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Xpert HPV as a Screening Tool for Anal Histologic High-Grade Squamous Intraepithelial Lesions in Women Living with HIV

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Keywords

HIV; Human papillomavirus; Anal Cancer; Women; Cancer Prevention

1. Introduction

Persons living with HIV (PLWH) have a markedly higher risk of developing squamous cell carcinoma of the anus (SCCA) than the general population. While the risk is highest in men who have sex with men (MSM) living with HIV, women living with HIV (WLWH) experience SCCA at rates of 10–29 cases per 100,000 person-years, with approximately

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eight times the rate of the general population.[1–3] These rates approach cervical cancer rates prior to the adoption widespread screening programs.

Like cervical cancer, anal cancer is caused by persistent infection with high-risk human papillomavirus (HR-HPV) leading to the development of anal cancer precursors known as high-grade squamous intraepithelial lesions (HSIL).[4–6] The performance of abnormal anal cytology as a screening tool for anal HSIL identified by high-resolution anoscopy (HRA), when defined as at least atypical squamous cells of undetermined significance (ASC-US), is limited with sensitivities of 69 to 93% and specificities of 32 to 59%.[7–9] The performance of anal cytology as a screening test varies with population screened, HIV infection, the proportional involvement of the anal canal with HSIL [7] and relies on consistent interpretation by cytopathologists.[10] The limited specificity of anal cytology may result in HRA procedures for many PLWH who do not have HSIL.

The addition of HR-HPV testing to cervical cytology can lengthen the interval between cervical cancer screen requirements in women ages 30 years and older [11]. HR-HPV co-testing has not yet been shown to improve the performance of anal cytology in MSM living with HIV, a population with a high prevalence of anal HR-HPV.[12, 13] In WLWH, the prevalence of anal HR-HPV, using clinical HPV assays, is lower with reported ranges of 40 to 76%, and anal HR-HPV testing may be a more useful screening tool than for MSM living with HIV.[14–16]

Xpert® HPV (Cepheid Inc, Sunnyvale, CA) is a simple-to-use, low-cost, test with point-of-care potential that performs DNA extraction and PCR in an enclosed cartridge providing results in about an hour. It provides semi-quantitative HPV DNA PCR results (as cycle threshold), and expanded HPV typing information reporting 14 HR-HPV types in 5 groups or channels (HPV 16, 18/45, 31/33/35/52/58, 51/59 and 39/68/56/66). Any channel with a cycle threshold of > 40 cycles (for HPV 16, 18/45) or > 38 cycles (all remaining channels) is considered negative. Xpert HPV includes a sample adequacy control (SAC) to control for insufficient sample quality and assay inhibition, by reporting the detection and cycle threshold of a human reference gene (hydroxymethylbilane synthase). It has been validated in cervical samples and has performed similarly to clinical HPV tests [17, 18] and performed similarly to Hybrid-Capture 2 assay (hc2 [Qiagen Corporation, Gaithersburg, MD]) in stored anal samples in a cohort of WLWH.[16]

Xpert HPV offers the promise of point-of-care screening for anal HSIL in WLWH. Xpert HPV's expanded grouped genotyping and cycle threshold (Ct) could represent an improvement in screening approaches if an optimized interpretation of these granular results could capture an acceptable proportion of HSIL in WLWH while reducing the number of women unnecessarily referred for HRA, i.e., improved specificity with only minor decreases in sensitivity. Kuhn et. al. demonstrated optimization of Xpert HPV specificity from 60% for cervical HSIL in South African WLWH to 77–86% with an accompanying decrease in sensitivity from 94% to 75–85% by removing less predictive channels in their screen and selecting alternate Ct cutoffs using receiver operator characteristic curve (ROC) analysis.[19] The risk of cervical HSIL and cancer differs by HPV genotype and highest with HPV 16.[20]

Xpert HPV has not been submitted for approval to the United States Food and Drug Administration (FDA).

We investigated using and optimizing Xpert HPV for anal HSIL screening using stored specimens from AIDS Malignancy Consortium 084 (AMC 084), a multicenter study of anal HSIL screening and outcomes in WLWH undergoing HRA.[21, 22]

2. Materials and Methods

2.1. Study Population and Regulatory Approvals

AMC 084 cross-sectionally evaluated anal HPV as a screening test for anal HSIL.[21, 22] AMC 084 accrued 256 WLWH who underwent anal swabs for cytology and HPV testing as well as HRA with directed biopsies at the enrollment visit to evaluate for the presence of histologic HSIL (hHSIL). All study samples were evaluated for the presence of HPV with two assays that have been validated for the detection of cervical HSIL: the DNA-based hc2 and mRNA-based Aptima HPV test (Hologic Incorporated, Marlborough, MA).

Women with documented HIV-1 infection ages 18 years and older were recruited at 12 AMC sites between 2014 and 2016. Eligibility and exclusion criteria are described elsewhere;[21] only WLHW without a prior history of anal HSIL or carcinoma by cytology or histology were enrolled. Study investigators who were certified by the AMC HPV Working Group for HRA performed all study procedures. AMC 084 was approved by the Institutional Review Board at each site. The additional analyses proposed here were approved by the National Cancer Institute's Cancer Therapy Evaluation Program, the Weill Cornell Medicine Institutional Review Board and the Stellenbosch University ethics committee.

Specimen collection of both anal swabs and biopsies in AMC 084 are described elsewhere.[21] Biopsy and cytologic specimens were submitted for local pathological interpretation using College of American Pathologists and the American Society for Colposcopy and Cervical Pathology Lower Anogenital Squamous Terminology (CAP-ASCCP LAST) project[23] and 2014 Bethesda System[24] terminology. There was central pathology review of histologic specimens at the University of California, San Francisco that ultimately determined the diagnosis of histological HSIL. hHSIL was defined as the presence of HSIL (also known as anal intraepithelial grade [AIN] 2 with p16 staining or AIN 3) in 1 or more anal histology samples.

After anal cytology was obtained and stored in a separate vial, another swab was prepared in 20mL of Anal PreservCyt® specimen per participant was sent to the AMC Biorepository. Aliquots of 8 mL and 1 mL were removed for analysis with hc2 and Aptima HPV, respectively, by the corresponding test manufacturer sites per the original AMC 084 protocol. The remaining PreservCyt specimens of participants who had consented for future analysis were frozen and stored. One mL of these samples was aliquoted and shipped to the Preiser Laboratory at Stellenbosch University (Cape Town, South Africa) under a material transfer agreement for analysis with Xpert due to the lack of availability of the cartridges in the United States at the time.

One mL of thawed fluid was placed into a Xpert cartridge and processed on the Xpert platform according to the manufacture's specifications. The additional genotypic and cycle threshold data were extracted using specialized software available in the laboratory provided by Cepheid.

2.2. Statistical Analysis

Agreement between Xpert and hc2, Aptima HPV, and abnormal anal cytology (combined result of ASC-US, LSIL, ASC-H, or HSIL) respectively was assessed by calculating interrater agreement and Cohen's kappa statistic. The test characteristics, including false omission rates, of Xpert and hc2, Xpert and Aptima, and Xpert and abnormal cytology to predict hHSIL were calculated. McNemar's exact test was used to compare the sensitivities and specificities between Xpert and hc2, Xpert and Aptima, and Xpert and abnormal anal cytology (ASC-US or more severe).

The ability of Xpert's channels (HPV 16, 18/45, 31/33/35/52/58, 51/59, 39/56/66/68) to predict the presence of prevalent hHSIL was assessed using chi-squared tests in univariate analysis and then using logistic regression for multivariate analysis. Various co-testing models, using both Xpert and cytology, were also assessed.

Various interpretive models of Xpert results were developed using the expanded grouped genotypic results and cycle threshold results using either logistic regression models and ROC analysis or recursive partitioning (RPART) in classification and regression tree (CART) [25] analyses. All statistical analyses were performed in R[26] using RStudio.[27]

2.2.1. ROC Analysis using Xpert Channels—Multiple logistic regression models were created with a focus on model parsimony. Using the model coefficients, predictive probabilities for hHSIL were calculated. ROC curves of predictive thresholds were plotted. A predictive cutoff was selected by choosing the geometric threshold on the curve nearest the top-left-most corner of the ROC graph, in an effort to maximize specificity preferentially to sensitivity. Xpert results were reinterpreted in the study population based on the selected cutoff and new tests characteristics were calculated for this optimized screening test to detect hHSIL.

2.2.2. RPART Analysis using Xpert Channels—A RPART fitted model was created using Xpert Channel data given the relative complexity of reinterpreting Xpert results based on certain patterns of channel positivity. This approach selects cut points based on prediction rather than maximizing sensitivity and/or specificity. We then the calculated the test performance parameters of this decision tree model's ability to predict prevalent hHSIL.

2.2.3. Cycle Threshold—Xpert channels' cycle thresholds (Ct) were subjected to ROC analysis to select different Ct cutoffs that may better discriminate the presence of hHSIL for a given channel. Xpert channel positivity was reinterpreted using the new Ct cutoffs and then we combined these reinterpreted channels with other unmodified channels to verify if alternate cutoffs produced improvements in test discrimination. These optimized channels were then included in a similar logistic regression modeling and ROC analysis approach described above to assess the performance of certain channel patterns to predict anal HSIL.

RPART analysis was employed to generate a decision tree model to assess whether more complex relationships existed between channel Cts.

Building on this analysis, Xpert channel Ct values were normalized using the Ct value for the SAC channel. All of the above optimization analyses were repeated to assess if normalization resulted in any change in the predictive performance of a modified assay.

3. Results

3.1. Study Population

Of the 276 WLWH providing consent for the study, 234 participants consented for future use of specimens and collected data. There was sufficient remaining PreservCyt specimen available for Xpert analysis in 208 of the 234 women. Xpert results were available for 195 participants for this analysis as the result of 3 Xpert cartridge errors, 2 Xpert platform errors, and lack of adequate SAC detection in 8 samples (Figure S1, Supplemental Digital Content). Among the 8 participants whose specimens failed Xpert SAC detection, all participants had interpretable cytology, valid hc2 and Aptima results, in one HR-HPV was detected by Aptima with benign anal biopsies, and another participant who had anal hHSIL. The characteristics of the sub-cohort with Xpert results did not differ significantly from those of the greater AMC 084 study cohort. 55 (28%) participants with Xpert results had prevalent hHSIL and 122 (62%) had abnormal anal cytology (Table S1, Supplemental Digital Content).

3.2. Agreement of Screening Tests

Anal HR-HPV was detected in 90 (47%), 85 (46%), 121 (61%) women by hc2, Aptima, and Xpert. The agreement of Xpert with cytology (ASC-US or more severe) (Cohen's $\kappa = 0.35$, 95% CI 0.21 to 0.49) was lower than that of Xpert with hc2 (Cohen's $\kappa = 0.59$, 95% CI 0.48 to 0.69) and that of Xpert with Aptima (Cohen's $\kappa = 0.64$, 95% CI 0.53 to 0.74) (Table 1).

3.3. Xpert Performance

The sensitivity (Sn) and specificity (Sp) of Xpert for hHSIL were 89% and 49% which were significantly different from those of hc2 at 76% and 65%, respectively, in the subcohort. The Sn of Aptima was not significantly different than Xpert at 80% but the Sp of Aptima exceeded that of Xpert at 67%. Only cytology had Sn and Sp that were not different from Xpert at 87% and 49%, respectively (Table 2).

Using a co-testing strategy requiring anal cytology more severe than ASC-US and detection of HR-HPV by Xpert had a respective Sn and Sp of 81% and 68% (Table 2) with a risk-ratio (RR) of 5.0 (95% CI 2.7, 9.3).

3.4. Prediction of hHSIL by Xpert

Unmodified Xpert was predictive of hHSIL (risk ratio [RR] 5.0, 95 % CI 2.3, 11.1). Xpert detection of HPV 16 was highly predictive of hHSIL in bivariate (RR 4.1, 95% CI 2.8, 6.0) and when included in multivariate analysis with the other channels (aOR 12.6; 95% CI 4.9, 35.4, Table S2, Supplemental Digital Content). Xpert detection of HPV 18/45 was the only

genotypic results that was not predictive in bivariate (RR 1.0; 95% CI 0.5, 1.8) or multivariate analysis (aOR 0.7, 95% CI 0.2, 2.0, Table S2, Supplemental Digital Content). Of 121 WLWH with detection of HR HPV by Xpert, 60 (50.0%) had detection in more than a single channel.

3.5. ROC Optimization

Multiple logistic regression models were explored and the most parsimonious was selected (Table 3). Predictive probabilities were calculated using the regression coefficients and were used to plot an ROC curve (Figure 1-A) and to select an optimized probability cutoff. Probabilities of hHSIL based on one or more Xpert channel results ≥ the cutoff are considered positives screens and are enumerated at and to the left of the vertical line on the x-axis in Figure 1-B. This produced an optimized Xpert assay with a Sn of 74.6% and Sp of 83.6% for anal hHSIL (Table 2).

ROC analysis was used to select optimized Ct cutoffs for each channel (including HPV 18/45, see HPV 18/45 Optimization, Supplemental Digital Content) but this did not markedly improve test performance when included with an unmodified Xpert test or in optimization models.

3.6. Recursive Partitioning

Recursive partitioning was used to produce a decision tree using Xpert's channel results which detected hHSIL with Sn of 64% and Sp of 91% (Figure S2, Supplemental Digital Content). Of note HPV 18/45 and 39/56/66/68 were excluded by this model. Generation of a decision tree using alternate channel Ct cutoff was generated (Figure 2) and predicted anal hHSIL with Sn of 74.6% and Sp of 86.4% (Table 2).

The ratio of Xpert channel Cts to same channel SAC Ct were calculated on the assumption that a lower SAC Ct corresponds to cytologic specimen with higher cellularity and therefore higher quantity of HPV DNA and analyzed with recursive partitioning. A test was not found with superior test characteristics compared with the other models.

4. Discussion

This study demonstrated that Xpert HPV can be useful to screen WLWH for anal hHSIL with similar performance to anal cytology with Sn around 90% and specificity around 50%. We demonstrated that the screening characteristics of Xpert can be improved using the genotypic and/or cycle threshold (DNA quantification) results to improve specificity to ≥82% while maintaining sensitivity ≥75%. A co-testing strategy using both abnormal cytology and unmodified Xpert did not perform better than other the HPV assays or the optimized Xpert assays. In all analyses detection of HPV 16 was most predictive of anal HSIL and is a clear indication for referral to HRA in WLWH.

This decreased sensitivity of these optimized tests will misclassify additional WLWH that had hHSIL while the increased specificity reduces the number of WLWH referred for HRA that do not have HSIL. Most hHSIL will not progress to cancer and hHSIL that does

progress will do so slowly.[4] Frequent repeat screening may detect missed prevalent HSIL and as well as newly incident HSIL.

While the best agreement was between Xpert and Aptima there was less agreement between Xpert and hc2 which differs from prior clinical studies comparing the two tests in anal specimens from South African WLWH[16] and as well as in cervical specimens from women referred for colposcopic evaluations.[17, 18] Any disagreement may be due to differences in test methodology: mRNA (Aptima), DNA-hybridation (hc2), versus DNA PCR (Xpert). Xpert detected more HR-HPV than hc2 or Aptima. The lower rate of agreement may also be due to differences in prevalent genotypes between South African WLWH and this AMC study population, differences in sample preparation, and differences in detected genotypes. Xpert targets HPV 66 but hc2 does not—although HPV 66 would be expected to provide only a very minor contribution to any differences detected.

Our analysis benefited from a large racially and ethnically diverse group of participants which approximates the HIV epidemic among WLWH in the United States and Puerto Rico.[28] The sample was not enriched from the use of a referral population or based on prior history of HPV disease. HRA provider metrics were monitored and there was central pathology review of histologic samples to ensure diagnostic quality. Furthermore, we did not compare Xpert to another HPV DNA real-time PCR test, instead, we compared it to two other HPV detection modalities: DNA hybridization (hc2), and mRNA amplification (Aptima).

AMC 084 was adequately powered to evaluate the various screening tests and strategies and not for analyses to optimize Xpert HPV. Another limitation is that we did not perform validation of our results. The cohort was limited to WLWH in the United States and Puerto Rico, and test performance would have to be separately validated and/or optimized in populations in which prevalent HPV genotypes and HSIL incidence are different. Although we did not use freshly collected samples for the analysis, the use of stored samples does not appear to be an issue given the high rates of detection of HR-HPV by Xpert.

While these results need to be validated, they demonstrate that Xpert may be used to rapidly screen WLWH for anal hHSIL, potentially as a point of care test. This would be useful for low resource settings that lack cytopathologic services but have the Xpert platform due to its wide dissemination in low and intermediate income countries for diagnosis and identification of drug-susceptible and drug-resistant tuberculosis.[29] We also demonstrated that the optimized Xpert models would result in a 44 to 50% relative reduction in positive screening tests with only 0.6 to 0.8% increases in anal hHSIL false omission rates compared with anal cytology. The improved specificity of these optimized tests reduces the number of WLWH without anal hHSIL who would be unnecessarily referred for HRA. A reduction in HRA burden allows for better utilization of HRA-trained providers particularly in settings where this is a limited resource.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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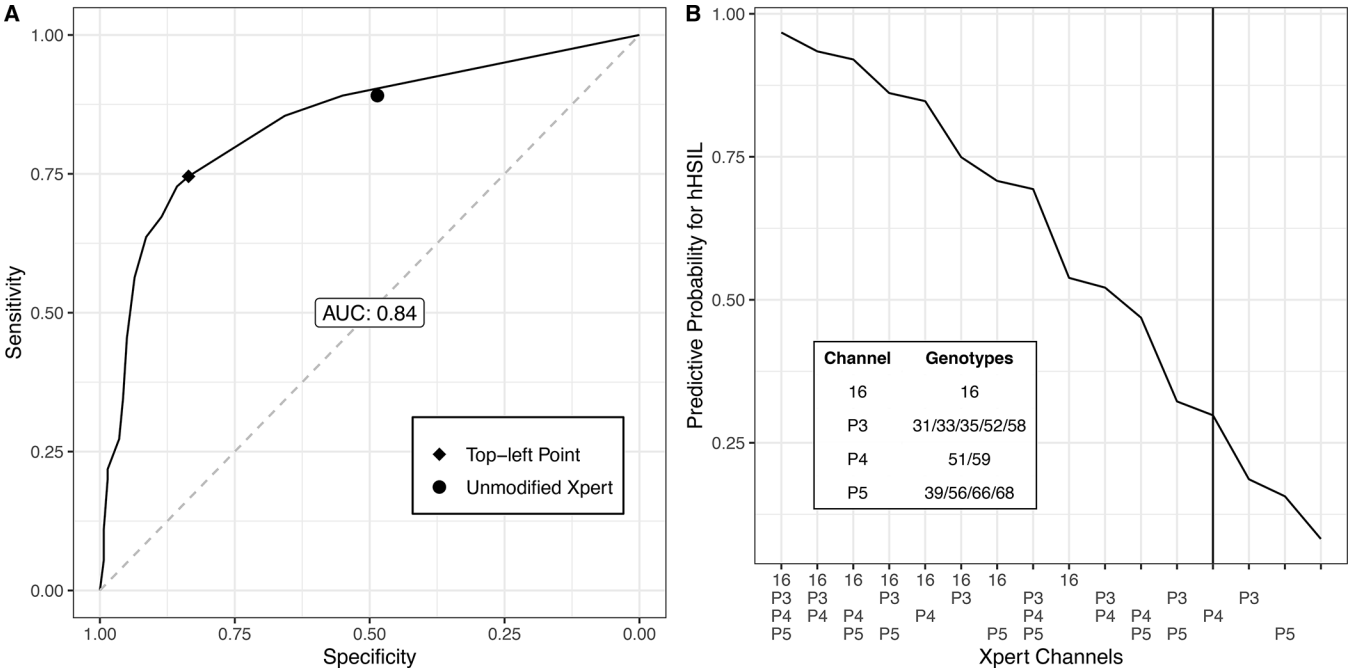

Figure 1.

A. ROC curve generated from predictive probabilities calculated using the coefficients of the logistic model in Table 3. The predictive cutoff selected ($p \geq 0.242$) is the top-left most point (◆) which corresponds to the vertical line in Figure 1-B. **B.** Predictive probabilities each permutation of channel results. All channel results permutations at and to the left of the vertical line are considered positive screens. For reference, the performance of unmodified Xpert to detect hHSIL is also plotted (●).

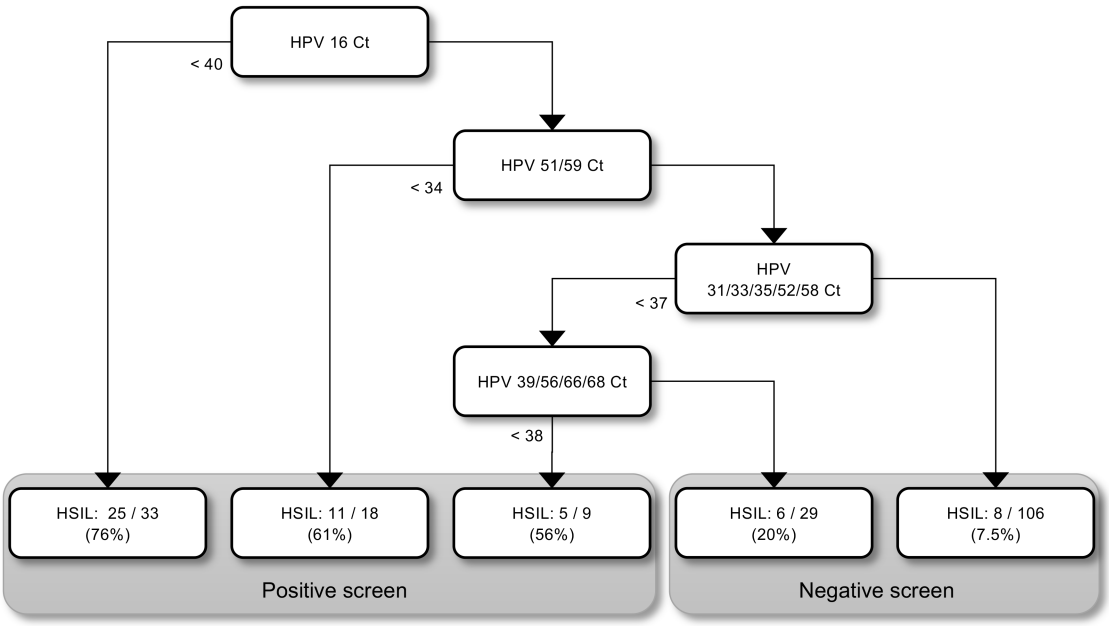

Figure 2.

Recursive partitioning decision tree using Xpert channel cycle thresholds (Ct). This decision tree predicts anal hHSIL with a sensitivity of 75% and specificity of 86%.

Table 1.

Agreement of Anal Cytology, hc2, and Aptima with Unmodified Xpert.

Xpert	**hc2**		**Aptima**		**Anal Cytology**	
Agreement, %	79.8		81.5		69.1	
Cohen's κ [95% CI]	0.59 [0.48 to 0.69]		0.64 [0.53 to 0.74]		0.35 [0.21 to 0.49]	
	+, *n*	−, *n*	+, *n*	−, *n*	+, *n*	−, *n*
Xpert +, *n*	85	34	81	30	86	29
Xpert −, *n*	5	64	4	69	29	44

Table 2.

Comparison of test performance. The significance of a McNemar's test comparing the difference of sensitivity (comparing results when hHSIL was present) and specificity (comparing results when hHSIL was absent) of an unmodified Xpert to anal cytology, hc2, and Aptima is indicated by asterisks. False omission rate is the complement of NPV (1 - NPV) and represents the proportion of WLWH with a respective negative screening test that are expected to have hHSIL.

	Abnormal Screen, n (%)	hHSIL Detected, *n*	Sensitivity, % (95% CI)	Specificity, % (95 % CI)	PPV, % (95% CI)	NPV, % (95% CI)	Relative Change in Prevalence of Abnormal Screen Compared to Cytology, %	False Omission Rate, %
Anal Cytology, *n=188*	115 (61)	45	87 * (74, 94)	49 * (40, 57)	39 (30, 49)	90 (81, 96)	*Ref*	9.6
Unmodified Xpert	121 (62)	49	89 (78, 96)	49 (40, 57)	41 (32, 50)	92 (83, 97)	2	8.1
Co-test Anal Cytology and Unmodified Xpert, *n=188*	86 (46)	42	81 (67, 90)	68 (69, 75)	49 (38, 60)	90 (83, 95)	−25	9.8
Xpert Optimized by Channel and ROC	64 (33)	41	75 (61, 85)	84 (76, 89)	64 (51, 76)	89 (83, 94)	−44	10.6
Xpert Optimized using Ct and Recursive Partitioning	60 (31)	41	75 (61, 85)	86 (80, 92)	68 (55, 80)	90 (83, 94)	−50	10.4
hc2, *n=193*	90 (47)	41	76 ** (62, 87)	65 *** (56, 73)	46 (35, 56)	87 (79, 93)	−25	12.6
Aptima, *n=184*	85 (46)	42	81 * (67, 90)	67 *** (59, 75)	49 (38, 60)	90 (82, 95)	−25	10.1

p-values:

* > 0.05

** ≤ 0.05

*** < 0.001.

Table 3.

Multivariate predictive logistic model by Xpert Channel.

Xpert Result	Participants with Result, *n (%)*	Prevalence hHSIL in in those with Result, *n (%)*	Adjusted Odds Ratio [95% CI]
16	33 (17)	25 (76)	13.1 (5.1, 36.5) *
31/33/35/52/58	69 (36)	31 (45)	2.6 (1.2, 5.7) **
51/59	32 (16)	20 (63)	4.8 (1.8, 12.7) **
39/56/66/68	54 (28)	27 (50)	2.1 (0.8, 4.8) ***

* $p \leq 0.001$

** $p \leq 0.02$

*** $p = 0.09$