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Comparison of Nylon-flocked swab and Dacron Swab Cytology for Anal HSIL Detection in Transgender Women, and Gay, Bisexual, and other Men Who Have Sex with Men.

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Abstract

Purpose: Anal histological High-Grade Squamous Intraepithelial Lesion (hHSIL) is an anal cancer precursor. Experts recommend Dacron-swab anal cytology as a primary screen for anal hHSIL, especially among HIV-infected and -uninfected MSM. Studies show Dacron cytology inaccurately predicts anal hHSIL, resulting in unnecessary diagnostic procedures. Nylon-flocked swabs are shown to trap pathogens and cells well. Thus, we compared test characteristics of anal cytology using nylon-flocked (NF) and Dacron swab collection protocols to predict anal hHSIL.

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Contributions:

All authors provided editorial support, revised, and approved the manuscript for publication. In addition, Dorothy Wiley conceived/ designed the research; acquired, analyzed, and interpreted data analyses; drafted the manuscript. Hilary Hsu designed the research approach, acquired and analyzed data. Martha Ganser provided detailed data analyses and iterative approaches to evaluating patterns. Jenny Brook provided detailed data analyses and iterative approaches to evaluating patterns. David Elashoff developed analytic approach, iteratively evaluated data patterns. Matthew Moran conceived/ designed specimen collection approach, acquired specimens, analyzed data, interpreted results. Stephen Young developed the laboratory approach, implemented quality assurance activities, interpreted data analyses. Nancy Joste developed histo- and cyto-pathology quality assurance activities, interpreted data analyses. Ronald Mitsuyasu directed care project medical director, interpreted data analyses. Teresa Darragh implemented histo-/cyto-pathology quality assurance activities, interpreted data analyses. David Morris interpreted data analyses. Otoniel Martínez-Maza interpreted data analyses. Roger Detels interpreted data analyses. J.Y. Rao implemented histo-/cyo-pathology quality assurance activities, interpreted data analyses. Robert Bolan interpreted data analyses. Eric Shigeno interpreted data analyses. Last, Ernesto Rodriguez interpreted data analyses.

IRB Status: Approved by the UCLA IRB under Protocol #13-000997

Methods: A single-visit, randomized clinical trial compared NF- and Dacron-swab anal cytology specimens to predict High-resolution Anoscopy and biopsy diagnosed anal hHSIL. Data for 326 gay, bisexual, and other men who have sex with men, and male-to-female transgender women contributed descriptive and tabular statistics on which unadjusted and fully-adjusted logistic regression models were constructed. Models estimated odds of hHSIL, test accuracy (AUC) and sensitivity, specificity, as well as positive and negative predictive values of abnormal NF- and Dacron-cytology to predict hHSIL.

Results: In the fully-adjusted model, sensitivity for NF- and Dacron-cytology were near equal (48% vs. 47%), but specificity was higher with NF cytology (76% vs. 69%). Comparisons of area under ROC-curves (AUC) showed NF-cytology alone predicted hHSIL better than the covariate model (AUC: 0.69 vs. 0.63, p=0.02), but NF- and Dacron-cytology comparisons showed no statistically significant difference (AUC: 0.69 vs. 0.67, p=0.3).

Conclusion: NF-cytology and Dacron-cytology provide modest sensitivity, but NF-cytology has higher specificity and accuracy, important to lowering costs of population-based screening.

Introduction

Invasive anal cancer (IAC) disproportionately affects HIV-infected gay, bisexual, and other men who have sex with men (MSM) where current rates are higher than general male population estimates (130 vs. 1.5 cases/100,000 person-years (PY) and some experts suggest rates are higher in HIV-uninfected MSM.[1–5] Rates now exceed invasive cervical cancer (ICC) rates when cervical cytology was introduced in the 1950s, ~50 cases/100,000 PY.[1,2,6]

Twelve human papillomavirus (HPVs) genotypes are necessary, but alone insufficient causes of human cancers.[7] Group 1 high-risk HPV carcinogens (Group1/hrHPVs) cause ICCs, IACs, and other anogenital and aerodigestive cancers.[7,8] Risk factors for IAC and anal histological High-Grade Squamous Intraepithelial Lesions (hHSILs), a precancer, include smoking, early-life or exclusive male-male sexual partnerships, receptive-anal intercourse (RAI), anal hrHPV infections, especially HPV16/18 among both men and women; and abnormal cervical cytology in women.[9–13] HIV-infection and other immunosuppressive conditions such as organ transplant; and lifetime history of syphilis, Chlamydia, gonorrhea, and anal warts are positively associated with IAC and anal hHSIL.[9–11]

HPV-infection and -disease are well described.[14,15] HPV infection may be asymptomatic, or show cytological and histological Low-Grade Squamous Intraepithelial Lesions (hLSIL) and hHSIL. hHSILs may regress, remain stable, and few progress to cancer.[11–13] Meta-analyses suggest IAC incidence sharply rose following introduction of combined-antiretroviral therapy (CART) for HIV. For example, pre- and post-CART-era data show a 3.6-fold increase in IAC incidence that is attributed to longer survival with HIV: 21.8/ vs. 77.8/100,000 person-years.[16] Progression from first clinical hHSIL detection to cancer may be 0.6%, annually.[17]

Efficacious screening to identify anal hHSILs is consistent with other U.S. secondary cancer prevention strategies. Currently, anal cytology is solely recommended by experts for anal cancer or hHSIL screening in high-risk populations.[18] Few head-to-head comparisons of

anal cytology collection strategies are published, especially where anal biopsy is performed universally. Currently, experts recommend Dacron-swab cytology specimen collection, where data show cytology samples marginally predict anal hHSIL.[18] To estimate prevalence of abnormal cytology; and to estimate the sensitivity, specificity, positive- and negative-predictive value (SSPN) of two anal cytology-collection protocols, we enrolled subjects in a single-visit, randomized-controlled trial.

Methods

Subject and Setting:

Between 2013–2016, 347 MSM and three male-to-female transgender women (TW) provided written-informed consent to participate in a single-visit, randomized-controlled trial of two cytology-collection protocols, Dacron- vs. Nylon-flocked (NF)-swab, and four HPV assays to predict hHSIL determined by High-Resolution Anoscopy (HRA)-guided biopsy, “Improving Screening Tools for Anal Cancer” study (ISTA). ISTA compared the test characteristics of two anal cytology-collection protocols with two high-threshold assays that measure Group1/hrHPVs using residual cytology specimens (https://clinicaltrials.gov/ct2/show/NCT02816879). In total, 325 MSM and one transwoman (n=326) showed complete cytology and biopsy data for cytology/histology. ISTA was approved by the UCLA Medical-IRB (#13–000997).

The study sample included community and Multicenter AIDS Cohort Study (MACS) participants. Previously, 67% (1541/2311) of MACS participants had received ≥1 anal (Dacron) cytology and HPV testing as part of a MACS substudy (2010 to 2015) described elsewhere.[19,20] Community recruitment employed fliers placed at community clinics, social service organizations, drug treatment centers, and housing projects in Los Angeles and Riverside Counties.

Study Design:

A block-randomization study design sorted the order of specimen collection in groups of four. Examination and self-reported data and test specimens were gathered at a single visit.

Study Procedures:

Cytology Collection: Four anal swab specimens were collected from each subject. Randomization determined swab order, then preservative within each swab type: Dacron vs. NF-swab; PreservCyt® (Hologic, Inc., Marlborough, MA) and SurePath® (Becton, Dixon, & Co (BD), Franklin Lakes, NJ) for Dacron swabs, and SurePath® and RNA preservative for NF-swabs. Swab *type* tested the hypothesis that large surface-area NF-swab (2120 mm^2) specimens better predicted hHSIL than Dacron-swab (129 mm^2) specimens (Figure 1). Differences between PreservCyt® and SurePath® for Dacron-swab cytology specimens were evaluated. RNA-preserved NF-swab specimens are reserved for future research.

Two cytology-collection protocols were evaluated. For first-collected Dacron-cytology, a lightly-moistened swab was blindly inserted through the anal verge ~5 cm, approximated to the anal wall, and rotated circularly over 30 seconds, withdrawn, and deposited into

PreservCyt®. To pass the NF-swab, a disposable anoscope (CooperSurgical, Inc., Trumbull, CT) was lightly lubricated across the leading edge (water-soluble lubricant) and inserted into the canal. After opening the verge, the obturator was removed and a dry rayon swab (Scopette Jr., Owens & Minor, Mechanicsville, VA) removed excess lubricant. Once introduced, the NF-swab (Copan Italia, Brescia, Italy) was approximated to the squamo-mucosal junction and twirled clockwise and counter-clockwise to collect cells and fluids, withdrawn, and deposited into SurePath®. Once placed, all subsequently-collected swabs were collected through the anoscope and deposited into SurePath® to prevent possible lubricant contamination. Swabs were agitated by hand and mechanically vortexed in preservative solution before being removed. Specimen containers were sealed, conveyed to one CLIA-certified laboratory, and evaluated (Tricore Reference Laboratories, Albuquerque, NM).

Dacron-swab collected into PreservCyt® and SurePath® were routinely tested for cytology initially (n=80) and Dacron/SurePath® was additionally evaluated randomly thereafter. Herein, paired Dacron-SurePath®/PreservCyt® specimens (n=145) evaluated the effect of preservatives on cytology.

Cytology findings were classified as *negative for intraepithelial lesions (NIL)*; *atypical squamous cells,* either *of undetermined significance* (ASC-US) or *cannot exclude HSIL* (ASC-H); LSIL, or HSIL. Anal cytology showing ≥ASC-US is regarded as *abnormal* in clinical practice.[25] Dacron-cytology/PreservCyt® specimens showing <1–2 nucleated squames/high-powered field (hpf) and NF-cytology/SurePath® samples showing <3–6 nucleated squames/hpf were evaluated as *unsatisfactory for evaluation* (unsatisfactory cytology).[26] No subject showed ASC-H or HSIL cytology *with* <hHSIL on biopsy. Thus, no composite diagnosis for HSIL was employed for these analyses. We hypothesized unsatisfactory cytology was costly and, thus, compared unsatisfactory to NIL cytology in thess analyses.

One experienced examiner performed **High-Resolution Anoscopy** for all subjects following cytology-specimen collection. A 5% acetic-acid soaked, gauze-padded swab, passed through an anoscope and subsequently withdrawn, allowed aceto-whitening of anal epithelium. The anoscope was reintroduced using 4% lidocaine cream/water-soluble lubricant. The anal canal was examined systematically using bright light and magnification. Up to 0.5 mL of 2% lidocaine/epinephrine (1:100,000) solution/quadrant was distributed evenly across the field for hemorrhoids obstructing the examination. Endoscopic or Tischler biopsy forceps were used to collect specimens into 10% neutral-buffered formalin. Monsel's solution was applied to achieve hemostasis, as indicated.

For both cytology and histology, board-certified cyto- and histo-pathologists used standardized procedures, blinded to clinical examination data. Cytology was classified using the Bethesda Classification System.[12,21,22], Histology specimens were classified according to international recommendations[23,24] and harmonized using expert recommendations:[12] Anal intraepithelial neoplasia (AIN) Grade-2, −2/3, and −3 are classified as hHSIL and AIN Grade 1 is classified as hLSIL.[12] When pathologists or providers were uncertain, block-positive $p16^{INK4a}$-immunostaining classified the specimen as hHSIL.[12] Subjects showing ≥1

AIN2, 2/3, or 3 lesions (hHSIL) were compared to those whose most severe finding was <hHSIL.[12,24]

Other covariates of interest included age (continuous), race (White, non-White), HIV-infection characteristics (HIV-uninfected; HIV-infected with <500 or >500 cells/mm3), smoking (former, current, never), and the number of male RAI partnerships reported for the two years prior to the examination (0, 1, 2–10, >10), and swab-collection order (first vs. not).

Statistical Analyses: Descriptive analyses, using Pearson Chi-square, Kruskal-Wallis, and Student's t-test statistics, evaluated individual associations between covariates and hHSIL in the data. A stratified analysis using logistic regression contrasted SSPN for first- and subsequent-ordered NF- and Dacron-cytology collection protocols; additionally, SSPN estimates, calculated using logistic regression, were summarized and adjusted for randomization order alone. To assess odds of hHSIL, we also explored the effects of unsatisfactory (vs. NIL) cytology for both swab protocols. Final logistic regression models adjusted for the effects of swab-randomization alone.

Fully-adjusted logistic regression models estimated the odds of predicting hHSIL using cytology showing ≥ASC-US (vs. NIL) findings from two swab-collection protocols, adjusting for effects of other covariates of interest (*other covariates*). The final fully-adjusted model evaluated ≥ASC-US vs NIL to predict hHSIL, adjusting for the effect of unsatisfactory cytology (vs. NIL) and *other covariates*. Odds ratios (ORs) with 95% confidence intervals (95% CI), and area under receiver-operating characteristic curves (AUCs) were estimated from the data. Fully-adjusted AUCs assess each swab-protocol's performance independent of the decision threshold to correctly classify those with and without hHSIL, adjusting for the effects of other covariates. The U-statistic evaluated differences between models. Last, to estimate the effect of preservative and slide-preparation method on Dacron- and NF-swab cytology to accurately predict hHSIL and <hHSIL, AUCs from fully-adjusted logistic regression models for 145 Dacron-swab specimens collected into each preservative were compared.

Results

Descriptive Analyses:

The mean age of participants was 55 (σ=11.5) years old, most were white MSM (72%), former (56%) or current (18%) tobacco smokers. Nearly 40% of subjects were HIV-uninfected and among HIV-infected participants (60%), one-third showed <500 CD4+ T-lymphocytes/mm^3 (CD4+ count). More than half reported no RAI partners over the two years prior to the examination. HIV-infected subjects were nearly twice as likely as the –uninfected to report minority race (p=0.04) and current smoking (p=0.009; Table 1).

Dacron-cytology showed a higher prevalence of ≥ASC-US than NF-cytology (Table 1). Dacron-cytology near equally showed NIL (37%), ≥ASC-US (34%), or unsatisfactory (29%). NF- cytology showed NIL (62%) three-times more often than ≥ASC-US (20%). Unsatisfactory specimens were more common among Dacron-cytology (29%) than NF-

cytology (18%), and prevalence of unsatisfactory cytology was positively associated with the number of Dacron swabs collected (r=0.36, p<0.0001), but NF-cytology was not (p=0.32). Unsatisfactory NF-cytology occurred about 10% more often among HIV-infected than uninfected subjects (p=0.04). The prevalence of unsatisfactory Dacron-swab cytology specimens collected randomly, first to fourth, were 11%, 15%, 35% and 48%. Nearly 14%, 20%, 15%, and 26% of first to fourth collected NF-swab cytology specimens were unsatisfactory. The odds of unsatisfactory were statistically significantly greater for third- and fourth-collected Dacron-cytology specimens, alone: OR $_{3^{rd}\text{ vs. }1^{st}}$=4.4 (1.7,11.3), OR $_{4^{th}\text{ vs. }1^{st}}$=7.5 (3.1,18.0).

All participants received ≥1 biopsy (μ=3.1 [σ=1.4], M=3, Range: 1–8), and 46% showed hHSIL. hHSIL-affected subjects reported more RAI partners over the 24 months before HRA (p=0.007). hHSIL was positively associated with ≥ASC-US and unsatisfactory cytology for NF- and Dacron-swab protocols (p-values≤0.0009, Table 1). Providers/pathologists infrequently requested p16-immunostaining: 62% (16/26) showed AIN2, and 15% (4/26) and 23% (6/26) showed LSIL or NIL, respectively.

Unadjusted Analyses: Age, race, and tobacco use were associated with HIV-infection. HIV-infected participants were younger than the –uninfected (52.9 vs. 57.3 years, p=0.002), and Whites were 1.7-fold less likely to be HIV-infected than minority participants (OR=0.6 (0.3, 1.0)). HIV-infected participants were more likely to report current tobacco use (23% vs. 11%) than comparators (Table 1).

hHSIL was not associated with age, race, smoking, or HIV-infection characteristics (Table 1). Subjects reporting 1, 2–10, or >10 partners showed 2.6-, 3.1-, and 5.7-fold higher odds of hHSIL, respectively, than those reporting none (p<0.05) (Table 1). The odds of hHSIL were positively associated with both ≥ASC-US and unsatisfactory cytology (vs. NIL), using either swab. The odds of hHSIL for Dacron-cytology ≥ASC-US was 2.7-fold greater than for NIL findings (OR=2.7 (1.6, 4.6)). NF-cytology ≥ASC-US showed 4.9-fold higher odds than NIL for hHSIL (OR=4.9 (2.6, 9.1)). Unsatisfactory-cytology findings (vs. NIL) using either Dacron- or NF-cytology showed higher odds of hHSIL: OR=1.9, (1.1, 3.3), and OR=2.0, (1.1, 3.6), respectively (Table 2).

Comparisons for Abnormal to NIL Cytology

Unadjusted Sensitivity, Specificity, Positive and Negative Predictive Value (SSPN) analyses showed abnormal Dacron-cytology was 1.5-fold more sensitive than NF-cytology as primary screening for anal hHSIL: 62% vs. 40% (p=0.02), respectively (Table 2, Supplementary Table 1A, 1B; Figure 2A, 2C). Specificity for *abnormal* Dacron-cytology was lower than for NF-cytology: 63% vs. 88% (p<0.0001). PPV for *abnormal* Dacron-cytology was 1.3-fold lower than NF-cytology (57% vs. 73%) (p<0.001). NPV estimates for both cytology-collection protocols were similar.

Fully-adjusted SSPN analyses: Adjusting for the effects of age, race, HIV-infection characteristics, swab-randomization order, and the number of RAI-sexual partners over the two years before the study visit, abnormal Dacron-cytology was more sensitive to predict hHSIL than NF-cytology, 47% vs. 42% (p=0.007). Specificity was no greater (70% vs. 81%

(p=0.1), respectively), albeit each showed specificity >50% (p-values<0.0001). PPV for Dacron-cytology ≥ASC-US (56%) was 1.2-fold lower than NF-cytology (65%) to predict hHSIL, and only NF-cytology showed PPV statistically significantly >50% (p=0.005).

Swab-collection order affected **abnormal** Dacron-cytology (vs. NIL) findings alone to predict hHSIL. NF-cytology showed similar sensitivity for first- or second-collected cytology specimen (40% vs. 40%), but Dacron-cytology sensitivity improved 1.2-fold when collected after NF-swab: 57% vs. 68%. Nonetheless, sensitivity for predicting hHSIL was >50% for subsequently-collected Dacron-cytology swabs alone (p=0.01). Specificity for NF-cytology was high for both first (83%) and subsequently-collected (93%) specimens and both estimates were >50% (Ho: specificity>0.5, p-values<0.0001). Dacron-cytology specificity for both first (61%) and subsequently-collected (65%) specimens was >50% (p-values<0.05), both lower than NF-cytology estimates. PPV estimates for first and successively-collected NF-cytology alone were >50%: 66% and 82% (Ho: Pr>50%, p=0.03, and p<0.0001), respectively.

Comparisons for Unsatisfactory and NIL Cytology: Unadjusted and fully-adjusted SSPN estimates comparing unsatisfactory to NIL for Dacron- and NF-cytology findings are reported in tables and graphs (Table 2, Supplementary Table 1A, 1B; Figure 2B, 2D). Prevalence of unsatisfactory were similar for Dacron and NF-swab cytology: (15% vs. 21%, p=0.6). Fully-adjusted estimates showed sensitivity for unsatisfactory cytology to predict hHSIL was ≤50% (p-values<0.0001). Specificity was lower for Dacron- than NF-swab: 79% vs. 85%, respectively (p-values<0.0001).

Effect of Preservative on Dacron-swab Cytology

Paired, randomly ordered Dacron-swab specimens intentionally separately collected into PreservCyt® and SurePath® suggested the accuracy of cytology to predict hHSIL (vs. <hHSIL) were similar. Accuracy estimates did not statistically significantly differ: AUC=0.698 vs. AUC=0.691 (p=0.83), respectively.

Fully-adjusted Accuracy Analyses to Predict hHSIL

Fully-adjusted analyses incorporated all Dacron/Preservcyt® and NF/SurePath® cytology data. Analyses suggested participants with ≥ASC-US on NF- and Dacron-cytology (abnormal) showed 5.2- and 2.6-fold higher odds of hHSIL (vs. NIL), respectively ($OR_{NF\text{-}cyt}$=5.2, (2.8, 9.6) and $OR_{Dacron\text{-}cyt}$=2.6 (1.5, 4.5)). Overall, models showed the accuracy of abnormal Dacron- and NF-cytology to predict hHSIL were closely approximated (p>0.3) and differed modestly. The adjusted model that included abnormal NF-cytology (vs. NIL) more accurately predicted hHSIL than the covariates alone: age, race, HIV-infection characteristics, swab-randomization order, and the number of RAI-sexual partners over the two years before the study visit: $AUC_{NF\text{-}cyt}$=0.69 (p=0.02) vs. $AUC_{covariate}$=0.63. However, abnormal Dacron-cytology did not: $AUC_{Dacron\text{-}cyt}$=0.67 (p=0.08). No statistically significant differences were detected for Dacron- or NF-swabs showing ≥ASC-US (vs. NIL) to predict hHSIL for either HIV-infected or –uninfected participants when compared to a covariate model alone (Supplementary Table 1A and 1B).

Discussion

This head-to-head evaluation of two anal cytology-collection protocols as a primary screen for anal hHSIL among HIV-infected and –uninfected MSM/TW shows higher sensitivity, but lower specificity and PPV for Dacron- over NF-cytology. The fully-adjusted analyses suggest only NF-cytology improved overall accuracy for predicting hHSIL when compared to covariates alone (swab-randomization order, sociodemographic, sexual-behavioral, and HIV-infection characteristics reported within 24 months of examination). Higher specificity translates to fewer false-positive NF-cytology tests, decreasing diagnostic follow-up, and lowering the cost of anal cytology screening for anal hHSIL.

Our adjusted sensitivity and specificity analyses for either swab fall within the range of published performance. Anal cytology instrumentation builds on >50 years of experience with cervical cytology-screening strategies: cervical cytopick; cotton, Dacron, rayon, and NF swabs; brooms, and cytobrushes, with and without spatulas.[27–35] Cervical cytology shows wider variation in sensitivity than specificity to predict hHSIL: 34%−96% (Median=64%) vs. 92%−98% (Median=96%), respectively.[36] Anal cytology studies largely describe screening outcomes alone, and few report complete HRA/histology data. Among those that do, the sensitivity and specificity for Dacron (anal) cytology to predict hHSIL varied widely: 19%[37] to 89%[38], and 40%[38–40] to 88%,[37] respectively. Research reports, meta-analyses, and systematic reviews suggest sensitivity and specificity of anal cytology ≥ASC-US modestly predicts hHSIL: 66%−95% and 32%−96%, respectively.[41–43] Comparatively, the sensitivity of cervical- or anal-cytology ≥ASC-US to predict cervical (91%) or anal (90%) hHSIL is high, but specificity for cervical (53%) is greater than abnormal anal cytology (33%).[43] Consequently, more false-positive tests may occur with abnormal anal cytology findings.[43]

Mechanistically, NF-swabs trap more cells, microorganisms, and fluid during sampling. NF-swab abrasiveness is demonstrated by the improved performance of Dacron-cytology collected after NF-cytology in this sample. One study of 23 NF-cytology swabs showed a higher number of cells per slide than Dacron-cytology (p=0.003) but no greater DNA quantity or quality using NF-swab.[44] Others report 5–10 and 1.6–2.0- fold higher yield of cells and DNA, respectively, using NF- than cotton-swab for cervicovaginal sampling.[45]

Some data suggest cytology and HRA-findings are modestly related. Four large studies performing both cytology and HRA with biopsy report 12% to 25% of subjects with NIL cytology have hHSIL.[37,38,40,46] Three of four report a substantial fraction of HRA-examiners miss hHSIL using HRA, with an average of 13% of subjects showing ASC-H or HSIL cytology with histological-NIL or not receiving biopsy during HRA.[37,38,40,46] Abnormal anal cytology is not associated with anal condyloma, a low-grade SIL, in other reports.[38,47]

Our analyses may be limited. Prevalence of unsatisfactory cytology was higher than expected, possibly related to the size of the NF-swab. Also, collecting cytology through an anoscope for either swab may impair swab-to-epithelium contact. Our provider reported subjects described NF swabbing as more abrasive (than Dacron). Our earlier published study reports fewer unsatisfactory NF- (7%, 7/58) and Dacron-cytology (14%, 8/58) using swabs

employed herein.[35] Nonetheless, published studies infrequently report or include unsatisfactory cytology in analyses. Several centers report 6–7% of anal cytology specimens are unsatisfactory, and other studies with large samples report an unsatisfactory prevalence ranging 10–17%.[48–51] The study protocol was developed and implemented as p16-immunostaining recommendations were published.[12] Testing herein was performed when the assay was requested to clarify diagnoses. While our accuracy estimates are adjusted for the effects of HIV-infection, including CD4+ T-lymphocyte counts, the study was not adequately powered to detect differences in test characteristics or accuracy (AUC) estimates for HIV-infected and –uninfected groups. Last, bias introduced by polychotomous, self-reported variables are difficult to predict.[52,53]

Our analyses suggest NF-cytology screening provides greater specificity than Dacron-cytology. NF-cytology ≥ASC-US showed two-fold higher odds of hHSIL than (abnormal) Dacron-cytology and, alone, NF-cytology demonstrated greater accuracy for predicting neoplasia than sociodemographic, sexual-behavioral, and HIV covariates alone. When screening-test findings are independent time over time, the probability of false negatives, missing anal hHSIL, decreases steadily when performed annually or semi-annually in high-risk populations, such as HIV-infected MSM/TW.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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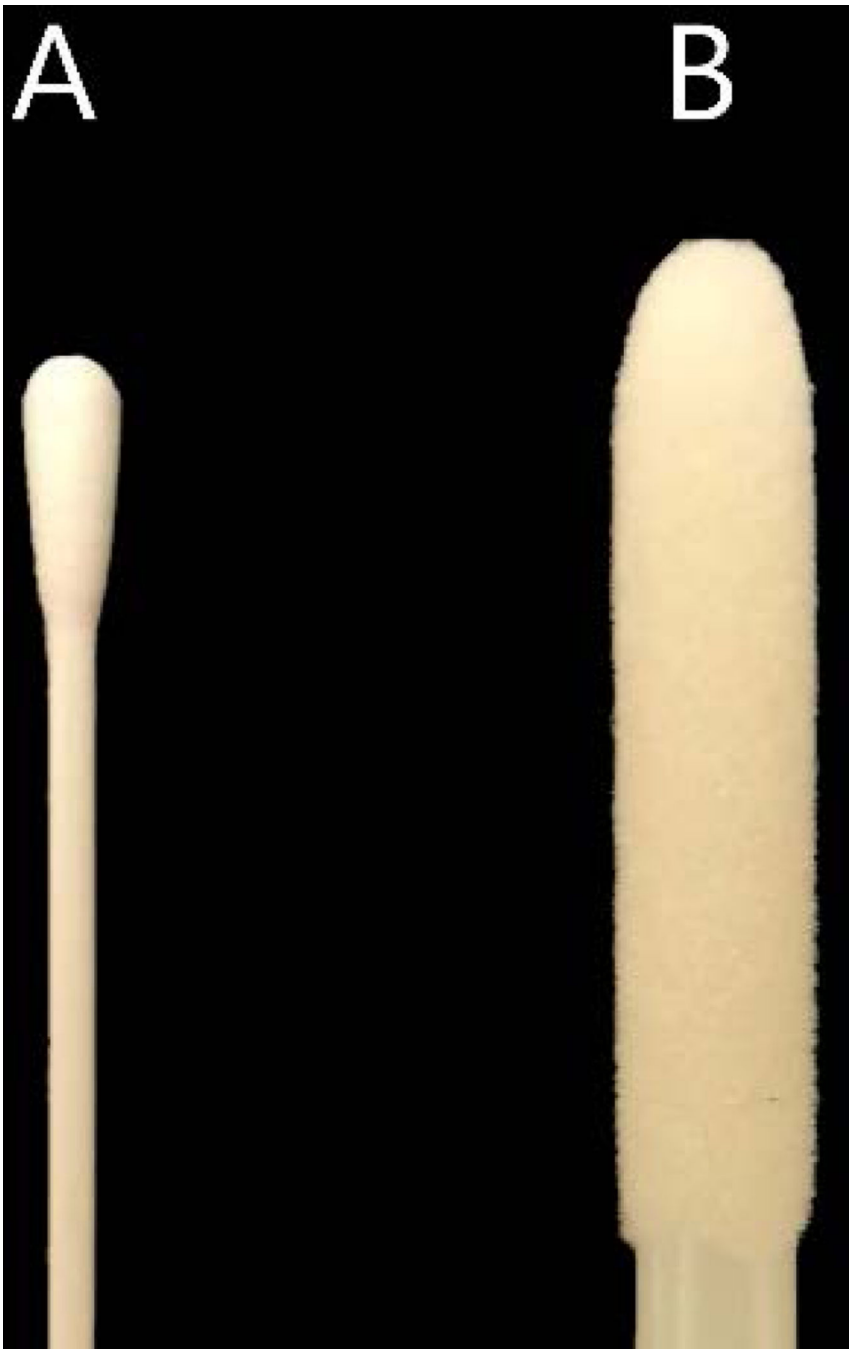
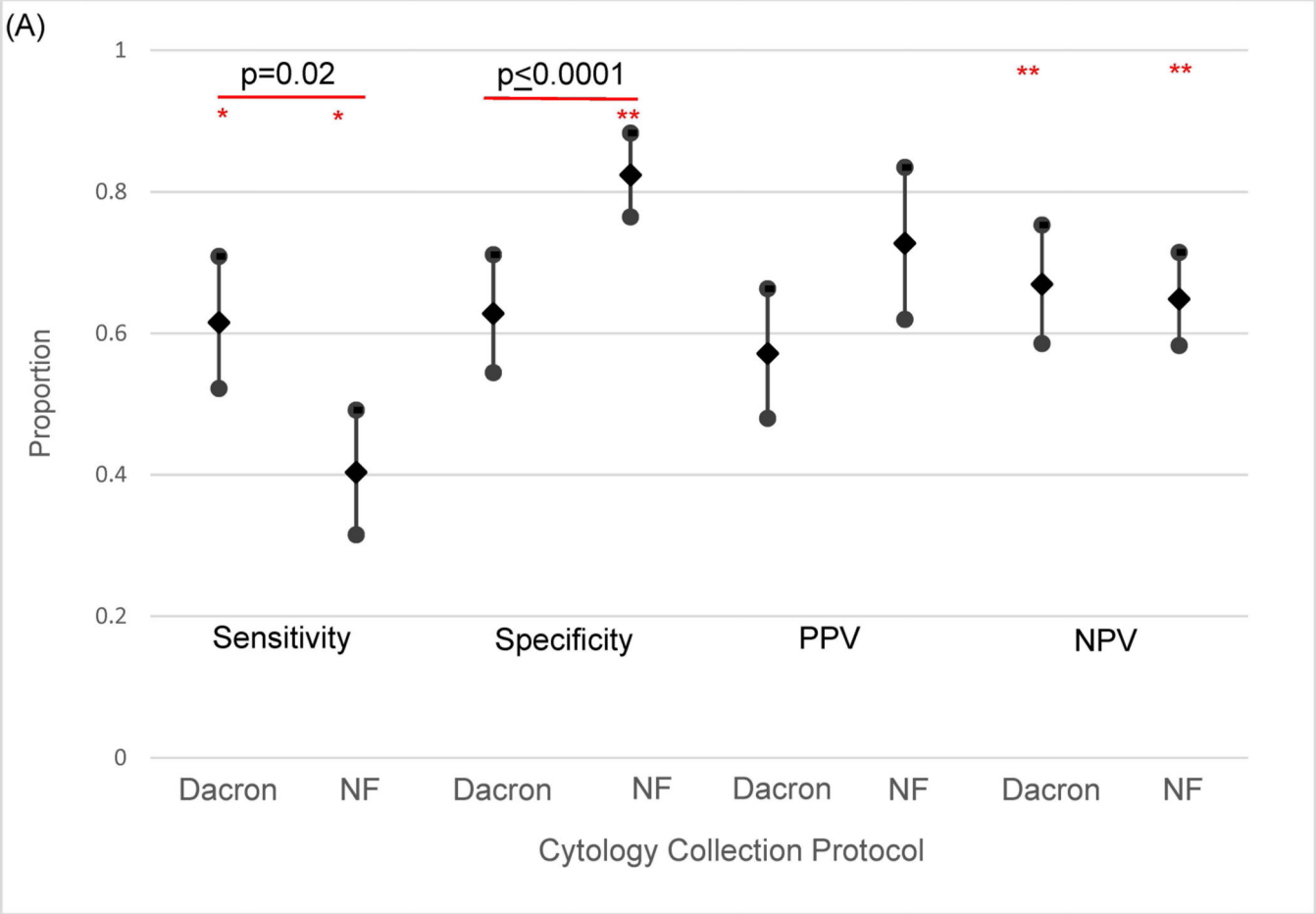
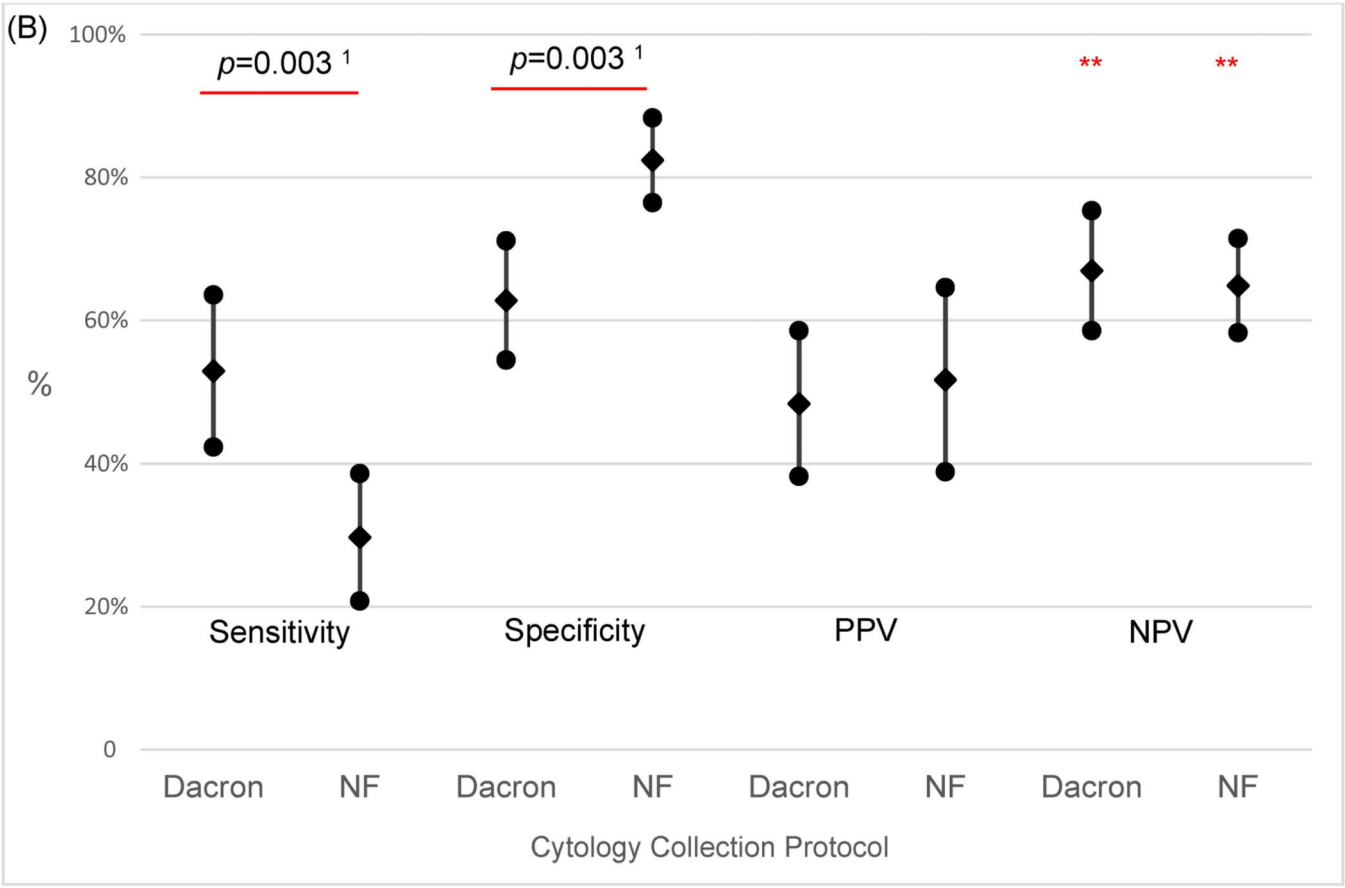
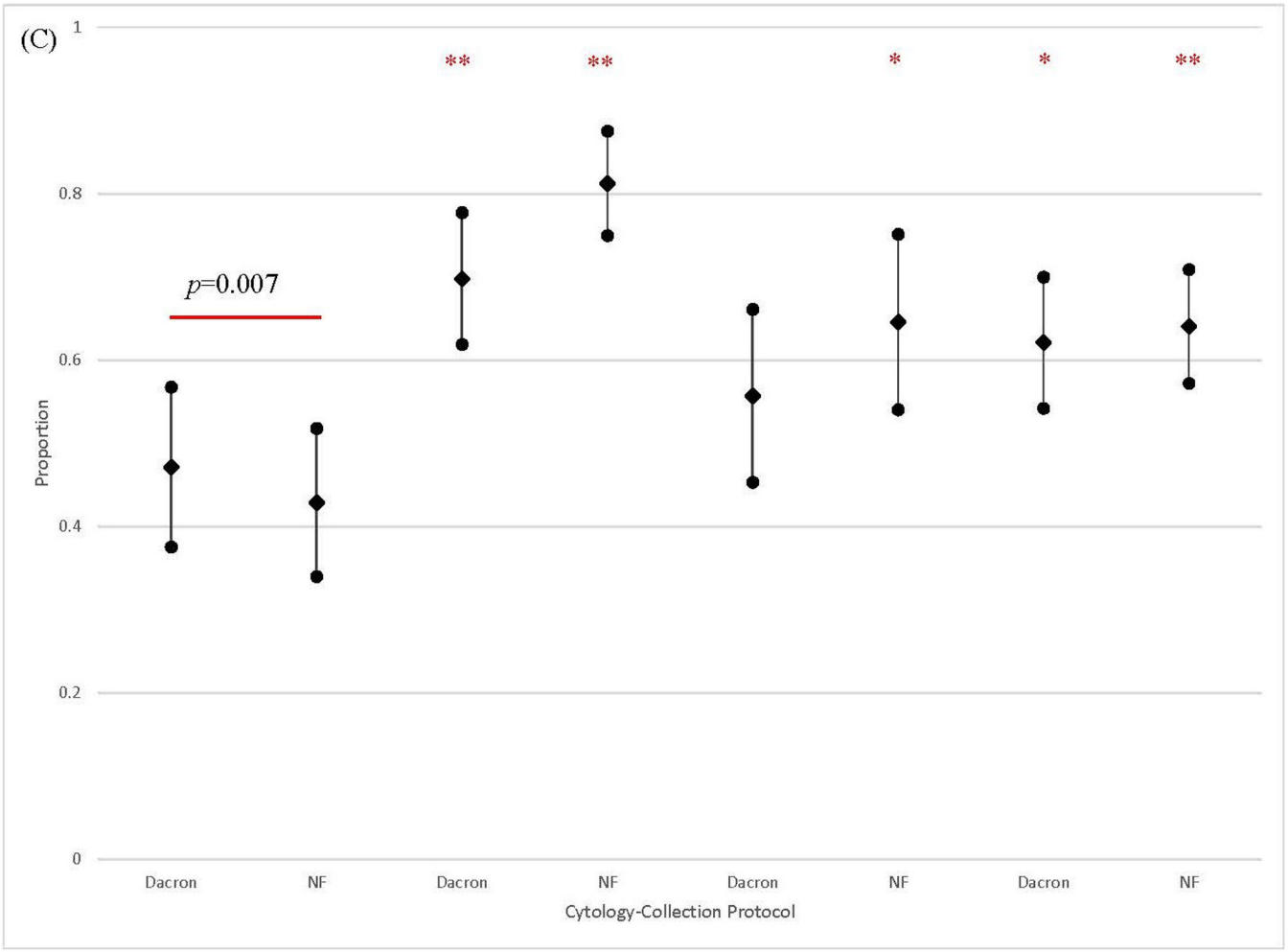


Figure 1:
Comparison of Dacron- and NF-swab for cytology collection protocols, with approximately 130 vs. 2120 mm^2 surface area, respectively.







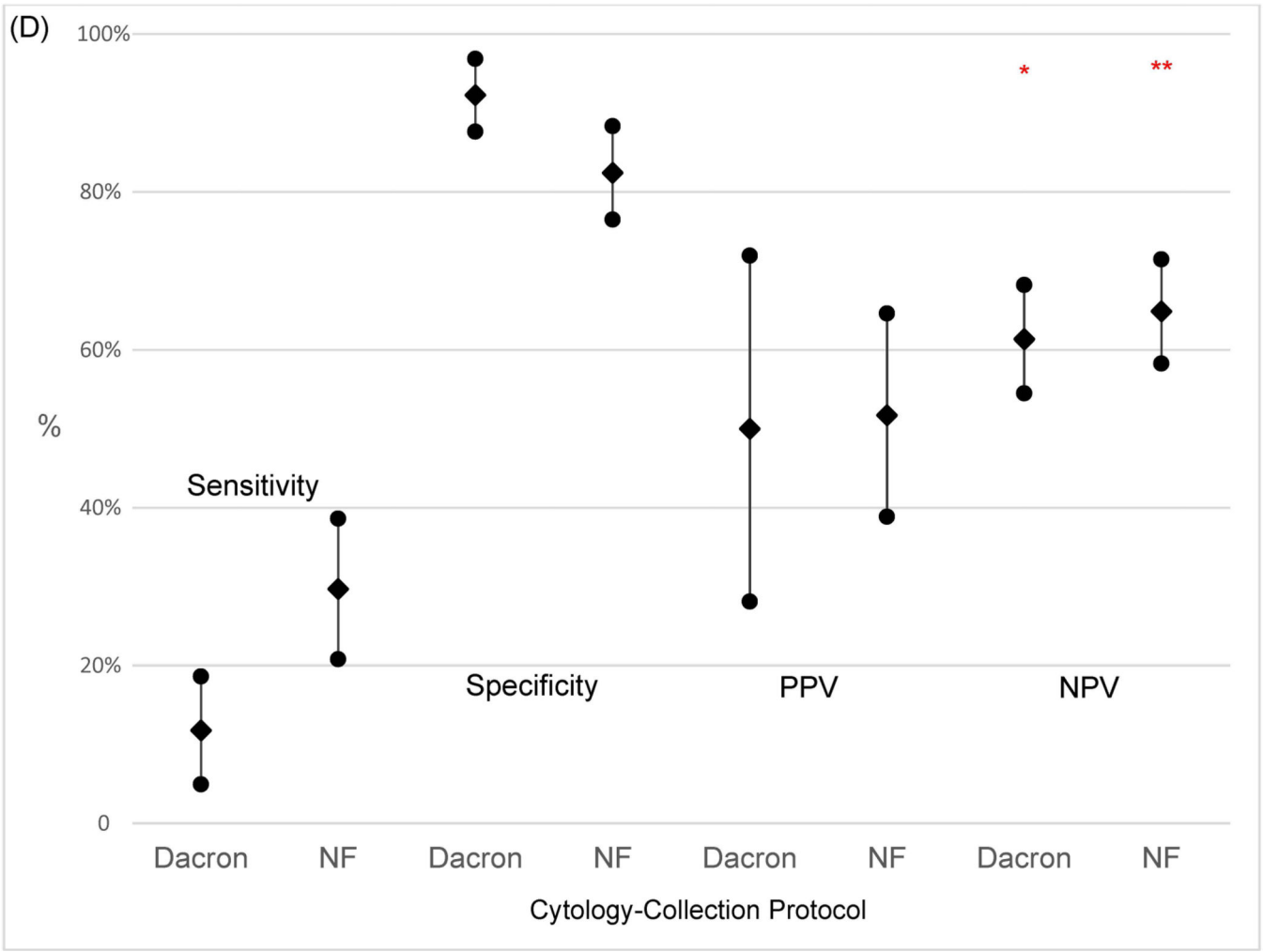


Figure 2 A-D:

Comparison of Sensitivity, Specificity, Positive and Negative Predictive Value Estimates to Predict Anal hHSIL for Nylon-flocked and Dacron Swab Cytology-Collection Protocols for 326 MSM/TW.

(2A): Unadjusted Comparison of Cytology Showing ≥ASC-US vs. NIL to Predict hHSIL.

(2B): Unadjusted Comparison of Cytology Showing unsatisfactory vs. NIL to Predict hHSIL.

(2C): Fully-Adjusted Comparison of Cytology Showing ≥ASC-US vs. NIL to Predict hHSIL.

(2D): Fully-Adjusted Comparison of Cytology Showing unsatisfactory vs. NIL to Predict hHSIL.

Table 1:

Randomized-Controlled Trial Comparing Two Cytology-Collection Protocols to Predict hHSIL for 326 MSM/TW.

	Total (n = 326)		HIV-Uninfected (n = 131)		HIV-Infected (n = 195)			≤hHSIL (n = 177)		hHSIL (n = 149)		
	Number	%	Number	%	Number	%	*p*-value**	Number	%	Number	%	*p*-value**
Randomization												0.86
Copan	168	51.53	66	50.38	102	52.31	0.73	92	51.98	76	51.01	
Dacron	158	48.47	65	49.62	93	47.69		85	48.02	73	48.99	
Tobacco Smoking												0.37
Never	84	25.77	31	23.66	53	27.18	0.01	49	27.68	35	23.49	
Former	182	55.83	85	64.89	97	49.74		100	56.5	82	55.03	
Current	60	18.40	15	11.45	45	23.08		28	15.82	32	21.48	
HIV Infection Characteristics												0.27
HIV-uninfected	131	40.18	131	100	0	0		78	44.07	53	35.57	
HIV+, CD4 ≥ 500 cells/mm^3	131	40.18	0	0	131	67.18		68	38.42	63	42.28	
HIV+, CD4 < 500 cells/mm^3	64	19.63	0	0	64	32.82		31	17.51	33	22.15	
Race												0.83
0 = Non-White	90	27.61	28	21.37	62	31.79	0.04	48	27.12	42	28.19	
1 = White	236	72.39	103	78.63	133	68.21		129	72.88	107	71.81	
Receptive Anal Intercourse Partnerships 24 months Before HRA												
0	186	57.06	85	64.89	101	51.79	0.16	147	66.1	69	46.31	
1	58	17.79	16	12.21	42	21.54		26	14.69	32	21.48	
2 to 10	60	18.40	22	16.79	38	19.49		26	14.69	34	22.82	
>10	11	3.37	4	3.05	7	3.59		3	1.69	8	5.37	
Missing	11	3.37	4	3.05	7	3.59		5	2.82	6	4.03	
Dacron-swab Cytology												0.0
NIL	121	37.12	47	35.88	74	37.95	0.5	81	45.76	40	26.85	
≥ASC-US	112	34.36	42	32.06	70	35.9		48	27.12	64	42.95	
Unsatisfactory	93	28.53	42	32.06	51	26.15		48	27.12	45	30.2	
Nylon-flocked Swab Cytology												<0.0001

	Total (n = 326)		HIV-Uninfected (n = 131)		HIV-Infected (n = 195)			≤hHSIL (n = 177)		hHSIL (n = 149)		
	Number	**%**	**Number**	**%**	**Number**	**%**	***p*-value****	**Number**	**%**	**Number**	**%**	***p*-value****
NIL	202	61.96	87	66.41	115	58.97	0.39	131	74.01	71	47.65	
≥ASC-US	66	20.25	24	18.32	42	21.54		18	10.17	48	32.21	
Unsatisfactory	58	17.79	20	15.27	38	19.49		28	15.82	30	20.13	
Variable	**Mean**	**Std.Dev.**	**Mean**	**Std.Dev.**	**Mean**	**Std.Dev.**	***p*-value***	**Mean**	**Std.Dev.**	**Mean**	**Std.Dev.**	***p*-value***
Age	54.73	11.46	57.54	12.38	52.84	10.41	0.00	55.63	11.4	53.66	11.48	0.12

*Student's T-test

**Pearson's Chi-square Test.

HIV indicates human immunodeficiency virus; hHSIL, histological high-grade squamous intraepithelial lesion; HRA, high-resolution anoscopy; NIL, negative for intraepithelial lesion; ASC-US, atypical squamous cells of unknown significance; std. dev, standard deviation.

Table 2:

Logistic Regression Model Comparisons for Two Cytology-Collection Protocols Predicting hHSIL for 326 MSM/TW.

Condition	≥ASC-US	Unsatisfactory	Randomization Order Alone	Test Characteristics		ROC Characteristics			
Test	OR* (95% CI)	OR* (95% CI)	OR* (95% CI)	Sens %	Spec %	AUC (95% CI)	*SE*	*p*	*p*
Unadjusted model									
Dacron-swab Cytology	2.7 (1.6, 4.6)	1.9 (1.1, 3.3)	-	43.0	72.9	0.61 (0.55, 0.67)	*0.03*	-	*ref*
Nylon-flocked Swab Cytology	4.9 (2.7, 9.1)	2.0 (1.1, 3.6)	-	52.3	74.0	0.65 (0.59, 0.70)	*0.03*	-	0.4
Randomization-Order Adjusted									
Randomization Order (Alone)						0.50 (0.45, 0.56)	*0.03*	*ref*	
Dacron-swab Cytology	2.7 (1.6, 4.6)	2.0 (1.1, 3.6)	0.9 (0.6, 1.4)	43.0	67.2	0.62 (0.56, 0.68)	*0.03*	0.002	*ref*
Nylon-flocked Swab Cytology	5.0 (2.7, 9.2)	2.0 (1.1, 3.6)	0.9 (0.6, 1.4)	43.0	74.0	0.65 (0.59, 0.71)	*0.03*	<.0001	0.5
Fully Adjusted*									
Covariates (Alone)*						0.63 (0.56, 0.69)	*0.03*	*ref*	
Dacron-swab Cytology	2.6 (1.5, 4.5)	2.1 (1.1, 3.8)	-	47.0	68.9	0.67 (0.61, 0.72)	*0.03*	0.08	*ref*
Nylon-flocked Swab Cytology	5.2 (2.8, 10.0)	2.1 (1.1, 4.0)	-	47.7	75.7	0.69 (0.64, 0.75)	*0.03*	0.02	0.3

*Adjusted for the effects of age, race, HIV-infection, smoking, number of male receptive-anal intercourse partnerships two years before examination, swab-collection order.

ASC-US indicates atypical squamous cells of unknown significance; ROC, receiver operating curve; OR, odds ratio; AUC, area under the ROC; SE, standard error; ref, reference.