HIGH-RISK HUMAN PAPILLOMAVIRUS TESTING OF PHYSICIAN- AND SELF-COLLECTED SPECIMENS FOR CERVICAL CANCER SCREENING AMONG FEMALE SEX WORKERS IN NAIROBI, KENYA

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A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of the Doctor of Philosophy in the Department of Epidemiology.

Chapel Hill
2013

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ABSTRACT

JIE TING: High-risk human papillomavirus testing of physician- and self-collected specimens for cervical cancer screening among female sex workers in Nairobi, Kenya
(Under the direction of Jennifer S. Smith, PhD, MPH)

A cervical cancer screening program based on high-risk human papillomavirus (hrHPV) testing of self-collected specimens (hrHPV self-testing) may help increase screening access in low-resource settings, thus reducing invasive cervical cancer (ICC) incidence in these regions. Little is known, however, about the performance of hrHPV testing with physician- collected versus self-collected specimens for cervical cancer screening among high-risk women in low-resource settings. In addition, to determine if a screening strategy is optimal for a given setting, the costs and benefits of each screening strategy must also first be compared.

From 2009-2011, 344 female sex workers (FSW) in Nairobi participated in a study to compare hrHPV physician- versus self-testing for cervical cancer screening. Participants must have been between 18-50 years, had an intact uterus, and were not in the second trimester of pregnancy or later.

HrHPV testing sensitivity for cytological high-grade squamous intraepithelial lesion or more severe (≥HSIL) was similar in physician- (86%) and self- collected specimens (79%). Specificity of hrHPV for ≥HSIL was also similar in physician- (73%) and self-collected (75%) specimens. To determine the optimal screening strategy for our FSW population, we compared screening efficiency (number of colposcopies required to detect one histological cervical intraepithelial neoplasia 2 or more severe, ≥CIN 2) of three

strategies (conventional cytology, hrHPV physician- and self-testing) for a once-in-a-lifetime cervical cancer screening. At a lower "willingness-to-pay" upper limit (number of colposcopies willing to conduct to detect a case of ≥CIN2) of <15 colposcopies per case of ≥CIN 2 detected, conventional cytology was the optimal strategy for our FSW population, given the available information.

Screening using hrHPV self-testing in high-risk populations such as our FSW can be a reliable tool for cervical cancer screening, comparing favorably with hrHPV physician-testing. HrHPV mRNA testing may still be more costly than cytology. However, a once-in-a-lifetime screening using highly sensitive hrHPV self-testing in a low-resource setting with infrequent screening may potentially increase the overall screening cost-effectiveness, compared with cytology. Our decision analysis nevertheless suggests that, given the current information, more data are still required to determine which screening strategy is most efficient for our FSW population.

ACKNOWLEDGEMENTS

This dissertation and all which it entailed particularly owe their thanks to Dr. Jennifer Smith. She was always supportive and solicitous of my betterment as a researcher, and as an individual. She encouraged me endlessly, and continued to believe when I found it difficult to do so. I am deeply indebted to her, and I hope my future undertakings do her mentorship and professionalism justice. This work and myself have also benefited greatly from members of my dissertation committee, to whom I wish to express my most sincere and unreserved thanks: Dr. Michael Hudgens for his expertise and advice on the statistical demands of this work, Dr. Evan Myers for his continued patience in coaching me in a new skill, Dr. Charlie Poole for his encouragement, guidance and emphasis on the highest standards of epidemiological practices, and Dr. Victor Schoenbach for his insightful critique and cold editorial eye. I have learnt much from these individuals in the course of my studies and research here, during which time they have each rendered me ready and active help. Without their knowledge and ceaseless assistance, this work would not have been possible. Finally, I must also thank my long-suffering parents, as well as my family and friends. They have all been a great source of strength, and have been tremendously kind and patient with my pre-occupation with completing this degree.

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LIST OF ABBREVIATIONS

APD	Adjusted prevalence difference
ASCUS	Atypical squamous cells of undetermined significance
CI	Confidence interval
CIN	Cervical intraepithelial neoplasia
CLD	Confidence limit difference
DAG	Directed acyclic graph
ELISA	Enzyme-linked immunosorbent assay
FSW	Female sex workers
HIV	Human immunodeficiency virus
HPA	Hybridization protection assay
HPV	Human papillomavirus
HR	High-risk
HSIL	High-grade squamous intraepithelial lesion
ICC	Invasive cervical cancer
LSIL	Low-grade squamous intraepithelial lesion
NPV	Negative predictive value
PCR	Polymerase chain reaction
PPV	Positive predictive value
RLU	Relative light units
S/CO	Signal-to-cutoff
STI	Sexually transmitted infection
TMA	Transcription mediated amplification

CHAPTER 1.
SPECIFIC AIMS

Specific Aim 1

Aim 1.1: To compare the sensitivity and specificity of high-risk HPV (hrHPV) mRNA testing of physician- and self-collected specimens to detect cytological high-grade squamous intraepithelial lesions or more severe (≥HSIL) in a population of female sex workers (FSW) in Nairobi, Kenya aged 18-50 years.

*Hypothesis***:** We hypothesize that the sensitivity of hrHPV mRNA testing of physician-collected specimens for ≥HSIL would be higher than that of self-collected specimens. Specificity of hrHPV mRNA testing of physician- and self-collected specimens would be similar.

Aim 1.2: To examine the risk factors for hrHPV mRNA positivity in physician- and self-collected specimens in our population of FSW in Kenya.

Specific Aim 2

Aim 2.1: To estimate the bounds of sensitivity and specificity (and corresponding 95% confidence interval, CI) of hrHPV mRNA testing of physician- and self-collected specimens for histological cervical intraepithelial neoplasia 2 or more severe (≥CIN 2) for our FSW population in Kenya.

Specific Aim 2.2: To evaluate the potential efficiency (measured as number of colposcopies required to detect one case of ≥CIN 2) of a once-in-a-lifetime cervical cancer screening in our population of FSW in Kenya. We consider three different screening strategies: conventional cytology, hrHPV mRNA testing of physician- and self-collected specimens.

CHAPTER 2.
BACKGROUND AND SIGNIFICANCE

Human Papillomavirus and Cervical Cancer

Invasive cervical cancer (ICC) is the third most common cancer in women worldwide, the second most common cancer in women in less-developed countries, and the leading cancer in women in sub-Saharan Africa. In Eastern Africa, the estimated annual incidence of ICC is still the highest in the world (43/100,000) (1).

Human papillomavirus (HPV) is one of the most common sexually transmitted infections (STIs) worldwide. HPV infection of the cervix is well established as the primary etiologic factor in ICC carcinogenesis (2). In women, HPV is also responsible for other anogenital cancers, including vulvar and vaginal cancers (3). The clinical classification of HPV types is according to their oncogenic potential. Among the 35 or so HPV types that infect the female genital tract, 14 are considered high-risk types (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 (4). Women with a persistent infection with one of these hrHPV types were shown to have increased risk for developing severe cervical dysplasia or ICC (4, 5). HPV types 16 and 18 alone accounted for 60-70% of ICC worldwide. Low-risk HPV types (types 6, 11, 26, 30, 32, 34, 40, 42-44, 53-55, 57, 61, 64, 67, 69-73, 81-86, 89 and JC 9710) on the other hand are weakly associated with ICC and precancer (6).

HPV prevalence among African women ranged from 18%-60%, with typically higher rates among HIV-seropositive women (7). In Kenya, prevalence of high-risk HPV

(hrHPV) infection was 44% in women attending family planning clinics in Nairobi (mean age 35 years, where prevalence of cytologically-confirmed high-grade squamous intraepithelial lesion or more severe (≥HSIL) was 7% (8). Among FSW in Mombasa (median age 28 years), prevalence of hrHPV infection was 56%, and that of ≥HSIL was 3% (9).

Constraints to Cytology-Based Cervical Cancer Screening

The lower incidence and mortality of ICC in developed countries is attributed to the implementation of effective conventional cytology screening programs (10-13). Such lower incidence and mortality rates are, however, not observed in low-resource regions such as Eastern Africa (1). Although facilities for opportunistic screening may be available (14), there are still insufficient infrastructure and resources to implement and effectively maintain screening programs (15). In Kenya in 2001-2002, only 3% of women were estimated to have had a Pap smear in the last three years (4% in urban areas, 2% in rural areas) (16), compared to 40% in all countries and 19% in less-developed countries (14).

Potential barriers to seeking a pelvic examination for cervical cancer screening include cultural reticence as well as residence in remote areas where healthcare services are not easily assessable (15, 17). Often, these barriers result in late use of screening services by women at high risk of cervical cancer, or by those already presenting with advanced disease (17). Frequent screenings can be costly and satisfactory coverage and follow-up of women with abnormal smears for treatment is difficult to attain (18).

The interpretation of smears is also inconsistent, due to the subjectivity with which the smears are read by cytopathologists (18, 19). In the ASCUS-LSIL Triage Study (ALTS), a multicenter randomized controlled-trial on management of atypical squamous cells of

undetermined significance (ASCUS) and low-grade squamous intraepithelial lesion (LSIL) cytological interpretations, the quality reviewer interpretation of LSIL concurred with the original interpretation in 68% of cases, and only 47% for HSIL (19). Finally, Pap smears have low sensitivity for detecting ≥CIN 2, with an average sensitivity of 50% (range 30-80%) (20, 21).

On the other hand, compared with conventional cytology, molecular HPV testing provides an objective test outcome and is highly reproducible (22). High test reproducibility has implications on the consistency of the number of true and false positives and negatives over time, given that other factors remained constant. The objectivity of test outcome also means that the benefits of screening are not unequally distributed based on the expertise, consistency or availability of cytopathologists. HrHPV testing is also more sensitive at detecting ≥CIN 2, although generally less specific, compared with conventional cytology (23). Higher sensitivity means higher negative predictive value (NPV) for ≥CIN 2 over a longer period of time, as risk of developing ≥CIN 2 is low following a negative hrHPV test result, thus potentially allowing for decreased number of screening visits and cost (24). The lower specificity of hrHPV testing compared with conventional cytology is because most HPV infections are transient, and only a minority of women will develop high-grade lesions or more severe (25).

Although prophylactic HPV vaccines are currently available for primary prevention of oncogenic HPV types (26, 27), secondary prevention by way of effective screening will remain an essential component of screening programs (28). Screening is especially relevant in low-resource countries such as Kenya, where screening strategies adopted in more developed countries may not be feasible due to logistical and cost issues (28-30). Therefore,

alternative screening methods such as HPV testing, which is less subject to inconsistencies and potentially allows for larger intervals between screenings, should be considered (31-35).

High-risk HPV mRNA Testing

In previous studies from Europe and North America, the sensitivity of HPV DNA testing to detect ≥CIN 2 was far superior to that of conventional cytology (96% versus 53%), although somewhat less specific (91% versus 96%) (23). Recent evidence show that molecular HPV testing based on mRNA detection could improve specificity for ≥CIN 2, compared with DNA detection (36).

HPV infection establishes itself at the basal epithelium of the cervix (37). Here, the early proteins E6 and E7 are expressed at low levels for viral genome maintenance and cell proliferation. As differentiation of the cells of the basal epithelium occurs, the HPV virion undergoes genome amplification, virus assembly and eventual release. Expression patterns of the virion also shift from early to late genes. Should genetic or epigenetic changes occur to cause progression to precursor lesions, the expression of E6/E7 is deregulated, resulting in the overexpression of these E6/E7 oncogenes. The E6/E7 mRNA expression of hrHPV types is thus vital to the development and progression of ICC (38). HrHPV E6/E7 mRNA overexpression could therefore be a more specific marker of precursor lesions which potentially warrants further medical attention, compared with hrHPV DNA, since the latter may also be found in low- and medium-grade lesions which could be due to a transient HPV infection. The hrHPV mRNA assay used in the present study detects HPV E6/E7 mRNA from 14 high-risk HPV types (36).

Three population-based studies, from France (39), Canada (35) and China (40), have so far been conducted to compare the performance of hrHPV mRNA testing with that of hrHPV DNA testing (Table 2.1). In these studies, the hrHPV mRNA testing had comparably high sensitivity for the detection of ≥CIN 2 compared with hrHPV DNA testing. HrHPV mRNA testing also appeared to be generally more specific than hrHPV DNA testing for the detection of ≥CIN 2. Similar sensitivity and higher specificity of hrHPV mRNA testing compared with that of hrHPV DNA testing for ≥CIN 2 have also been observed in women referred for colposcopy.

These data demonstrate the feasibility of hrHPV E6/E7 mRNA testing as a valuable biomarker for detecting ≥CIN 2, comparing favorably against the more widely used hrHPV DNA testing and cytology. The potential of hrHPV mRNA testing should therefore be further explored in variable populations and settings. We are not aware of studies of hrHPV mRNA testing as a possible tool for cervical cancer screening in Africa.

Self-Collected Specimens for High-Risk HPV and Cervical Lesion Detection

HPV testing of self-collected specimens has been demonstrated in previous studies to be a viable option for circumventing the barriers to cytology- based screening programs, such as the need for an initial pelvic examination (18, 28, 41-43). A meta-analysis of 18 studies found a high level of agreement (kappa statistic, $\kappa = 0.66$; 95% CI: 0.50-0.82) between self- and physician- collected specimens for the detection of hrHPV DNA (44). The sensitivity and specificity of hrHPV testing of self-collected specimens (hrHPV self-testing) to detect ≥CIN 2 differed by population and country (Table 2.2).

In previous studies, sensitivity of hrHPV testing of physician-collected specimens (hrHPV physician-testing) to detect ≥CIN 2 ranged from 82 to 97%, while that of self-collected specimens ranged from 49 to 92% (Table 2.2). Specificity ranged from 52 to 92% using physician-collected specimens, and 53-89% using self-collected specimens. In all previous studies, sensitivity of hrHPV physician-testing for ≥CIN 2 was generally higher than that of self-testing. Specificity, however, appeared similar in hrHPV physician- and self-testing across all studies.

Four previous studies have evaluated hrHPV physician- and self-testing (15, 45-47) in African populations. Only one of these studies, conducted on previously unscreened women from South Africa, compared hrHPV physician- and self-testing to detect ≥CIN 2 (47) (Table 2.2). In this study, the sensitivity of hrHPV self-testing for ≥CIN 2 was similar to that of conventional cytology (66% and 68%, respectively), but lower than that of physician-testing (84%). Specificity of hrHPV physician- and self-testing, as well as of conventional cytology for ≥CIN 2 were similar (83%, 85% and 88%, respectively).

Acceptability of self-collection has been surveyed (15, 28, 48, 49). In Rakai, Uganda, women favored self-collection performed during home visits over collection through a pelvic examination (>85% versus 50% acceptability) (15, 28). Self-collection was also generally accepted in Thailand and China, although some women had reservations about the safety of the device (48, 49).

Benefits and Cost Implications of Different Cervical Cancer Screening Strategies in Low-Resource Settings

Randomized controlled trials provide the most accurate estimate of screening efficacy of various cervical cancer screening strategies. However, these trials are expensive and

require screening of large populations to generate a measurable effect. Also, these trials may not be able to adequately assess all types screening strategies (50). Simulation modeling can consider differences in population characteristics, test sensitivity and specificity, costs, and benefits of different tests or strategies. Results of modeling can potentially inform specific policy questions regarding the optimal cervical cancer prevention strategy for a particular setting, as well as the affordability of each setting for a screening strategy and follow-up re-screening or treatment (50). Relatively few studies have assessed screening costs and benefits of different cervical cancer screening strategies, including hrHPV-based testing, in low-resource countries (51-55), and to date, only one study, from South Africa, has evaluated screening costs and benefits of hrHPV self-testing in a low-resource setting (52).

Direct quantitative comparisons of results of screening benefits and costs from different studies are challenging due to model choice and parameter assumptions, as well as to the imprecision surrounding the estimates of costs and outcomes (50). Parameters for population characteristics, as well as test sensitivity and test specificity may also vary by settings. Nevertheless, previous studies on low-resource settings in general found that for screening one to three times during a woman's with screening coverage below 25%, improvements in the sensitivity of screening tests will have minimal population impact on lifetime cervical cancer risk. However, if high screening coverage rates can be achieved in settings with infrequent screening, small changes in test sensitivity (range 65-95%) can potentially have a larger impact on lifetime cervical cancer risk and overall screening cost-effectiveness; changes in specificity (range 70-96%), on the other hand, would have less impact. On the other hand, in developed countries, overall screening cost-effectiveness is heavily affected by small changes in test specificity, due to greater screening coverage,

frequent screening and more aggressive follow-up strategies. Previous studies also found that the decision among cytology, hrHPV-based testing, and visual inspection in low-resource settings will be most affected by the following factors: whether or not screening and treatment can be accomplished in fewer visits, costs required for each test, and test sensitivity (50).

One challenge of such model based analyses is that they are invariably subject to uncertainty (imprecision) in the parameters used as inputs in the model (56). If inadequately assessed, such parameter uncertainty can lead to the adoption of a suboptimal screening strategy with consequent allocation of limited resources.

Table 2.1 Comparison of sensitivity and specificity of the high-risk HPV mRNA and high-risk HPV DNA testing to detect ≥CIN 2

Reference	Country	Population	Mean/ median age	N	Sensitivity of hrHPV testing for ≥CIN 2 (%)[1] (95% CI)		Specificity of hrHPV testing for ≥CIN 2 (%)[1] (95% CI)	
					HrHPV mRNA	HrHPV DNA	HrHPV mRNA	HrHPV DNA
Monsonego (2011) (39)	France	Population-based	20-65	4,429	92 (86-98)	97 (93-100)	92 (91-93)	86 (85-87)
Ratnam (2011) (35)	Canada	Population-based	36	1,373	100 (56-100)	100 (56-100)	88 (87-90)	85 (83-87)
Wu (2010) (40)	China	Population-based	35	2,095	100 (87-100)	89 (71-98)	91 (90-92)	85 (83-86)
Castle (2007) (37)[2]	USA	Clinical specimens	NA	527	92 (85-97)	92 (85-97)	54 (49-59)	47 (42-52)
Ovestad (2011) (57)[3]	Norway	Referral	37	528	98 (89-100)	100 (93-100)	38 (27-49)	18 (10-28)
Waldstrom (2011) (58)[2]	Denmark	Referral	42	325	88 (75-95)	94 (83-99)	78 (73-83)	64 (58-70)
Clad (2011) (59)	Germany	Referral	NA	424	92 (88-95)	91 (87-94)	75 (68-81)	61 (54-68)
Reuschenbach (2010) (60)	Germany	Referral	36	205	92 (85-96)	93 (87-97)	65 (53-76)	56 (44-67)
Dockter (2009) (36)	France	Referral	NA	753	91 (85-95)	95 (90-98)	56 (52-60)	47 (43-51)
Szarewski (2008) (61)	UK	Referral	30	949	95 (92-97)	100 (98-100)	42 (38-46)	28 (25-32)

Table 2.1 continued

HrHPV: high-risk human papillomavirus; ≥CIN 2: cervical intraepithelial neoplasia 2 or more severe; CI: confidence interval; NA: not available
[1]Unless otherwise indicated, hrHPV mRNA testing was performed using the APTIMA HPV Assay (Hologic Gen-Probe, CA), which detects 14 hrHPV types (16,18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) (36), and hrHPV DNA testing was performed using the Hybrid Capture 2 High-Risk HPV DNA Test (Qiagen, CA), which detects 13 hrHPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) (62)
[2]HrHPV DNA testing was performed using the Linear Array HPV Genotyping Test (Roche Diagnostics, IN), which detects 13 hrHPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) and 24 low-risk HPV types (6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73,81, 82, 83, 84, IS39 and CP6108) (63)
[3]HrHPV DNA testing was performed using the Amplicor Human Papillomavirus Test (Roche Diagnostics, IN), which detects 13 hrHPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) (63)

Table 2.2 Comparison of sensitivity and specificity high-risk HPV DNA testing of physician- and self-collected specimens to detect ≥CIN 2

Reference	Country	Population	Mean/ median age	N	Sensitivity of hrHPV DNA testing for ≥CIN 2 (%)[1] (95% CI)		Specificity of hrHPV DNA testing for ≥CIN 2 (%)[1] (95% CI)	
					Physician-collection	Self-collection	Physician-collection	Self-collection
Belinson (2003) (64)	China	Population-based	41	8,497	97 (95-99)	87 (84-91)	80 (79-81)	77 (76-78)
Salmeron (2003) (18)	Mexico	Population-based	41	1,147	93 (86-97)	71 (61-80)	92 (91-92)	89 (89-90)
Wright (2000) (47)	South Africa	Previously unscreened women	39	1,365	84 (73-98)	66 (53-77)	81 (80-85)	81 (79-83)
Bhatla (2009) (28)	India	Women with gynecological complaints	36	546	90 (81-99)	80 (68-92)	92 (89-94)	88 (85-91)
Garcia (2003) (65)[2]	Arizona, Mexico, Peru	Women at colposcopy clinic	37	334	82 (75-90)	49 (39-59)	67 (61-73)	73 (52-79)
Sellors (2000) (42)	Canada	Women at colposcopy clinic	32	200	98 (91-100)	86 (75-94)	52 (44-61)	53 (45-62)
Hillemanns (1999) (41)	Germany	High-risk women	NA	247	92 (81-98)	92 (81-98)	72 (66-78)	61 (55-68)

HrHPV: high-risk human papillomavirus; ≥CIN 2: cervical intraepithelial neoplasia 2 or more severe; CI: confidence interval

[1]Unless otherwise indicated, hrHPV DNA testing was performed using the Hybrid Capture 2 High-Risk HPV DNA Test (Qiagen, CA), which detects 13 hrHPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) (62)

[2]HPV DNA testing performed by polymerase chain reaction (PCR) amplification using the PGMY 09/11 L1 consensus primer system, followed by detection of 27 HPV types (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51-59, 66, 68, 73, 82, 83 and 84) (65)

CHAPTER 3.
METHODS

Study design

Study population and recruitment process

Between August 2009 and March 2011, FSW attending the Korogocho clinic for sexually transmitted diseases in Nairobi were invited to participate in a cervical cancer screening study. The clinic, jointly managed by the University of Nairobi, provides medical care including free cervical cancer screening and treatment for sexually transmitted infections (STIs) for FSW in the Korogocho slum area. The clinic also provides counseling on the risks of commercial sex work and ways by which these risks may be reduced.

Women were informed of the study by community peer leaders during "baraza" public meetings. Potential participants were advised that participation was completely voluntary, and that their care at the clinic would not be affected should they choose to decline participation. Participants must be between 18-50 years of age. Women were not eligible if they had undergone hysterectomy or were in the second trimester of pregnancy or later. A total of 350 FSW aged 18-49 years provided written informed consent and were subsequently enrolled.

Specimen collection and processing

At the clinic during screening, participating women were administered a questionnaire to collect sociodemographic, reproductive, and sexual behavior data. Then,

each participant in private self-collected a cervico-vaginal specimen for hrHPV mRNA testing, using the APTIMA Cervical Specimen Collection and Transport cytobrush (Hologic Gen-Probe, San Diego, CA) according to pictorial instructions. The cytobrush was then swirled in the APTIMA specimen transport medium (STM) and discarded. The participant then underwent a pelvic examination, during which a physician collected one cervical sample for hrHPV mRNA testing, using a Cervex-Brush (Rovers Medical Devices, The Netherlands), which was then swirled in the PreservCyt medium (Hologic Gen-Probe, San Diego, CA) and discarded. The physician collected a second cervical sample to conduct a conventional Pap smear. The physician- and self-collected specimens were stored at -20ºC until their transport to Hologic Gen-Probe in San Diego for hrHPV mRNA and STI testing. Blood samples were also drawn from each participant for HIV testing and CD4 count assessment in the University of Nairobi.

HrHPV mRNA testing

HrHPV mRNA testing of physician- and self-collected specimens was conducted using the APTIMA HPV Assay (Hologic Gen-Probe, San Diego, CA) by Hologic Gen-Probe in San Diego, according to manufacturer's instructions, without knowledge of the Pap smear or other study results. The APTIMA HPV Assay is a qualitative nucleic acid amplification test which detects E6/E7 mRNA of 14 hrHPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68), but does not identify the HPV type. The assay involves three main steps, all taking place in a single tube. First, target mRNA capture by target-specific oligomers linked to magnetic microparticles; second, amplification of the isolated target mRNA using Transcription Mediated Amplification (TMA); third, detection of the amplification products

by chemiluminescent-labeled probed in the Hybridization Protection Assay (HPA). The hybridized probe signals are then measured in relative light units (RLU). An internal control added to all samples at the target capture step minimizes the risk of false-negative results (66).

Before specimen testing, physician-collected specimens in PreservCyt solution were transferred to a tube containing APTIMA STM (Self-collected specimens were already in APTIMA STM). The APTIMA STM lyses the cells, releasing mRNA, and protects them from degradation. When the APTIMA HPV Assay is performed, the Assay captures only single-stranded nucleic acids to ensure that only hrHPV mRNA, but not DNA, is detected. The sequence-specific regions of the capture oligomers hybridizes to specific regions of the target hrHPV mRNA. The capture oligomer-hrHPV mRNA complex is then captured out of solution by lowering the temperature of the reaction to room temperature. The capture oligomer also contains a string of deoxyadenosine residue. The lowering of the temperature allows hybridization between the deoxyadenosine region on the capture oligomer and the poly-deoxythymidine molecules attached to magnetic particles. The captured target hrHPV mRNA bound to the magnetic particles are pulled to the side of the reaction tube by magnets. The supernatant is aspirated, and the particles are washed to remove residual specimen that potentially act as amplification inhibitors (66).

In the second step of the APTIMA HPV Assay, the hrHPV mRNA is amplified using TMA. TMA is a transcription-based nucleic acid amplification method which uses two enzymes, namely the MMLV reverse transcriptase and T7 RNA polymerase. The MMLV reverse transcriptase generates a DNA copy of the target hrHPV mRNA sequence, which

contained a promoter sequence for T7 RNA polymerase. From the DNA copy template, T7 RNA polymerase generates multiple copies of hrHPV mRNA amplicon (66).

In the third step, the hrHPV mRNA amplicon are detected by HPA, using single-stranded nucleic acid probes complementary to the amplicon, and which contain chemiluminescent labels. These labeled probes hybridize to the amplicon. Hybridized probes are differentiated from unhybridized ones using the Selection Reagent, which inactivates the label on the unhybridized probes (66).

Light emitted from the labeled RNA-DNA hybrids is measured as photon signals in a luminometer and reported as RLU. Results of the assay are interpreted based on the analyte signal-to-cutoff (S/CO). An internal control, added during the target capture, monitors the target capture, amplification and detection steps. The signal emitted by the internal control is distinguished in each step from the HPV signal by the different light emission kinetics from probes with different labels. The hrHPV mRNA amplicon is detected using probes with slower light emission (glower), while the internal control amplicon is detected using a probe with a more rapid light emission (flasher). HrHPV mRNA testing results are interpreted as positive, negative or invalid, as determined by the internal control RLU and the analyte S/CO (Table 3.1) (66).

A run of the APTIMA HPV Assay is invalidated and subsequently repeated when any of the following occurred: more than one invalid negative calibrator replicate, more than one invalid positive calibrator replicate, an invalid negative control or an invalid positive control. A negative calibrator is buffered solution, a positive calibrator non-infectious HPV 16 *in vitro* transcript at 1,000 copies per mL in buffered solution, a negative control lysed, inactivated HPV-negative cultured cells in buffered solution and positive control lysed,

inactivated HPV-negative and HPV-positive cultured cells at 25 cells per mL in buffered solution (66).

STI testing

The physician- and self-collected specimens were also tested for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* with the APTIMA Combo 2 assay, for *Trichomonas vaginalis* with the APTIMA TV assay and for *Mycoplasma genitalium* with the APTIMA research use only assay, using the same target capture, TMA and HPA steps as hrHPV mRNA detection. STI testing was also performed according to the manufacturer's instructions, without knowledge of the Pap smear or other study results. Serum was tested for HIV antibodies by enzyme-linked immunosorbent assay (ELISA), with positive results confirmed by a second ELISA. Peripheral blood CD4 cells were enumerated for HIV-seropositive women.

Cervical cytology

Cytological smears were evaluated at the University of Nairobi and classified according to the 2001 Bethesda System (TBS 2001) for cervical cytology. All smears were independently read by two cytopathologists blinded to HPV and STI testing results. For discrepant cases, the final diagnosis was made based on the consensus of the reviewing cytopathologists. Study participants were notified of their Pap smear results two weeks after their screening visit. Women with LSIL or ASCUS were instructed to undergo a repeat cytology four months later. Women with HSIL or atypical squamous cells of undetermined significance with possibility of high-grade changes (ASCUS-H) were immediately referred to

a colposcopy-directed biopsy. In the event of histological ≥CIN 2, women received standard care and treatment at Kenyatta National Hospital. Women who had <CIN 2 were considered disease negative for statistical analyses.

Statistical methods

For specific aim 1.1: Sensitivity and specificity hrHPV mRNA physician- and self-testing specimens for ≥HSIL

Of the 350 FSW recruited, 6 women were missing at least one hrHPV testing result (specimens missing for 1 woman, self-collected specimens from 5 women invalid for hrHPV mRNA testing) and were excluded from subsequent analyses, resulting in a final sample size of 344. Median unbiased estimates and their mid-*P* 95% confidence intervals (CI) were computed for sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of hrHPV mRNA testing of physician- and self-collected specimens for the detection of ≥HSIL (67).

For specific aim 1.2: Risk factors for hrHPV positivity in physician- and self-collected specimens

Potential risk factors for hrHPV positivity in physician- and self-collected specimens were determined, and directed acyclic graphs (DAG) (68) were constructed and analyzed with online software (69) to identify minimally sufficient sets of adjustment variables to reduce confounding in binomial regression estimates of the prevalence difference of hrHPV positivity between categories of potential risk factors. Agreement between hrHPV positivity in physician- and self-collected specimens was measured by the kappa statistic. Risk factors analyses of hrHPV positivity in physician- and self-collected specimens were restricted to women with normal cervical cytology.

For specific aim 2.1: Estimation of bounds of sensitivity and specificity of hrHPV testing for ≥CIN 2

Using data on sensitivity and specificity of hrHPV testing for ≥HSIL from our FSW study (70) and those of ≥HSIL for ≥CIN 2 from the South African study (47), we estimated sensitivity and specificity bounds of hrHPV physician- and self-testing in our study of FSW for ≥CIN 2 detection. The estimation is described in detail in the Appendix.

For specific aim 2.2
A. Decision model

We developed a decision model using TreeAge Pro™ 2012 (TreeAge Software Inc., Williamstown, MA, USA) to estimate the potential efficiency of a once-in-a-lifetime cervical cancer screening among Kenyan FSW using three screening strategies: conventional cytology, hrHPV physician- and self-testing (Figure 1). Screening efficiency was defined as the number of colposcopies required to detect one case of ≥CIN 2. This definition was not based on total screening cost, as we did not have the resources to estimate costs. Furthermore, recommendations of screening strategy need not necessarily be based on cost-effectiveness findings (71). Colposcopy referrals were used as a surrogate for screening program cost in our analysis. We also assume that the cost of each screening test is approximately equivalent. We constructed a decision model by which women who had ≥HSIL at cytological screening or who tested hrHPV mRNA positive in either the physician- or self-collected specimens were referred to colposcopy. We modeled whether a woman with or without ≥CIN 2 would be referred for colposcopy, under each screening strategy.

B. Model parameters

We obtained estimates of population age distribution, HIV prevalence and of the sensitivity and specificity of hrHPV physician- and self-testing for ≥HSIL detection from our FSW study in Kenya (70). We obtained estimates for sensitivity and specificity of ≥HSIL for ≥CIN 2 detection from published data on unscreened South African women (median age 39 years) (47). The prevalence of ≥HSIL was 4.1% (95% confidence interval, CI: 2.3-6.6%) in our FSW study (70) and 3.1% (95% CI: 2.3-4.1%) in the South African study (47). Prevalence of ≥CIN 2 in the South African study was 4.1% (95% CI: 3.2-3%) (47) .

Using data on sensitivity and specificity of hrHPV testing for ≥HSIL from our FSW study (70) and those of ≥HSIL for ≥CIN 2 from the South African study (47), we estimated sensitivity and specificity bounds of hrHPV physician- and self-testing for ≥CIN 2 detection in our study of FSW. The estimation is described in detail in the Appendix.

We parameterized sensitivity as a function of specificity and of the diagnostic odds ratio (72) to take into account the inverse relationship between sensitivity and specificity. We used the ratio of sensitivity of hrHPV physician- to self-testing for ≥HSIL in our FSW study to reflect the difference in sensitivity of hrHPV physician- and self-testing for ≥CIN 2. The same was done to reflect the different in specificity. The performance of hrHPV physician- relative to self-testing for ≥HSIL in our study of FSW was consistent (higher sensitivity with physician- compared with self-testing, similar specificity) with that of previous studies comparing hrHPV DNA physician- and self-testing for ≥CIN 2 in resource-low settings (18, 28, 47, 64).

We ascribed a lognormal distribution to the diagnostic odds ratio of ≥CIN 2 detection by conventional cytology (using ≥HSIL as screening threshold). Sensitivity and specificity of

hrHPV mRNA testing of physician- and self-collected specimens for ≥CIN 2 were first sampled from uniform distributions. We specified the upper and lower limit of these uniform distributions with our estimated upper and lower bound for sensitivity and specificity. These sampled values were then ascribed a beta distribution, where the mean was the sampled value and the standard deviation was the mean of the approximate standard error of the lower and upper bound estimate. Parameter estimates and distributions used in our model are shown in Table 1.

Monte Carlo simulation

Monte-Carlo simulations were performed to calculate the probability of each screening strategy being optimal at different levels of willingness-to-pay. Probabilistic sensitivity analysis was first performed, by randomly drawing a value from each parameter distribution (screening test sensitivity and specificity), thereby allowing for the evaluation of the combined uncertainty about the parameters in the model (73). This procedure was repeated 10,000 times (M=10,000).

For each set of parameters drawn, 1,000 observations (N=1,000) were generated to account for the random variation in individual-level outcomes (e.g. age, HIV-serostatus and whether or not a woman had ≥CIN 2) (74). For each of these trials of N=1,000 observations, the number of colposcopies performed and the number of women whose ≥CIN 2 was detected by colposcopy were estimated for each screening strategy, and the net benefit (NB) is calculated across a range of willingness-to-pay limit.

In a cost-effectiveness analysis, a cost-effectiveness NB of one option (i.e treatment or intervention) is compared to another. This cost-effectiveness NB is defined as: $NB = \lambda \times$

$E - C$ (where C is the total cost and E the total effect of each option), and is calculated across a range of the decision-maker's willingness to pay for one unit gain in health outcome, λ. An option is cost-effective if and only if the NB for a given $\lambda > 1$ (75). In assessing the NB of different screening strategies for our FSW study, C represents the number of colposcopies performed E the number of women whose ≥CIN 2 was detected by colposcopy and λ the number of colposcopy the decision maker is willing to conduct to detect one case of ≥CIN 2.

For each trial of N=1,000 observations, the screening strategy with the highest NB was identified. The proportion of the M=10,000 interations in which a screening strategy had the highest NB at a given λ was then used to construct an acceptability curve for each screening strategy by plotting these proportions on the y-axis and the corresponding λ on the x-axis (Figure 2) (75). Since we defined willingness-to-pay as the number of colposcopies willing to conduct to detect one ≥CIN 2, this is also equivalent to the inverse of the positive predictive value (PPV) for ≥CIN 2 among women with ≥HSIL or positive hrHPV physician- or self-testing result.

Table 3.1 Interpretation of APTIMA HPV Assay result for the detection of high-risk HPV mRNA (66) in female sex workers, Kenya

APTIMA HPV Assay result	Criteria
Positive	Analyte S/CO[1] ≥0.05 Internal control ≤2,000,000 RLU Analyte ≤13,000,000 RLU
Negative	Analyte S/CO <0.05 Internal control ≤2,000,000 RLU Internal control ≥ Internal control cutoff[2]
Invalid	Analyte S/CO <0.05 and Internal control < Internal control cutoff OR Internal control >2,000,000 RLU OR Analyte >13,000,000 RLU

HPV: human papillomavirus; S/CO: signal-to-cutoff; RLU: relative light unit

[1]Analyte S/CO = analyte RLU/analyte cutoff, where analyte cutoff = (mean analyte RLU of the valid negative calibrator replicates) + (0.09 x mean analyte RLU of the valid positive calibrator replicates)

[2]Internal control cutoff=0.5 x (mean internal control RLU of the valid negative calibrator replicates)

CHAPTER 4.
HIGH-RISK HUMAN PAPILLOMAVIRUS mRNA TESTING OF PHYSICIAN- AND SELF-COLLECTED SPECIMENS FOR CERVICAL LESIONS DETECTION IN HIGH-RISK WOMEN, KENYA

Overview

Little is known about the performance hrHPV physician- and self-testing or risk factors for hrHPV mRNA positivity in physician- versus self-collected specimens. We compared the performance of hrHPV mRNA physician- and self-testing to detect ≥HSIL and examined risk factors for hrHPV mRNA positivity in FSW in Nairobi.

From 2009-2011, 344 FSW participated in this cross-sectional study. Women self-collected a cervico-vaginal specimen. A physician conducted a pelvic examination to obtain a cervical specimen. Physician- and self-collected specimens were tested for hrHPV mRNA and sexually transmitted infections using APTIMA nucleic acid amplification assays. Cervical cytology was conducted using physician-collected specimens and classified according to the Bethesda criteria.

Overall hrHPV prevalence was similar in physician- and self-collected specimens (30% versus 29%). Prevalence of ≥HSIL was 4% (N=15). Overall sensitivity of hrHPV mRNA testing for detecting ≥HSIL was similar in physician- (86%; 95% CI=62-98%, 13 cases detected) and self-collected specimens (79%; 95% CI=55-95%, 12 cases detected). Overall specificity of hrHPV mRNA for ≥HSIL was similar in both physician- (73%; 95% CI=68-79%) and self-collected (75%; 95% CI=70-79%) specimens. HrHPV mRNA

positivity in both physician- and self-collected specimens appeared higher in women who were younger (<30 years), had *Trichomonas vaginalis* or *Mycoplasma genitalium* infections, or had >8 years of educational attainment.

Self-collected specimens for hrHPV mRNA testing appeared to have similar sensitivity and specificity as physician-collected specimens for the detection of ≥HSIL among high-risk women.

Introduction

Successful implementation of Papanicolaou (Pap) smear screening programs has drastically reduced ICC incidence and mortality in developed countries (11, 13). However, Pap screening programs have been difficult to implement in low-resource settings due to limited infrastructure and access to trained cytopathologists and clinicians (30). Consequently, a region with low screening coverage such as Eastern Africa still has among the highest estimated annual incidence of ICC in the world (34/100,000) (1).

A primary screening approach based on testing for the central etiological risk factor for cervical cancer, hrHPV infection, in self-collected specimens could help increase access to screening in low-resource settings. hrHPV testing of self-collected specimens can be integrated into a two-stage cervical cancer screening process (76). Women with positive hrHPV testing result could be re-screened using a second, more specific test (e.g Pap smear, visual inspection with acetic acid, colposcopy) or referred directly to treatment (47). As primary hrHPV self-testing does not require an initial gynecologic examination, hrHPV self-testing as an initial screen could be an advantage in low-resource settings if the appropriate follow-up of women with positive HPV results can be assured. Furthermore, women's

acceptability of self-collection has generally been positive in various geographical settings worldwide (77).

Very few studies have evaluated hrHPV self-testing testing for cervical cancer screening in low-resource settings and, to date, all have used HPV DNA testing (28, 47, 78). Recently developed diagnostic testing allows for the detection of hrHPV mRNA, which may be a more specific marker than hrHPV DNA for clinically significant disease (39), and has not yet been implemented in a high-risk, low-resource setting.

We present here results comparing the performance of hrHPV mRNA physician- and self-testing to detect high-grade cervical lesions in high-risk FSW in Kenya. We also examined risk factors for hrHPV mRNA positivity in our population of FSW.

Materials and Methods

Study population

From August 2009 to March 2011, FSW attending the Korogocho clinic in Nairobi, Kenya were invited to participate in this study to compare the performance of physician- and self-collected specimens for cervical cancer screening with hrHPV mRNA testing. The clinic provides counseling and medical care including screening and treatment for cervical cancer as well as STIs for FSW in the Korogocho slum area.

Women were informed of the study by community peer leaders during "baraza" public meetings. Women were not eligible if they had undergone hysterectomy or were in the second trimester of pregnancy or later. A total of 350 FSW aged 18-49 years provided written informed consent and were subsequently enrolled.

At screening, participating women were administered a questionnaire to collect sociodemographic, reproductive, and sexual behavior data. Of the 350 FSW recruited, 6 women were missing hrHPV mRNA testing results and were excluded from subsequent analyses, resulting in a final sample size of 344.

Sample collection and laboratory analyses

Each woman self-collected a cervico-vaginal specimen using the APTIMA Cervical Specimen Collection and Transport cytobrush (Hologic Gen-Probe Incorporated, San Diego, CA) according to pictorial instructions. The cytobrush was then swirled in the APTIMA specimen transport medium and then discarded. During a pelvic examination, the physician collected one cervical sample from each woman using a Cervex-Brush (Rovers Medical Devices, The Netherlands), which was then swirled in the PreservCyt (Hologic Gen-Probe Incorporated, San Diego, CA) medium and then discarded. The physician then collected a second cervical sample to conduct a conventional Pap smear.

Cytological smears were evaluated at the University of Nairobi and classified according to the 2001 Bethesda System for cervical cytology. All smears were independently read by two cytopathologists blinded to HPV and STI testing results. For discrepant cases, the final diagnosis was made based on the consensus of the reviewing cytopathologists. Study participants were notified of their Pap smear results two weeks after their screening visit. Women with LSIL or ASCUS were instructed to undergo a repeat cytology four months later. Women with HSIL or ASCUS-H were immediately referred to a colposcopy-directed biopsy. In the event of histological ≥CIN 2, women received standard care and

treatment at Kenyatta National Hospital. Women who had <CIN 2 were considered disease negative for statistical analyses.

HPV and STI testing

The physician- and self-collected specimens were transported to Hologic Gen-Probe in San Diego for HPV and STI testing. Laboratory testing for HPV in our study was by the APTIMA HPV Assay (Hologic Gen-Probe Incorporated, San Diego, CA) which qualitatively detects E6/E7 mRNA of 14 hrHPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68). The hrHPV mRNA assay comprises three main steps, namely target capture, transcription-mediated amplification (TMA) of the target, and finally target detection by hybridization with complementary probes linked to chemiluminescent labels (66).

The physician- and self-collected specimens were also tested for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* with the APTIMA Combo 2 assay, for *Trichomonas vaginalis* with the APTIMA TV assay and for *Mycoplasma genitalium* with the APTIMA research use only assay, using the same target capture, TMA and hybridization steps as hrHPV mRNA detection. All assays were performed according to the manufacturer's instructions, without knowledge of the Pap smear or other study results.

Serum was tested for HIV antibodies by ELISA, with positive results confirmed by a second ELISA. Peripheral blood CD4 cells were enumerated for HIV-seropositive women. The HIV ELISA and CD4 assays were conducted in University of Nairobi.

Statistical analyses

Agreement between hrHPV positivity in physician- and self-collected specimens was measured by the kappa statistic. Median unbiased estimates and their mid-*P* 95% confidence intervals (CI) were computed for sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of hrHPV testing of physician- and self-collected specimens for the detection of ≥HSIL (67). Potential risk factors for hrHPV positivity in physician- and self-collected specimens were determined, and directed acyclic graphs (DAG) (68) were analyzed with online software (69) to identify minimally sufficient sets of adjustment variables to reduce confounding in binomial regression estimates of the prevalence difference of hrHPV positivity between categories of potential risk factors. All statistical analyses were performed using SAS 9.2.

Results

Participant characteristics

Overall prevalence of hrHPV was similar in physician- (30%) and self-collected specimens (29%) (Table 4.1). HrHPV prevalence in both physician- and self-collected specimens was slightly higher in women <30 years than in older women. Prevalence of any abnormal cytology (≥ASCUS) in the population was 19%, and was similar in women ≥30 years (21%) than in younger women (17%).

Performance of hrHPV mRNA testing of physician- and self-collected specimens

The overall sensitivity of hrHPV physician-testing for ≥HSIL appeared similar to that of self-testing (Table 4.2). HrHPV physician-testing detected only 1 ≥HSIL case more than

that of self-testing (N=13 versus N=12) (Table 4.3). This ≥HSIL case, which was negative by hrHPV self-testing, was also disease negative by histology. Overall specificity for ≥HSIL appeared similar in both hrHPV physician- and self-testing (Table 4.2).

The NPV for hrHPV mRNA testing of physician- and self-collected specimens was 97-99% overall and in both age groups. The PPV was 12-13% overall, and varied by age group, being somewhat lower in women <30 years (9-10%) than in older women (17-18%). The agreement between hrHPV physician- and self-testing was κ=59% (95% CI: 49-68) overall, κ=76% (95% CI: 32-100) in women with ≥HSIL and κ=55% (95% CI: 45-65) in women with <HSIL.

Of the 15 women with ≥HSIL, 14 underwent colposcopy-directed biopsy. One woman could not be traced and was lost to follow-up. Twelve had histological ≥CIN 2, and 2 were considered disease negative (both had normal histology). Of the 12 ≥CIN 2 cases, 10 were hrHPV positive in both physician- and self-collected specimens. The remaining 2 ≥CIN 2 cases were hrHPV negative in both physician- and self-collected specimens.

Risk factors for hrHPV mRNA positivity in physician- and self-collected specimens with normal cervical cytology

Adjusted prevalence differences (APDs) of hrHPV positivity in both physician- and self-collected specimens were generally ≤5% (Table 4.4). APD was >5% in women who were younger (<30 years), had *T. vaginalis* or *M. genitalium* or had more education (>8 years). In physician-collected specimens, APD was also >5% in women who reported less frequent condom use with sexual clients and regular partners, as well as women who charged more per transaction. In self-collected specimens, APD was also >5% in HIV-seropositive women and in women with CT/GC infection.

The 95% confidence limit differences (difference between upper and lower 95% confidence limits, CLD) (79) for the APDs were generally between 0.19 and 0.25 (Table 4.4). The least precise APD estimates (95% CLD >0.25) in both physician- and self-collected specimens were those relating to HIV-seropositivity, *C.trachomatis/ N. gonorrhoeae*, *T. vaginalis* and *M. genitalium* positivity, to the number of regular sexual partners, and to frequency of condom use with regular sexual partners.

Discussion

Physician- and self-collection for hrHPV testing demonstrated a high sensitivity for the detection of ≥HSIL. HrHPV self-testing in our population of FSW with high hrHPV prevalence appeared to have similar sensitivity and specificity for ≥HSIL as that of hrHPV physician-testing. We also found that prevalence of hrHPV positivity in both physician- and self-collected specimens was somewhat higher in women who were <30 years, had *T. vaginalis* or *M. genitalium* infection, or had higher educational attainment.

The prevalence of hrHPV positivity in our study (30%) was lower than that of hrHPV DNA positivity in another FSW population in Mombasa (56%) (9) which had higher HIV prevalence than in our study. HrHPV positivity in our study was also lower than hrHPV DNA positivity in a similar FSW cohort in Nairobi (54%) which had a lower median age (23 years) than our study (80). Compared with non-FSW populations in Africa, hrHPV positivity in our study was higher than hrHPV DNA positivity in studies which had a higher median age (47) or lower HIV prevalence (15) than in our FSW study.

The overall sensitivity of hrHPV physician- and self-testing for ≥HSIL in our study appeared similar, consistent with a previous study comparing HC2 hrHPV DNA testing of

physician- and self-collected specimens for ≥CIN 2 (41). However, the small number of women with ≥HSIL (N=15) in our study made comparing sensitivity estimates of hrHPV physician- and self-testing in our population of high-risk FSW somewhat difficult. Previous studies using HC2 hrHPV DNA testing in Africa (47) and in other settings (28, 78) have also found higher overall sensitivity for ≥CIN 2 using physician- compared with self-collected specimens. Our finding of similar overall specificity of hrHPV testing for ≥HSIL when using physician- or self-collected specimens was also consistent with previous studies that used HC2 testing (28, 47, 78).

Prevalence of ≥HSIL in our study (4%) was similar to what was found in other African studies (9, 47). We found that prevalence of ≥HSIL in women <30 years in our study was similar to that in women ≥30 years (4% versus 5%) and was thus not notably different in this group of high-risk FSW in Kenya. The comparability of ≥HSIL detection by hrHPV physician- and self-testing in women <30 years in our study (Table 4.3) suggests that self-collected specimens for hrHPV testing could also be a viable option for cervical cancer screening in high-risk FSW populations 18-29 years of age in Kenya.

HrHPV self-testing in our study also detected as many ≥CIN 2 cases as did that of hrHPV physician-testing. Our results suggest that in low-resource areas where Pap screening is not routinely available, hrHPV self-testing can be used to identify high-risk women at high risk of ≥CIN 2.

In both physician- and self-collected specimens, the somewhat higher (APD >0.05) and more precise (confidence limits difference ≤0.25) estimates of hrHPV positivity in younger women was consistent with earlier findings from FSW populations, where younger age was a strong risk factor for hrHPV DNA positivity (81-83). The relatively higher and

more precise hrHPV positivity estimates in women who had higher educational attainment was, on the other hand, in contrast to previous studies on FSW populations, where lower educational attainment strongly predicted hrHPV DNA positivity (81, 84).

Women with *M. genitalium* or *T. vaginalis* infection also appeared to have higher APD of hrHPV mRNA positivity, although the estimates were imprecise (95% CLD range: 0.38 to 0.49). Nevertheless, higher hrHPV DNA positivity had been found in women with *T. vaginalis* infection in earlier studies (85, 86). Data on *M. genitalium* infection as a risk factor for HPV infection are scarce. There was no evidence of increased hrHPV DNA positivity with *M. genitalium* infection in women with normal cervical cytology (87). On the other hand, among women with abnormal cytology, higher hrHPV DNA positivity was found in those who had a mycoplasma infection (88). Inconsistencies between our results and those of earlier studies could be due to differences in populations, methods of assessing and categorizing variables, hrHPV test (DNA versus mRNA) and sampling variability.

Our study has several advantages that improved the validity of the comparison of hrHPV physician- and self-testing. Firstly all cervical smears were independently read by two cytopathologists, followed by consensus of reviewing cytopathologists for discrepant cases to ensure accurate cytological diagnoses. Secondly, a woman's physician- and self-collection of specimens were performed on the same day, enabling direct comparison of hrHPV mRNA testing results of these two sample types.

.One limitation of our study was that histological results were obtained only for women who had cytological ≥HSIL. As women who were Pap smear normal and hrHPV positive were not systematically referred to colposcopy, histological ≥CIN 2 could therefore not be used as reference standard for evaluating test sensitivity and specificity, due to the

potential for verification bias (89). Although cytological ≥HSIL and LSIL often correspond to the histologic diagnoses of, respectively, ≥CIN 2 and CIN 1, previous studies estimated that at least 70% of ≥HSIL cases and up to 30% of LSIL cases have ≥CIN 2 (90).

In terms of public health ramifications, our results of the performance of self- versus physician-collected specimens for hrHPV testing were from a high-risk population of FSW in a resource poor setting, and therefore not necessarily generalizable to lower risk populations. Also, one feasibility concern of using self-collected specimens for HPV testing in primary screening is that a woman who tested HPV positive may not return for follow-up screening or treatment (47). Other commonly reported issues include difficulty in using the brush for self-collection or in understanding the instructions, and contamination of the self-collection brush (77). Despite potential limitations, findings from a meta-analysis (44) supported an increased usage of self-collection in epidemiological studies. Future research should address if self-collection will improve screening coverage in underserved women in low-resource settings.

HrHPV self-testing may not be sufficiently specific to be a stand-alone test for cervical cancer screening (8). However, consistent with earlier studies (28, 47, 78), our findings showed that hrHPV testing, including hrHPV self-testing, have high NPV and can sensitively identify high risk women at greatest risk for high-grade lesions without an initial gynecologic examination. The limited resources may then be channeled into clinical follow-up such as re-screening using a different test of the women who were positive by hrHPV self-testing based on specific local capacity.

Table 4.1 Sociodemographic and sexual behavior characteristics of 344 female sex workers in Kenya, 2009-2011

Characteristic	Overall (N=344)		Age <30 (N=197)	Age ≥30 (N=147)
	n	median or %	median or %	median or %
Age (years) (range)		28 (18-49)	25 (18-29)	35 (30-49)
HrHPV mRNA (physician-collection)				
Negative	241	70.1	69.0	71.4
Positive	103	29.9	31.0	28.6
HrHPV mRNA (self-collection)				
Negative	246	71.5	70.0	73.5
Positive	98	28.5	30.0	26.5
Cervical cytology				
Normal	279	81.1	82.7	79.0
ASCUS[a]	14	4.1	3.0	5.4
LSIL	36	10.5	10.7	10.2
≥HSIL	15	4.3	3.6	5.4
HIV[b]				
Seronegative	259	76.0	86.3	61.8
Seropositive	82	24.0	13.7	38.2
CD4 count/mm^3 [c] (range)		478 (152-1391)	476 (160-1269)	492 (152-1391)
Sexually transmitted infections				
Chlamydia	13	3.8	6.1	0.7
Gonorrhea	8	2.3	3.0	1.4
Trichomonas vaginalis	25	7.3	6.1	8.8
Mycoplasma genitalium	44	12.8	15.7	8.8
Education (years) (range)		8 (0-16)	8 (0-16)	8 (0-15)
Marital status[b]				
Single/never married	150	43.7	54.6	29.2
Divorced/widowed/separated	190	55.4	44.4	70.1
Married/cohabitating	3	0.9	1.0	0.7
Age at first sexual intercourse (years) (range)		16 (10-25)	16 (10-22)	16 (10-25)

Number of sexual clients per week (range)		10 (2-40)	10 (2-40)	10 (2-40)
Number of regular sexual partners (range)		1 (0-10)	1 (0-10)	1 (0-7)
Condom use with sexual clients[b]				
Most of the time/always	253	73.8	76.0	73.7
Sometimes/half the time	70	20.4	19.4	21.8
Never/rarely	20	5.8	4.6	7.5
Condom use with regular sexual partners[d]				
Most of the time/always	60	24.6	19.5	32.6
Sometimes/half the time	21	8.6	10.7	5.3
Never/rarely	163	66.8	69.8	62.1
Charge per transaction (Ksh) (range)[e]		200 (50-5,000)	250 (50-5,000)	200 (50-1,500)

HrHPV: High-risk HPV; ASCUS: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; Ksh: Kenyan shillings

[a]Includes atypical glandular cells of undetermined significance (AGUS) (n=2)

[b]Numbers do not add up to total due to missing values: HIV serostatus (n=3); marital status (n=1); condom use with sexual clients (n=1)

[c]Among HIV seropositive women (n=82)

[d]Among women with regular sexual partners only (n=244)

[e]200 Ksh is equivalent to 2.50 USD

Table 4.2 Performance of high-risk HPV mRNA testing of physician- and self-collected specimens for the detection of cytological high-grade cervical lesions in 344 female sex workers in Kenya, 2009-2011

Collection method	Sensitivity/specificity for ≥HSIL[1]	Overall (N=344)	Age <30 (N=197)	Age ≥30 (N=147)
Physician-collection	Sensitivity of hrHPV mRNA (95% CI)	86% (62, 98)	84% (47, 99)	86% (52, 99)
	Specificity of hrHPV mRNA (95% CI)	73% (68, 79)	72% (65, 78)	75% (68, 82)
Self-collection	Sensitivity of hrHPV mRNA (95% CI)	79% (55, 95)	71% (33, 95)	86% (52, 99)
	Specificity of hrHPV mRNA (95% CI)	75% (70, 79)	73% (66, 79)	78% (70, 84)

HrHPV: high-risk HPV; 95% CI: 95% confidence interval
[1]Median-unbiased estimate of sensitivity and specificity; mid-*P* 95% CI

Table 4.3 High-risk HPV mRNA testing results of physician- and self-collected specimens stratified by age and cytology in 344 female sex workers in Kenya, 2009-2011

Cytology	HrHPV mRNA test result N (hrHPV+ in physician-collection, hrHPV+ in self-collection)		
	Overall	**Age <30**	**Age ≥30**
Normal	279 (68, 61)	163 (44, 41)	116 (24, 20)
ASCUS[a]	14 (8, 10)	6 (3, 4)	8 (5, 6)
LSIL	36 (14, 15)	21 (8, 9)	15 (6, 6)
≥HSIL[b]	15 (13, 12)	7 (6, 5)	8 (7, 7)
Total	344 (103, 98)	197 (61, 59)	147 (42, 39)

HrHPV: High-risk HPV; ASCUS: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion

[a]Includes atypical glandular cells of undetermined significance (AGUS) (n=2)

[b]Includes squamous cell carcinoma (SCC) (n=1)

Table 4.4 Association of potential risk factors with hrHPV mRNA positivity among 279 female sex workers with normal cytology in Kenya, 2009-2011

Risk factors	**Normal (N=279)[a]**	**HrHPV mRNA positivity (physician-collection) (N=68)**			**HrHPV mRNA positivity (self-collection) (N=61)**		
	n	**n**	**CP**	**APD (95% CI) [b]**	**n**	**CP**	**APD (95% CI) [b]**
Age (years)							
≥30	116	24	0.21	0	20	0.17	0
<30	163	44	0.27	0.06 (-0.04,0.16)	41	0.25	0.08 (-0.02,0.17)
HIV[c]							
Seronegative	227	53	0.23	0	46	0.20	0
Seropositive	51	15	0.29	0.01 (-0.17,0.20)	15	0.29	0.11 (-0.08,0.29)
STI[d]							
Chlamydia/gonorrhea							
Negative	261	60	0.23	0	55	0.21	0
Positive	18	8	0.44	0.01 (-0.29,0.28)	6	0.33	0.10 (-0.18,0.37)
Trichomonas vaginalis							
Negative	259	60	0.23	0	56	0.22	0
Positive	20	8	0.40	0.19 (-0.06,0.43)	5	0.25	0.09 (-0.12,0.31)
Mycoplasma genitalium							
Negative	244	57	0.23	0	51	0.21	0
Positive	35	11	0.31	0.09 (-0.11,0.29)	10	0.29	0.06 (-0.13,0.25)
Education (years) [e]							
≤8	208	47	0.23	0	41	0.20	0
>8	71	21	0.30	0.06 (-0.06,0.19)	20	0.28	0.09 (-0.03,0.20)
Marital status[f]							
Single/never married	122	37	0.30	0	31	0.25	0
Divorced/widowed/separated	156	31	0.20	-0.09 (-0.20,0.01)	30	0.19	-0.03 (-0.14,0.08)

Age at first sexual intercourse (years)[g]							
≥16	174	44	0.25	0	39	0.22	0
<16	105	24	0.23	-0.02 (-0.13,0.08)	22	0.21	-0.02 (-0.11,0.08)
Number of sexual clients per week [e]							
≤10	158	40	0.25	0	34	0.21	0
>10	121	28	0.23	-0.01 (-0.11,0.09)	27	0.22	0.04 (-0.05,0.14)
Number of regular sexual partners[h]							
≤1	227	54	0.24	0	47	0.21	0
>1	52	14	0.27	0.02 (-0.11,0.15)	14	0.27	0.05 (-0.08,0.18)
Condom use with sexual clients[i]							
≥Most of the time	202	47	0.23	0	44	0.21	0
<Most of the time	76	21	0.28	0.06 (-0.06,0.17)	17	0.22	0.01 (-0.09,0.12)
Condom use with regular sexual partners[j]							
≥Most of the time	43	9	0.21	0	10	0.23	0
<Most of the time	155	42	0.27	0.12 (-0.02,0.28)	36	0.23	0.05 (-0.10,0.19)
Charge per transaction (Ksh) [e]							
≤200	160	35	0.22	0	29	0.18	0
>200	119	33	0.28	0.06 (-0.06,0.16)	32	0.27	0.05 (-0.06,0.16)

HrHPV: high-risk HPV; STI: sexually transmitted infection; Ksh: Kenyan shillings; CP: crude prevalence; AP: adjusted prevalence; APD: adjusted prevalence difference; CI: confidence interval

[a]Analyses restricted to women with normal cervical cytology and valid specimens for hrHPV mRNA testing (n=279)

[b]Each minimally sufficient adjustment set identified by directed acyclic graph (DAG)

[c]One woman missing HIV serostatus; estimates adjusted for age, STI, average number of sexual clients per week, number of regular sexual partners, condom use with sexual clients and condom use with regular sexual partners

Table 4.4 continued

[d]Estimates adjusted for age, other STI, average number of sexual clients per week, number of regular sexual partners, condom use with sexual clients and condom use with regular sexual partners
[e]Estimates adjusted for age
[f]One woman missing marital status; the three women presently married were categorized under divorced/widowed/separated; estimates adjusted for age
[g]Estimates adjusted for age and education
[h]Estimates adjusted for age and marital status
[i]One woman missing data on condom use with sexual clients; estimates adjusted for age, HIV serostatus, STI, education, marital status
[j]Among women with ≥1 regular sexual partners only (n=198); estimates adjusted for age, HIV serostatus, STI, education, marital status

CHAPTER 5.
IMPACT OF UNCERTAINTY IN RELATIVE TEST PERFORMANCE BETWEEN CYTOLOGY, PHYSICIAN- AND SELF-COLLECTED HIGH-RISK HPV TESTING ON ESTIMATED SCREENING EFFICIENCY IN KENYA: A SIMULATION

Overview

The costs and benefits of each cervical cancer screening strategy must be considered to determine the optimal screening strategy for a low-resource setting. We estimated the potential efficiency (measured as colposcopies required per ≥CIN 2 detected) of a once-in-a-lifetime cervical cancer screening among female sex workers (FSW) in Kenya using three strategies: conventional cytology, high-risk (hr) HPV physician- and self-testing.

We estimated bounds of sensitivity and specificity of hrHPV physician- and self-testing for ≥CIN 2 from our study of FSW in Kenya and from published South African data. We constructed a decision model of FSW in Kenya, and performed probabilistic sensitivity analyses to identify the proportion of simulations where a given screening strategy was optimal at a given "willingness-to-pay" (number of colposcopies willing to conduct to detect a case of ≥CIN2) limit.

The estimated sensitivity bounds for ≥CIN 2 of hrHPV physician-testing (53-91%) were similar to those of self-testing (53-99%). The estimated specificity bounds were also similar for physician- (71-73%) and self-testing (71-75%). At a willingness-to-pay of <15 colposcopies per ≥CIN 2 detected, the probability of cytology being optimal was >80%. At a willingness-to-pay of 20-40 colposcopies per ≥CIN 2 detected, the probability of

all three strategies being optimal was ≤50%. Above 40 colposcopies per ≥CIN 2 detected, the probability of conventional cytology being optimal decreased to 10-20%.

At a willingness-to-pay limit of <15 colposcopies per ≥CIN 2 detected, conventional cytology was the optimal screening strategy, given the available information. At a higher willingness-to-pay limits, the probability that hrHPV testing being optimal exceeded that of conventional cytology. However, due to relative imprecision of the sensitivity and specificity of hrHPV testing for ≥CIN 2, more data (e.g extending the model to include costs and estimated impact of each screening strategy on cervical cancer mortality) is likely required to determine which screening strategy is most efficient at higher willingness-to-pay limits.

Introduction

Cytology-based screening programs effectively prevent invasive cervical cancer (ICC) (11). Such programs, however, are generally difficult to implement in low-resource regions due to limited access to trained clinicians and cytologists (30). Testing for high-risk human papillomavirus (hrHPV) infection, the necessary cause of ICC, could improve the sensitivity of screening programs to detect high-grade cervical intraepithelial neoplasia (≥CIN 2) and result in a high negative predictive value for ≥CIN 2 (23, 91). Molecular hrHPV testing also has greater reproducibility compared with conventional cytology (22). If provided via self-collected specimens, access to screening in low-resource regions could potentially be more available. High sensitivity of a screening test must also be balanced against test specificity, since a false-positive screening test may lead to colposcopy or even treatment, incurring extra costs and exposing the woman to a more invasive procedure (92).

Identification of the most efficient screening strategy requires comparison of the impact of different screening tests' sensitivity and specificity for ≥CIN 2 screening benefits and costs (74, 93). Here we compare the screening efficiency of three cervical cancer screening strategies, as well as the uncertainty surrounding the choice among them, in a female sex worker study from Kenya (70). We define screening efficiency as the number of colposcopies required to detect a case of ≥CIN 2. Screening strategies considered are conventional cytology, hrHPV testing of physician-collected specimens (hrHPV physician-test) and that of self-collected specimens (hrHPV self-test).

We first estimate bounds of sensitivity and specificity of hrHPV physician- and self-testing for ≥CIN 2 detection from our study of female sex workers (FSW) (70) and other published data from South Africa (47). We then identify the optimal screening strategy, in terms of screening efficiency at a given "willingness-to-pay" (number of colposcopies willing to conduct to detect one ≥CIN 2) limit, for a once-in-a-lifetime cervical cancer screening in a female sex worker population in Kenya.

Materials and Methods

Study population

The FSW study in Nairobi, Kenya has been previously described (70). Briefly, in 2009-2011, a total of 343 FSW (median age 28 years) were enrolled in a study to compare the performance of hrHPV mRNA testing of physician- and self-collected specimens for the detection of cytological high-grade squamous intraepithelial lesion or more severe (≥HSIL). Each participating woman first self-collected a cervico-vaginal specimen in a clinical setting using standardized illustrations. Next, during a pelvic examination, cervical samples from

each woman were collected by a physician for hrHPV mRNA testing and for conventional cytology. Women with ≥HSIL were immediately referred to colposcopy and directed-biopsy upon indication. The physician- and self-collected specimens were tested for hrHPV mRNA by the APTIMA Assay (Hologic Gen-Probe Incorporated, San Diego, CA).

Decision model

We developed a decision model using TreeAge Pro™ 2012 (TreeAge Software Inc., Williamstown, MA, USA) to estimate the potential efficiency of a once-in-a-lifetime cervical cancer screening among Kenyan FSW using three screening strategies: conventional cytology, hrHPV physician- and self-testing (Figure 5.1). Screening efficiency was defined as the number of colposcopies required to detect one case of ≥CIN 2. This definition was not based on total screening cost, as we did not have the resources to estimate costs. Furthermore, recommendations of screening strategy need not necessarily be based on cost-effectiveness findings (71). Colposcopy referrals were used as a surrogate for screening program cost in our analysis. We also assume that the cost of each screening test is approximately equivalent. We constructed a decision model by which women who had ≥HSIL at cytological screening or who tested hrHPV mRNA positive in either the physician- or self-collected specimens were referred to colposcopy. We modeled whether a woman with or without ≥CIN 2 would be referred for colposcopy, under each screening strategy.

Model parameters

We obtained estimates of population age distribution, HIV prevalence and of the sensitivity and specificity of hrHPV physician- and self-testing for ≥HSIL detection from our

FSW study in Kenya (70). We obtained estimates for sensitivity and specificity of ≥HSIL for ≥CIN 2 detection from published data on unscreened South African women (median age 39 years) (47). The prevalence of ≥HSIL was 4.1% (95% confidence interval, CI: 2.3-6.6%) in our FSW study (70) and 3.1% (95% CI: 2.3-4.1%) in the South African study (47). Prevalence of ≥CIN 2 in the South African study was 4.1% (95% CI: 3.2-5.3%) (47) .

Using data of i) hrHPV testing to detect ≥HSIL and ii) ≥CIN 2 diagnosis in women with ≥HSIL from our FSW study (70), as well as iii) cytological testing (≥HSIL) to detect ≥CIN 2 from the South African study (47), we estimated sensitivity and specificity bounds of hrHPV physician- and self-testing for ≥CIN 2 detection in our study of FSW. The estimation is described in detail in the Appendix.

We parameterized sensitivity as a function of specificity and of the diagnostic odds ratio (72) to take into account the inverse relationship between sensitivity and specificity. We used the ratio of sensitivity of hrHPV physician- to self-testing for ≥HSIL in our FSW study to reflect the difference in sensitivity of hrHPV physician- and self-testing for ≥CIN 2. The same was done to reflect the different in specificity. The performance of hrHPV physician- relative to self-testing for ≥HSIL in our study of FSW was consistent (higher sensitivity with physician- compared with self-testing, similar specificity) with that of previous studies comparing hrHPV DNA physician- and self-testing for ≥CIN 2 in resource-low settings (18, 28, 47, 64).

We ascribed a lognormal distribution to the diagnostic odds ratio of ≥CIN 2 detection by conventional cytology (using ≥HSIL as screening threshold). Sensitivity and specificity of hrHPV mRNA testing of physician- and self-collected specimens for ≥CIN 2 were first sampled from uniform distributions. We specified the upper and lower limit of these uniform

distributions with our estimated upper and lower bound for sensitivity and specificity. These sampled values were then ascribed a beta distribution, where the mean was the sampled value and the standard deviation was the mean of the approximate standard error of the lower and upper bound estimate. Parameter estimates and distributions used in our model are shown in Table 1.

Monte Carlo simulation

Monte-Carlo simulations were performed to calculate the probability of each screening strategy being optimal at different levels of willingness-to-pay. Probabilistic sensitivity analysis was first performed, by randomly drawing a value from each parameter distribution (screening test sensitivity and specificity), thereby allowing for the evaluation of the combined uncertainty about the parameters in the model (73). This procedure was repeated 10,000 times (M=10,000).

For each set of parameters drawn, 1,000 observations (N=1,000) were generated to account for the random variation in individual-level outcomes (e.g. age, HIV-serostatus and whether or not a woman had ≥CIN 2) (74). For each of these trials of N=1,000 observations, the number of colposcopies performed and the number of women whose ≥CIN 2 was detected by colposcopy were estimated for each screening strategy, and the net benefit (NB) is calculated across a range of willingness-to-pay limit.

In a cost-effectiveness analysis, a cost-effectiveness NB of one option (i.e treatment or intervention) is compared to another. This cost-effectiveness NB is defined as: $NB = \lambda \times E - C$ (where C is the total cost and E the total effect of each option), and is calculated across a range of the decision-maker's willingness-to-pay for one unit gain in health

outcome, λ. An option is cost-effective if and only if the NB for a given $\lambda > 1$ (75). In assessing the NB of different screening strategies for our FSW study, C represents the number of colposcopies performed, E the number of women whose ≥CIN 2 was detected by colposcopy and λ the number of colposcopy the decision maker is willing to conduct to detect one case of ≥CIN 2.

For each trial of N=1,000 observations, the screening strategy with the highest NB was identified. The proportion of the M=10,000 interations in which a screening strategy had the highest NB at a given λ was then used to construct an acceptability curve for each screening strategy by plotting these proportions on the y-axis and the corresponding λ on the x-axis (Figure 5.2) (75). Since we defined willingness-to-pay as the number of colposcopies willing to conduct to detect one ≥CIN 2, this is also equivalent to the inverse of the positive predictive value (PPV) for ≥CIN 2 among women with ≥HSIL or positive hrHPV physician- or self-testing result.

Results

Bounds of sensitivity and specificity of hrHPV testing for ≥CIN 2

The estimated sensitivity bounds of hrHPV physician-testing for ≥CIN 2 (45-91%) in our study of FSW were similar to that of self-testing (45-9%). The estimated specificity bounds were also similar for physician- (71-74%) and self-testing (73-75%), and were much narrower than those of sensitivity (Table 5.2).

The difference between the upper and lower 95% confidence limits (confidence limit difference, CLD) (79) for the true sensitivity of hrHPV testing to detect ≥CIN 2 was similar for physician- and self-testing (CLD=0.68 for both hrHPV physician- and self-testing). The

CLD of the 95% confidence limit for the true specificity was also similar for physician- and self-testing (CLD=0.10 for physician- and CLD=0.11 for self-collected specimens) (Table 5.2), and are lower than those for sensitivity.

Comparison of screening efficiency and associated uncertainty

Figure 5.2 shows an acceptability curve: the proportion of simulations that an individual screening strategy was optimal (i.e, having the highest NB) at a given willingness-to-pay limit. Given the available information, at a willingness-to-pay limit of <15 colposcopies per case of ≥CIN 2 detected, conventional cytology has >80% probability of being optimal. At a willingness-to-pay of between 20 and 30-40 colposcopies per case of ≥CIN 2 detected, the probability of all three strategies being optimal was between 8-50%. Above 40 colposcopies per case of ≥CIN 2 detected, the probability of conventional cytology being optimal decreased to between 10-20%. Also, above this willingness-to-pay limit, the probability of hrHPV physician-testing being optimal increased to between 55-60%, while that of hrHPV self-testing remained at between 25-30%.

Prevalence of ≥CIN 2 by age

Using maximum likelihood estimation (Appendix), the estimated prevalence of ≥CIN was 4.3% (95% CI: 3.3-5.3%). This prevalence of ≥CIN 2 appeared to vary by age, and estimated to be higher among older compared with younger women (>4% in women ≥30 years, <4% in women <30 years) women (Table 5.3).

Discussion

At a willingness-to-pay limit of <15 colposcopies per case of ≥CIN 2 detected, conventional cytology was the optimal screening strategy, for a once-in-a-lifetime cervical cancer screening in a population of FSW in Kenya. At a willingness-to-pay limit of between 20 to 40 colposcopies per case of ≥CIN 2 detected, the probability of any of the three strategies being optimal was ≤50%. At a willingness-to-pay limit of >40 colposcopies per case of ≥CIN 2 detected, the probability of hrHPV physician-testing being optimal increased to between 55-60%, while that of hrHPV self-testing and conventional cytology was between 15-30%. Our analysis suggests that at relatively higher willingness-to-pay limits, more research may be required to determine which screening strategy is most efficient.

The estimated sensitivity bounds of hrHPV physician-testing for ≥CIN 2 in our population of FSW in Kenya were somewhat wide (45-91%). Sensitivity estimates of hrHPV mRNA testing for ≥CIN 2 from population-based studies from Canada (35), France (39) and China (40) were higher than our estimated bounds (92-100%). Our estimated specificity bounds (71-75%) were relatively lower, however, than other population-based estimates (88-92%) (35, 39, 40). Our lower specificity is likely due to the higher overall hrHPV prevalence in our FSW (30%) as compared with previous studies, where overall hrHPV prevalence ranged from 10-12% (35, 39, 40). Although specificity of hrHPV testing for ≥CIN 2 will decrease with increasing hrHPV prevalence, the PPV for ≥CIN 2 among women positive for hrHPV will not decrease (94). Compared with other low-resource settings, the estimated ≥CIN 2 prevalence in our study of FSW (4.3%, median age 28 years) appeared similar to that in South Africa (4.1%, median age 39 years)(47) and China (4.4%, mean age 40 years) (64), albeit lower than that in India (7.8%, median age 36 years) (28).

HrHPV physician- or self-testing were the optimal screening strategy at higher limits of willingness-to-pay (>30 colposcopies to detect one case of ≥CIN 2 for physician-collected specimens and >60 self-collected specimens). We are unable, however, to compare our results directly with previous cost-effectiveness analyses of different screening strategies due to different modeling approaches and outcomes being considered (e.g different definition of screening cost) (51, 52, 95). Nevertheless, our finding that a screening strategy with greater sensitivity was optimal only at higher willingness-to-pay limits was qualitatively similar to previous cost-effectiveness findings, where increased sensitivity was associated with higher screening cost relative to benefits (95). Compared with conventional cytology, hrHPV testing in our analysis also has a potentially higher sensitivity, but lower specificity to detect ≥CIN 2, consistent with previous findings comparing hrHPV DNA with conventional cytology (18, 47). Greater sensitivity unaccompanied by improved specificity can explain our finding that hrHPV testing increased only with higher willingness-to-pay limits. Nevertheless, in previous analyses on low-resource settings with infrequent screening which accounted for actual screening costs as well as the impact of screening on lifetime cancer risk and mortality, even small increments in test sensitivity can increase overall screening cost-effectiveness. Changes in test specificity, on the other hand, were much less influential on screening cost-effectiveness (50).

We defined screening efficiency as the number of colposcopies conducted per ≥CIN 2 detected, without accounting for the actual cost of screening, thus limiting the comparability of our results with other cost-effectiveness data on low-resource regions (51, 52). However, the screening strategies considered in this study have already been successfully implemented in our population of FSW. Considering the costs and benefits in terms of the number of

colposcopies required to detect a case of ≥CIN 2 can inform us of the practicality of screening by hrHPV without triage by a more specific test, compared with conventional cytology. Furthermore, colposcopies per case of ≥CIN 3 detected was used as the primary measure of harm by the American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology when developing screening guidelines, as colposcopies can cause physical discomfort and potentially lead to more invasive procedures with greater harms (24).

An advantage of our analysis is that since the screening strategies evaluated have been successfully employed in our FSW population, we did not have to make assumptions on the availability of screening tests and feasibility of implementing a screening strategy. Further, as our decision model ascribed a distribution to the input parameters, we accounted for the imprecision surrounding not only the sensitivity and specificity of screening tests, but also that surrounding the screening outcomes (e.g cytological finding of ≥HSIL or positive for hrHPV testing) of individual participants. This allow decisions to be made based on the decision maker's preferred maximum limit for screening cost, in terms of number of colposcopies (74, 75, 96).

This study has several limitations. We found only one African study, from South Africa (47), which had complete data on cervical histology stratified by cytology that we could use to estimate sensitivity and specificity bounds of hrHPV testing to detect ≥CIN 2. The sensitivity and specificity of conventional cytology for ≥CIN 2 in the South African study may have differed from those in our FSW study. However, ≥HSIL prevalence in the South African study (4.1%, 95% CI: 3.2-5.3%) (47) was similar to that in our FSW study (3.1%, 95% CI: 2.3-4.1%) (70). Further, we assumed perfect adherence to follow-up to

colposcopy of women with ≥HSIL or positive hrHPV testing results, and, implicitly, 100% sensitivity of colposcopy and receipt of appropriate treatment for ≥CIN 2 lesions. Also, given the high hrHPV prevalence in our FSW (70), many women who were hrHPV positive and referred to colposcopy may have low-grade cervical lesions, or not have any cervical abnormality at all. As a positive screening test result can potentially have emotional impact on a woman, the effect of screening strategies on the overall quality of life should also be considered in future analyses when comparing screening strategies (95).

In conclusion, our findings potentially have implications for other low-resource settings with a similarly high-risk population as our FSW which are considering a once-in-a-lifetime screening program. Our estimated specificity of hrHPV testing for ≥CIN 2 was somewhat lower than that of conventional cytology. However, previous data found that despite a lower specificity, the PPV for ≥CIN 2 among women with hrHPV test positive results is likely higher in populations with high hrHPV prevalence and lack of previous screening, as is generally the case with most resource-low settings (94). Given the available data and the imprecision of sensitivity and specificity estimates of hrHPV testing for ≥CIN 2 in our analysis, however, our results suggest that further empiric data is needed to determine the optimal screening strategy at higher willingness-to-pay limits, and that extension of the model to incorporate total costs and estimated impact of each screening strategy on ICC mortality is needed. We may then compare the effect of imprecision surrounding estimates of costs and mortality to that of test performance, on the efficiency of each screening strategy (value-of-information, (97)). Such value-of-information assessment potentially informs us of the important areas in which to focus future research and limited research resources.

Table 5.1 Parameter estimates, distributions, confidence intervals utilized in the simulation model of cervical cancer screening efficiency in female sex workers in Kenya

Variable	Estimate	Distribution	95% CI	Reference
Prevalence of women age ≥30 years	43%	Beta	38-48	(70)
Prevalence of HIV-seropositivity				
<30 years	14%	Beta	9-19	(70)
≥30 years	38%	Beta	30-46	(70)
Prevalence of ≥CIN 2				
HIV-seronegative women	2%	Beta	1-5	(98)
HIV-seropositive women	7%	Beta	3-15	(98)
Conventional cytology[1]				
Sensitivity for ≥CIN 2	52%	Beta	38-65	(47)
Specificity for ≥CIN 2	99%	Beta	98-100	(47)
Ratio of sensitivity of self- to physician-collected hrHPV mRNA testing for ≥CIN 2				
Sensitivity	1.08	Lognormal	0.79-1.50	Assumption (70)
Specificity	0.98	Lognormal	0.90-1.08	Assumption (70)
Follow-up of women with abnormal results	100%			Assumption

CI=confidence interval; CIN=cervical intraepithelial neoplasia; HSIL=high-grade squamous intraepithelial neoplasia; hrHPV= high-risk human papillomavirus infection
[1]Cytologic threshold of ≥HSIL

Table 5.2 Estimated bounds of sensitivity and specificity of high-risk HPV mRNA testing of physician- and self-collected specimens for ≥CIN 2 detection among female sex workers in Kenya

Specimen type	Sensitivity of hrHPV mRNA for ≥CIN 2					Specificity of hrHPV mRNA for ≥CIN 2				
	Lower bound	**SE**	**Upper bound**	**SE**	**95% CI[1]**	**Lower bound**	**SE**	**Upper bound**	**SE**	**95% CI[2]**
Physician-collected specimens	45%	0.08	91%	0.06	32-100%	71%	0.02	74%	0.03	68-78%
Self-collected Specimens	45%	0.08	91%	0.06	32-100%	73%	0.02	75%	0.02	68-79%

HrHPV=high-risk human papillomavirus; CIN= cervical intraepithelial neoplasia; SE=standard error; 95% CI= 95% confidence interval

[1]Critical value $c_{\alpha/2}$ used to calculate the 95% CI was 1.645

[2]Critical value $c_{\alpha/2}$ used to calculate the 95% CI was 1.750

Table 5.3 Approximate prevalence of ≥CIN 2, stratified by age, among female sex workers in Kenya

Age (years)	**Prevalence of ≥CIN 2**
<25	3.5%
25-29	3.7%
30-39	4.2%
≥40	4.5%

CIN=cervical intraepithelial neoplasia

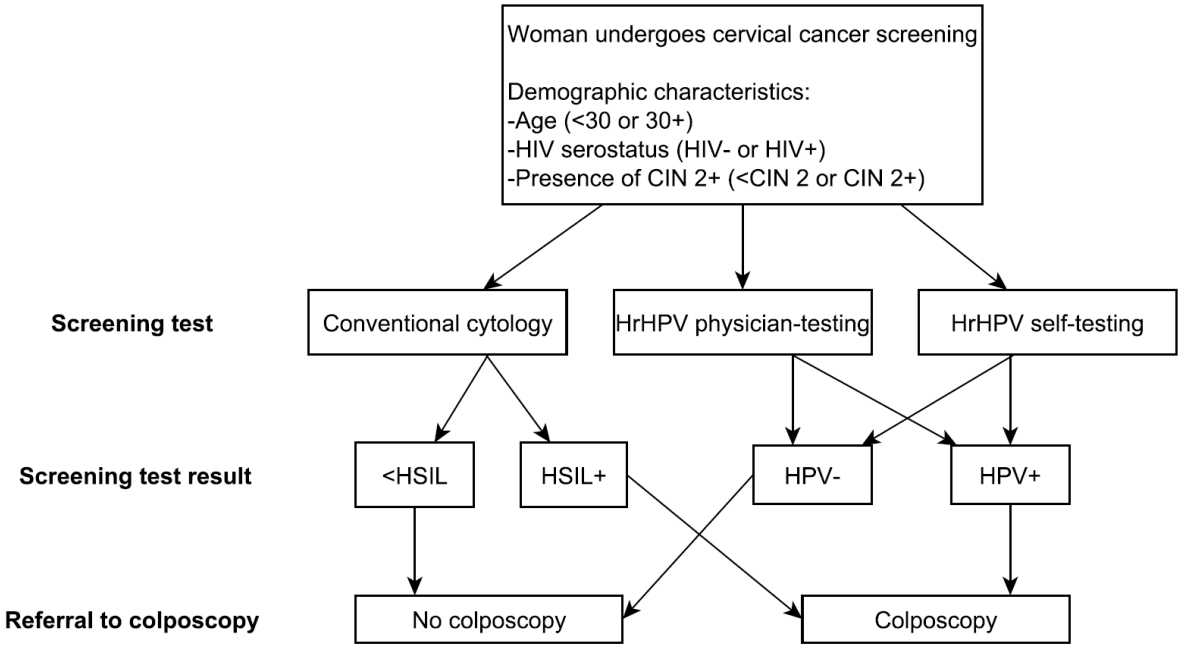

Figure 5.1 Decision model for cervical cancer screening in female sex workers in Kenya. A woman undergoes a once-in-a-lifetime cervical cancer screening by cytology, high-risk human papillomavirus (hrHPV) physician- or self-testing. All women are referred to colposcopy in the event of cytological high-grade squamous intraepithelial lesions or more severe (≥HSIL) or positivity for hrHPV physician- or self-testing. Inputs for age, HIV, ≥HSIL as well as sensitivity and specificity of hrHPV physician- and self-testing for ≥HSIL are obtained from our female sex workers study in Kenya (70). Inputs for histological cervical intraepithelial neoplasia or more severe (≥CIN 2) prevalence stratified by HIV-serostatus, as well as the sensitivity and specificity of ≥HSIL for ≥CIN detection are from published data from South Africa (47, 98). Inputs for sensitivity and specificity of hrHPV physician- and self-testing for ≥CIN 2 are estimated from our female sex workers study and from published data from South Africa (47). A true-positive outcome is when a screening test correctly identifies a woman with true ≥CIN 2 for colposcopy (a screening benefit). The false-positive outcome is referring a woman with <CIN 2 to colposcopy (a screening burden).

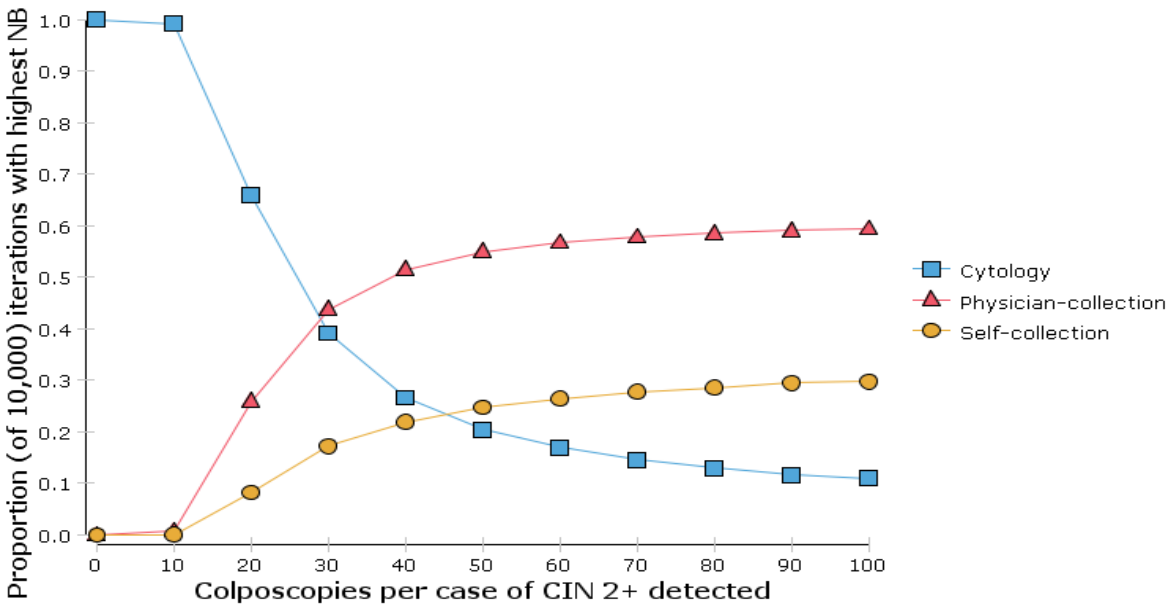

Figure 5.2 Acceptability curve for the three cervical cancer screening scenarios.
Each line shows the proportion of the 10,000 iterations (each with 1,000 women) in which that screening strategy had the highest net benefit (NB) at a value of willingness-to-pay. Inputs: Cytology=conventional cytology, sensitivity 52% (95% CI: 38-65%) and specificity 99% (95% CI: 98-100%) for ≥CIN 2; Physician-collection=high-risk (hr) HPV physician-testing, sensitivity bounds 45-91% (95% CI: 32-100%) and specificity bounds 71-74% (95% CI: 68-78%) for ≥CIN 2; Self-collection=hrHPV self-testing sensitivity bounds 45-91% (95% CI: 32-100%) and specificity bounds 73-75% (95% CI: 68-79%) for ≥CIN 2.

CHAPTER 6.
CONCLUSIONS

Summary of Findings

Sensitivity and specificity of hrHPV self-testing for ≥HSIL were similar to those of physician-testing in our population of FSW. Our finding of similar sensitivity was contrary to our hypothesis, where we anticipated higher sensitivity when using physician- compared with self-collected specimens. Although our estimates are imprecise, they nevertheless suggest that hrHPV self-testing has the potential to offer a simple, private and convenient self-test which can help increase access to screening in low-resource regions.

The estimated sensitivity bounds of hrHPV physician-testing for ≥CIN 2 (49-100%) for our FSW population were similar to those of self-testing (43-96%). Specificity bounds were also similar for physician- (71-74%) and self-testing (72-75%), and were narrower than those of sensitivity. We believe that our lower estimated specificity bounds compared with previous studies (88-92%) (35, 39, 40) was due to our FSW having a higher overall hrHPV prevalence (30%) than that in previous studies (10-12%) (35, 39, 40). A previous systematic review found specificity of hrHPV testing for ≥CIN 2 to decrease with increasing hrHPV prevalence (94). Nevertheless, our finding of lower specificity should not be a deterrent to use of hrHPV-based screening in settings with high hrHPV prevalence. The reason is that the PPV for ≥CIN 2 among women with hrHPV test positive results is likely higher in

populations with high hrHPV prevalence and lack of screening, as in most low-resource settings (94).

Presenting our findings of the decision analysis in the form of an acceptability curve (Figure 5.2) allows decisions to be made based on the decision maker's preference for the maximum limit of screening cost (willingness-to-pay). We found that at a lower willingness-to-pay limit of <15 colposcopies per case of ≥CIN 2 detected, conventional cytology was the optimal screening strategy for a once-in-a-lifetime cervical cancer screening in a population of FSW in Kenya. At higher willingness-to-pay limits, however, our results suggest that, given the current information, more data are still required to determine which screening strategy is most efficient.

Public Health Significance

In contrast to the experience of developed countries in the past three decades, no significant reduction in ICC incidence has been achieved in low-resource countries due to lack of effective screening programs (1, 99). Kenya at this time does not have an established nationwide cervical cancer screening program. A once-in-a-lifetime cervical cancer screening in high-risk populations in low-resource settings such as our FSW in Kenya using a simple, affordable, and accurate test can help begin to reduce ICC incidence and mortality in these regions.

A recent randomized controlled trial in India reported that compared with no screening (control group), a one-time screening with hrHPV DNA testing in participants aged 30-59 years was associated with a significant decrease in ICC incidence (hazard ratio: 0.5, 95% CI=0.3-0.7) and mortality (hazard ratio: 0.5, 95% CI 0.3-0.8). By contrast, no significant reductions in ICC

incidence (hazard ratio: 0.8, 95% CI=0.5-1.1) or mortality (hazard ratio: 0.9, 95% CI=0.6-1.3) was found in the conventional cytology testing group, compared with no screening (99).

A potential limitation to HPV testing in low-resource settings is that HPV testing is relatively more expensive (US $20-$30 per test) and typically takes 3.5 to 6 hours to process, depending on the test used. HPV testing also requires a sophisticated laboratory infrastructure (99). However, data from China showed that a hrHPV DNA test (*care*HPV, Qiagen, CA), if provided via self-collection, has the lowest direct non-medical cost, in terms of savings in transportation time and out-of-pocket expenses, compared with other screening strategies (100). Findings from our FSW study as well as those of previous studies (Table 2.2) on the performance hrHPV self-testing showed that self-collection can be a reliable tool for cervical cancer screening.

Results of our decision analysis showed that hrHPV mRNA testing is potentially more costly than conventional cytology (in terms of the number of colposcopies required) due to relatively higher sensitivity but lower specificity. However, an evaluation of different cervical cancer screening strategies in South Africa found a once-in-a-lifetime screening of women aged 35 with HC2 hrHPV DNA testing of physician- or self-collected specimens followed by treatment on the second visit of screen positive women was cost-effective (according to the Commission on Macroeconomics and Health, which defined strategies with a cost-effectiveness ratio that is less than the per capita gross domestic product as “very cost-effective”), compared with conventional cytology (52). Furthermore, for single lifetime screening, once screening cost and impact of screening on lifetime cancer risk and mortality were accounted for, small increments in test sensitivity could potentially increase overall screening cost-effectiveness in settings with infrequent screening. Changes in test specificity on the other hand have less impact

on screening cost-effectiveness, in settings with high disease prevalence and very infrequent screening (50).

Strengths

Apart from a South African study (47), this study is the only other one to compare hrHPV physician- and self-testing for cervical cancer screening in Africa (Table 2.2). Our study is the first to use hrHPV mRNA testing in Africa, and also the only so far to compare the risk factors of hrHPV mRNA positivity in physician- and in self-collected specimens.

For the second specific aim of this dissertation, we used data from our study of FSW and a population of South African women (with similar ≥HSIL prevalence as in our FSW study) to estimate bounds for sensitivity and specificity of hrHPV physician- and self-testing for ≥CIN 2. As all three screening programs (conventional cytology and hrHPV physician- and self-testing) have already been successfully carried out in our FSW study, we also did not have to make assumptions on the availability of screening tests or the feasibility of implementing a screening strategy in our population of FSW.

Limitations

The small number of ≥HSIL cases (N=15) in our FSW study could have obscured potential significant differences in sensitivity of hrHPV mRNA testing for ≥HSIL when using physician- compared with self-testing and resulted in the imprecision of our estimates. This finding of similar sensitivity was consistent with a previous study that used hrHPV DNA testing to detect ≥CIN 2 in high-risk women in Germany (41). In other previous studies, however, the

sensitivity of hrHPV DNA for ≥CIN 2 was consistently higher when using physician-testing, compare with self-testing (Table 2.2).

Our study of FSW also did not have cervical histology data for every woman, since only women with cytological ≥HSIL were referred to colposcopy-directed biopsy. Thus, in specific aim 1.1 of this dissertation, ≥HSIL was used as screening outcome, instead of histological ≥CIN 2, potentially limiting the comparability of our results with those of previous studies (Table 2.2).

A limitation of the APTIMA HPV Assay used in our study to detect hrHPV mRNA is that the assay could not determine if a negative test result interpretation was due to an absence of hrHPV or an absence of cells altogether. Although the assay is very sensitive and can detect hrHPV mRNA expression levels in as few as one infected cell per reaction, it did not have cellular control that would test for the presence or adequacy of a specimen (66). Specimen inadequacy is a potential concern especially where it involved self-collected specimens, as we could not account for a woman's adherence to the instructions for self-collection. However, in our study, the prevalence of hrHPV among women with valid physician- (30%) and self-collected specimens (29%) were similar, suggesting that self-collected specimens were potentially as reliable as physician-collected specimens for detecting hrHPV.

Self-collected specimens are not currently an approved specimen type for the APTIMA HPV Assay. The difference in the cellular contents between physician-collected cervical specimens and self-collected cervico-vaginal specimens may help explain why some of the self-collected specimens (N=5) were invalid for hrHPV mRNA testing. Inhibitory substances may have been present in these self-collected specimens, perhaps from vaginal substances applied by women (L. Guzenski, Hologic Gen-Probe, personal communication). However, it was not possible to determine the exact cause of the invalid test results for these specimens.

As with any study that depends on self-reported data, we could not verify the accuracy of self-reported information on important risk factors of hrHPV positivity (e.g frequency of condom use and number of sexual clients) obtained via interview. Self-reported data would be inaccurate if study participants incorrectly recalled or refused to disclose information on risk factors, or if participants intentionally responded incorrectly to a question about their sexual history. However, interviews were conducted by trained nurses, and participating women were ensured of their confidentiality so that they may feel more comfortable in disclosing personal information.

For our decision analysis, we used a decision model incorporating certain population characteristics and test sensitivity and specificity. However, more than one model structure may be actually be possible for our research aim (specific aim 2.2), and different models can result in variation in the outcomes under evaluation (97).

Finally, the findings of our study may be generalizable only to similar populations of high-risk women with high hrHPV prevalence in low-resource settings, who meet the study participation criteria (aged between 18 and 50 years, have an intact uterus and were not in the second trimester or later). Also, in our study of FSW, each woman's physician- and self-collected specimens were sampled during the same clinic visit. Although this is an advantage of our study as it allowed for direct comparison of hrHPV testing results of these two sample types, comparisons of our findings with those of other studies where physician- and self-collection were conducted at different times (e.g self-collection performed at home, physician-collection at the clinic on a different day) should consider the possibility that results may be affected by these factors.

Future Research Directions

HrHPV testing of self-collected specimens can potentially help increase screening coverage in low-resource areas. However, more data on hrHPV self-testing for cervical cancer screening in low-resource settings are needed, as currently available data are limited (Table 2.2).

Although our estimates were imprecise, prevalence of hrHPV in physician- or self-collected specimens appeared to be higher in women with *M. genitalium* or *T. vaginalis* infection (Table 4.4). Our finding regarding the potential association between *T. vaginalis* and hrHPV infection was consistent with previous data (86), but the role of *M. genitalium* or *T. vaginalis* as risk factors of hrHPV infection is still unknown. Longitudinal data are needed to determine if infection with *M. genitalium* or *T. vaginalis* increases a woman's risk of hrHPV acquisition or persistence.

In our study of FSW, physician- and self-collected specimens were sampled in the same clinic visit, and participating women were given uniform instructions for self-collection. We found that in this setting, hrHPV self-testing was feasible and performed well in detecting cervical lesions, compared with physician-testing. Future studies should examine whether our findings are reproducible outside a research setting. More data are needed in low-resource communities to assess interest and willingness of self-collection, especially among asymptomatic healthy women (28). The participation rate of home-based self-collection and effect of different types of patient education on self-collection should also be assessed.

Lastly, data on costs and benefits of hrHPV self-testing for cervical cancer screening in a low-resource population with high hrHPV prevalence are very limited (52). Further assessment of the test performance as well as total costs and benefits of a cervical cancer screening program based on hrHPV self-testing in low-resource settings should be done. Also, screening tests and

facilities may not be equally available in all settings. Therefore, future analyses on costs and benefits of hrHPV self-testing should consider and provide results for potentially available screening strategies, taking into account country-specific circumstances.

APPENDIX

Estimation of bounds of sensitivity and specificity of hrHPV testing for ≥CIN 2

The following is a estimation of the bounds of sensitivity and specificity of hrHPV testing for ≥CIN 2, using data on i) hrHPV testing to detect cytologic ≥HSIL (Table A1) and ii) cervical histologic and hrHPV testing results for women with cytologic ≥HSIL (Table A2) obtained from our Kenyan FSW study (70), as well as iii) ≥HSIL and histologic ≥CIN 2 (Table A3) from a previous study conducted in Cape Town, South Africa (47).

Table A1. High-risk HPV mRNA testing results by cervical cytology

	≥HSIL	**<HSIL**	**Total**
HrHPV+	a	b	$a+b$
HrHPV-	c	d	$c+d$
Total	$a+c$	$b+d$	$a+b+c+d$

HrHPV=High-risk human papillomavirus; HSIL=high-grade squamous intraepithelial lesion

Table A2. Cervical histology and high-risk HPV mRNA testing results among women with cytologic ≥HSIL

	≥CIN 2	**<CIN 2**	**Total**
≥HSIL, hrHPV+	e	f	a
≥HSIL, hrHPV-	g	h	c
Total	$e+g$	$f+h$	$a+c$

CIN=cervical intraepithelial neoplasia; hrHPV=high-risk human papillomavirus HSIL=high-grade squamous intraepithelial lesion

Table A3. Cervical cytology results by histology

	≥CIN 2	**<CIN 2**	**Total**
≥HSIL	i	j	$i+j$
<HSIL	k	l	$k+l$
Total	$i+k$	$j+l$	$i+j+k+l$

HSIL=high-grade squamous intraepithelial lesion; CIN=cervical intraepithelial neoplasia

Maximum likelihood estimation was used to draw inference about the sensitivity and specificity of hrHPV testing for ≥CIN2. We let $\pi_1, \pi_2 \ldots \pi_8$ denote the probabilities corresponding to the eight possible test outcomes (Table A4) should a woman be given all three screening tests. We assume that in our Kenyan FSW study (70), the data on histologic ≥CIN 2

were missing at random (MAR) (101), as cervical histology was available for women with cytologic ≥HSIL but not for women with <HSIL. Under the MAR assumption, the log likelihood for the observable data in Tables A1, A2 and A3 is:

$$logL = \ b \cdot log(\pi_5 + \pi_7) + d \cdot log(\pi_1 + \pi_3) + e \cdot log(\pi_2) + f \cdot log(\pi_6) + g \cdot log(\pi_4)$$

$$+h \cdot log(\pi_8) + i \cdot log(\pi_4 + \pi_8) + j \cdot log(\pi_2 + \pi_6) + k \cdot log(\pi_3 + \pi_7) + l \cdot log(\pi_1 + \pi_5)$$

As can be seen from the log-likelihood, parameters π_2, π_4, π_6 and π_8 are identifiable from the observable data, while parameters π_1, π_3, π_5 and π_7 are only partially identifiable (although the terms $[\pi_1 + \pi_3]$, $[\pi_1 + \pi_5]$, $[\pi_3 + \pi_7]$ and $[\pi_5 + \pi_7]$ are each identifiable). As a result, the maximum likelihood estimators (MLEs) of the sensitivity and specificity are not unique.

Table A4. Possible outcomes of screening by high-risk HPV testing, cervical cytology and cervical histology in female sex workers in Kenya

	Testing result		
Parameter	**HrHPV**	**≥CIN 2**	**≥HSIL**
π_1	negative	negative	negative
π_2	negative	negative	positive
π_3	negative	positive	negative
π_4	negative	positive	positive
π_5	positive	negative	negative
π_6	positive	negative	positive
π_7	positive	positive	negative
π_8	positive	positive	positive

HrHPV=High-risk human papillomavirus; CIN=cervical intraepithelial neoplasia; HSIL=high-grade squamous intraepithelial neoplasia

Let $\hat{\pi}_1, \hat{\pi}_2 \ldots \hat{\pi}_8$ denote the resulting estimates of $\pi_1, \pi_2 \ldots \pi_8$. The estimated sensitivity and specificity of hrHPV testing for ≥CIN 2 are:

$$\text{Sensitivity} = \frac{\hat{\pi}_7 + \hat{\pi}_8}{\hat{\pi}_3 + \hat{\pi}_4 + \hat{\pi}_7 + \hat{\pi}_8}$$

$$\text{Specificity} = \frac{\hat{\pi}_1 + \hat{\pi}_2}{\hat{\pi}_1 + \hat{\pi}_2 + \hat{\pi}_5 + \hat{\pi}_6}$$

To obtain the lower sensitivity bound (i.e the smallest MLEs), the log-likelihood was first maximized under π_7 constrained to a range of values (e.g. $\pi_7 = 0, 0.01, 0.02, \ldots 0.98, 0.99, 1.00$),

since π_8 and the denominator for calculating sensitivity ($\pi_3 + \pi_4 + \pi_7 + \pi_8$) are identifiable. The smallest value of π_7 where the log-likelihood was the same as when π_7 was not constrained was used to determine the minimum value for sensitivity. Similarly, the largest value of π_7 where the log-likelihood equals that under π_7 unconstrained was used to determine the maximum value for sensitivity (i.e the largest MLEs). Analogous methods were used to obtain the lower and upper specificity bounds, with the log-likelihood maximized under π_1 constrained to a range of values.

Standard errors for the upper and lower bound sensitivity estimates were estimated using the delta method (102). To take into account the uncertainty due to sampling variability, we constructed the approximate 95% CI for the true sensitivity by (103):

$$[C_L, C_U] = [\hat{\beta}_l - c_{\alpha/2}\mathrm{se}(\hat{\beta}_l),\ \hat{\beta}_u + c_{\alpha/2}\mathrm{se}(\hat{\beta}_u)]$$

where $\hat{\beta}_l$ is the lower bound sensitivity estimate, $\hat{\beta}_u$ the upper bound sensitivity estimate, and $\mathrm{se}(\hat{\beta}_l)$ and $\mathrm{se}(\hat{\beta}_u)$ are the corresponding estimated standard error. The critical value $c_{\alpha/2}$ was calculated as the solution to the following equation (103):

$$\min\left[\Phi(c_{\alpha/2}) - \Phi\left\{-c_{\alpha/2} - \frac{\hat{\beta}_u - \hat{\beta}_l}{\mathrm{se}(\hat{\beta}_u)}\right\}, \Phi\left\{c_{\alpha/2} + \frac{\hat{\beta}_u - \hat{\beta}_l}{\mathrm{se}(\hat{\beta}_l)}\right\} - \Phi(-c_{\alpha/2})\right] = 1 - \alpha$$

where $\Phi(\cdot)$ is the cumulative distribution function of a normal standard variate. Analogous methods were used to estimate the standard errors and 95% CI of the true specificity.

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