Étude comparative randomisée entre deux méthodes d'auto-prélèvement vaginal pour la recherche de papillomavirus humain (HPV) en milieu sec : bâtonnets secs VS cartouche FTA

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Thèse préparée sous la direction du Professeur Patrick Petignat

« ÉTUDE COMPARATIVE RANDOMISÉE ENTRE DEUX MÉTHODES D'AUTO-PRÉLÈVEMENT VAGINAL POUR LA RECHERCHE DE PAPILLOMAVIRUS HUMAIN (HPV) EN MILIEU SEC : BÂTONNETS SECS VS CARTOUCHE FTA »

Thèse
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par

Rosa Pinto Catarino

de

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Table des matières

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1. Introduction

Le cancer du col utérin reste un problème de santé publique important, en particulier dans les pays en développement, où plus de 80% des cancers du col sont diagnostiqués [1].

Cette thèse de doctorat a pour objet la détection du papillomavirus humain (HPV) pour la prévention du cancer du col utérin.

En premier lieu, nous allons parler du cancer cervical, du HPV et de la relation de causalité entre les deux. Puis, après avoir décrit les différentes méthodes pour la détection du HPV et son impact sur la santé publique, nous présenterons les résultats pertinents d'une étude qui a eu comme objectif d'évaluer la faisabilité de deux méthodes d'auto-prélèvement pour la détection du HPV. L'étude en version anglaise sera disponible dans son intégralité à la fin de la discussion. Dernièrement, on exposera la situation en Suisse en termes de prévalence du HPV et du cancer cervical ainsi que des méthodes de dépistage.

2. Le cancer du col de l'utérus

L'incidence du cancer du col utérin varie considérablement dans les différentes parties du monde. Il existe une grande différence entre les pays en développement et les pays développés, où les programmes de dépistage par cytologie ont montré une réduction de plus de 50% des cas de cancer cervical. Cependant, dans les pays à faibles ressources, où les programmes de dépistage cytologique sont difficiles à mettre en œuvre, l'incidence du cancer du col de l'utérus reste élevée.

Selon les dernières statistiques [2], le cancer cervical est le quatrième cancer le plus fréquent chez les femmes dans le monde (528'000 nouveaux cas par année) et le deuxième cancer le plus répandu dans les pays en développement (445'000 nouveaux cas par année). C'est une cause majeure de morbidité et de mortalité chez les femmes dans les pays en développement, causant 230'158 décès par an [2].

Le cancer du col se développe à partir des lésions précancéreuses. Ce procès peut durer 15 à 20 ans chez des femmes dotées d'un système immunitaire normal [3]. C'est un cancer qui n'est pas hormonodépendant et son incidence augmente progressivement jusqu'à 70 ans avec une incidence maximale à 48 ans. Son principal facteur de risque est l'infection au virus HPV de haut risque.

Dans les pays en développement, la majorité des cancers est détectée à un stade avancé, où le traitement n'est plus possible ou disponible. Une corrélation claire existe entre le taux de survie à 5 ans et le produit intérieur brut (PIB) : plus le PIB est bas, plus le taux de survie est faible [2].

3. Le papillomavirus humain

3.1 Présentation du papillomavirus humain

Les HPVs sont des virus à ADN appartenant à la famille des *Papillomaviridae*, qui sont capables d'infecter les humains par transmission directe via un contact cutané ou muqueux. Les lésions sont fréquentes et le plus souvent bénignes. Ces virus ont une capside icosaédrique à symétrie cubique. Leur génome est une molécule d'ADN bicaténaire circulaire d'environ 8000 paires de bases.

Plus de 170 types de HPV ont été identifiés à ce jour. Selon la classification proposée par l'International Agency for Research on Cancer (IARC), les HPVs peuvent être répartis en trois groupes selon leur potentiel oncogène: Groupe 1, contient les HPVs à haut risque (HPV16, 18, 31, 33, 35, 39, 45, 51,52, 56, 58 et 59), groupe 2A, inclut les HPVs probablement cancérigènes (HPV68) et groupe 2B, englobe les HPVs possiblement cancérigènes (HPV 26, 30, 34, 53, 66, 67, 69,70, 73, 82, 85 et 97) [4].

L'infection à HPV est l'infection sexuellement transmissible la plus fréquente. Le pic de prévalence de l'infection se situe entre 20 et 25 ans et la prévalence baisse considérablement après 30 ans, car la plupart des infections sont transitoires sans aucune conséquence clinique [5].

3.2 Le lien entre le papillomavirus humain et le cancer du col de l'utérus

Le HPV est le principal facteur de risque de survenue d'un cancer du col de l'utérus. En effet, lorsque l'infection subclinique persiste chez environ 10% des femmes infectées, il y a un risque élevé de développer des lésions précancéreuses du col utérin qui peuvent évoluer vers un cancer invasif. Ces lésions précancéreuses peuvent être classifiées selon Richart. Cette classification comporte

3 grades de dysplasie : CIN1 ou dysplasie légère, CIN2 ou dysplasie modérée, CIN3 ou dysplasie sévère et le carcinome invasif.

L'ADN du HPV peut être retrouvé dans près de 99% des cancers du col [6]. En particulier le HPV16 et le HPV18 sont les plus communs et provoquent environ 75% des cas de cancer du col utérin [7]. Cependant, la plupart des infections ne causent pas la maladie.

Le fait que le procès de carcinogénèse est assez long, cela permet la détection et le traitement des lésions précancéreuses qui évitera l'évolution en cancer. Cette prévention secondaire est actuellement faite par frottis cytologique (ou Pap test) et le traitement local par conisation dans les pays développés ou par « cold coagulation » ou cryothérapie dans les pays à faibles ressources.

Des nouveaux vaccins ont été développés pour protéger contre certains génotypes de HPV, ce qui a permis une avance majeure dans la prévention de lésions précancéreuses et cancéreuses liées aux HPV. Ces vaccins ont une action prophylactique et ne soignent pas l'infection. Pour cette raison, ils doivent être administrés chez de jeunes femmes n'ayant pas encore eu contact avec le HPV.

Depuis août 2015, le vaccin fait désormais partie des programmes de vaccination nationaux de quatre-vingt-huit pays. Néanmoins, la mise en œuvre à grande échelle de cette vaccination fait encore défaut dans les pays en développement et ne remplacera pas la nécessité du dépistage du cancer cervical. En Afrique subsaharienne, seulement trois pays proposent ce programme de vaccination : l'Afrique du Sud, l'Ouganda et le Rwanda [8]. Des programmes de vaccination pilotes existent dans trente-huit autres pays en développement.

3.3 Différentes méthodes pour la détection du HPV et impact sur la santé publique

Dans les pays à faibles ressources, il y a plusieurs problèmes et défis associés au dépistage du cancer utérin. La raison principale associée à l'échec du dépistage et par conséquent une haute prévalence du cancer du col, est lié à la complexité du processus de dépistage et les obstacles inhérents à ces pays. La pauvreté, le manque d'information sur le dépistage et le cancer, l'absence de programmes de dépistage organisés, le manque d'infrastructures de soins et le manque de praticiens formés sont les principaux obstacles à la mise en œuvre des programmes de dépistage [9].

Le test HPV pourrait apporter pour les pays en développement des solutions viables quant aux obstacles inhérents au dépistage cytologique. La sensibilité du test HPV pour la détection des néoplasies cervicales intraépithéliales de grade 2 ou plus sévère (CIN2+) est deux fois supérieure à celle de la cytologie [10-12]. En outre, le dépistage via auto-prélèvement HPV offre l'opportunité d'améliorer l'accès au dépistage à ceux qui généralement n'y participent pas, de réduire les coûts, et d'augmenter la participation au dépistage [13, 14]. L'auto-prélèvement HPV est reconnu pour être aussi fiable que les prélèvements cervicaux faits par le médecin pour la détection du HPV et des CIN2+ [15-18]. De plus, le dépistage par test HPV a été associé à une diminution de la mortalité dû au cancer du col utérin dans les pays à faibles ressources [19, 20].

Une grande variété de dispositifs de collecte a été évaluée dans les différentes études sur l'auto-prélèvement HPV. Les dispositifs les plus courants sont les tampons, brosses cervico-vaginales, bâtonnets et lavage cervico-vaginal. Dans la pratique actuelle, les prélèvements vaginaux sont stockés dans un milieu liquide,

ce qui requiert une manipulation minutieuse par les patientes qui doivent éviter de le renverser, sans compter les risques d'inflammabilité et de toxicité liés à ces milieux. Une seconde contrainte est la nécessité d'une température stable pour le transport et le stockage du prélèvement avant analyse. Ce milieu est difficile et couteux pour un usage courant dans les pays en développement.

Les milieux de transport à sec sont une alternative intéressante, permettant un rangement facile et un transport à température ambiante. Les bâtonnets secs constituent une méthode peu couteuse qui a démontré avoir un bon accord avec la technique habituel de prélèvement en milieu liquide [21].

3.4 Etude comparative entre plusieurs méthodes de prélèvement

Le but de cette étude était de comparer l'acceptabilité et la performance entre deux milieux de transport à sec pour la détection du HPV, le bâtonnet sec (s-DRY) et la cartouche FTA (s-FTA). Le s-FTA est aussi un milieu sec qui immobilise et stabilise les acides nucléiques à partir d'échantillons frais appliqués. Les performances des deux méthodes à sec ont été comparées à la performance obtenue par le prélèvement fait par le médecin en milieu liquide, PreservCyt (Dr-WET)

Principaux résultats

- ✓ La prévalence du HPV pour les types à haut risque était de 62,3% détectée par s-DRY, 56,2% détectée par le Dr-WET et 54,6% détectée par s-FTA, en utilisant le même test pour la détection du HPV (Anyplex II HPV28).

- ✓ Les sensibilités pour la détection de lésions intraépithéliales de bas grade ou pire (LSIL+) étaient les suivantes : 64,0% pour s-FTA, 84,6% pour s-DRY et 76,9 % pour Dr-WET.
- ✓ L'accord global entre s-FTA et s-DRY était de 70,8% (kappa = 0,34)
- ✓ L'accord global entre l'auto-prélèvement HPV et dr-WET était de 82,3% (kappa = 0,56).
- ✓ La méthode d'auto-prélèvement HPV préférée des patientes était le s-DRY (40,8% contre 15,4% ont choisi le s-FTA).
- ✓ En ce qui concerne les coûts, la carte FTA était 5 x plus chère que le bâtonnet (~5 dollars américains (USD) / par carte *vs* 1 USD/par bâtonnet).

Discussion

La diminution de la détection HPV dans les spécimens s-FTA comparée à s-DRY ou Dr-WET est probablement attribuable à l'insuffisance de cellules présentes dans le spécimen recueilli ou à un transfert incomplet et insuffisant de matériel de la brosse à la carte et ceci bien que la couleur de la carte ait changé.

Bien que la carte FTA offre certains avantages, cette méthode n'est pas aussi sensible que d'autres méthodes de collecte et est associée à un coût plus élevé (~5 USD par carte vs ~1 USD par bâtonnet). Ceci n'englobe pas le prix du kit total qui comprend la brosse pour la collecte et un punch pour l'extraction. En plus de ces inconvénients, la méthode serait difficile à mettre en œuvre dans un contexte à faibles ressources en raison de sa complexité. De plus, le transfert d'un échantillon prélevé avec une brosse sur la surface d'une carte FTA pourrait aboutir à un échantillon non représentatif.

En conclusion, les avantages des milieux secs sont attrayants en raison de

leur accessibilité et simplicité. Notre étude a tenté de valider la technique la plus optimale pour le dépistage du HPV. Nous avons constaté que la carte FTA est non seulement moins sensible que les bâtonnets, mais est aussi plus chère et semble donc inappropriée pour les pays à faibles ressources. Sur la base des résultats de cette étude, les bâtonnets secs apparaissent comme la méthode la plus adaptée pour être utilisée dans les milieux à faibles ressources.

4. La situation en Suisse

4.1 Le cancer du col en Suisse et dépistage

Le cancer du col de l'utérus est un cancer rare en Suisse. Chaque année en Suisse, environ 5'000 femmes sont diagnostiquées avec une lésion précancéreuse du col de l'utérus. Ce sont le plus souvent des femmes jeunes. Un cancer cervical invasif va être développé chez 240 femmes par année et environ 90 femmes en décèdent.

Depuis les fin des années soixante, le dépistage du cancer du col utérin a été promu par les gynécologues et on estime qu'il a réduit son incidence d'environ 50 à 60% en Suisse [22]. Le pays dispose d'un système de dépistage opportuniste basé essentiellement sur l'invitation du gynécologue pour un «contrôle annuel», où un frottis cervical est effectué. Le taux de couverture du programme national est relativement élevé (76%) [23].

Les dernières recommandations de dépistage du cancer du col utérin par la Société suisse de gynécologie et d'obstétrique (SSGO) suggèrent des frottis Pap chaque deux ans chez les femmes âgées de 21 jusqu'à 30 ans. A partir de 30 ans et ce jusqu'à 70 ans le frottis devrait être effectué tous les 3 ans si les 3 derniers frottis étaient normaux [24].

4.2 Le Papillomavirus humain en Suisse

En Suisse, les recommandations nationales de dépistage du cancer du col de l'utérus n'intègrent pas le test HPV dans le dépistage primaire. Il est maintenant temps de changer ces recommandations vu qu'on dispose actuellement de raisonnablement de preuves pour considérer l'introduction du test HPV chez les femmes âgées de 30 ans et plus.

La prévalence du HPV en Suisse n'est pas connue avec précision en raison de l'absence de données objectives. Une étude intégrant plus de 680 frottis endocervicaux de femmes ayant consulté leur médecin pour un examen préventif à Zurich et environs a trouvé une prévalence de 8.1% pour le HPV à haut risque [24]. Une autre étude qui s'est déroulée à Genève, regroupant des femmes qui ne se font pas dépister depuis au moins 3 ans (étude Depist), a montré une prévalence de HPV de 23.7% (les résultats de cet étude sont en cours de publication).

Concernant la vaccination contre le HPV, cette dernière est recommandée à toutes les filles et tous les garçons et les jeunes femmes et hommes âgés de 11 à 26 ans.

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6. Article original

Abbreviations

CC: Cervical Cancer

CIN 2+: cervical intraepithelial neoplasia grade 2 or worse

Dr-WET: physician-collected samples in specimen transport medium for HPV-testing

HPV: Human Papillomavirus

Self-HPV: self-sampling for HPV-testing

s-FTA: self-collected samples in the FTA cartridge for HPV-testing

s-DRY: self-collected samples with vaginal dry swabs for HPV-testing

Abstract

Background: Human papillomavirus (HPV) self-sampling (self-HPV) is valuable in cervical cancer screening. HPV testing is usually performed on physician-collected cervical smears stored in liquid-based medium. Dry filters and swabs are an alternative. We evaluated the adequacy of self-HPV using two dry storage and transport devices, the FTA cartridge and swab.

Methods: A total of 130 women performed two consecutive self-HPV samples. Randomization determined which of the two tests was performed first: self-HPV using dry swabs (s-DRY) or vaginal specimen collection using a cytobrush applied to an FTA cartridge (s-FTA). After self-HPV, a physician collected a cervical sample using liquid-based medium (Dr-WET). HPV types were identified by real-time PCR. Agreement between collection methods was measured using the kappa statistic.

Results: HPV prevalence for high-risk types was 62.3% (95%CI: 53.7–70.2) detected by s-DRY, 56.2% (95%CI: 47.6–64.4) by Dr-WET, and 54.6% (95%CI: 46.1–62.9) by s-FTA. There was overall agreement of 70.8% between s-FTA and s-DRY samples (kappa = 0.34), and of 82.3% between self-HPV and Dr-WET samples (kappa = 0.56). Detection sensitivities for low-grade squamous intraepithelial lesion or worse (LSIL+) were: 64.0% (95%CI: 44.5–79.8) for s-FTA, 84.6% (95%CI: 66.5–93.9) for s-DRY, and 76.9% (95%CI: 58.0–89.0) for Dr-WET. The preferred self-collection method among patients was s-DRY (40.8% vs. 15.4%). Regarding costs, FTA card was five times more expensive than the swab (~5 US dollars (USD)/per card vs. ~1 USD/per swab).

Conclusion: Self-HPV using dry swabs is sensitive for detecting LSIL+ and less expensive than s-FTA.

Impact: Our findings suggest that dry swabs should be preferred for self-HPV screening.

Keywords: Self-HPV, FTA cartridge, dry swabs, wet swabs, cervical cancer, human papillomavirus (HPV)

Résumé

La détection de l'ADN du virus du papillome humain (VPH, en anglais human papillomavirus, HPV) est une méthode efficace pour dépister le cancer du col utérin. Cette détection peut être effectuée par le médecin ou par la patiente elle-même (auto-prélèvement HPV). Une fois le prélèvement HPV fait, ce dernier peut être conservé soit en milieu liquide, soit en milieu sec avant de procéder à l'analyse. Le milieu sec offre comme avantage, d'être simple d'utilisation et un coût peu élevé, toutefois l'optimisation des milieux secs doit encore être étudiée. Cette étude a eu pour objectif d'évaluer l'acceptabilité et la performance de deux milieux secs, la carte FTA et le bâtonnet sec auprès de 130 patientes.

Chaque participante a effectué deux auto-prélèvements HPV; soit un bâtonnet placé en milieu sec (self-DRY (s-DRY)), soit un prélèvement appliqué sur une carte FTA (self-FTA (s-FTA)). La randomisation déterminait l'ordre dans lequel les tests ont été réalisés. Les patientes étaient ensuite invitées à un examen colposcopique, où un prélèvement cytologique et HPV cervical en utilisant un milieu liquide (Dr-WET) a été réalisé. La méthode PCR a été utilisée sur chacun des prélèvements pour identifier les types d'HPV. Les participantes ont rempli un questionnaire comprenant des questions démographiques et leur préférence quant à la méthode d'échantillonnage. La concordance entre les méthodes de prélèvement et les types d'HPV ont été calculés à l'aide du coefficient kappa (κ), la sensibilité et spécificité ont été évaluées en prenant la cytologie comme référence.

La sensibilité était plus faible pour s-FTA que pour s-DRY en utilisant les résultats cytologiques (LSIL +) comme « gold standard » (64,0% vs 84,6%). L'accord global entre s-FTA et s-DRY était de 70,8% (kappa = 0,34) et entre l'auto-prélèvement

HPV et Dr-WET de 82,3% (kappa = 0,56). La méthode d'auto-prélèvement HPV préférée des patientes était le s-DRY (40,8% contre 15,4% ont choisi le s-FTA).

Sur la base des résultats de cette étude, les bâtonnets secs peuvent être un considérable atout pour le dépistage cervical dans les milieux à faibles ressources.

Introduction

The burden of cervical cancer (CC) remains very significant, especially in developing countries, and accounts for around a quarter of a million deaths annually worldwide (1). In the developed world, screening programs have shown a reduction in CC cases. On the contrary, in low-resource settings, cytology-based screening programs are difficult to implement.

Human papillomavirus (HPV) testing appears to overcome some inherent barriers of cytological screening. Detection sensitivity for cervical intraepithelial neoplasia grade 2 or worse (CIN 2+) of HPV testing is twice that of cytology (2-4). A single round of HPV testing is associated with a significant decrease in CC mortality in low-resource countries (5, 6). Furthermore, screening via self-collected samples improves access to healthcare, reduces time and costs, and contributes to increased screening attendance (7, 8). Self-sampling has also been proven as reliable as physician-obtained cervical samples for HPV and CIN2+ detection (9-12).

A great variety of collection devices is being used in studies on self-sampling for HPV-testing (self-HPV). The most common devices are tampons, swabs, cervicovaginal brushes, and cervicovaginal lavage. In current practice, samples for HPV testing are stored in a liquid-based medium, which requires careful handling owing to its flammability and toxicity. The need for stable transport and storage temperatures makes such testing methods difficult and costly to provide in developing countries. Furthermore, despite women's high acceptance of self-HPV, they remain concerned about the validity of the method and are afraid of spilling the transport medium during the sampling procedure and transport (9, 13-15).

Dry storage and transport might be a valuable option. The FTA elute cartridge (Whatman Inc., Clifton, NJ, USA) is a dry carrier that immobilizes and

stabilizes nucleic acids from fresh samples. This biohazard-free paper is chemically treated with proprietary reagents that lyse cells upon contact, denaturing proteins. The FTA cartridge contains an indicating dye that changes color when a sample is applied, thereby confirming that the procedure has been performed properly. Moreover, easy storage and transport at room temperature is possible.

Evidence shows good agreement for detection of HPV DNA between samples collected in the FTA cartridge (s-FTA) and in a liquid-based medium (13, 15-19). Despite its advantages, the FTA cartridge is inconvenient in that DNA from the cytobrush used for specimen collection can be only partly transferred to the cartridge.

Alternatively, vaginal dry swabs (s-DRY) are inexpensive and are not usually associated with a great loss of cellular material for analysis. Studies have shown that self-HPV swabs can be successfully transported in a dry state at ambient temperature without compromising specimen integrity, and that there is good agreement (70–90%) for HPV detection between dry and wet swabs (20, 21).

Although the feasibility of both the FTA cartridge and s-DRY as self-collection methods for HPV detection has been compared in several studies to standard swab collection in a liquid medium, to our knowledge, the relationship between performance of the FTA cartridge and s-DRY for HPV detection has never been addressed. Our goal is to evaluate the acceptability and performance of two dry storage and transportation devices, s-FTA and s-DRY, and comparing them with the current standard of HPV testing using physician-collected samples and a specimen transport medium (Dr-WET).

Material and methods

Study population

From March 2014 through February 2015, we enrolled women from the colposcopy clinic of Geneva University Hospitals, Switzerland. Women referred to the colposcopy clinic may come for a routine test, follow-up of an HPV infection with genotypes 16 and 18 or follow-up of cervical disease.

Women were eligible if they were over 30 years old, and if they understood the study procedures and voluntarily agreed to participate by signing an informed consent form. Pregnant women, those with a history of hysterectomy, and who did not consent to participate, were excluded. Of the 150 women initially recruited, 20 (13.3%) were excluded since they did not meet the inclusion criteria or they refused to participate (Figure 1). A sample of 130 women was included in the study. The ethics committee of Geneva approved the study (CER: 14-011).

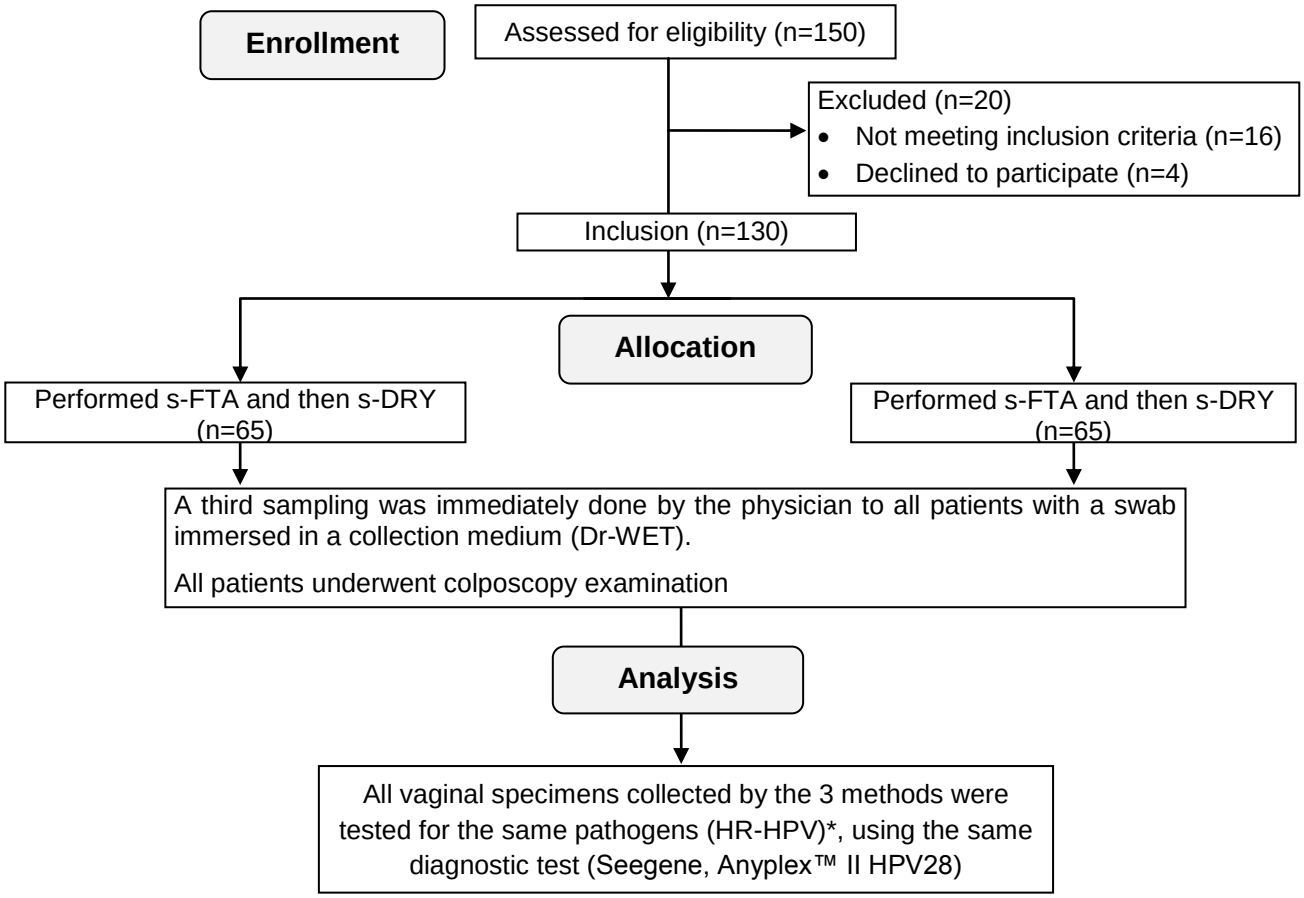
Study procedure

All eligible women were invited to collect two self-samples (s-FTA and s-DRY). A physician subsequently collected a third cervical sample with a swab immersed in a collection medium (ESwab™; COPAN Italia, Brescia, Italy) for HPV testing (Dr-WET). The sequence of the two self-HPV tests was randomized to avoid potential bias that might favor the “first” test. A research nurse gave oral instructions to participants, who were instructed to wash their hands before the specimen collection procedure. Each participant received a package containing a specimen collection kit. The Rovers® Viba-Brush® (Rovers Medical Devices B.V., Oss, The Netherlands) was used for self-collection with the FTA cartridge, and the mid-

turbinate flocked vaginal swab (FLOQSwabs™; COPAN Italia) used for self-collection with the s-DRY method.

Figure 1.

Flowchart of study participants



Abbreviations: Dr-WET = physician-collected samples using specimen transport medium; s-DRY = self-collected samples using dry swab without transport medium; s-FTA = self-collected samples using a cytobrush applied to an FTA cartridge
*Oncogenic genotypes include types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73, 82

Recommendations were to hold the brush or swab by the end of its handle and insert the brush or swab into the vagina, avoiding contact with the external genitalia, until resistance was felt (at least 6 cm). While gently maintaining pressure, participants were instructed to turn the brush or swab three to five times.

Subsequently, the brush was to be applied to the FTA cartridge by pressing it onto the middle of the indicated sample area and then rotate the brush three to five times across that area. The FTA elute matrix contains an indicating dye that changes color from purple to white when a sample has been applied correctly. Three small circles of approximately 3.0 mm diameter were cut from the center section of the FTA card with a disposable sterile carbon steel surgical blade, and placed into a 1.5-ml microcentrifuge tube. A new sterile blade was used for each card.

On the other hand, the flocked swab (s-DRY) was simply inserted into its plastic sleeve for storage and transport. During the subsequent colposcopy consultation, a physician also collected a sample for HPV testing.

After all specimen collection, participants completed a self-administered questionnaire to collect sociodemographic data and query acceptance of the test. The FTA-card punches, flocked swabs, and ESwabs were immediately forwarded to the laboratory for analysis.

DNA isolation and extraction

Elution from FTA card: The 3.0-mm FTA punch discs were washed in 500 µl of sterile water and pulse vortexed three times for 5 s. The water was removed from each tube and the punch discs were transferred to a new microfuge tube containing 200 µl distilled water, which was then transferred to a heating block at 95 °C for 30 min. The sample was then removed, pulse vortexed three times for 5 s, and centrifuged for 30 s to separate the matrix from the eluate. The eluted material was transferred to a new tube and stored at –20 °C until DNA extraction.

Material recovery from dry swab sample (s-DRY) : Each sample was placed into 1 ml of sterile phosphate-buffered saline (PBS), and each tube was pulse vortexed three times for 15 s before removing and discarding the swab collection device. Samples were then stored at −20 °C until extraction.

Material recovery from ESwab sample (Dr-WET) : Tubes containing the Dr-WET sample were also pulse vortexed three times for 15 s, and then the swab was discarded. Samples were then stored at −20 °C until automated extraction.

DNA extraction: The entire volume of recovered material was transferred into appropriate tubes and the volume adjusted to 600 µl with PBS. DNA was then extracted on an *m*2000*sp* instrument (Abbott Molecular, Des Plaines, IL, USA), according to manufacturer instructions. The DNA elution volume was 100 µl/sample.

HPV and genotyping: HPV analyses were performed using the Anyplex II HPV28 (H28) Detection test (Seegene, Seoul, South Korea). The H28 is a semi-quantitative real-time multiplex PCR 30 assay for screening and genotyping 28 HPV genotypes. This test uses Dual-Priming Oligonucleotides (DPO™) and Tagging Oligonucleotide Cleavage and Extension (TOCE™) technologies, allowing simultaneous detection of 19 high-risk HPVs (including types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73 and 82) and nine low-risk HPVs. The 19 high-risk HPVs detected in the test include HPV types defined by the *International Agency for Research on Cancer* as Group 1 (carcinogenic to humans), Group 2A (probably carcinogenic to humans), and Group 2B (possibly carcinogenic to humans*)*. Analysis was done as recommended by the manufacturer.

Statistical analysis

A sample size of 130 women was needed to validate differences in test performance of 10% or more, assuming a 40–50% prevalence of HPV infection in the selected population. Agreement between the collection methods according to cytological results was measured using the kappa statistic (κ) and corresponding standard deviation (SD). The proportion of positive agreement (PPA) between paired s-FTA and s-DRY samples was calculated using 2a/(f1 + g1), where a is the number of samples that were positive for HPV in both dry samples, f1 is the number of samples that were positive for s-FTA, and g1 is the number of samples that were positive for s-DRY. The same was done for self-collection methods (combined results of s-FTA and s-DRY) and Dr-WET.

Sensitivity and specificity for detection of high-risk HPV using Dr-WET as the gold standard was reported, as well as sensitivity and specificity of the three sampling methods for abnormal Pap smear. Because of the small number of high-grade squamous intraepithelial lesions (HSIL) or carcinomas in our population, we assessed sensitivity and specificity for low-grade squamous intraepithelial lesion or worse (LSIL+). The two-tailed McNemar's test was used for mutual comparison of sensitivity and specificity. Positive and negative predictive values were also calculated.

Most of the patients performed cytology as part of the routine consultation. If cytology was not performed, we used cytological results obtained in the 12 months prior to the patient recruitment date in the data analysis.

Data were analyzed using Stata Statistical Software Release 13 (StataCorp LP, College Station, TX, USA).

Results

Sample characteristics

One hundred and thirty patients were included in the study. The median age of participants was 42 years [interquartile range (IQR) was 34–50], and the majority had a partner (77.7%). Sociodemographic data are represented in Table 1. The median delay between sampling and laboratory processing was 44 (IQR = 26–60) days.

Table 1
Sample sociodemographic characteristics (n=130)

Variable	N. (%)
Age, median (IQR), y	42 (34-50)
Age groups, y	
30-39	50 (38.5)
40-49	47 (36.1)
≥50	33 (25.4)
Marital Status	
Without a partner	29 (22.3)
With a partner	101 (77.7)
Education	
Unschooled	2 (1.7)
Primary education	23 (19.7)
Secondary education	47 (40.2)
Tertiary education	45 (38.4)
Number of child (mean ± SD)	1.3±1.1

Abbreviations: IQR = Interquartile range N. = number; SD = standard deviation; y = years.

Overall HPV detection with the three collection methods and HPV genotype distribution

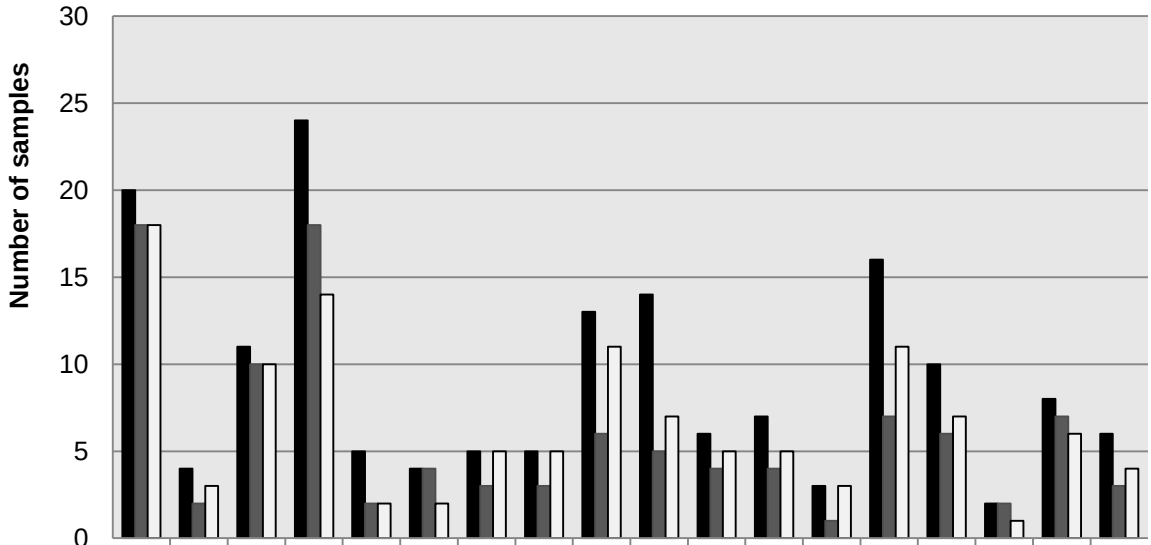
HPV prevalence for high-risk types was 62.3% (95% CI: 53.7–70.2) detected by s-DRY, 56.2% (95% CI: 47.6–64.4) by Dr-WET, and 54.6% (95% CI: 46.1–62.9) by s-FTA. Infection with HPV types 16 and 18 alone was identified in 4.6% of

participants, and infection together with other high-risk types in 13.8% of cases. Infection with high-risk genotypes other than types 16 and 18 was found in 50% of women.

The distribution of HPV genotypes is shown in Figure 2 for high-risk types and in Figure 3 for low-risk types. In general, a larger number of positive samples for each genotype were detected more often in s-DRY samples compared with the other collection methods.

Figure 2

Number of high-risk human papillomavirus (HPV) genotypes detected by each sample collection method.

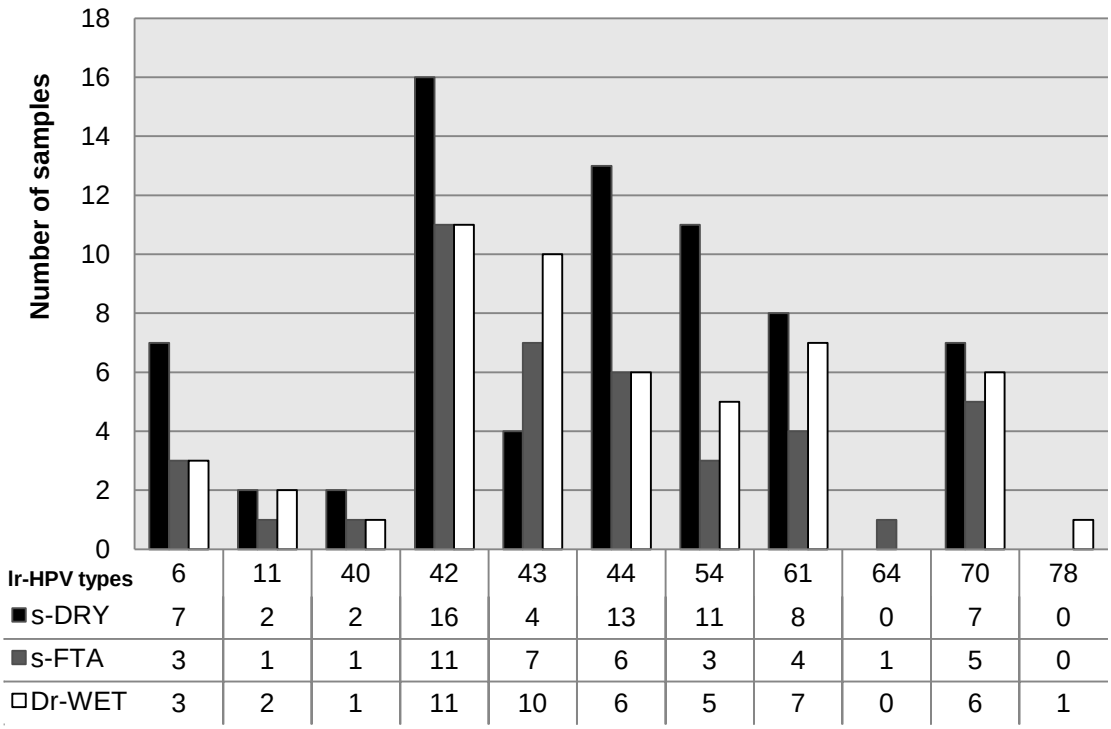


hr-HPV types	16	18	31	33	35	39	45	51	52	53	56	58	59	66	68	69	73	82
s-DRY	20	4	11	24	5	4	5	5	13	14	6	7	3	16	10	2	8	6
s-FTA	18	2	10	18	2	4	3	3	6	5	4	4	1	7	6	2	7	3
Dr-WET	18	3	10	14	2	2	5	5	11	7	5	5	3	11	7	1	6	4

Abbreviations: Dr-WET = physician-collected samples using specimen transport medium; s-DRY = self-collected samples using dry swab without transport medium; s-FTA = self-collected samples using a cytobrush applied to an FTA cartridge; hr-HPV = high-risk human papillomavirus.

Figure 3

Number of low-risk HPV genotypes detected by each sample collection method.



lr-HPV types	6	11	40	42	43	44	54	61	64	70	78
s-DRY	7	2	2	16	4	13	11	8	0	7	0
s-FTA	3	1	1	11	7	6	3	4	1	5	0
Dr-WET	3	2	1	11	10	6	5	7	0	6	1

Abbreviations: Dr-WET = physician-collected samples using specimen transport medium; s-DRY = self-collected samples using dry swab without transport medium; s-FTA = self-collected samples using a cytobrush applied to an FTA cartridge; lr-HPV = Low risk human papillomavirus.

Agreement between collection methods according to cytological results

Cytological results obtained in the 12 months prior to the patient recruitment date or during the study were considered in the data analysis (Tables 2 and 3). We found cytological diagnoses for 119 of the 130 patients enrolled. There were 61 normal diagnoses, 1 squamous cell carcinoma, 5 HSIL, 20 LSIL, 9 atypical squamous cells-cannot rule out high grade squamous intraepithelial lesion (ASC-H), 3 atypical glandular cells (AGC) and 20 atypical squamous cells of undetermined significance (ASC-US). Overall agreement between s-FTA and s-DRY samples when LSIL+ was present was 69.2% ($\kappa = 0.27$), and the PPA was 78.9%.

Table 2

Agreement and HPV positivity for each collection method, overall (n=130) and according to cytology results (n=119)

Cytology analysis	N. of Samples - Self-HPV					% Positive		Agreement (%)		Kappa (95% CI)
	Total	s-FTA+/ s-DRY+	s-FTA+/ s-DRY-	s-FTA-/ s-DRY+	s-FTA-/ s-DRY-	s-FTA cartridge % (95% CI)	s-DRY % (95% CI)	Overall	PPA	
Overall	**130**	**70**	**9**	**29**	**22**	**60.8 (52.2-68.7)**	**76.2 (68.1-82.7)**	**70.8**	**78.7**	**0.34 (0.18-0.50)**
NILM	61 (51.3)	26	2	17	15	45.9 (34.0-58.3)	72.1 (59.8-81.9)	68.3	72.2	0.38 (0.18-0.59)
ASC-US	20 (16.8)	14	4	0	2	90.0 (68.7-98.4)	70.0 (47.9-85.7)	80.0	87.5	0.41 (-0.01-0.83)
AGC	3 (2.5)	1	1	0	1	66.7 (20.2-94.4)	33.3 (56.3-79.8)	66.7	66.7	0.40 (-0.37-1.0)
ASC-H	9 (7.6)	7	1	1	0	88.9 (54.3-99.9)	88.9 (54.3-99.9)	77.8	87.5	-0.13 (-0.30-0.05)
LSIL	20 (16.8)	13	1	3	3	70.0 (47.9-85.7)	80.0 (57.8-92.5)	80.0	86.7	0.47 (-0.04-0.90)
HSIL	5 (4.2)	2	0	3	0	40.0 (11.6-77.1)	100 (51.1-100)	40.0	57.1	0
CAN INV	1 (0.8)	0	0	1	0	0 (0-83.3)	100 (16.8-100)	0	0	0
LSIL+	**26 (21.8)**	**15**	**1**	**7**	**3**	**61.5 (42.5-77.6)**	**84.6 (66.5-93.9)**	**69.2**	**78.9**	**0.27 (-0.07-0.61)**

Abbreviations: N. = number; NILM = Negative for intraepithelial lesion or malignancy; ASC-US = Atypical squamous cells of undetermined significance; ASC-H = Atypical squamous cells-cannot rule out high grade; AGC = Atypical glandular cell; LSIL = Low-grade squamous intraepithelial lesion; LSIL+ = Low-grade squamous intraepithelial lesion or worse; HSIL = High-grade squamous intraepithelial lesion; CAN INV = Invasive cancer; CI = confidence interval; PPA = Proportion of Positive Agreement.

Overall agreement between self-HPV and physician-collected samples when LSIL+ was present was 89.7% (κ = 0.61), and the PPA was 93.0%. Overall agreement between FTA cards and Dr-WET samples was 83.1%, and kappa was 0.64 (0.50–0.77). Overall agreement between s-DRY and Dr-WET samples was 85.2%, and the kappa was 0.64 (0.50–0.78).

Table 3

Agreement and HPV positivity for each collection method, overall (n=130) and according to cytology results (n=119)

Cytology analysis	N. of Samples - Self-HPV* vs. Dr-WET					% Positive		Agreement (%)		Kappa (95% CI)
	Total	Self+/ Dr-WET+	Self+/ Dr-WET -	Self-/ Dr-WET +	Self-/ Dr-WET -	Self-collection* % (95% CI)	Dr-WET % (95% CI)	Overall	PPA	
Overall	**130**	**85**	**23**	**0**	**22**	**83.1 (75.7-88.6)**	**65.4 (56.9-73.0)**	**82.3**	**88.1**	**0.56 (0.41-0.70)**
NILM	61 (51.3)	34	12	0	15	75.4 (63.2-84.6)	55.7 (43.3-67.5)	80.3	85.0	0.58 (0.39-078)
ASC-US	20 (16.8)	15	3	0	2	90.0 (68.7-98.4)	75.0 (52.8-89.2)	85.0	90.9	0.50 (0.05-0.95)
AGC	3 (2.5)	1	1	0	1	66.7 (20.2-94.4)	33.3 (56.3-79.8)	66.7	66.7	0.40 (-0.37-1)
ASC-H	9 (7.6)	8	1	0	0	100 (65.5-100)	88.9 (54.3-99.9)	88.9	94.1	0
LSIL	20 (16.8)	15	2	0	3	85.0 (63.1-95.6)	75.0 (52.8-89.2)	90.0	93.8	0.69 (0.31-1)
HSIL	5 (4.2)	5	0	0	0	100 (51.1-100)	100 (51.1-100)	100	100	1
CAN INV	1 (0.8)	0	1	0	0	100 (16.8-100)	0 (0-83.3)	0	0	0
LSIL+	**26 (21.8)**	**20**	**3**	**0**	**3**	**88.5 (71.86.0)**	**76.9 (56.0-89.0)**	**89.7**	**93.0**	**0.61 (0.23-1)**

Abbreviations: Dr-WET = physician-collected samples using specimen transport medium; N. = number; NILM = Negative for intraepithelial lesion or malignancy; ASC-US = Atypical squamous cells of undetermined significance; ASC-H = Atypical squamous cells-cannot rule out high grade; AGC = Atypical glandular cell; LSIL = Low-grade squamous intraepithelial lesion; LSIL+ = Low-grade squamous intraepithelial lesion or worse; HSIL = High-grade squamous intraepithelial lesion; CAN INV = Invasive cancer; CI = confidence interval; PPA = Proportion of Positive Agreement.
*Comprises results from Self-HPV using both collection methods (s-DRY and s-FTA).

Number of oncogenic HPV genotypes detected

Agreement between the s-FTA versus Dr-WET, s-DRY versus Dr-WET, and s-FTA versus s-DRY for the number of oncogenic HPV types is shown in Table 4. Overall agreement between s-FTA versus Dr-WET was 70.8% with kappa = 0.56, between s-DRY

versus Dr-WET was 72.3% with kappa = 0.61, and between s-FTA versus s-DRY was 54.6% with kappa = 0.35. Detection of three or more oncogenic HPV types was more common in s-DRY samples (17.7%) relative to s-FTA and Dr-WET samples (4.6% and 8.4%, respectively).

Table 4
Comparison between number of oncogenic HPV genotypes* detected in the three different samples (s-FTA vs. Dr-WET; s-FTA vs. s-DRY; s-DRY vs. Dr-WET)

	N. of hr-HPV types detected in Dr-WET samples					**N. of hr-HPV types detected in s-DRY samples**				
N. of hr-HPV types detected in s-FTA samples	0	1	2	3+	Total	0	1	2	3+	Total
0	**48**	7	3	0	58	**38**	15	2	3	58
1	9	**29**	9	4	51	9	**20**	13	9	51
2	0	5	**9**	1	15	0	3	**7**	5	15
3+	0	0	0	**6**	6	0	0	0	**6**	6
Total	57	1	21	11	130	47	38	22	23	130
N. of hr-HPV types detected in s-DRY samples										
0	**43**	3	1	0	47					
1	12	**26**	0	0	38					
2	1	5	**15**	1	22					
3+	1	7	5	**10**	23					
Total	57	41	21	11	130					

Abbreviations: N.= number
*Oncogenic genotypes include types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73, 82 (Seegene, Anyplex™ II HPV28)
Overall agreement between s-FTA vs. Dr-WET was 70.8% and the kappa 0.56±0.06; overall agreement between s-DRY vs. Dr-WET was 72.3% and the kappa 0.61±0.05; overall agreement between FTA vs. s-DRY was 54.6% and the kappa 0.35±0.05.

Clinical performance of self-collection and physician-collection methods

Sensitivity of the s-FTA method using the Dr-WET sample results as the gold standard was 85.4% (95% CI: 76.4–91.5), compared with 96.5% (95% CI: 90.1–98.8) for the s-DRY method ($p = 0.02$). On the other hand, we found specificities of 82.2% (95% CI: 68.7–90.7) and 62.2% (95% CI: 47.6–74.9) ($p = 0.05$) for the s-FTA and s-DRY methods, respectively (Table 5).

Using the cytological results as gold standard (LSIL+), the s-FTA had a sensitivity of 64.0% (95%CI:44.5–79.8) and specificity 38.1% (95%CI:29.8–49.4); s-DRY sensitivity was 84.6% (95%CI: 66.5–93.9) with specificity 28.0% (95%CI:19.9–37.8); Dr-WET sensitivity was 76.9% (95%CI:58.0–89.0) with specificity 37.6% (95%CI:28.5–47.8).

Table 5
Clinical performance of self-collection methods using Dr-WET results as gold-standard and performance of self- and physician-collection methods using pathological cytological results as gold-standard (LSIL+)

Variables	**s-FTA and s-DRY performances, using Dr-WET results as gold-standard**			
	Sensitivity (95% CI)	**Specificity (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**
s-FTA	85.4 (76.4-91.5)	82.2 (68.7-90.7)	89.9 (81.3-94.8)	75.5 (61.9-85.4)
s-DRY	96.5 (90.1-98.8)	62.2 (47.6-74.9)	82.2 (74.2-89.0)	90.3 (75.1-96.7)
p value*	0.020	0.050	.	.

Variables	**s-FTA, s-DRY and Dr-WET performances, using pathological cytological results as gold-standard (LSIL+)**		
	Sensitivity (95% CI)	**Specificity (95% CI)**	**PPV (95% CI)**
s-FTA	64.0 (44.5-79.8)	39.1 (29.8-49.4)	22.2 (14.2-33.1)
s-DRY	84.6 (66.5-93.9)	28.0 (19.9-37.8)	24.7 (16.9-34.6)
p value*	0.059	0.008	
Dr-WET	76.9 (58.0-89.0)	37.6 (28.5-47.8)	25.6 (17.3-36.3)
p value* (vs. FTA)	0.180	0.800	.
p value* (vs. s-DRY)	0.160	0.020	.

Abbreviations: CI = confidence interval; s-DRY = vaginal dry swabs: PPV = Positive Predictive Value; NPV = Negative Predictive Value; Dr-WET= physician-collected samples using specimen transport medium; s-DRY = self-collected samples using dry swab without transport medium.
*The *p*-value was calculated with McNemar's test.

Sample preferences and opinion about the collection methods

Sample preferences and opinion about the collection methods are represented in Table 6. The preferred self-collection method among participants was s-DRY (40.8% vs. 15.4%), and a larger number of women reported feeling very confident using it (48.5%). One hundred and seventeen (90.0%) women affirmed that they were prepared to self-collect a vaginal sample at home. Half of the participants (50.0%) expressed a preference for HPV testing by self-collection at home rather than going to a clinic for a pelvic exam and cytology testing.

Table 6

Sample preferences and opinions regarding collection methods (n=130)

Variable	FTA cartridge N. (%)	Dry swab N. (%)
Which one of the two self-collection methods do you prefer?		
I have a preference for:	20 (15.4)	53 (40.8)
No preference	57 (43.8)	
Do you that both self-collection methods have the same reliability?		
I don't know	86 (66.7)	
Yes	24 (18.6)	
No	18 (14.0)	
If not, which one do you think is more effective to screen cervical lesion?		
The most effective method is:	14 (10.8)	6 (4.6)
I don't know	78 (60.0)	
Do you think that the self-collection was painful?		
Not painful at all	98 (75.4)	108 (83.1)
Slightly painful	25 (19.2)	16 (12.3)
Moderately painful	6 (4.6)	4 (3.1)
Very painful	1 (0.8)	2 (1.5)
In a scale of 0-4, how painful was the procedure? (mean±sd)	1.3±0.60	1.2±0.58
Are you confident that you have correctly performed the self-collection?		
Not sure at all	3 (2.3)	3 (2.3)
Slightly confident	24 (18.5)	14 (10.8)
Moderately confident	49 (37.7)	49 (37.7)
Very confident	51 (39.2)	63 (48.5)
In a scale of 0-4, how confident are you about the procedure? (mean±sd)	1.2±0.41	1.4±0.58
How do you classify the methods' complexity?		
Easy	91 (70.0)	111 (85.4)
Moderate	32 (24.6)	17 (13.1)
Complex	7 (5.4)	2 (1.5)
Which one of the tests would you recommend to your family and friends?		
I would recommend the:	13 (10)	43 (33.1)
Both	56 (43.1)	
Any	2 (1.5)	
I don't know	16 (12.3)	
Would you be ready to do the sample self-collection at home using written instructions?		
No	13 (10.0	
Yes	117 (90.0)	
If yes, with which one of the methods?		
I would do self-collection at home with:	8 (6.2)	32 (24.6)
I don't know	77 (59.2)	
In the future would you rather do self-collection for HPV testing or continue going to the doctor for a Pap test?		
Pap Test with doctor	63 (48.5)	
Self-Collection for HPV testing	63 (48.5)	

Abbreviations: n. = number; SD = standard deviation.

Discussion

To our knowledge, this was the first study to compare acceptability and analytic performance between two dry storage and transportation devices for HPV detection, the FTA cartridge and dry swab.

Overall, detection of HPV was significantly less frequent with the s-FTA method relative to s-DRY or Dr-WET, using the same test for HPV detection (Anyplex II HPV28). This finding is consistent with a previous trial in Barcelona (16) but contradicts the findings of other studies (13, 15, 18, 22) in which s-FTA appeared to be a suitable dry transport carrier. However, in the former studies, the collection method or storage medium to which the s-FTA sample was compared differed from ours. In a study by Gonzalez et al. (18), there was increased HPV detection of any type in samples collected in an s-FTA medium than in those in a PreservCyt medium (54.5% versus 45.8%). The reduced detection of HPV in s-FTA samples in our study is most likely attributable to insufficient cellular material collected by the patient or inadequate transfer of material from the brush onto the card.

In our study, sensitivity was much lower for the s-FTA method than the s-DRY method with use of cytological results as the gold standard (64.0% vs. 84.6%), even when a real-time PCR method was used for HPV testing in both collection methods. Indeed, previous studies have demonstrated that s-FTA is a favorable method for HPV diagnosis in the context of GP5/6-PCR use, but not Hybrid Capture 2 (13, 18). In a study by Geraets et al. (16), in which the FTA-based self-collection method was compared with physician-collected cervical samples stored in a liquid medium, a 98% sensitivity in CIN 2+ detection for the physician-collected sample using GP5+/6+ testing was found, which is comparable to the combination of self-collection with s-FTA and SPF10 HPV detection (sensitivity 95.9%). These results indicate that clinical performance of HPV detection is determined by both the sample collection method and test used.

Overall, several studies have shown substantial agreement for PCR HPV detection and genotyping between cervical cells collected using a liquid-based medium and specimens collected using FTA cartridges, among both self-collected and clinician-collected samples (13-15, 18, 22). In Geraets et al. (16), overall agreement was 89.0% between HPV test results with the s-FTA method and corresponding physician-collected samples, giving a kappa of 0.73 (95% CI: 0.63–0.84). These results are consistent with ours. However, if we compare the s-FTA samples with the s-DRY method, agreement was much less than expected (70.8%; kappa = 0.34; 95% CI: 0.18–0.50).

This study has some limitations that must be addressed. First, the self-collected samples were always done before the physician-collected sample, creating a bias in favor of the self-HPV. Second, it is uncertain that our findings can be generalized to other populations with low HPV prevalence; our study was based on adult women in a colposcopy clinic with a high prevalence of HPV infection. Third, the sample size was small. For this reason, these findings cannot be generalized based on this study alone. Fourth, we did not have histology results for HPV-positive women to confirm the disease status and therefore we used cytological results as reference. Finally, we do not have information on diagnostic procedures and further management of women recruited in this study.

The strengths of our study include the possibility for comparing the s-FTA, s-DRY and Dr-WET methods within categories of cytological diagnoses and, more specifically, for evaluating the performance of s-FTA and s-DRY among women with an LSIL+ diagnosis. Furthermore, by randomizing the sequence of the two self-collection methods, we did not favor one method over the other.

Although the FTA card may have some advantages such as the assurance of correct test performance by means of the color indicator, it is not as sensitive as other collection methods and has a higher cost. Indeed, by comparing the regular prices of the

card (Whatman Indicating FTA™ Elute Micro Card) with the dry swab (COPAN FLOQSwabs™), we found that the card is five times more expensive (~5 USD per card vs. ~1 USD per swab). This does not even consider the price of the entire kit, which includes the brush for collection and puncher for extraction. In addition to these disadvantages, the method would be difficult to implement in a context of low human and material resources, owing to laborious sample processing. In the present study, testing was done according to strict standard laboratory procedures to avoid PCR contamination. Punching of the FTA card is not only expensive, it is labor-intensive, and cross-contamination can readily occur if rigorous techniques are not used. In this study, a disposable surgical blade was used and we have no reason to believe that this would affect card performance, as this technique has been previously used without compromising the results (17). Furthermore, transferring a sample collected with a brush onto the surface of an FTA card might result in a nonrepresentative sample. Additionally, in cases of high-grade CIN (with greater HPV DNA integration), HPV copies per cell tend to be reduced and dysplastic cervical cells may be less likely to be transferred onto a solid substrate like FTA (14). Since the sensitivity for detecting low- and high-grade cervical dysplasia using the FTA card is unsatisfactory, its use should be reconsidered in light of better existing alternatives.

In summary, the benefits of dry carriers are appealing, owing to accessibility and simplicity. The present study used a cost-effective strategy to promote use and validate the most optimal technique for HPV screening. Contrary to the optimistic results of other studies (14, 15, 17, 18, 22), we did not find the FTA method to be as promising for HPV testing. We found that the FTA cartridge is not only less sensitive than swabs but is also more expensive than other methods. In our view, the FTA method is inappropriate for use in low-resource settings and may only be slightly appealing for self-HPV testing in developed countries, because of a pleasing modern design that may help reassure

women and motivate them to perform self-sampling at home. Based on the findings of this study, dry swabs might be a great asset for cervical cancer screening, within low-resource settings.

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