the primary screening method for cervical cancer in areas with enough resources. The low-cost, simple *care*HPV™ test is therefore recognized as a potential tool for future large scale cervical cancer screening projects.

However, the current *care*HPV™ test utilizes DCM preservative solution as the sample carrier, which has limitations of large sample volume, risk of leakage, and restriction of storage and transportation. Exploring for a solid alternative to the current liquid-base sampling medium would help resolve this problem and greatly increase the coverage of screening. This study found that using the FTA card as sample carrier could gain comparable accuracy with the conventional DCM preservative solution both for physician and women themselves collected samples, with the added benefit of simplicity of sample transportation and storage to this screening method. Moreover, FTA card demonstrated a high reliability between different sampling operators and a good agreement with the liquid sample carrier. Furthermore, previous studies demonstrated good performance of FTA card application in situations of humid tropical climate, which also provided this solid sample carrier a promising future for population-based cervical cancer screening in different geographic areas (Gustavsson et al., 2011; Phongsavan et al., 2012).

Previous literature showed that although self-collection and clinician collection were equally comfortable and convenient, the Chinese participants still preferred clinician collection because of lack of trust in the results of self-collection (Guan et al., 2012), but the Lao women preferred self-sampling (Yoshida et al., 2013). Promisingly, we found that FTA card gained comparable accuracy of detecting CIN2+ lesions between self-sampling and physician-sampling ($p>0.05$), and gained a higher specificity than the DCM preservative solution for self-collected samples. This provided an encouraging future for the self-sampling method in population-based screening project. However, it still should be noted that although the distribution of high-risk HPV genotypes was fairly equivalent across different genital sites, viral loads were largely variable. As shown in Table 3 and other study, in the CIN2+ cases, high-risk HPV viral load for cervix samples collected by physician were much higher than that collected by women themselves (Zhang et al., 2014). This may due to vaginal other than cervical samples are more easily to be collected by self-sampling, and the viral load of vagina samples was much lower than that of cervix samples. Moreover, previous study showed that the low-risk HPV positive rate was highest in lower vagina samples and lowest in cervix samples, but low-risk HPV seldom lead to CIN2+ lesions and were not detected by *care*HPV™ test (Zhang et al., 2014). Therefore, another study recommended raising the cutoff viral load of *care*HPV™ to 2.0 RLU/PC for physician-sampling and remaining 1.0 RLU/PC for self-sampling to gain a better tradeoff of sensitivity and specificity of this screening test (Kang et al., 2014). Future studies are required to validate this recommendation.

Since FTA card has demonstrated a high acceptability and a good accuracy for cervical cancer screening among Chinese women (Guan et al., 2012; Zhang et al., 2014), FTA card based self sample collection and centralized sample detection therefore is considered an acceptable potential method for cervical cancer screening. As dried material on a solid carrier is neither hazardous nor inflammable, applying genital self-samples on FTA card can solve storage and transportation problems encountered in low-resource areas. With implementation of education programmes about the validity of self-collection that target general population and further optimization of the experimental workflow, self-collection by the FTA card in combination with centralized *care*HPV™ test could be used for cervical cancer screening in low- and middle-income countries. We plan to develop a sample collection kit for cervical cancer screening, including operating instruction, sampling glove, brush, FTA card, label, and an envelope. Women can self-sample at home using the FTA card, and mail the sample to the regional central laboratory for sample analysis by the *care*HPV™ test. Doctors would only call back those with positive result for colposcopy examination. Consequently, limited healthcare resources could be centralized to high-risk populations and maximally increases the coverage of the current screening initiative with the lowest cost.

This study was the first to explore experimental workflows for the *care*HPV™ test by using the FTA card as sample carrier. It parallel compared the clinical accuracy of detecting CIN2+ lesions for different sampling operators (self vs. physician collection) and sampling medium (liquid vs FTA card), which would provide important scientific data for future investigations. This study has some limitations that must be addressed as well. First, we selected the referral population that was triaged for colposcopy, which may have a higher HPV infection rate than the general population. The sample size was also small, resulting in a smaller number of endpoints. Both may result in a relatively lower specificity than reports in previous literatures. Second, we didn't consider the sequence of sample collection, taking four samples with a short time interval might have had an impact on the results. Finally, there are several types of cervical cytological sampling brushes, but this study only used cervical sampler (Qiagen) for sample collection. Future comparisons of different cytological samplers need to be investigated to identify the most appropriate sampler for the best accuracy.

In conclusion, the FTA card is a promising sample carrier for cervical cancer screening. With appropriate education programmes to improve the validity of self-collection and further optimization of the experimental

workflow, FTA card based self-collection in combination with centralized *care*HPV™ testing can help expand the coverage of cervical cancer screening in low-resource areas.

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References

Arbyn M, Ronco G, Anttila A, et al (2012). Evidence regarding human papillomavirus testing in secondary prevention of cervical cancer. *Vaccine*, **30**, 88-99.

Gage JC, Ajenifuja KO, Wentzensen N, et al (2012). Effectiveness of a simple rapid human papillomavirus DNA test in rural Nigeria. *Int J Cancer*, **131**, 2903-9.

Gonzalez P, Cortes B, Quint W, et al (2012). Evaluation of the FTA carrier device for human papillomavirus testing in developing countries. *J Clin Microbiol*, **50**, 3870-6.

Guan Y, Castle PE, Wang S, et al (2012). A cross-sectional study on the acceptability of self-collection for HPV testing among women in rural China. *Sex Transm Infect*, **88**, 490-4.

Guan Y, Gravitt PE, Howard R, et al (2013). Agreement for HPV genotyping detection between self-collected specimens on a FTA cartridge and clinician-collected specimens. *J Virol Methods*, **189**, 167-71.

Gustavsson I, Lindell M, Wilander E, et al (2009). Use of FTA card for dry collection, transportation and storage of cervical cell specimen to detect high-risk HPV. *J Clin Virol*, **46**, 112-6.

Gustavsson I, Sanner K, Lindell M, et al (2011). Type-specific detection of high-risk human papillomavirus (HPV) in self-sampled cervicovaginal cells applied to FTA elute cartridge. *J Clin Virol*, **51**, 255-8.

IARC (2005). Cervix Cancer Screening IARC Handbooks of Cancer Prevention 10.

Kang LN, Jeronimo J, Qiao YL, et al (2014). Optimal positive cutoff points for careHPV testing of clinician- and self-collected specimens in primary cervical cancer screening: an analysis from rural China. *J Clin Microbiol*, **52**, 1954-61.

Lenselink CH, de Bie RP, van Hamont D, et al (2009). Detection and genotyping of human papillomavirus in self-obtained cervicovaginal samples by using the FTA cartridge: new possibilities for cervical cancer screening. *J Clin Microbiol*, **47**, 2564-70.

Phongsavan K, Gustavsson I, Marions L, et al (2012). Detection of human papillomavirus among women in Laos: feasibility of using filter paper card and prevalence of high-risk types. *Int J Gynecol Cancer*, **22**, 1398-406.

Qiao YL, Sellors JW, Eder PS, et al (2008). A new HPV-DNA test for cervical-cancer screening in developing regions: a cross-sectional study of clinical accuracy in rural China. *Lancet Oncol*, **9**, 929-36.

Ronco G, Dillner J, Elfstrom KM, et al (2014). Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. *Lancet*, **383**, 524-32.

Sankaranarayanan R, Esmy PO, Rajkumar R, et al (2007). Effect of visual screening on cervical cancer incidence and mortality in Tamil Nadu, India: a cluster-randomised trial. *Lancet*, **370**, 398-406.

Yoshida T, Nishijima Y, Hando K, et al (2013). Primary study on providing a basic system for uterine cervical screening in a developing country: analysis of acceptability of self-sampling in Lao PDR. *Asian Pac J Cancer Prev*, **14**, 3029-35.

Zhang SK, Ci PW, Velicer C, et al (2014). Comparison of HPV genotypes and viral load between different sites of genital tract: the significance for cervical cancer screening. *Cancer Epidemiol*, **38**, 168-73.

Zhao FH, Lin MJ, Chen F, et al (2010). Performance of high-risk human papillomavirus DNA testing as a primary screen for cervical cancer: a pooled analysis of individual patient data from 17 population-based studies from China. *Lancet Oncol*, **11**, 1160-71.