THE PREVENT ANAL CANCER (PAC) SELF-SWAB STUDY

NCT Registration Number: NCT03489707

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PROTOCOL

THE PREVENT ANAL CANCER (PAC) SELF-SWAB STUDY

Sponsor: National Cancer Institute of the National Institutes of Health
Official title: Annual anal sampling using DNA screening to identify men who have sex with men at increased risk for anal cancer

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PROTOCOL CHANGE HISTORY				
Date Submitted	**Date Approved**	**Version**	**Revisions**	**Author**
2/3/2019	3/4/2019	none	1. Pilot interviews	Nyitray
3/28/2019	5/10/2019	1.1	1. Remove text in approved consent and protocol about liquid in collection vial.	Nyitray
7/3/2019	7/19/2019	1.1	1. Pilot 2: Pilot self-swab kit with a second test group before enrollment begins.	Nyitray
3/27/2019	12/17/2019	2.0	1. Start enrollment for 400 participants in Milwaukee 2. Changes based on pilot study findings 3. Change online consent to face-to-face 4. Remove Pap test in last 12 months as an exclusion criterion 5. Changes to specimen delivery 6. Co-I contact info updated 7. Add transmen to Inclusion Criteria 8. Changes to swabbing protocol and processing 9. Changes to CASI questions 10. Edits for clarity 11. Stratification removed from stratification scheme	Nyitray
1/3/2020	5/20/2020	2.0	1. Add Spanish language material (no protocol change needed)	Nyitray
5/20/2020	8/25/2020	3.0	1. COVID-19-Induced Consent Change 2. Remote consenting instituted due to COVID-19. 3. Consent ICF changed to reflect changes in the consenting process and related changes to the Baseline and Post Swab CASIs.	Nyitray

			4. Screening limit increased to 1200 and consenting limit raised to 500 to accommodate no shows between remote consenting and Visit 1. 5. COVID-19 questions added to surveys in addition to minor wording changes to some survey questions. 6. Co-investigator Dr. Elizabeth Chiao worksite updated. 7. Insurance requirement removed. 8. Use of voicemail added. 9. AIDS Resource Center of Wisconsin's new name Vivent Health added. 10. Biometric measurements moved to clinic location	
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6/17/2021	7/27/2022	4.0	1. Recruitment material, training medical students, ICF-protocol consistency, added survey questions, and Spanish translation	Nyitray
01/03/22	1/3/2022	4.1	1. Add analysis of de-identified data. 2. Add language for blood draws at HRA as previously approved by MCW IRB 3. Allow up to 4 calls to ppts at the 12 month time point to encourage participation.	Nyitray

01/05/2022	1/10/2022	4.2	1. New Recruitment material	Nyitray
1/30/2022	2/7/2022	4.3	Press Release used to support recruitment.	Nyitray
2/23/2022	5/18/2022	4.4	Letter to ppts about ANCHOR results	Nyitray
Various	5/25/2022 7/15/2022 11/14/2022	n/a	Three amendments to increase numbers of total screened and consented.	Nyitray
06/20/2023	7/10/2023	4.5	1. Add MUSC as getting a de-identified dataset 2. New survey to participants to ask about interest in follow-up study	Nyitray

SITES PARTICIPATING IN THE STUDY

1. Medical College of Wisconsin in Milwaukee is the lead site of the study.
2. Anal Dysplasia Program – MCW/Froedtert Hospital in Milwaukee will conduct anal canal swabbing, Digital Anal Rectal Exams (DARE), and high-resolution anoscopy (HRA).
3. The Inclusion Clinic – MCW/Froedtert Hospital in Milwaukee will conduct anal canal swabbing and DARE.
4. Vivent Health in Milwaukee will conduct anal canal swabbing and DARE.
5. Sixteenth Street Community Health Centers in Milwaukee will conduct anal canal swabbing and DARE.
6. Holton Street Clinic in Milwaukee will conduct anal canal swabbing and DARE.
7. MD Anderson Cancer Center will provide observations of initial HRA procedures and consultation.
8. University of Texas Health Sciences Center at Houston, Texas will provide consultation.
9. Moffitt Cancer Center and Research Institute in Tampa, Florida will conduct human papillomavirus (HPV) genotyping.

10. Queen Mary University of London, UK will conduct host DNA and HPV DNA methylation testing.

11. Molecular Biology Laboratory, Centre of Translational Oncology, Instituto do Câncer do Estado de São Paulo in São Paulo, Brazil will conduct HPV variant testing.

PROTOCOL ROSTER

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STUDY SYNOPSIS

A randomized, non-blinded study of compliance with annual home-based vs clinic-based DNA screening to detect anal canal precancers among 400 Milwaukee HIV+ and HIV- men who have sex with men (MSM) and transgender persons.

This is a prospective, randomized, two-arm clinical study to evaluate compliance with annual home-based vs clinic-based DNA screening of anal canal exfoliated cells among Milwaukee HIV+ and HIV- MSM and transpersons aged ≥25 years. At study entry, persons randomized to arm 1 will receive a home-based collection kit in the mail at 0 and 12 months and those in arm 2 will attend a clinic where a clinician will collect the exfoliated cell specimen at 0 and 12 months. Then, persons will receive HRA-directed biopsy to assess precancerous lesions by study arm. We hypothesize that a majority of persons will comply with annual screening with increased compliance among persons in the home-based arm vs clinic-based arm.

We will test our hypothesis with 3 specific aims:

1) Determine persons' compliance with annual anal HPV DNA specimen collection. The primary endpoint is compliance with swabbing at both baseline and 12 months.
2) Determine factors associated with annual screening compliance.
3) Establish the proportion of persons in each arm who agree to have HRA.

The current research will deliver:

- The first estimates of compliance with an annual biomarker screening program among MSM and transpersons;
- The first estimates of the relative ability of home-based screening to increase annual compliance;
- Evidence of the influence that home-based vs clinic-based screening has on the uptake of HRA-directed biopsy.

The proposed research could indicate that annual HPV DNA screening and subsequent HRA are acceptable to MSM and transpersons; thus, we will determine how high-risk persons are identified for HRA in light of limited HRA resources. The duration of each participant's activities is expected to be 12 months. The study is expected have participant activity from 2019 to 2023.

OBJECTIVES:

Primary Objective: To determine compliance with annual DNA-based screening among Milwaukee HIV+ and HIV- MSM and transpersons, aged ≥ 25 years.

Secondary Objectives:

- To determine estimates of the relative ability of home-based screening to increase annual compliance.
- To estimate the influence that home-based vs clinic-based screening has on the uptake of HRA-directed biopsy.
- To estimate the association between high-risk HPV persistence and anal high-grade squamous epithelial lesions.
- To estimate the association between host and viral genome methylation and anal high-grade squamous epithelial lesions.

LIST OF ABBREVIATIONS

CAB	Community Advisory Board
CAIR	Center for AIDS Intervention Research
CASI	Computer-Assisted Self-Interview
CTEP	Cancer Therapy Evaluation Program
DARE	Digital Anal Rectal Exam
HBM	Health Belief Model
HPV	Human papillomavirus
HRA	High-Resolution Anoscopy
MSM	Men having sex with men

BACKGROUND

MSM are at much greater risk of anal cancer which is the most commonly diagnosed HPV-associated anogenital cancer other than cervical cancer.[1] While total anal cancer incidence among men and women is rare (~1.8/100,000), the rate has almost tripled in the last three decades[2, 3] and is dramatically higher among both HIV- and HIV+ MSM (e.g., 131/100,000 for HIV+).[4, 5] Also, African-American males with HIV have a disproportionate risk for anal cancer and worse survival than non-Hispanic white males with HIV.[6] However, if anal cancer is detected at an early stage, the 5-year survival rate sharply increases[6] while delayed diagnosis can result in sphincter dysfunction and permanent colostomy.[7] As with cervical cancer, if anal cancer precursor lesions are successfully removed, then morbidity and mortality will likely decline.

Persistent anal HPV infection is the primary cause of anal cancer which is a common cancer among MSM. Anal cancer screening (either Digital Anal Rectal Exam (DARE) or Pap cytology followed by high-resolution anoscopy) is standard of care for HIV+ MSM and recommended by expert opinion for HIV- MSM and is increasingly offered by clinics. But most HIV+ and HIV- MSM do not screen. Since home-based self-sampling increases rates of cervical cancer screening and self-sampling is acceptable to MSM, it is important to investigate home-based screening for anal cancer among MSM.

In addition, data on anal cancer incidence among transpersons is sparse due to lack of data collection on gender identity; however, there are data indicating that HPV prevalence among MSM and transwomen is comparable. In addition, there are data indicating a similarly high (or higher) HIV prevalence among transwomen compared to MSM. Finally, there are no data to our knowledge on anal HPV among transmen, including transmen who have sex with men.

No one has assessed compliance with annual home-based anal canal self-sampling which is required to detect 12-month persistent HPV infections. Cervical cancer screening protocols increasingly rely on a molecular biomarker (e.g., HPV DNA testing). DNA collection works well with home-based kits, but one-time DNA screening is of no value for MSM and transwomen given their high HPV prevalence; however, an experiment of *repeated* DNA screening can detect persistent HPV infection. High-risk HPV DNA persistence helps predict appearance and lack of spontaneous clearance of precancerous lesions.[16, 17] Identifying persons at highest risk for precancerous lesions is particularly needed since there is very poor infrastructure for HRA in the US and elsewhere.[18]

There has been no testing of annual home-based screening for high-risk HPV DNA at the anal canal. Annual self-screening for high-risk DNA tests enables the detection of high-risk HPV persistence which should increase the lower specificity of a single DNA screening for precancerous lesions.[57] Screening at 0 months and 12 months is not an example of sequential screening since there is only one outcome of the two tests: a positive or negative result for persistent high-risk HPV.

We will take advantage of the ability to test another biomarker by also testing for host DNA and HPV DNA methylation using the same specimens that will be tested for HPV DNA. Once the specimen is returned to MCW, it will be aliquoted into five cryovials. The first will be used for

HPV DNA genotyping, the second will be used for host DNA and HPV DNA methylation testing while the third will be archived. DNA methylation testing of HPV and human genes accurately predicts cervical HSIL.[59] Methylation patterns of HPV-16 and the host tumor suppressor gene EPB41L3 will be studied to determine if higher levels of methylation are associated with HSIL.[60]

Within cervical cancer screening, the transition to DNA tests from Pap tests also provides increased opportunity for self-screening since DNA specimens are more feasible to collect than Pap specimens given their greater sensitivity.[21] Home-based self-swab programs may also be cost-effective and also increase access for those less likely to visit clinics[22-24] due to men's greater tendency than women to avoid doctors and avoid ano-genital exams due to embarrassment.[25-28] Unease may be more acute among African Americans who may have heightened embarrassment.[29]

Our prospective design and use of a highly sensitive assay also present us the opportunity to detect deficiencies in specimen collection from either participant or clinician (as measured by the presence of β-globin). Specimen adequacy for self-collected and clinician-collected cytology are comparable, ≥80%,[30, 31] and given increased sensitivity of HPV DNA collection,[21] we expect DNA specimens would yield better adequacy.[32, 33] To enhance quality control, we will compare the adequacy of sample collection between providers and sites after 40 samples have been collected. Our ability to compare specimen adequacy of home-based self-collected vs clinician-collected annual specimens is itself a strong contribution to the literature; however, we emphasize that Aim 1 and 2, compliance with a screening protocol and factors associated with compliance, are in no way dependent on the adequacy of the specimen. The compliance findings will inform the development of repeated anal cancer screening protocols deemed efficient and accurate; thus, no matter the biomarker, health care providers and researchers will find the current study results important as benchmarks for annual anal cancer screening.

To our knowledge there has been no assessment of a mailed, culturally-competent home-based anal canal self-collection kit for persons at increased risk for anal cancer. Under the direction of PI Nyitray, our prior anal cancer-screening R21 (PI, Nyitray; NCI 1R21CA181901-01A1) recruited 200 MSM. While only 26% of these men reported prior anal cancer screening, 93% of men said they would use a swab to collect a home-based anal sample, and of those, 98% said they would do it at least annually. Thus, there is compelling evidence for MSM doing home-based repeated screening but there are no empirical data.

We chose annual screening 1) based on our studies estimating that 12% of MSM will have 12-month persistence with high-risk HPV (suggesting an increased risk for anal cancer and thus needed HRA),[19] 2) given expert opinion on anal screening intervals for HIV+ MSM,[20] and 3) because it is a familiar interval that may encourage repeat screening.

While our primary objective is to determine compliance with annual anal cancer screening, we want to understand 1) non-modifiable factors to better understand which vulnerable populations to target for future interventions and 2) modifiable factors (beliefs, attitudes, and knowledge) so that future studies might develop interventions addressing those determinants. We are using the Health Belief Model (HBM),[34] as a framework to understand the determinants of screening. If there are other determinants pointing to constructs not in the HBM, our CASI will allow us to identify those constructs and then address those determinants in future investigations. Given the

personal nature of some questions on the surveys, we are using a CASI platform to increase the likelihood of candid and accurate responses. CASIs are considered highly reliable survey methods for collecting sensitive data within multi-lingual and multi-cultural cohorts.[47-50]

In our prior R21, over 95% of 173 men reported that they could do annual anal swabbing to screen for anal cancer (unpublished data). Also, HIV+ and HIV- men showed no difference in their *intentions* to adopt annual swabbing (95% and 97%, respectively, p=0.51) as did men regardless of age, race, ethnicity, marital status, or concern about anal cancer. However, we believe HIV status will influence *actual* compliance since most (77%) believed HIV increases the risk for anal cancer; thus, we propose now to directly measure screening *behavior* to elucidate our findings of *intentions* to screen. As another example, co-Investigator Schick, et al. found an increased return of anal samples from women reporting receptive anal sex.[54] Our R21 data among MSM shows no such difference in intentions to do self-sampling based on receptive anal sex. We now intend to test that association among MSM and transpersons. Knowing the factors that increase compliance within a behavioral framework will help create interventions that increase screening uptake among persons at increased risk for anal cancer.

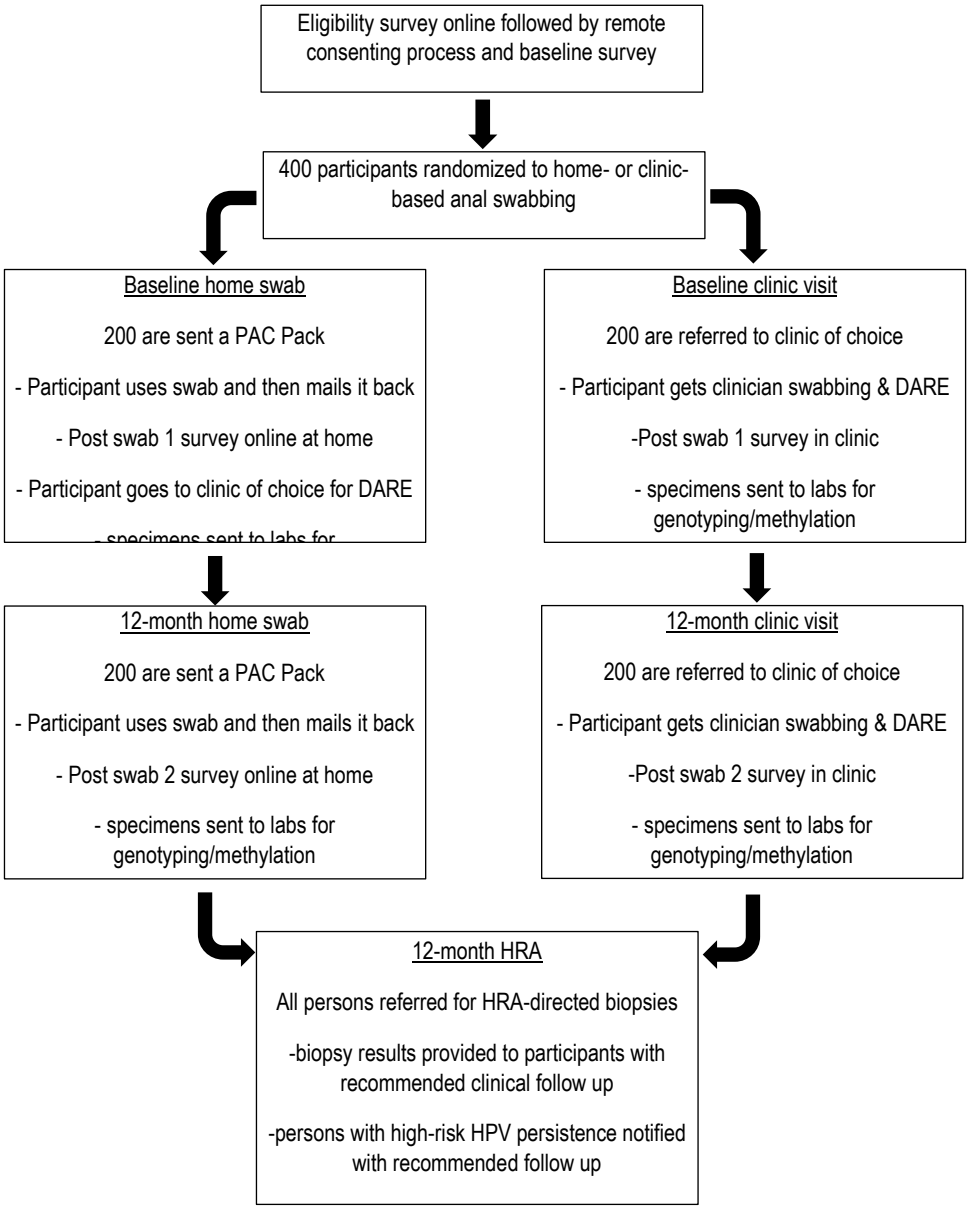
In addition to a biomarker, a procedure like HRA with directed biopsy is needed to identify precancerous lesions or cancer in the anal canal; however, some persons may refuse to undergo the invasive procedure of HRA with biopsy. The studies are equivocal with regard to HRA acceptability among MSM with one finding significant pain and bleeding with HRA,[55] and a second finding little.[56] The current study will assess a real-world scenario where a certain proportion of persons, even though potentially compliant with annual screening, may decide to forgo HRA.

We are keenly aware that introduction of the more invasive HRA may also influence those who choose to consent and those who are compliant with repeated sampling; thus, we will collect reasons for declining consent and data on those lost to follow up.

Our proposed experimental design in Aim 1 and 2, plus our prior work in HPV natural history and anal cancer screening positions us to identify 12-month persistence among persons and make important observations about which of these HPV-persistent persons complied with HRA.[19, 61] With this design we can efficiently test important questions: Will MSM and transpersons comply with annual biomarker screening? Will home-based sampling increase compliance? What other factors are associated with repeated annual screening? What proportion of persons will agree to clinic-based HRA-directed biopsy (the definitive test for precancerous lesions) after home-based self-sampling? What HPV DNA biomarkers efficiently detect anal high-grade lesions? Answers to these questions support our long-term goal of reducing morbidity and mortality from anal cancer given an increasing incidence of disease and very limited impact of HPV vaccination on adults given very low uptake to date.[8]

STUDY SCHEMA

Figure 1. The PAC Self-Swab Study visits overview



PARTICIPANT RECRUITMENT AND ENROLLMENT

Participants must meet all stated eligibility criteria.

Inclusion Criteria

- Be ≥ 25 years of age
- Sex at birth is male or gender identity is a transgender person
- Acknowledge sex with men in the last 5 years, or identify as gay or bisexual
- Understand and be willing to give informed consent
- Be willing to be randomized and able to comply with the protocol
- Spanish and/or English speakers/readers, and
- HIV+ or HIV-

Exclusion Criteria

- Not acknowledge sex with men in the past five years and not identify as gay or bisexual
- Use of anticoagulants other than Aspirin or NSAIDS
- Prior diagnosis of anal cancer
- Plans to move within 12 months
- Not Milwaukee metro residents
- Not willing to attend one of the designated study clinics at baseline, or
- Inability to give informed consent.

Persons who have had HPV vaccination will not be excluded.

Recruitment Procedures

We will recruit participants to engage in a study on anal cancer prevention (with no mention of self-sampling or HRA) in order to attract participants with diverse views about self-sampling and HRA. Study recruiters will use six strategies:

1. Social media banner ads, e.g., Grindr, Growlr, Jack'd, and Facebook, to reach MSM and transpersons outside of clinics and thus possibly benefitting more from home-based screening;[62]
2. Engagement in clinic waiting rooms serving MSM and transpersons (e.g., Vivent Health) for persons not using social media or websites;
3. Distribution of promotional materials in targeted businesses and non-profits;[63, 64]
4. Word of mouth;
5. Presentations to community groups;
6. Engagement of participants in a voluntary referral program.

The PAC Referral Program will help direct qualified candidates to the study by incentivizing consented study participants to refer partners, friends and family to be a part of the PAC Study. Specifically:

1) Study staff will provide already-consented participants with information about the referral program:
    a. The referral program is designed to spread the word about PAC Study recruitment to men who have sex with men and transgender persons who have sex with men.

b. The referral program and the study are entirely voluntary.
c. If study participants want to help recruit, they can pass along up to five coupons to their partners, friends and family. The study participant may be active in the study or have completed the study.

2) Each coupon is stamped with a unique number. Staff members will record which coupon numbers were given to which participants.
3) Participants can distribute a digital image or hard copy of the coupons to refer persons to the PAC Study Eligibility Survey in REDCap.
4) The referred persons then engage in study activities as anyone else with the exception that they will provide the coupon code to a staff person when attending a consenting session. Study team members will ask 'how did you hear about us?' or 'who can we thank for referring you to the study?"
5) The participant who referred the person and provided a coupon to person will receive $20 for each coupon redeemed up to a maximum of five coupons or a total of $100. Form of payment, e.g., debit card, gift card or cash app, is up to local site. Sites may decide to pay the referrer after each coupon is received or at regular intervals (e.g., bimonthly).
6) The $20 incentive is received by the referee as long as an eligible referent attends a consenting session. The referent does not have to consent to the study for the referee to receive the incentive.

It is our goal that 38% of participants are African American and 18% are Latino which reflects the racial and ethnic demographics of the City of Milwaukee. It is also our goal that 50% are HIV+. We will record data on:

- Recruitment strategies and process data on item distribution
- Number of website hits and calls
- Number who decline consenting
- Reasons for declining (including HRA)
- Reasons for ineligibility, and
- Those not keeping appointments.

We will collect data from study eligibility before consent occurs, e.g., age and sexual orientation, and then retain those data with no identifying information to describe who was more likely to decline participation. These data will include name, phone number, and email address to allow study staff to invite persons who are eligible to consider enrolling in the study. After consenting is complete, we will collect full contact information. Persons who do not provide contact information necessary for the study, i.e., name, mailing address, email address, and primary phone number will not be officially enrolled.

Given that neither the feasibility of a mailed HPV self-sampling kit nor the optimal form and procedures for such a kit have been established, we will employ a 6-10 member community advisory board (CAB) for guidance.[65] Comprised of both HIV-positive and HIV-negative MSM and transpersons and agency representatives who serve them, we will partner with the CAB to ensure study materials are culturally competent for middle-aged, older, and racial and ethnic minority MSM and transpersons. After training on anal cancer and study objectives, the CAB will provide guidance on recruitment, recruitment materials, internet webpage design, kit design, kit

dissemination, and interpretation of results. It will convene for an inaugural dinner during the first 6 months with regular meetings thereafter.

Eligibility Survey, Consenting and Enrollment Procedures

Interested persons will complete eligibility questions on a HIPAA-protected secure web platform. Those not eligible will be directed to medically-vetted website that includes information about anal cancer (e.g., http://www.thebottomline.org.au/). Those eligible will be contacted by staff to arrange a remote consenting session.

The consent form will include detailed information on study activities, including anal canal swabbing and HRA-directed biopsies, and the rationale behind the study. For example, participants will be told that an anal canal screening program of this type would only be useful if persons provided swabs over a period of one year; thus, we are asking them to provide a swab at the beginning of the study and 12 months later.

After consenting, participants will provide contact data including name, phone number, alternate phone number, street address, email address, preferred method of contact and alternate contact information in case primary phone, email, or street address changes. Then, persons will complete the baseline survey.

Those persons with no web access will be encouraged to call study offices for study and eligibility information after which they will be invited for consenting at study offices in which social distancing will be observed and personal protective equipment will be employed. Persons who decide not to consent will be thanked for their time and will be asked if they would like to be directed to a website with information about anal cancer, e.g., http://www.thebottomline.org.au/. After collection of contact information, all participants will be asked to complete a baseline CASI remotely. Persons who successfully complete select CASI questions, i.e., those deemed crucial to study validity, will be considered officially enrolled.

PARTICIPANT RANDOMIZATION

Persons officially enrolled will be randomized in a 1:1 allocation to either the home- or clinic-based swabbing. Blocked randomization is not necessary due to sample size. The Houston Data Coordinating Center will prepare randomization lists using Stata Version 13 (College Station, TX). The randomization process will occur for a consented participant who has satisfactorily completed the baseline CASI. Given obvious differences of each arm, randomization cannot be blinded to study staff or participants although participant data including study arm will be blinded to lab staff.

PARTICIPANT ACTIVITIES

Self-Swabbing Kits

After completing the CASI baseline survey, participants will get information about receiving a swabbing kit in the mail or making a clinic appointment. Participants in arm 1 will be sent a kit within 2 weeks while those in arm 2 will be asked to attend a clinic appointment in the next 2 weeks. Persons in arm 2 will be provided with names and contact information for a participating clinic and asked to call that clinic to set up an appointment. If they have not made an appointment within 48 business hours, they will be contacted up to three times and asked to make the appointment. Clinic or study staff (depending on clinic) will then contact participants within 24 business hours if the participant does not show for an appointment. Clinic staff will attempt to contact participants 3 times over one month before being classified as non-compliant for Visit 1.

A mock-up of the arm 1 self-swabbing kit will be pre-tested with the CAB. The kit box will not be identifiable as having health-related contents. Shipments will be logged, and the arm 1 participant will be notified upon shipment. Participants not confirming kit receipt within 7 days will be prompted about the kit receipt. The kit will include return packaging, instructions for returning the sample, self-sampling instructions, a biohazard bag, a swab, and a vial of 2 mL of standard transport media (STM) pre-labeled with a participant unique ID, and kit # (i.e., kit 1 or 2). STM is classified as a non-hazardous substance. To assess specimen exposure to extreme temperatures during mail transit, the kit will be packaged within insulation (e.g., Styrofoam) and include a temperature-monitoring device (LogTag Recorders, Auckland, New Zealand).

Home-based participants' kits will include instructions for self-swabbing written at a 6th-grade reading level in either English or Spanish. The instructions will be modeled after already-tested self-sampling instructions[67, 68] and presented to the CAB.

After receipt of the kit, participants will be instructed to wash hands with soap and water. The instructions will ask the participant to get into a comfortable position (e.g., one foot on a toilet), hold the swab in their dominant hand, pull back a buttock with the other hand and then insert the swab gently into the anal canal about 2-3 inches. The participant will then twirl the swab, count slowly to 10, and remove the swab, all the while applying swab pressure to the anal canal walls. After collecting the specimen, persons will place the swab into the tube, and then place the tube into the biohazard bag.[30, 69] Kit instructions will ask participants to record the date of the swabbing. Participants will be asked to wait until 24 hours after last receptive anal sex before self-swabbing.

Participants will be asked to return the kit within 3 days by overnight mail to the MCW Tissue Bank. The receipt date will be logged at the MCW Tissue Bank. When MCW receives the kit, the participant will be sent a link to complete the post swab 1 CASI online. Study staff will contact participants who have not returned kits within 2 weeks of kit delivery. Study staff will attempt to contact participants 3 times over one month after kit delivery before recording them as non-compliant.

All persons in the home-based arm 1 will be asked to attend a study clinic of their choice to receive height, weight, and waist circumference measurements, a clinician swab and a DARE to rule out prevalent anal cancer. If there is a finding on DARE, then standard clinical practices will be followed.

Clinic-Based Swabbing

Participants in Arm 2 will attend an appointment at a participating clinic for biometric measurements (height, weight, and waist circumference) anal canal swabbing, DARE, and computer-assisted self-interviews. Persons will be asked to attend appointments within two weeks after randomization. Appointments will be made that are convenient for the participant and attended by a highly-experienced clinician who will collect exfoliated cells with a swab from the participant using the Darragh swabbing protocol (e.g., twirling the swab, counting slowly to 10, slowly removing the swab, and applying pressure to the anal canal walls)[70] and the same brand of swab as home-based kits. The study staff will put the swabs in an insulated cooler and return them to MCW for immediate processing.

The PI or study coordinator will observe the sampling protocol of the first 3 participants at each clinical site and re-observe on an annual basis.

All persons in the clinic-based arm will receive a DARE after the clinician swabbing to rule out prevalent anal cancer. If there is a finding on DARE, then standard clinical practices will be followed.

Specimen Storage

All swabs will be stored at the MCW Tissue Bank in O-ringed cryovials after processing by Tissue Bank staff.

Swabbing 2 at 12 months and HRA Scheduling

Procedures for kits and clinic appointments at 12 months will mirror baseline procedures. All persons in arm 1 will be reminded 11.5 months after baseline of the shipment of kit #2 or if in arm 2, the need to schedule clinic appointment #2 for clinician-based swabbing. Reminders and communication to all participants will mirror procedures typically used by medical clinics, e.g., birthday greetings and up to four reminders. At the same time, all persons will be scheduled for HRA at the Froedtert/MCW Anal Dysplasia Program. We will make up to four attempts to schedule a person for HRA. For a person who declines the HRA, we will request the reason(s) why. Persons in both arms who are lost to follow up after consenting will still be solicited for a 12-month swabbing and HRA.

Computer-Assisted Self-Interviews

To accomplish the Aim 2 objective, *to assess factors independently associated with MSM and transpersons who return a swab at baseline and 12 months*, we will design CASI questionnaires capturing demographics, attitudes, and behavioral data in the baseline survey, after the 0- and 12-month swabbing time points, and after HRA. All participants will complete CASIs in English or Spanish (after translation and back translation) on the HIPAA-compliant secure web platform REDCap (Vanderbilt University). Each CASI will include questions adapted from validated cervical cancer screening survey. For adaptation, the items were modified with the input of co-Investigators, study staff and cognitive interviews with the target community. The surveys have subscales that can be used independently if any of the psychometric properties of the subscale indicate that it is not a reliable or valid measure of the construct.[72, 73] Since participants will complete CASIs in the study spanning the time from baseline CASI to post-HRA CASI, changes in participants' attitudes to swabbing or HRA will be captured.

The baseline survey conducted remotely after consenting will record demographics of the study entry population including anal cancer anxiety, prior experience with anal cancer, prior experience with other anal diseases, self-sampling acceptability, prior anal cancer screening (including HRA), benefits/barriers of anal cancer screening, and sexual behavior.

Participants will complete another CASI after each swabbing. Participants will be asked about the experience of self-sampling/clinician sampling and DARE within the framework of HBM constructs.[44, 71] Experience with DARE will be asked of Arm 1 participants at the end of their clinic DARE visit. Other CASI items will cover kit mailing, kit return, kit appearance, kit materials, and trust in the clinician (depending on study arm). The CASIs will include anxiety-related questions about anal cancer screening and the annual screening interval.

Participants will complete a final short CASI to assess the acceptability of HRA like perceived barriers (embarrassment) in addition to prior anoscopy experiences, the perception of pain, and knowledge of HRA availability.

Persons in either arm who are uncomfortable using computers or do not have access to technology will be assisted by staff in study offices to complete the CASI (or provided a mailed paper copy if a participant in the home-based arm prefers). In our prior R21, ~2% of participants required such assistance.

To determine the proportion of consented persons who would be interested in a follow-up or renewal anal cancer study, all persons who consented to the parent study will be contacted near study end. Persons will be contacted by the email or phone number provided by them in the parent study. If necessary, non-respondents will be contacted up to 3 times starting with their preferred method of contact for the first two contacts and switching to another method for the third contact. After asking the one question in this effort (see Renewal Survey file), we will record a Yes or No in the appropriate REDCap field to record their interest in a future study.

Incentives

Regardless of the return of a swab, all participants will receive a nominal incentive ($35) for completion of the Post-Swab 1 CASI and $45 for the Post Swab 2 CASI after the second swabbing. The HRA incentive of $50 includes completion of the Post-HRA CASI.

HRA

All persons will receive HRA at the Anal Dysplasia Program of the Medical College of Wisconsin by a highly trained and experienced high-resolution anoscopist. A DARE will be done on all participants. For DARE and HRA, all findings will be recorded systematically. All suspicious lesions will be documented and biopsied. If no lesions are seen on HRA, then two biopsies will be taken from two different quadrants of the anal canal to support confirmation of the anoscopists' negative finding for lesions. A senior high-resolution anoscopist will observe the HRA and HRA-directed biopsies of the HRA clinician for 5 persons. We will also monitor HSIL rates, investigate unexpected variation in HSIL rates, and assesses need for further training and observations. Importantly, the primary outcomes for all 3 aims do not rely on HRA results. The clinician will also treat external anal disease or refer for treatment, triage internal anal disease if necessary, and ask to be notified if a participant has any anus-related concerns in the following week.

A blood draw will be done during the HRA visit. The blood specimen will allow testing for factors in serum, that may be associated with anal precancers.

For DARE and HRA follow up, persons will receive usual care. HBM constructs will then be used in a brief CASI focusing on the acceptability of HRA.

After the study is finished, we will telephone participants once a year for two years and ask about screening for anal cancer that the person may have done on their own and the results of that screening.

Medical Records

All participants will have their medical record reviewed for HPV-associated procedures or conditions. From the medical records of persons with HIV, we will abstract viral load and CD4 count information. Findings will be systematically recorded using a standardized data extraction form. All persons who are not verified as HIV-positive through medical records will receive an HIV test at the baseline clinic visit and one year later at HRA. Standard clinical practices will be followed for providing HIV test results and any needed subsequent care.

Results Given to Participants

Given that the HRA will identify any occult disease in the participant, the genotyping and methylation results will not be given to participants with the following exception: participants with *persistent high-risk HPV* will be notified by either study staff or the clinician and counseled to adhere to regular contact with a knowledgeable physician to assess future anal cancer risk. HPV genotyping and methylation status are not currently used to drive clinical decisions for anal health care. At study start, all persons will receive a DARE to rule out palpable masses or other symptoms that may indicate malignancy; however, the DARE will be delayed for 2-4 weeks after study start for persons in the home-based arm so that the proportion of persons compliant with home-based swab 1 specimen collection are not biased by the DARE.

BURDEN OF TIME ON PARTICIPANTS

Both arms:
Eligibility CASI– 5 minutes
Consenting – 30 minutes
Baseline CASI – 15 minutes
Travel to clinic for HRA and DARE – varies by mode of transportation
HRA and DARE – 15 minutes
HRA CASI – 5 minutes

Arm 1:
Home-based swab 1 – 10 minutes
Home-based swab 1 CASI – 10 minutes
Travel to clinic at baseline for DARE and anal swabbing – varies by mode of transportation
DARE and anal swabbing – 10 minutes
DARE satisfaction survey – 5 minutes
Home-based swab 2 – 10 minutes
Home-based swab 2 CASI – 10 minutes

Arm 2:
Travel to clinic for swab 1 and DARE – varies by mode of transportation
Clinic-based swab 1 and DARE – 10 minutes
Clinic-based swab 1 CASI – 10 minutes
Travel to clinic for swab 2 – varies by mode of transportation
Clinic-based swab 2 – 10 minutes
Clinic-based swab 2 CASI – 10 minutes

LABORATORY ASSAYS

HPV Genotyping

All Aim 1 specimens will be batch sent to Moffitt Cancer Center for processing (81 specimens at a time will be sent which is equal to one box and one LiPA25 run). DNA will be extracted from all specimens using the robotic MDx Media Kit (Qiagen), according to the manufacturer's instructions. The HPV SPF_{10} PCR-DEIA-LiPA line probe assay system will be utilized to detect 25 HPV genotypes by reverse hybridization technology. We will add control DNA from cervical cell lines with high (CaSki) and low (SiHa) HPV-16 copy numbers as well as lines that are HPV-negative (C33A) and will perform PCR to detect the presence of human beta-globin.

HPV Variant Testing

To avoid persistence misclassification, all persons with type-specific high-risk HPV concordance at 0 and 12 months will have their specimens classified according to HPV-type variant. A variant analysis will be done by the Molecular Biology Laboratory, Centre of Translational Oncology, Instituto do Câncer do Estado de São Paulo, São Paulo, Brazil.[74, 75]

Methylation Testing

The second aliquot of each cryovial will be sent to Queen Mary University of London for host DNA and viral DNA methylation testing. The cryovial will be sent on wet ice and labeled only with the participant's unique ID.

Pathology Readings

Biopsies specimens will be retained in the MCW Tissue Bank before forwarding to an MCW pathologist experienced in reading anal canal biopsies. The pathologist will use the most recent published criteria for describing the presence of dysplastic lesions.

Biopsies will be interpreted by a single experienced pathologist using a 2-tiered system (low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL), and further classified by the applicable AIN subcategorization (anal intraepithelial neoplasia 1 (AIN1), AIN2 and AIN3).[76] Low-grade AIN will be defined as the presence of AIN 1. High-grade AIN will be defined as the presence of AIN 2 or AIN 3. We plan to conduct p-16 staining on any histology samples that demonstrate a potential diagnosis for AIN 2/3. We anticipate 25% will need p-16 staining. The interpretation will drive clinical care and be available within 3 weeks of specimen collection. Biopsies will also be assessed for HPV genotypes.

EPIDEMIOLOGICAL AND STATISTICAL CONSIDERATIONS

Analysis

Participants will be randomized to either Arm 1 or Arm 2. For Aim 1 our primary analysis is to compare the proportion of those who comply with 0 and 12-month swabbing in the home- and clinic-based arms. Our primary endpoint, compliance, is categorized as "Yes" if a swab is returned at 0 and 12 months and "No" otherwise. The null hypothesis is that the proportion of those complying in each arm is the same. Under the intention-to-treat principle, all participants who are randomized are included in the analyses (primary and exploratory). We will compare the proportion of persons who are compliant in the two arms using a Mantel-Haenszel test for difference in proportions in a stratified design (α=0.05). For sensitivity analysis we will also do a per protocol analysis comprised of compliant participants.

Participants will be considered non-compliant for either baseline or 12-month swab if they do not provide a swab within 30 days after kit mailing or clinic appointment. Persons randomized to arm 1 who "crossover" and attend a doctor's visit for anal cancer screening, including DNA collection, without returning a home-base kit, will be counted as non-compliant.

For Aim 2, to determine factors associated with compliance, we will use purposeful modeling strategies.[77] We will perform a bivariate logistic regression for each factor, and those factors with a p-value less than 0.25 in the bivariate analysis will be included in a multivariable logistic regression model. Given that we expect 144 and 108 12-month swabs returned from arm 1 and arm 2, respectively, we can safely examine up to 10 factors simultaneously in the model and maintain the recommended minimum of 10 events per variable in the multivariable model.[77] We will pay particular attention to the aforementioned HBM constructs, e.g., self-efficacy, to ascertain their ability to explain variance in compliance. Associations in the multivariable model will be considered significant with a *p*-value of <0.05. Adjusted and unadjusted odds ratios will be

reported with 95% CIs. We will regress compliance on covariates after adjusting for study arm, HIV status, race, and age (i.e., the randomization strata) and potential confounders identified with Directed Acyclic Graphs[78] including insurance status, socioeconomic status and ethnicity.

For Aim 3, we will estimate the proportion who agree to HRA (with 95% confidence interval). After genotyping results are available, we will assess the proportion of persons agreeing and not agreeing to HRA who have high-risk persistence. Analysis of HRA CASI data will focus on HRA acceptability and use purposeful modeling strategies as with Aim 2 CASI analyses. Exploratory analysis of de-identified data may also be done by collaborators including the University of Chicago and Medical University of South Carolina.

Sample Size Estimation

Our Aim 1 outcome is compliance with swabbing at both baseline and 12 months. We will compare study arms using a Mantel-Haenszel test for difference in proportions in a stratified design. Assuming a large sample size in each arm (~200), we can approximate a lower bound for our power assuming a chi-squared distribution with 1 degree of freedom.[79] Given swab return data in cross-sectional studies, we anticipate ~80% of persons will return the home-based swab at baseline.[30, 31] We estimate 10% loss to follow up at 12 months (n=180) and 80% of persons will return a home-collected swab (144) for 72% compliance at 12 months (i.e., 144/200).

Table 1. Power to detect difference in proportion of DNA samples received: home-based vs. clinic-based

Proportion of home samples	Proportion of clinic samples	Difference	Power
0.63	0.43	0.20	0.89
0.72	**0.54**	**0.18**	**0.84**
0.70	0.56	0.14	0.63
0.65	0.52	0.13	0.55

Using the same algorithm with cervical cancer screening compliance data,[80] we estimate 70% of clinic-based attendees at baseline and 60% at 12 months also with 10% loss to follow up (200*0.9 = 180; 180*0.60 = 108 or 108/200 = 54% compliance). Using intent to treat, if 144 home-collected swabs (72%) and 108 clinic-collected swabs (54%) are returned at 12 months, and assuming two-sided tests and alpha of 0.05, we have 84% power to detect a difference (0.18) between arms (table 1) for Aim 1. Power was computed with PASS 14.

Aim 1 is the primary driver of the sample size of 400 which also provides ample power for assessing factors associated with compliance in Aim 2 as shown in table 2.

Table 2. Power to detect odds ratio for varying proportion exposed in clinic arm

	Proportion exposed in clinic arm (e.g., HIV+)		
Odds Ratio	**0.4**	**0.5**	**0.6**
1.50	0.52	0.52	0.49
1.75	0.79	0.79	0.75
2.00	0.93	0.92	0.89
2.25	0.98	0.98	0.96

Threats to Validity

For Aim 1 selection bias and loss-to-follow-up are threats to validity;[81] thus, we will recruit without reference to home-based screening or HRA to lessen the population of persons with heightened concern and reservations about self-swabbing or HRA. Recruitment will use diverse strategies to select a broad spectrum of MSM and transpersons. Those strategies will be modified if enrolled persons are not racially and ethnically diverse; however, since study participants may still be different than non-participants we will ask participants about enrollment motivation including potential concern about study procedures like HRA.

For Aim 2 our primary CASI instruments will be adapted from validated cervical cancer screening instruments given the absence of validated anal cancer screening instruments. The CASI format will keep information bias and missing data to a minimum. If there are excessive missing data for a covariate, we will use the SAS procedure MI to impute missing values.

For Aim 3, we are keenly aware that selection bias and loss-to-follow-up are threats to validity; thus, questions during eligibility screening and consenting will capture data on reasons for declining study participation and the baseline CASI will provide rich data on those who do not return at 12 months. Specimen validity may suffer if poorly collected or exposed to extreme temperature, but studies find self-swabs generally have high adequacy.[68]

PUBLICATION OF RESEARCH FINDINGS

We have budgeted for three publications although we anticipate >12 publications. Community presentations will deliver study results to Milwaukee communities. Presentations of results will occur at HPV-related conferences.

ETHICAL AND REGULATORY CONSIDERATIONS

IRB Approval

The principles of Institutional Review Board (IRB) approval and informed consent described in Department of Health and Human Services (DHHS) regulations for the Protection of Human Subjects regulations (45 CFR Part 46)] must be followed. IRB approval of the protocol and the informed consent form must be given in writing.

The Investigator(s) must receive a copy of the letter of approval from the IRB, which specifically approves the protocol and informed consent, before participant enrollment. The IRB must also approve any significant changes to the protocol and documentation sent to the Investigator(s). The IRB must review the research project at least once every 365 days during the project. Continuing approval of the project must be given in writing and provided to the Investigator(s).

Records of all study review and approval documents must be kept on file by the Investigator and are subject to inspection during or after completion of the study. Adverse events must be reported to the IRB according to local procedures. The IRB should receive notification of completion of the study and final report within 3 months of study completion and termination. The Investigator will maintain an accurate and complete record of all submissions made to the IRB, including a list of all reports and documents submitted.

Changes to the Protocol

Any change or addition to this protocol requires a written protocol amendment that must be approved by the MCW IRB and the Investigator before implementation. A copy of the written approval of the IRB must be sent to the Investigator.

Participant Confidentiality

The Investigator will grant monitor(s) and auditor(s) from the NCI and regulatory authorities access to the participants' data. The participant's confidentiality will be maintained and will not be made publicly available to the extent permissible by the applicable laws.

Records to be Kept

Information linking the participants' identity to the study ID will be kept in locked file cabinets. Separate cabinets will contain informed consents.

All data will be in password-protected digital format. Names, unique ID number, and contact information will be kept in separate digital files from all other data. All study-related outcome data will only include the unique ID number coded identifiers. All computers will require a password to access files. Computer-assisted self-interviewing will be used so participants can confidentially report sensitive behaviors. No information that could lead to personal identification of participants will be included in any of the reports or given to any non-authorized person. Staff will be trained on how to review electronic medical records for evidence of HIV status and HPV disease and procedures. Medical record data will be entered directly into a digital abstraction form. Laptops will also have strong data encryption installed as per requirements at MCW.

Specimen Archiving

As an optional procedure to participants, we will store remaining samples in a specimen bank after study testing is complete for future use at the Medical College of Wisconsin and collaborating institutions. They will be stored without any personal information that could link them to a participant. The samples will have a code. The study staff will have the codes linking a participant to the samples stored in a locked, private area. Samples may be used for evaluation of infectious diseases or markers of infection or disease and will not be destroyed until the specimen cannot be used for these purposes. The results of this testing would not be available to a participant or a participant's physician and would not alter participant care in any manner.

Adverse event reporting

No adverse events are expected. The procedure of inserting the scope into the anal canal (HRA) may be embarrassing for some persons. The anal speculum can be uncomfortable, and the vinegar solution can cause some irritation in the anus. Some bleeding occurs with every biopsy. Serious bleeding is rare, about 1 in 1000 biopsies. If serious bleeding occurs, a simple procedure may be necessary to stop the bleeding such as burning or cauterizing the area. An injection of lidocaine may be given prior to a biopsy. It is possible to experience pain or dizziness from the injection. Rarely, an allergic reaction could occur. Events will be graded according to Common Terminology Criteria for Adverse Events v5 (CTCAE)

We will follow the Cancer Therapy Evaluation Program (CTEP) guidelines for reporting of adverse events (although we are employing no therapeutic interventions). Reportable safety events will be submitted to the MCW Institutional Review Board (IRB) per institutional guidelines. The study team, including PI, Study Coordinator, and other study staff will have weekly meetings. At each weekly meeting after enrollment starts, the Study Coordinator will provide the following information: number of participants entering the study, status with respect to meeting recruitment targets, the percentage of participants completing swab 1 and 2, number of drop-outs, reasons for drop-out, and number of participants undergoing HRA. Information about any adverse events also will be presented including potential anxiety from persistent HPV results or harm from directed biopsies. These participants will be managed using standard of care practices. By examining this information, the team will keep abreast of critical issues regarding recruitment and data integrity. Study staff also will notify at least one supervisor immediately if at any point a participant shows the need for urgent treatment (e.g., diagnosis of cancer). This type of information will be communicated immediately, with consultation with clinician co-Investigators about an appropriate course of action. In the event of an injury that results from the research, neither financial compensation nor the costs of medical treatment will be available through the research project. However, necessary facilities, emergency treatment, and professional services will be available to the participants, just as they are to the general community. Annual feedback will be provided to the NCI and MCW.

Criteria for Termination of the Trial

Since there are only 2 visits and an HRA visit, the trial cannot be terminated until these activities have been completed. For example, a participant who is not compliant with a swabbing visit but does attend an HRA is an important data point for determining the primary outcome of compliance.

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APPENDICES

APPENDIX I: STUDY SCHEDULE

Time table of activities																				
	2017-2018				2018-2019				2019-2020				2020-2021				2021-2022			
(shaded blocks indicate the activity will occur in that quarter)	9-11	12-2	3-5	6-8	9-11	12-2	3-5	6-8	9-11	12-2	3-5	6-8	9-11	12-2	3-5	6-8	9-11	12-2	3-5	6-8
Complete human subjects' approvals																				
Transfer of grant from Houston to Milwaukee																				
Install/meet with CAB to discuss responsibilities																				
Edit/finalize protocol, interview questions, home kit																				
Train/observe clinician staff in study procedures																				
Pilot/analyze pilot/revise full protocol																				
Recruit participants and deliver protocol – swab 1																				
Genotype first 200 specimens from time 0																				
Deliver protocol – swab 2; conduct HRAs																				
Genotype 200 specimens from time 0 and 200 from time 12																				
Perform epidemiological and statistical analyses																				
Genotype last 200 specimens from time 12																				
Prepare and submit manuscripts																				

APPENDIX II: INFORMED CONSENT FOR ANAL STUDY AND TISSUE BANKING
APPENDIX III: INSTRUCTION FOR SELF SWABBING

Here's what you do in 5 easy steps:

1) Read the instructions for anal self-swabbing.

2) Open the white USPS Priority Mail box and use the swab inside according to the anal canal swabbing instructions.

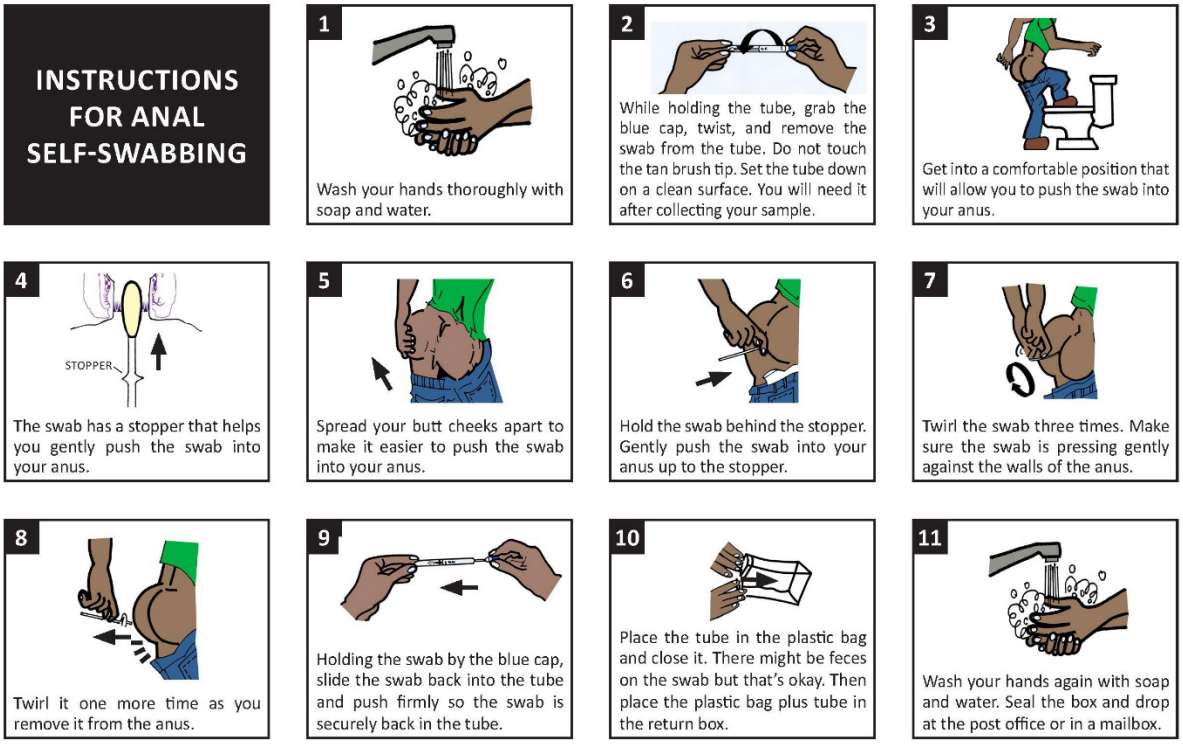
3) There is a blue LogTag temperature recorder in the box. Please do not handle it or remove this from the box. It should be sent back with the swab.

4) When the anal swab and tube is back in the white box, use the adhesive strip to close and seal the white USPS Priority Mail box. (You can throw away the larger blue box that it came in.)

5) Within a day or two, put the white USPS Priority Mail box in any post office box or take it to the Post Office. Postage is prepaid.

Call Dr. Alan Nyitray at 414-955-7701 if you have questions about the PAC Pack.
Call 911 if you have an emergency.

INSTRUCTIONS FOR ANAL SELF-SWABBING



APPENDIX IV: INSTRUCTION FOR CLINICAL SWABBING

Clinical anal canal swabbing will be performed at the Milwaukee clinics identified above. The participant should undress so that the buttocks are exposed, and either bend over the exam table or lay in the left lateral decubitus position. The examiner should use one hand to spread the buttocks and expose the anal verge.

Procedure for obtaining an anal swab specimen

A study-supplied swab will then be gently inserted by the clinician into the anus up to the swab's "stopper" or until the swab touches the wall of the rectal vault. If there is difficulty inserting the swab, the participant can also help to pull back their buttocks with the swab then inserted in the canal. With pressure on the distal end of the swab rotate it and twirl it with firm pressure on the anal canal walls. Do this while counting slowly to 10, and then slowly removing the swab from the canal while rotating the swab one more time. Do not retract the buttocks when the swab is close to the verge to ensure that it is sampled as well. The swab will then be inserted into the supplied tube containing 2 mL of STM. [84]

APPENDIX V: HIGH RESOLUTION ANOSCOPY (HRA) AND ANAL BIOPSIES

The patient will be positioned for anal evaluation to determine any anogenital lesions or pathology. A mixture of an anesthetic cream (e.g. 4% lidocaine cream) and water-soluble lubricating jelly should be used as a lubricant. A digital anal rectal exam (DARE) should then be performed palpating the entire anal canal, distal colon, and perianus, noting any condylomas or lesions.

The procedure for HRA is as follows:

- Insert the anoscope, remove obturator, and place a cotton swab wrapped in gauze soaked in 5% acetic acid into the anus.
- Remove the anoscope over the swab and leave swab in place for 1 to 2 minutes.
- Remove the swab and re-insert the anoscope. Carefully examine the anal canal with the anoscope.
- Re-apply acetic acid frequently to ensure adequate detection of lesions and verify that all aspects of the Anal Transformation Zone (AnTZ) have been visualized.
- If acetowhitening is noted, note vascular characteristics, if present.
- Lugol's solution (iodine) may be used as desired to aid in identifying areas of possible LSIL and HSIL near the squamocolumnar junction.
- Biopsy abnormal appearing areas (HRA-directed biopsies) clinically suspicious for HSIL; areas suspicious for LSIL may also be biopsied if more severe lesions are not seen. Local anesthetic (e.g., 1% lidocaine with or without epinephrine or .5% bupivicaine) may be used at the provider's discretion prior to biopsy.
- If there are no lesions suspicious for LSIL or HSIL, then the anoscopist will biopsy two quadrants of the anal canal (see Appendix VI).
- Attain hemostasis prior to removal of the anoscope at the discretion of the provider (with pressure, Monsel's solution, or silver nitrate).
- An external genital exam should be performed to note the presence of condyloma and other abnormalities.
- Apply acetic acid for one minute to perianal area and examine carefully with the anoscope.

- Biopsy any external (perianal) areas clinically suspicious for HSIL, using a local anesthetic (e.g., 1% lidocaine with or without epinephrine or 0.5% bupivacaine) prior to biopsy. Perianal condyloma or LSIL can be biopsied at the discretion of the provider.
- Participants with signs or symptoms consistent with proctitis or sexually transmitted infections other than HPV should be referred for appropriate diagnosis and treatment.

APPENDIX VI: BIOPSY PLAN FOR CASES OF "NO LESIONS"

The anal mucosa should be divided into 4 quadrants: Anterior, Left, Posterior and Right. Then a biopsy of normal appearing mucosa from two quadrants should be biopsied. Biopsies from different quadrants must go in separate formalin containers and get separate pathologic interpretations.

APPENDIX VII: HRA BIOPSY PREPARATION

Preparation of biopsies will be done by MCW Tissue Bank and follow standard procedures used for epidermal biopsies.

APPENDIX VIII: ELIGIBILITY CASI

APPENDIX IX: CONTACT INFORMATION FORM

APPENDIX X: BASE-LINE CASI

APPENDIX XI: POST SWAB 1 CASI

APPENDIX XII: ARM 1 DARE SATISFACTION CASI

APPENDIX XIII: POST SWAB 2 CASI

APPENDIX XIV: POST HRA CASI

APPENDIX XV: TISSUE BANK PROCESSING

APPENDIX XIV: PILOTING