WOMEN'S INTERAGENCY HIV STUDY
SECTION 39: CERVICAL CANCER SCREENING SUBSTUDY

A. STUDY PURPOSE

This study is designed to compare the accuracy of different molecular assays for cervical cancer screening. There are two arms to the study: (i) New HIV+ WIHS Participants and (ii) Non-WIHS Colposcopy Patients *(which does not involve WIHS participants)*. This MOO is focused on the new HIV+ WIHS Participants who will be enrolled at the UAB-MS (Alabama only), Atlanta, and UNC WIHS sites.

B. SPECIFIC AIMS

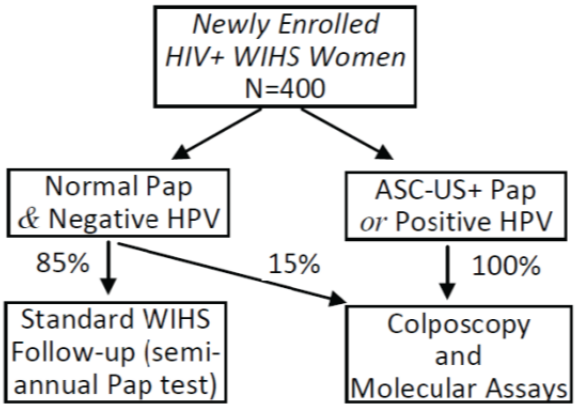
Aim 1: To determine the relative sensitivity/specificity/positive predictive value (PPV)/negative predictive value (NPV) of promising molecular assays for detection of CIN-2+ and CIN-3+ as an adjunct to Pap tests in HIV+ colposcopy patients.

Aim 2: To determine the optimal cervical cancer screening approach in HIV+ women that can be achieved using these assays alone or in combination with one another or in combination with HC2 (hybrid capture 2 high-risk HPV DNA test).

Aim3: To estimate cost-effectiveness of these several approaches for cervical cancer screening in HIV+ women.

C. RESEARCH DESIGN AND METHODS

1. STUDY DESIGN



Briefly, HIV+ WIHS women having their baseline visit at the UAB (excluding MS), Atlanta, and UNC WIHS sites (N=400) will have standard monolayer / liquid Pap tests (PreservCyt / ThinPrep) and be tested in "realtime" (within two weeks of enrollment) for oncHPV using the FDA approved cobas HPV Test at a CLIA-certified laboratory (UAB pathology), prepared from exfoliated cervical cells stored in the liquid Pap preservative (PreservCyt). *Women with either (i) an ASC-US+ Pap or (ii) positive HPV test at enrollment will undergo colposcopy, as will (iii) 15% of those negative by both tests*. Based on this strategy we will capture nearly all CIN-2+ prevalent at baseline. Thus, the main outcome will be pre-cancer / cancer at enrollment defined as CIN-2+ or CIN-3+ (a more stringent endpoint), assessed in separate analyses. Following this one visit, each of the WIHS women who participate in the CCSS return to standard WIHS follow-up and testing, unless they are referred to colposcopy (see figure above and text below).

In the second arm of the study *(which does not involve WIHS subjects)*, 650 HIV+ colposcopy patients will be enrolled through colposcopy clinics affiliated with WIHS clinical sites at UCSF, Georgetown, and Albert Einstein College of Medicine (exploiting the WIHS infrastructure). These women will provide two liquid Pap tests, which will be used for monolayer Pap and Cobas HPV testing, as well as testing in the other molecular assays. Enrollment of colposcopy clinic patients will greatly increase the number of CIN-2+ and CIN-3+ cases in the study, and these women can be enrolled rapidly (in just over two years). Colposcopy patients are typically referred because of an abnormal Pap and, thus, their data can be used to study new assays as an adjunct to Pap tests. Adjuncts to Pap testing are very important. However, in combination with the new HIV+

WIHS women, we can also accurately estimate the individual sensitivity, specificity, PPV, and NPV of each of the new molecular assays for cervical cancer screening, as well as assess these assays in combination with one another. This is a highly efficient study design which will achieve statistical power similar to a routine screening population of nearly triple the size.

In addition to (i) the Cobas HPV test, the molecular assays will include (ii) cellular markers of E6 activity/proliferation (p16/ki-67 cytology; CINtec+), (iii) cellular markers of aberrant S-phase induction (MCM2/Top2A cytology; BD ProExC), (iv) detection of oncHPV E6/E7 oncogene mRNA expression (PreTect HPV-Proofer), and (v) two additional HPV DNA assays – the HC2 test, and, second, a semi-quantitative PCR assay able to detect >40 HPV types. All Pap tests and histology will be centrally reviewed by an expert pathology panel, and all molecular assays will be centrally conducted.

2. INCLUSION CRITERIA

Eligibility for the newly enrolled WIHS women includes: (i) ≥18 years of age; (ii) able and willing to give informed consent; (iii) able to complete the interview in English or Spanish; (iv) willing to provide gynecologic specimens; (v) HIV+; and (vi) with an intact cervix (no history of hysterectomy).

3. CONSENT

At the WIHS baseline visit, eligible participants will be approached for CCSS recruitment by a WIHS staff person in a private room before the start of her examination. The study staff person will explain the CCSS to the participant and obtain informed consent prior to any CCSS procedures. To ensure participants understand the study information, potential participants will be asked to describe the study to the staff member, including the purpose, risks, and benefits. The WIHS locator form should indicate whether informed consent for the CCSS was obtained.

D. CCSS PROCEDURES

The WIHS participants will have a standard monolayer Pap test (ThinPrep) and be tested in "real-time" (within 2 weeks of enrollment) for oncHPV using the FDA-approved cobas HPV Test at a CLIA-certified laboratory, prepared from exfoliated cervical cells stored in a liquid Pap preservative (PreservCyt). Women with either (i) an ASC-US+ Pap or (ii) positive HPV test at enrollment will undergo colposcopy, as will (iii) 15% of those negative by both tests.

After the general physical examination, women undergo vaginal speculum examination. First, two liquid Pap test specimens (PreservCyt/ThinPrep, Hologic, Bedford, MA) will be obtained, as follows. The PreservCyt vials are labeled #1 (first sample) and #2 (second sample).

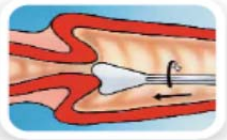
Specifically, the two liquid Pap tests (PreservCyt/ThinPrep, Hologic, Bedford, MA) will be collected using standard methods (see attached diagram) using a plastic spatula and an endocervical brush. As described in the *diagram below*:

i. If desired, use lukewarm water to warm and lubricate the speculum. Water-soluble gel lubricant may also be sparingly applied to the posterior blade of the speculum if necessary.
ii. Note which PreservCyt vial is labeled **#1 (first sample, s-code 120**), and which is labeled **#2 (second sample, s-code 121**).
iii. Use the plastic Ayre spatula and with two 360^0 turns over the ectocervix maintain tight contact with the mucosal surface to obtain exfoliated cervical cells.
iv. Rinse the spatula as quickly as possible into the PreservCyt Solution vial by swirling the spatula vigorously in the vial 10 times. Discard the spatula.

v. Using the endocervical brush, insert the brush into the cervix until only the bottom-most fibers are exposed. Slowly rotate 1/4 or 1/2 turn in one direction. DO NOT OVER-ROTATE.
vi. Rinse the brush as quickly as possible in the PreservCyt Solution by rotating the device in the solution 10 times while pushing against the PreservCyt vial wall. Swirl the brush vigorously to further release material. Discard the brush.
vii. Tighten the cap so that the torque line on the cap passes the torque line on the vial.
viii. **Then repeat** as above for the second PreservCyt/ThinPrep specimen (vial labeled #2), using a new plastic Ayre spatula and cytobrush.

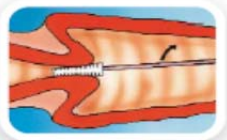
Quick Reference Guide
Endocervical Brush/Spatula Protocol

THE ThinPrep PAP TEST



Obtain...
an adequate sampling from the ectocervix using a plastic spatula. If desired, use lukewarm water to warm and lubricate the speculum. Water-soluble gel lubricant sparingly applied to the posterior blade of the speculum can be used if necessary.[1] Select contoured end of plastic spatula and rotate it 360 degrees around the entire exocervix while maintaining tight contact with exocervical surface.

Rinse...
the spatula as quickly as possible into the PreservCyt® Solution vial by swirling the spatula vigorously in the vial 10 times. Discard the spatula.



Obtain...
an adequate sampling from the endocervix using an endocervical brush device. Insert the brush into the cervix until only the bottom-most fibers are exposed. Slowly rotate 1/4 or 1/2 turn in one direction. DO NOT OVER-ROTATE.

Rinse...
the brush as quickly as possible in the PreservCyt Solution by rotating the device in the solution 10 times while pushing against the PreservCyt vial wall. Swirl the brush vigorously to further release material. Discard the brush.

Tighten...
the cap so that the torque line on the cap passes the torque line on the vial.

Record...
the patient's name and ID number on the vial.

Record...
the patient information and medical history on the cytology requisition form.

Place...
the vial and requisition in a specimen bag for transport to the laboratory.

Labeling:

(i) *The PreservCyt tubes* - will be labeled with the barcoded CCSS stickers and the labels must show which specimen was obtained fir**st** (#1, s-code 120) and which second (#2, s-code 121), because we will alternate which assays are conducted using the first vs the second specimen to minimize the possibility that variation in sampling order might affect the results. All women with ASC-US+ or a positive cobas HPV Test, as well as 15% of women negative in both tests, will be referred to colposcopy. Standard procedures will be used, except that colposcopist will obtain at least two biopsies from acetowhite lesions, even when the colposcopic impression is low grade or metaplasia.

Transport of PreservCyt tubes:

Emory – Using the same schedule as the non-CCSS WIHS Pap specimens, ship to UAB at least once per week.

UAB – Using the same schedule as the non-CCSS WIHS Pap specimens, transport the labeled tubes to the Cytology Laboratory at UAB.

Advance shipment notification and a manifest should be faxed to the UAB laboratory. A manifest should also be included in the shipment. The manifest should include at least the following information:

- Site number: 81=UNC; 82=Atlanta; 84=Birmingham.
- Shipper's contact person, phone, email, mailing address.
- Total number of Pap smear slides in shipment.
- List eight-digit WIHSIDs and associated two-digit WIHS visit numbers and collection dates for samples in the shipment.
- Local database tracking number on external label, if used.
- Clearly indicate the fax number to send confirmation of shipment receipt.
- Courier name, tracking number, ship date, and expected arrival date.
- S-code: s-code 120 (first specimen); s-code 121 (second specimen).

Shipments should be sent to UAB Monday through Wednesday only, using a courier and delivery option that can track the shipment; sites should contact UAB in advance to make special arrangements for shipment on other days of the week. UAB is open to accept shipments 8:00 am to 5:00 pm, Monday to Friday. UAB will confirm receipt of shipment by returning a signed and dated fax. Sites are responsible for tracking shipments to UAB and contacting UAB if a clinical report is not received within two weeks of shipment arrival.

Contact Allison Wrenn, UAB Cytology Supervisor, allisonwrenn@uabmc.edu.

Shipments should be sent to the following address:

Allison Wrenn CT(ASCP)
UAB Cytology Laboratory
Hospital Support Building - HSB 100
508 20th Street South
Birmingham, Alabama 35294
TELEPHONE: (205) 934-2025
FAX: (205) 975-7056

Real-Time System for WIHS Subject Triage to Colposcopy: Both Pap and Cobas HPV test results will be sent to the WIHS site as soon as available from the UAB CLIA certified pathology laboratory.

Abnormal Pap test – This represents routine colposcopy, so no special effort or procedures are involved. Approximately 25% of new WIHS enrollees are expected to have ASC-US+ and be triaged to colposcopy.

Oncogenic HPV+ with Normal Pap – Approximately 10% of HIV+ subjects are expected to have oncogenic HPV+ but a normal Pap test. These women are to be triaged to colposcopy for research purposes.

15% of Normal women (approximately 1/7th) – Every seventh woman with normal Pap and HPV test results enrolled in this substudy should be triaged to colposcopy.

Colposcopy WIHS Women: At the time of CCSS colposcopy visit, the WIHS clinician will complete the WIHS L14 form. These questions relate to visual and colposcopic evaluation of the lower genital tract including the cervix, vagina, vulva, perianal area and anus. At least two biopsies should be taken from acetowhite tissues and from all areas that are clearly lesional – given strong evidence that multiple biopsies improve accuracy. If there are no lesions present, then do not biopsy.

Reimbursement: At the completion of the baseline CCSS visit, participants will be reimbursed $25 in cash by the study staff. They will receive another $25 in cash if they are triaged to colposcopy and complete the procedure, with payment given after the procedure.

Pap Specimen Handling: The two liquid Pap specimens (PreservCyt/ThinPrep, Hologic, Bedford, MA) will be labeled with the participants CCSS number and date and shipped overnight to the University of Alabama (UAB) Department of Pathology. Upon arrival at UAB, each liquid Pap specimen will be gently vortexed to evenly distribute exfoliated cells, and then aliquoted for each assay. Residual material will be stored for future studies (as mentioned in the consent form). This information will also be entered into the web-based Tracking and Data Management System. A paper manifest will also need to be included in each shipment.

Data Collections Forms: The CCSS consent form will be kept locked up and separate from all other study materials. All other data collection forms and specimens will only be identified by WIHSID number.

Pathology Review:

Pap test – A standard ThinPrep monolayer cervical cytologic Pap test will be prepared per manufacturer's recommendations. All Pap tests will be screened at UAB by two cytotechnologists, with those found to be abnormal (ASC-US+) additionally screened by two independent UAB cytopathologists and, if necessary, adjudication of any discrepant results by a third. For quality control purposes, 10% of normal specimens will also be screened by a cytopathologist.

Histology – Histologic specimens, similarly, will be screened by two pathologists; a pathologist at the collaborating clinical site and a pathologist at UAB, with adjudication by an additional UAB pathologist. Each review will be conducted in a blinded fashion, without knowledge of the findings of other reviewers. On occasions when the adjudicator's findings do not correspond with either of the prior reviews, each of the UAB pathologists will confer to determine a final diagnosis.

If a biopsy is obtained, it is critical that the diagnostic histology slide be obtained and sent to UAB or, if that is not acceptable to the pathologist, that a high definition digital image is transmitted to UAB for centralized review.

Molecular Assays: The cobas HPV test (Roche Molecular Systems, CA), and the Hybrid Capture 2 test (HC2; Qiagen Inc, Germany) will each be conducted in CLIA-certified laboratories at UAB and Albert Einstein College of Medicine (AECOM), respectively. The CINTech+ P16/Ki67 cytology (mtm Laboratories AG, Germany), BDProExC MCM-2 / TOP2A cytology (BD Diagnostics, NJ), and PreTect HPV-Proofer oncHPV E6/E7 (Norchip AS, Norway), will each be conducted by the companies themselves. MY09/MY11 HPV PCR will be conducted by the well-established research laboratories of Drs. Robert Burk (AECOM) and Joel Palefsky (UCSF).

Follow-up:

Histopathology and treatment information – The WIHS L15 and L16 forms, containing the histopathology results from the colposcopy and any treatment information, should be completed within six months of enrollment into the CCSS.

Obtaining Histologic Slide or Image – The original histologic slide used in diagnosis or a digitalized image should be retrieved and sent to Dr. Isam-Eldin Eltoum at UAB [see address below]. If a digitalized image is sent but the interpretation at UAB is lower grade than the local interpretation (e.g., CIN-2 instead of CIN-3), or the image is poor, then the original histologic specimen will need to be requested and sent to Dr. Eltoum – even if the difference in diagnosis is only a single grade of neoplasia. If the UAB interpretation is the same or higher grade, then the slide does not need to be sent.

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Supplies: The CCSS will supply: (i) all forms, (ii) liquid Pap test vials prefilled with PreservCyt Solution (20cc in a 50cc vial), cytobrushes, and plastic Ayre spatulas for specimen collection; (iii) shippers for liquid Pap tests, (iv) shippers for histology slides, (v) FedEx acct info for shipping, and (vi) an online specimen tracking system.

FedEx Information:

Account #252636714. Using the online FedEx ticket form there is a space in section #4 BILLING DETAILS for the FedEx account number and underneath there is a space that says "Your Reference" where you should enter #9526-5817 so that the Einstein billing office knows it relates to the Cervical Cancer Screening Study (CCSS). The specimens should be sent for Next Day, Morning Delivery.