PART B
STUDY DESCRIPTION

TITLE OF PROTOCOL	Development of a two-stage cervical cancer screening algorithm for Botswana
Principal Investigator	Rebecca Luckett

B1. PURPOSE OF PROTOCOL

Cervical cancer screening programs vary across settings and there is no clear guidance for effective screening programs for HIV-positive women. Evaluating the performance of algorithms that include human papillomavirus (HPV) DNA testing as first stage screening in high HIV prevalence settings like Botswana is essential for establishing an evidence-based strategy for cervical cancer screening in HIV-positive women.

The proposed study seeks to evaluate sensitivity, specificity, and positive predictive value (PPV) of two different two-stage cervical cancer screening algorithms for HIV-positive women in Botswana: HPV followed by Papanicolaou (Pap) smear, and HPV followed by visual inspection with acetic acid (VIA). The specific aims are:

1. To determine the sensitivity, specificity, and PPV of a two-stage screening algorithm that utilizes HPV testing followed by Pap smear for the detection of high-grade cervical dysplasia, using colposcopy with histology as the gold standard.
2. To determine the sensitivity, specificity, and PPV of a two-stage screening algorithm that utilizes HPV testing followed by VIA for the detection of high-grade cervical dysplasia, using colposcopy with histology as the gold standard.

B2. SIGNIFICANCE AND BACKGROUND FOR THE STUDY

Cervical cancer is the fourth leading cause of cancer death in women worldwide and is the leading cause of cancer death in women in Botswana.[i,ii,iii] The disease burden in Botswana is impacted by the high prevalence of HIV (22% among people aged 15-49 years), which is a well-established risk factor for cervical cancer.[iv,v,vi] Cervical cancer is largely preventable and treatable where screening programs and treatment are available.[vii,viii,ix,x] The majority of cervical cancers are associated with infection with high-risk HPV subtypes (HPV).[xi,xii,xiii] Among HIV-positive women, persistent HPV positivity and infection with multiple subtypes are strong risk factors for cervical cancer.[xiv] HPV prevalence is variable, ranging from 15-45%, with higher prevalence in HIV-positive women.[xv,xvi,xvii] HPV 16, 18, and 45 are the high-risk subtypes most commonly associated with cervical cancer in Africa. In Botswana, HPV subtype prevalence in HIV positive women with high grade precancerous cervical lesions include 16, 18, 35, 58, 61; infections with multiple subtypes is common.[xviii,xix,xx,xxi,xxii]

Cervical cancer screening strategies are most effective when based on local evidence and tailored to the population and resource infrastructure.[xxiii,xxiv] Available screening tests include HPV testing, cytology [liquid-based or Pap smear], and VIA. HPV testing has a high sensitivity (93-100%) and specificity (80-90%), but low PPV (12-23%) for pre-invasive disease.[xxv,xxvi,xxvii,xxviii] Cytology is operator dependent, with variable sensitivity (43-94%) and specificity (78-98%), but PPV of up to 90%.[xxix,xxx,xxxi] VIA sensitivity (17-100%) and specificity (8-95%) also are variable, and in resource-poor settings without HPV testing or histopathology can reduce cervical cancer mortality.[xxxii,xxxiii,xxxiv] There is increasing evidence that primary HPV testing is the most effective screening strategy.[xxxv,xxxvi,xxxvii,xxxviii] Primary testing with HPV alone, followed by treatment, creates a high likelihood of overtreatment and harm to women who desire future fertility. In addition, primary HPV testing followed by colposcopy is not feasible in settings like Botswana where there are an insufficient number of trained colposcopists. To address these two issues, two-stage screening strategies, consisting of HPV testing followed by either cytology or VIA, have been advocated.[xxxix,xl,xli] HPV testing followed by cytology results in increased sensitivity and specificity.[xlii] In a single study from Cameroon in a population with a high prevalence

of HIV (14.2%), HPV testing followed by Pap smear has been shown to have superior sensitivity and specificity compared to HPV testing followed by VIA.[xliii]

Botswana s screening strategy is similar to low-resource countries in the region. Given logistical and implementation challenges associated with a pathology-dependent screening test,[xliv] a screen-and-treat approach with VIA was introduced in Botswana in 2011.[xlv] Currently the standard of care in Botswana is for women to be screened either with a Pap smear or VIA alone. If she has an abnormal Pap smear result, she is referred for colposcopy; if she has an abnormal VIA result she is either offered cryotherapy or a loop electrosurgical excision procedure, depending on the size of the lesion. In developed countries, screening protocols have incorporated HPV DNA testing as it has a higher sensitivity in detecting women at risk of developing cervical cancer. Botswana plans to roll out HPV DNA testing as it has unique resources that allow for a more resource-intensive and effective strategy. However, because HPV DNA testing is sensitive but not specific, HPV testing alone would result in overtreatment of women who are unlikely to develop cervical cancer. Fortunately, pathology services have improved dramatically since 2011, and Pap smear may be an appropriate follow-up after a positive HPV test. VIA is also becoming more available nationally. An efficient, high-impact, two-stage strategy with primary HPV testing followed by either Pap or VIA can now be introduced nationally to reduce overtreatment and potential harm to women. Comparing these two strategies will provide essential data to inform national cervical cancer screening policy in Botswana and the surrounding region.

B3. DESCRIPTION OF RESEARCH PROTOCOL

A. Study Design – Overview, Methods, Procedures

Study Population and HPV Screening

The proposed study will enroll 300 women for HPV testing in order to evaluate 81 HPV-positive women (see Sample Size calculations below). The first 100 enrollments will be nested within an existing cross-sectional pilot study designed to determine the uptake, acceptability, and feasibility of testing women for HPV using self-collected samples. The goal of this 100-person pilot study is to inform the future scale-up of primary HPV screening, by answering the question of whether self-collected swabs are as good as provider-collected swabs. These 100 participants will self-collect a vaginal specimen and undergo a concomitant provider-collected specimen for HPV testing; a provider-collected approach will be used for the subsequent 200 women who enroll for this proposed study. Co-enrollment with the existing pilot study leverages the existing study and facilitates use of the same staff and resources to ultimately accrue 300 women at minimal cost.

Research question

Which two-stage cervical cancer screening algorithm is more effective for HIV-positive women in Botswana: HPV followed by Papanicolaou (Pap) smear or HPV followed by visual inspection with acetic acid (VIA)?

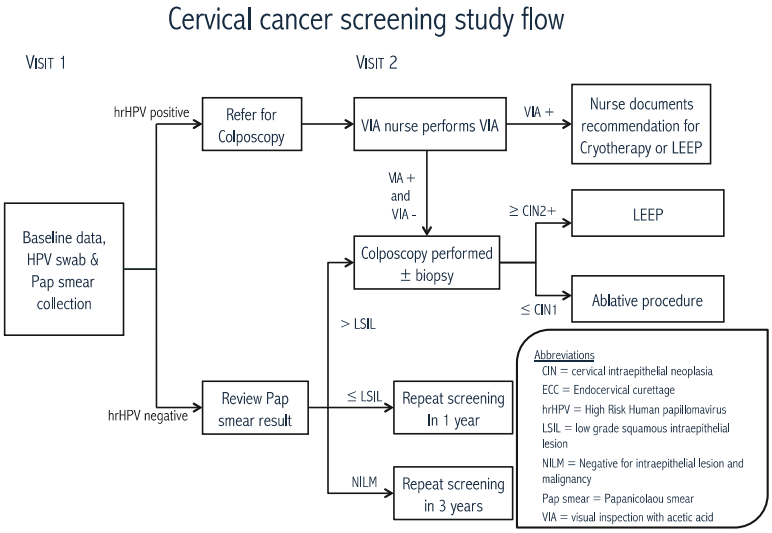
Study Procedures

For all 300 participants, HPV testing will be performed with either the commercially-available Cepheid Xpert® HPV Assay or the commercially-available Roche Cobas® HPV Assay for provider- and participant-collected samples, as per the protocol of the HPV pilot study. The pilot study currently utilizes a Cepheid GeneXpert machine on loan from the National Health Laboratory (NHL), and the duration of its availability is unknown. Should the GeneXpert machine become unavailable, the Roche Cobas® machine at the NHL will be used for HPV testing. After the HPV pilot study ends, the remaining 200 participants will be enrolled and screened in a similar manner using a provider-collected approach. This study introduces Pap smear collection for all participants at the time of provider-collected HPV specimen. Pap smear will be prepared using standard technique at the site of collection.

Participants who test HPV-negative will have their Pap smear sent to the NHL for staining and pathologist evaluation. If the Pap smear is abnormal, they will be referred to colposcopy per current Botswana Cervical Cancer guidelines.

Per the protocol of the HPV pilot study, participants who test HPV-positive will be asked to return for colposcopy and will undergo further diagnosis and treatment for cervical cancer per national guidelines. Participants who have positive HPV screening will have: 1) Pap smear sent to the NHL for review and 2) VIA at the colposcopy visit but performed prior to colposcopy. At the second visit - prior to the colposcopy a trained nurse will conduct VIA using the Botswana standard protocol. After application of acetic acid to the cervix, the nurse will record visual results as positive or negative. If VIA is positive based on assessment of the lesion(s), the nurse will record a recommendation for either cryotherapy or loop electrosurgical excision procedure (LEEP). Since all of these HPV-positive participants will undergo colposcopy, the participants will not be informed of the VIA results, as neither cryotherapy nor LEEP will be administered based on the VIA results. Rather, the participants will proceed to colposcopy as planned in the HPV pilot study (and as will be continued for the women participating only in this study) and results of colposcopy will determine further diagnosis and treatment. This design leverages the existing HPV pilot study and enables us to assess the utility of both HPV/Pap and HPV/VIA two-stage algorithms while providing the highest-quality follow-up to cervical cancer screening abnormalities in Botswana.

Participants will thus have a total of two study-related encounters if they are HPV positive one for initial screening and the one for the second-stage of screening and colposcopy. Participants who are HPV negative will only have the one study-related initial screening visit. Participants will also be asked if they want to be contacted in the future regarding participation in future research related to cervical screening and treatment. If the participant agrees, they will check yes for future contact in the consent form.



The study site is only in Botswana; however, statistical analysis will be done with support from staff at BIDMC.

A detailed data monitoring and safety plan has been developed. All adverse events will be reviewed by the PI and co-investigators. All serious adverse events will be reviewed by the PI, co-investigators and a designated safety monitor, Dr. Surbhi Grover.

B. Statistical Considerations

Sample Size

The goal of a two-stage algorithm is to increase PPV while maintaining sensitivity and specificity, and thus reduce overtreatment. Sample size assumptions were based on preliminary data, existing literature, and patient behavior regarding clinical follow-up. Among the first 25 women recruited in the parent study, the incidence of HPV-positivity was 30%. In a population with relatively high HIV prevalence, the PPV of an HPV-positive test for high-grade cervical dysplasia was 24%.[xlvi] Our sample size calculation was targeted to detect an improvement in PPV of 25% between HPV testing alone and either two-stage algorithm (a PPV of 49% for the two-stage algorithms). Assuming a two-sided test and an alpha of 0.05, a sample size of 81 participants will yield over 80% power to detect the specified difference. Clients receiving care at the Infectious Disease Care Clinic (IDCC) tend to return for clinical follow-up appointments, because they come to IDCC each month to obtain their free anti-retroviral medications. Clients seeking care at the gynecology outpatient clinic may be less integrated into routine care. Thus, we anticipate that approximately 10% of HPV-positive participants will not return for colposcopy. The proposed study will aim to enroll 300 participants to yield 90 who are HPV positive 81 of whom we expect to return for colposcopy.

Data analysis

All data will be analyzed using SAS 9.4 (Cary, NC) at Beth Israel Deaconess Medical Center. Descriptive statistics will be presented as mean with standard deviation, median with interquartile range, or proportion, based on data type and distribution. Data will be compared using the appropriate parametric or non-parametric test for continuous variables and Chi-square or Fisher s exact test for categorical variables. All tests will be two-sided and a p-value <0.05 will be considered statistically significant.

We will calculate sensitivity, specificity and PPV of HPV/Pap smear and HPV/VIA to detect high-grade cervical dysplasia amongst HPV-positive participants. We will also evaluate associations between HPV type, cytology, VIA result, and histopathology results.

C. Subject Selection

Inclusion and Exclusion Criteria

Inclusion criteria for the study are:
1) ≥25 years of age
2) HIV-positive
3) Competent to understand study procedures and give informed consent.

Exclusion criteria are:
1) Currently pregnant (as diagnostic procedures for cervical cancer are often deferred during pregnancy)
2) Currently menstruating or having persistent vaginal discharge
3) Previous hysterectomy
4) Previous diagnosis of cervical cancer

B4. POSSIBLE BENEFITS

Participants will benefit from this study by being tested for HPV, which is a test not currently available in regular care in Botswana. Participants who test positive for HPV may be identified as at higher risk for cervical cancer earlier than standard care, which may allow for more timely management.

Potential benefits to Botswana include determination of the effectiveness of two–stage screening algorithms for cervical cancer. Two-stage screening strategies have been widely adopted in other settings as they increase the specificity and positive predictive value of pre-invasive cervical disease, and thus reduce over-treatment of insignificant screening findings. The study will provide valuable operational data as the country prepares to scale up HPV testing as part of its Comprehensive Prevention and Control Strategy. Finally, study results will serve to estimate the prevalence of HPV subtypes, which may inform HPV vaccination policies and assessments.

B5. POSSIBLE RISKS AND ANALYSIS OF RISK/BENEFIT RATIO

The potential risks encountered during the study are: 1) HPV screening, 2) Sample collection, 3) Colposcopy and 4) Loss of confidentiality

1) HPV screening: The major risk for participants in this study is the concern and worry they might experience while awaiting HPV test results. Participants will receive counseling before and after HPV screening to answer questions that they may have or allay any worries. Participants who test positive for HPV might feel uncomfortable, anxious or depressed about their result and there may be stigma associated with their diagnosis. Trained personnel will give counseling to both participants and their partners to assist with any of these issues. All participants with HPV infection will receive standard of care follow-up and treatment as outlined.

2) Specimen collection: There may be some temporary discomfort associated with self-collected and provider-collected vaginal swabs. Study personnel will be trained how to minimize any adverse events and any such event that occurs will be reported to both the Site Coordinator and the Principal Investigator.

3) Colposcopy: Current standard of care in Botswana for patients with Pap smear results of high grade or persistent low grade are referred for colposcopy. Based on this standard of care, we are referring all patients with positive HPV results to colposcopy. Colposcopy allows for evaluation of the cervix under a microscope to detect any abnormal lesions. Patients with abnormal lesions will undergo biopsy as indicated in routine colposcopy procedure. Though colposcopy can be uncomfortable, it is a routine step in cervical cancer screening, as follow-up for abnormal results, and the benefit of diagnosing cervical cancer or pre-invasive cervical disease outweigh the risk of the procedure.

4) Loss of confidentiality: To protect patient privacy, all data will be handled in a confidential manner. Moreover, participants will be assigned a unique study identification number. The analytic dataset will only contain de-identified data.

Minimizing/alleviating risk:
Informed consent and strict confidentiality will be rigorously enforced to minimize risks to participants and their partners. All study personnel will receive training in the informed consent process.

Participants will be provided with a phone number whereby a member of the clinic staff and the Principal Investigators, Dr Rebecca Luckett and Dr. Doreen Ramogola-Masire, may be contacted to answer questions or in case of emergency, psychological distress or any questions regarding their informed consent.

Risk/Benefit Ratio:
Any woman seeking cervical cancer screening in Botswana will require a pelvic exam for any available screening modality currently available in Botswana (Pap and VIA), and thus provider-collected HPV testing does not introduce a new discomfort. The benefits to the participants of more sensitive diagnosis of pre-invasive cervical disease with HPV testing outweigh the discomfort of sample collection.

In this study, patients who are HPV positive will be referred for colposcopy. The benefit of colposcopy for the patient is evaluation of an abnormal cervical cancer screening test with the opportunity for diagnosis. It is standard of care for detection of pre-invasive and invasive cervical disease following an abnormal screening test in many settings. While it may be slightly more uncomfortable than a pelvic exam in the case that a biopsy is taken, it does not pose a risk of serious physical injury to a patient. While it may introduce an element of anxiety or psychological distress, measures will be taken to address any patient concerns prior to and during the procedure. The benefit of diagnosis of cervical disease outweighs the risk of the procedure.

Finally, the pelvic exam for colposcopy will be slightly longer due to the introduction of nurse evaluation with VIA prior to colposcopy. This discomfort is brief and temporary. The benefit to determining the effectiveness of VIA is essential for guiding national cervical cancer screening guidelines in Botswana, and will benefit a larger population in the long-run.

B6. RECRUITMENT AND CONSENT PROCEDURES

Recruitment
Participants will be recruited from patients presenting for care at the HIV treatment and gynecology outpatient clinics at Princess Marina Hospital (PMH), the tertiary care referral hospital in the capital of Botswana, Gaborone All women over the age of 25 who present to the clinics during study implementation will be informed that there is a study evaluating cervical cancer screening with primary HPV testing, and will be given information about HPV and cervical cancer.

Patients will not pay to participate in this study. All testing, procedures and office visits will be covered by the study.

Consent
Study personnel will invite interested women to a private clinic room and will thoroughly explain the study and the informed consent document. Information provided will include other available screening modalities in Botswana and the positive and negative predictive value of each screening modality. Additionally, patients will be given information about the utility of two-stage screening algorithms in terms of increasing the specificity of screening, and thus reducing overtreatment. After verification of eligibility, and potential participant confirmation of on-going interest in participation, a written, informed consent will be administered.

The process of obtaining informed consent will be finished before collection of data or specimens. Study personnel will exclude any potential participant who is unable to understand the informed consent.

Consent forms will be kept in a locked office in Gaborone. All study staff involved in recruiting and consenting participants will have completed the Human Research Subjects certification program before the beginning of the study. Participants will be provided with contact information for Principal Investigators, Dr Rebecca Luckett and Dr. Doreen Ramogola-Masire, for any questions or concerns.

Subject Protection

Study participants will be assured that their participations is completely voluntary and will not affect their clinical care. Patients declining participation will still receive standard screening from their provider, including VIA or a Pap smear. Only patients who can provide informed consent will be included.

B7. STUDY LOCATION

Privacy & Physical Setting

Patients will be consented in a private consultation room in the clinic at which they are seeking care at Princess Marina Hospital.

Examinations will be performed in private examination rooms at Princess Marina Hospital. Participants will only be contacted on the telephone number that they provide to the study. Prior to discussing study issues over the phone the patient will be asked if they are in a suitable location to receive results. All specimens will be stored at Princess Marina Hospital and data will be de-identified for data analysis. Data analysis will occur at BIDMC.

B8. DATA SECURITY

We will keep all electronic data in a password protected database, stored on a password protected device. Any paper with study data will be kept in a locked office belonging to a member of the study team. All data will be reported in aggregate such that participants cannot be identified.

B9 Multi-Site Studies

Is the BIDMC the coordinating site? ☐ Yes ☒ No

Is the BIDMC PI the lead investigator of the multi-site study? ☒ Yes ☐ No

B10 Dissemination of Research Results

We plan to share the results of these findings with the Ministry of Health of Botswana so that the valuable information gathered can be utilized to guide national cervical cancer prevention programming. We also plan to present the data at national and international meetings and publish the results in a peer-reviewed journal.

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