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Accuracy of screening tests for cervical precancer in women living with HIV in low-resource settings: a paired prospective study in Lusaka, Zambia

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ABSTRACT

Objective This study aimed to provide evidence to improve cervical screening for women living with HIV (WLHIV). We assessed the accuracy of screening tests that can be used in low-resource settings and give results at the same visit.

Methods and analysis We conducted a paired, prospective study among consecutive eligible WLHIV, aged 18–65 years, receiving cervical cancer screening at one hospital in Lusaka, Zambia. The histopathological reference standard was multiple biopsies taken at two time points. The target condition was cervical intraepithelial neoplasia grade 2 and above (CIN2+). The index tests were high-risk human papillomavirus detection (hrHPV, Xpert HPV, Cepheid), portable colposcopy (Gynocular, Gynius) and visual inspection with acetic acid (VIA). Accuracy of stand-alone and test combinations were calculated as the point estimate with 95% CIs. A sensitivity analysis considered disease when only visible lesions were biopsied.

Results Women included in the study had well-controlled HIV infection (median CD4 count=542 cells/mm^3) and all except one were on antiretroviral therapy. Among 371 participants with histopathological results, 27% (101/371) women had CIN2+ and 23% (23/101) were not detected by any index test. Sensitivity and specificity for stand-alone tests were: hrHPV, 67.3% (95% CI 57.7% to 75.7%) and 65.3% (95% CI 59.4% to 70.7%); Gynocular 51.5% (95% CI 41.9% to 61.0%) and 80.0% (95% CI 74.8% to 84.3%); and VIA 22.8% (95% CI 15.7% to 31.9%) and 92.6% (95% CI 88.8% to 95.2%), respectively. Combining tests did not improve test accuracy measures. All test accuracies improved in sensitivity analysis.

Conclusion The low accuracy of screening tests assessed might be explained by our reference standard, which reduced verification and misclassification biases. Better screening strategies for WLHIV in low-resource settings are urgently needed.

Trial registration number NCT03931083.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The 2021 WHO guidelines recommend that women living with HIV (WLHIV) receive screening for human papillomavirus (hrHPV) genotypes at 3–5 years intervals, followed by a triage test to determine whether treatment is needed but this is based on low and moderate certainty evidence.

WHAT THIS STUDY ADDS

⇒ This study among WLHIV in Lusaka, Zambia evaluated three screening tests that allow same-day treatment; hrHPV test, portable colposcopy (Gynocular) and visual inspection with acetic acid (VIA), using strict methods to reduce verification and misclassification biases. The test accuracy of the different screening was poor, with sensitivities and specificity for stand-alone tests: hrHPV, 67.3% and 65.3%; Gynocular 51.5% and 80.0%; and VIA 22.8% and 92.6%; respectively.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our findings have implications for research and cervical cancer screening policies among WLHIV if test accuracy in this high-risk population has been overestimated by verification and misclassification biases in a majority of existing studies. Methodologically robust studies are crucial to inform cervical cancer screening practices and policies for the successful implementation of a cervical cancer elimination plan in sub-Saharan Africa, where 85% of women with cervical cancer and HIV live.

INTRODUCTION

The WHO strategy to eliminate cervical cancer aims to improve prevention and treatment among women living with HIV infection (WLHIV).[1] A conditional recommendation for WLHIV suggests testing for high-risk human papillomavirus (hrHPV) followed by an additional screening test based on moderate certainty evidence.[1] Cervical cancer remains the leading cause of cancer-related death among women in sub-Saharan African (SSA) countries, where more than half of cervical cancer cases are attributable to HIV.[2] Increased life expectancy on

antiretroviral therapy (ART) increases the number of women with persistent hrHPV infection, which may progress to cervical precancer and cancer.[3–5]

In low-resource settings, tests that give same-day results and lead to decisions about treatment are preferred. An evaluation of alternative same-day screening tests among WLHIV, using methods that minimise verification biases, has not yet been conducted.[1 6 7] Colposcopy is the cornerstone of visual assessment for cervical cancer screening, used in screening pathways of high-resource countries but is rarely accessible in low-resource settings where most WLHIV live. In low-resource settings, visual inspection with acetic acid (VIA) is commonly used,[5 6] but with low accuracy, particularly for WLHIV.[7 8] The WHO strategy recommends molecular tests to detect hrHPV, which were reported to have a sensitivity of 91.6% (95% CI 88%.1 to 94.1%) among WLHIV in a systematic review.[7] Our objectives were to assess the accuracy of molecular and visual screening tests (hrHPV testing, portable colposcopy using the Gynocular and VIA).

METHODS

This study followed a published protocol[9] and is reported according to the Standards for Reporting Diagnostic accuracy studies (STARD) 2015 guideline (online supplemental appendix S1). More details on our methodology have been previously described.[9]

Study design and participants

We conducted a single-site, paired (all women received all tests) prospective test accuracy study among WLHIV in Lusaka, Zambia. Nurses from the cervical cancer screening clinic came to the adjacent HIV clinic, informed them about cervical screening and invited them to volunteer for assessment of eligibility for the study. Consecutive eligible participants received a detailed explanation on the study in a private room where they had the possibility to ask questions. Written information about the study was available in English and local languages. Women who wished to participate, provided written consent and those who declined were encouraged to have standard care. We enrolled women aged 18–65 years with confirmed HIV infection who had ever had sex, gave written consent and agreed to return for a 6-month follow-up visit. We excluded women with a history of cervical cancer or total hysterectomy and those vaccinated against HPV. Women enrolled were a consecutive series who fulfilled eligibility criteria and for whom the research staff could complete all study procedures.

Procedures

Two nurses and one research assistant collected and tested specimens. To ensure independent test results and prevent bias, two different nurses performed procedures in separate rooms and findings were documented on separate case-report forms. Clinical team members did not discuss results, and participants were asked not to communicate findings to staff. After consent, a nurse recorded medical history and sociodemographic information. Blood tests for HIV RNA viral load (Cobas HIV-1/2 Qual; Roche Molecular Systems, New Jersey, USA) and CD4 cell count (Pima CD4 Analyzer, Alere, Waltham, USA) were taken at baseline.

Reference standard

The target condition was the histological presence of cervical intraepithelial neoplasia grade 2 and above (CIN2+) or high-grade squamous intraepithelial lesions (HSIL) at baseline or 6-month follow-up. A study nurse took biopsies during the colposcopy examination. If lesions were seen, she took at least two biopsies from those that looked the most severe. If no lesions were seen, she took four biopsies from clock-face positions 3, 6, 9 and 12 o'clock within the transformation zone. The nurse received training in colposcopy and biopsy taking from a gynaecologist based at the International Agency for Research on Cancer (IARC) and a local senior gynaecologist (online supplemental appendix S2). To further reduce detection bias in the reference standard, we took a second set of biopsies from each woman 6 months later to identify cases of disease missed at baseline. Biopsies were assessed histologically at two independent laboratories in South Africa and Zambia. An expert gynaecological pathologist in each laboratory, blinded to the clinical findings, examined all biopsies and classified them using the Bethesda squamous intraepithelial lesion system.[10] They reviewed all histopathology results via teleconference and reached an agreement on diagnosis. Any sample with CIN2 or ambiguous findings was tested with p16 immunostaining.[10 11] We dichotomised histopathological findings into low-grade and HSIL by the lower anogenital squamous terminology definitions and the WHO Classification of Tumours of Female Reproductive Organs.[10]

Index tests

A trained nurse (online supplemental appendix S2) did a speculum examination and collected specimens, followed by VIA. An endocervical sample was taken using a single-use cytobroom and immediately placed into ThinPrep PreservCyt solution (Hologic, Marlborough, Massachusetts, USA) (online supplemental appendix S2). During screening, an additional swab from the posterior vaginal fornix was also taken and tested for *Trichomonas vaginalis*. The research assistant processed the *T. vaginalis* and hrHPV specimens within 2–4 hours of collection using the GeneXpert platform (Cepheid, Sunnyvale, California, USA) at the study site, as per the manufacturers' instructions. The Xpert HPV test detects 14 hrHPV subtypes, categorised for reporting as HPV16, HPV18/45 (subtypes 18 and/or 45) and HPV other (any of subtypes 31, 33, 35, 39, 51, 52, 56, 58, 59, 66 and 68).

VIA examination followed IARC methodology (online supplemental appendix S2).[12] As per local guidelines, VIA nurses categorised indeterminate findings as abnormal. In a separate room, a different nurse performed a

colposcopic examination using the Gynocular (online supplemental appendix S3) following methods described in the IARC colposcopy manual.[13] We used the Swede score to standardise the documentation of findings on visual inspection with a score from 0 (abnormality not seen) to 2 (most severe)[14] (online supplemental appendix S4).

Treatment

Women who tested positive for VIA, CIN2+ or HSIL during screening were offered treatment as clinically indicated. Women with lesions eligible for cryotherapy or thermoablation could be treated at the time of screening by a trained cervical cancer screening nurse. Larger lesions would be treated with loop electrosurgical excision procedure, which would require a follow-up visit with a gynaecologist. Women with histopathologically confirmed cervical cancer were referred to the University Teaching Hospital in Lusaka for treatment. Women with confirmed *T. vaginalis* on swab were offered treatment with a 7-day course of metronidazole, as per local guidelines.

Interpretations of results

The histological presence of CIN2+ or HSIL at baseline or 6-month follow-up was considered as the disease outcome in the primary analysis. New cases of CIN2+ at follow-up were considered as diagnoses that had been missed at baseline. A positive hrHPV test result was defined as the detection of any of the 14 subtypes detected by the Xpert HPV test. VIA findings were dichotomised as positive (abnormal, suspicious of cancer or indeterminate) and negative (normal). We used receiver operating characteristic curve analysis to calculate the area under the curve (AUC) for each level of the Swede score as assessed by Gynocular colposcopy. We then used the Youden cut-off in the primary analysis, optimising both sensitivity and specificity. In an additional analysis, we used cut-offs maximising either sensitivity (≥90%) or specificity (≥90%).

Sample size and statistical analyses

We required a sample of 350 participants based on estimates of precision for the sensitivity and specificity of Gynocular, hrHPV and VIA as stand-alone tests for detecting CIN2+ lesions with approximately a 10%–15% margin of error (online supplemental appendix S5). We aimed to recruit 450 women to allow for incomplete data.

In our analyses, we used consensus agreement of the reference standard. We assessed agreement between the two pathologists for the reference standard using Cohen's kappa coefficient (κ). The accuracy measures used to assess Gynocular, VIA and hrHPV tests were sensitivity and specificity, positive and negative predictive values, positive and negative likelihood ratios, false positive and false negative rates, and diagnostic ORs (DORs). Screening test accuracy measures were estimated with 95% Wilson CIs. Using the same approach, we evaluated the accuracies of two tests used together. We considered the combination positive if both single tests were positive and negative otherwise. This mimics the clinical scenario where the second test is used to decide whether treatment is required (triage test).[1]

We also described test accuracy measures in subgroups defined by age (<25, 26–35, 36–45, >46 years and menopausal status), parity, ART status, coinfection with *T. vaginalis*, methods of contraception and CD4 cell count. Sensitivity and specificity were calculated for each subgroup. Estimated sensitivity values were compared with those found in the reference category by calculating the sensitivity ratio. To investigate the occurrence of effect modification by patient characteristics on the association between the diagnostic test and disease status, we used univariable and multivariable logistic regression models and tested for the interaction between the diagnostic test and patient characteristics on disease status. We considered the following patient's characteristics: age, menopause, parity, ART status, *T. vaginalis* at baseline, methods of contraception, HIV RNA, CD4 cell count, history of treatment for precancer and education level. Adjustment was performed considering all before mentioned patient characteristics as predictors and performing a stepwise model selection based on the Akaike information criterion.

We conducted the following sensitivity analyses to investigate the influence of unverifiable assumptions. First, we explored a possible training effect by assessing the first 10% of participants separately. Second, we explored the impact of the COVID-19 pandemic by conducting primary analyses separately on women who finished the study before 28 March 2020 (study ceased due to the pandemic). Third, we assessed the impact of missing or indeterminate results in the reference standard or screening test results by considering them first as positive cases, then as negative. Finally, acknowledging that biopsy and HPV tests may be performed and interpreted differently, we conducted analyses using a reference standard from a hypothetical scenario in which a biopsy was taken only from visible lesions and using different categories of hrHPV test results.

Role of the funding source

The funders did not contribute to the study design, data collection, analysis, interpretation or writing of the manuscript.

RESULTS

Flow of participants

Between May 2019 and March 2021, we assessed 413 women, enrolled 376 and included 375 in the analysis (1 woman was found to have had a total hysterectomy; figure 1). We had valid reference standard results for 371 women. VIA and hrHPV tests were performed on the 375 enrolled with Gynocular examination conducted on 373. The follow-up period for deriving the reference standard was 6 months. There were no adverse events. Follow-up was completed for 104 women when national COVID-19

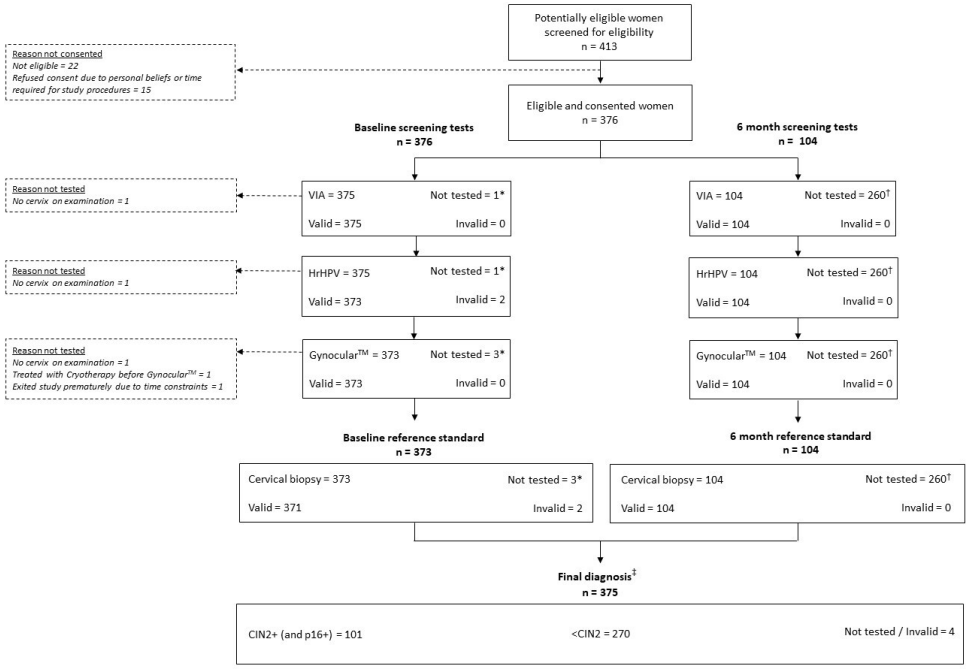

Figure 1 Flow of participants diagram to show the number of women receiving screening tests and reference standard, and analysed in the study. Data are n=number of women. *One women was excluded from the analysis as she did not receive any of the study screening tests - this brings the number of women included in the final diagnosis to 375. †Did not receive further tests as the study stopped following the COVID-19 pandemic. ‡The final diagnosis used in the analyses considers histopathological diagnosis for all women at baseline or 6-month follow-up, disease (CIN2+) was considered as present when biopsies from at least one time point were positive. CIN2+, cervical intraepithelial neoplasia grade 2 and above; <CIN2, cervical intraepithelial neoplasia grade 1 and below; HrHPV, high risk human papillomavirus; p16+, expression of cell cycle regulatory protein 16INK4A; VIA, visual inspection of the cervix with acetic acid.

restrictions on research studies meant that we had to stop the study. From March to December 2021, the official end of the study, we focused our efforts on ensuring that women who needed treatment received it. Study participants were contacted by phone and physically, by a peer educator, and invited to return to the clinic for treatment. However, despite all efforts, many women were unable to return. The study was officially closed in December 2021 as the pandemic persisted.

Patient characteristics

The full baseline characteristics are in table 1. At enrolment, participants had a median age of 37 years (IQR 31–44) and median parity of three (IQR 2–5). Most were not using any contraception (62%, n=231), did not smoke (99%, n=373) or use insunko[15] (a smokeless, carcinogenic tobacco product that can be used vaginally; 95%, n=355), and did not drink alcohol (88%, n=331). Most had never undergone cervical cancer screening (71%, n=267); VIA was the modality among those who had received screening. Seven women (2%) had received previous cryotherapy treatment. Almost all were on ART (99%, n=374) and had well-controlled HIV infection (median CD4 count=542 cells/mm^3). Women with histological CIN2+ were more likely to have a CD4 cell count <200 per mm^3 (7/101, 7%) than women without (5/270, 2%) and viral load ≥50 copies/mL (22/101, 22% and 36/270, 13%, respectively). We report baseline characteristics from routinely available data of all women aged 18–65 years seen at Kanyama HIV clinic in online supplemental appendix S6.

Disease spectrum

A consensus diagnosis of CIN2+ was made in 101 of 371 women with valid histology results (27.2%), of which 44 were CIN2, 56 were CIN3, and 1 was invasive cancer. The pathologists' agreement for determining CIN2+/HSIL was 71% (κ=0.37) at baseline and 82% (κ=0.46) at follow-up. Despite efforts to link all women with CIN2+ to care (online supplemental appendix S7), only 64/101 received treatment. Of these, 50 did not attend follow-up, 4 had positive histology at follow-up and 10 had negative histology results at follow-up. Prevalence of hrHPV was 43.5% (163/371) and *T. vaginalis* 19% (70/371) (online supplemental appendix S8).

Stand-alone screening test accuracy

Of 101 women with CIN2+, 23 (22.8%) had a negative result on all three screening tests (table 2). The stand-alone test with the highest point estimate for sensitivity was hrHPV testing (67.3%, 95% CI 57.7% to 75.7%) (figure 2, table of results in online supplemental appendix S9). Specificity was 65.3% (95% CI 59.4% to 70.7%). Women with CIN2+ were almost four times more likely to test positive for hrHPV than those without (DOR hrHPV 3.9, 95% CI 2.4 to 6.3). Using the Swede score, the AUC

Table 1 Baseline characteristics

Patient characteristics	Overall, n=375	CIN2+, n=101	<CIN2, n=270	Not tested/invalid, n=4
Age				
Median (IQR)	37 (31–44)	36 (28–42)	37 (31–44)	46 (42–54)
Missing	7	2	5	0
Menopause				
No	321 (86%)	90 (89%)	229 (85%)	2 (50%)
Yes	54 (14%)	11 (11%)	41 (15%)	2 (50%)
Marital status				
In a relationship/married	244 (65%)	70 (69%)	173 (64%)	1 (25%)
Separated/divorced	69 (18%)	12 (12%)	55 (20%)	2 (50%)
Single	12 (3%)	4 (4%)	8 (3%)	0 (0%)
Widowed	50 (13%)	15 (15%)	34 (13%)	1 (25%)
Employment				
Working	157 (42%)	39 (39%)	116 (43%)	2 (50%)
Not working	218 (58%)	62 (61%)	154 (57%)	2 (50%)
Education				
Did not finish secondary	308 (82%)	86 (85%)	219 (81%)	3 (75%)
Finished secondary	54 (14%)	12 (12%)	41 (15%)	1 (25%)
More than secondary	13 (4%)	3 (3%)	10 (4%)	0 (0%)
Income* (Kwacha/month)				
None	216 (58%)	61 (60%)	153 (57%)	2 (50%)
1–500	50 (13%)	16 (16%)	34 (13%)	0 (0%)
501–1000	74 (20%)	15 (15%)	57 (21%)	2 (50%)
1001–2500	32 (9%)	9 (9%)	23 (9%)	0 (0%)
2501–5000	3 (1%)	0 (0%)	3 (1.1%)	0 (0%)
Smoking				
No	373 (99%)	101 (100%)	268 (99%)	4 (100%)
Yes	2 (1%)	0 (0%)	2 (1%)	0 (0%)
Alcohol				
No	331 (88%)	92 (91%)	235 (87%)	4 (100%)
Yes	44 (12%)	9 (8.9%)	35 (13%)	0 (0%)
Insunko				
No	355 (95%)	95 (94%)	256 (95%)	4 (100%)
Yes	20 (5%)	6 (6%)	14 (5%)	0 (0%)
Age at sexual debut				
Median (IQR)	17 (16–19)	17 (15–19)	17 (16–19)	17 (16–18)
Missing	3	0	3	0
Contraception use				
No	231 (62%)	66 (65%)	161 (60%)	4 (100%)
Yes	144 (38%)	35 (35%)	109 (40%)	0 (0%)
Gravidity				
Median (IQR)	4 (2–5)	3 (2–5)	4 (2–5)	3 (2–6)
Parity				
Median (IQR)	3 (2–5)	3 (2–4)	4 (2–5)	3 (2–6)
On ART				
No	1 (0.3%)	0 (0%)	1 (0.4%)	0 (0%)

Continued

Table 1 Continued

Patient characteristics	Overall, n=375	CIN2+, n=101	<CIN2, n=270	Not tested/invalid, n=4
Yes	374 (100%)	101 (100%)	269 (100%)	4 (100%)
CD4 cell count (cells/mm3)				
Median (IQR)	542 (418–759)	532 (406–742)	543 (421–763)	1111 (928–1123)
Missing	2	0	1	1
HIV RNA load (copies/mL)				
<50	316 (84%)	79 (78%)	234 (87%)	3 (100%)
50–1000	29 (8%)	10 (10%)	19 (7%)	0 (0%)
1001–10 000	11 (3%)	6 (6%)	5 (2%)	0 (0%)
10 001 and more	18 (5%)	6 (6%)	12 (4%)	0 (0%)
Missing	1	0	0	1
History of previous cervical screening				
No	267 (71%)	71 (70%)	194 (72%)	2 (50%)
Yes	108 (29%)	30 (30%)	76 (28%)	2 (50%)
History of treatment for precancer				
No	368 (98%)	99 (98%)	265 (98%)	4 (100%)
Yes	7 (2%)	2 (2%)	5 (2%)	0 (0%)

Data are n=number of participants (%).
*Income in 3 months before enrolment.
ART, antiretroviral therapy; CD4, cluster of differentiation 4; cells/mm3, cell per cubic millimetre; CIN2+, cervical intraepithelial neoplasia grade 2 and above; <CIN2, cervical intraepithelial neoplasia grade 1 and below; copies/mL, copies per millilitre; hrHPV, high-risk human papillomavirus; VIA, visual inspection of the uterine cervix after application of 3%–5% acetic.

for Gynocular was 0.69 (95% CI 0.63 to 0.75) (online supplemental appendix S10). When dichotomised using the Youden index (Swede score 3), the test had a sensitivity of 51.5% (95% CI 41.9% to 61.0%), a specificity of 80.0% (95% CI 74.8% to 84.3%) and DOR of 4.25 (95% CI 2.6 to 6.9, figure 2, online supplemental appendix S9). When using the Swede score 1 (threshold yielding sensitivity≥90%), we reached a sensitivity of 97.0% (95% CI 92.0% to 99.0%) with a specificity of 3.3% (95% CI 1.8% to 6.2%). When using the Swede score 6 (threshold yielding specificity≥90%), specificity reached 94.1% (95% CI 90.6% to 96.3%) with a sensitivity of 29.7% (95% CI 21.7% to 39.2%). VIA had the lowest sensitivity (22.8%, 95% CI 15.7% to 31.9%) and highest specificity (92.6%, 95% CI 88.8% to 95.2%), with a DOR of 3.7 (95% CI 1.9 to 7.1).

Sensitivity analyses

We did not detect a strong training effect (online supplemental appendix S11a). Test accuracy measures were

Table 2 Tests results and CIN status

Screening test Gynocular	VIA	hrHPV	No Neoplasia n=128	CIN1 n=141	CIN2 n=44	CIN3 n=56	Cancer n=1
neg	neg	neg	82 (64%)	62 (44%)	18 (41%)	5 (8.9%)	0 (0%)
pos	neg	neg	9 (7%)	14 (10%)	5 (11%)	4 (7%)	0 (0%)
neg	pos	neg	3 (2%)	2 (1%)	1 (2%)	0 (0%)	0 (0%)
neg	neg	pos	28 (22%)	35 (25%)	9 (20%)	16 (29%)	0 (0%)
pos	pos	neg	2 (2%)	1 (1%)	0 (0%)	0 (0%)	0 (0%)
neg	pos	pos	0 (0%)	2 (1%)	0 (0%)	0 (0%)	0 (0%)
pos	neg	pos	2 (2%)	16 (11%)	5 (11%)	16 (29%)	0 (0%)
pos	pos	pos	2 (2%)	8 (6%)	6 (14%)	15 (27%)	1 (100%)

Women with three valid tests are included in this table, total=370. Data are n=number of participants; (%).
CIN1, cervical intraepithelial neoplasia grade 1; CIN2, cervical intraepithelial neoplasia grade 2; CIN3, cervical intraepithelial neoplasia grade 3; hrHPV, high-risk human papillomavirus; neg, test negative; pos, test positive; VIA, visual inspection of the uterine cervix after application of 3%–5% acetic.

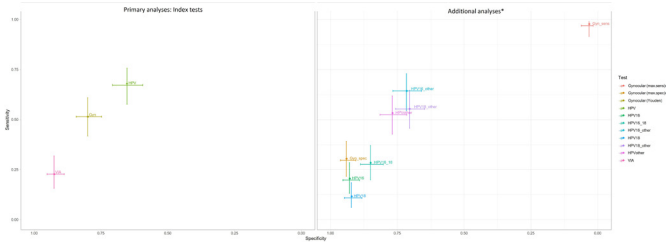

Figure 2 Sensitivity and specificity of single test screening strategies for prevalent CIN2+. *Secondary analysis (Gynocular) sensitivity analysis (HPV subtypes). Gyn, Gynocular; Max.spec, using a threshold that maximises specificity; Max.sens, using a threshold that maximises sensitivity; HPV16, human papillomavirus subtype 16; HPV18, human papillomavirus subtypes 18 and 45; HPVother, human papillomavirus other high-risk subtypes pooled –31, 33, 35, 39, 51, 52, 56, 58, 59, 66 and 68; hrHPV, high risk human papillomavirus; VIA, visual inspection of the uterine cervix after application of 3%–5% acetic.

similar whether or not participants stopped the study because of the COVID-19 pandemic (online supplemental appendix S11b), and results replacing missing and indeterminate test results and reference standards did not substantially affect estimates of accuracy (online supplemental appendix S11c). Using different categories of HPV subtypes showed similar results, with the best combination being HPV16 with 'other' (sensitivity 64.4%, 95% CI 54.6% to 73.0%, specificity 71.6%, 95% CI 66.0% to 76.7% and DOR 4.6, 95% CI 2.8% to 7.4%, table 2). In the hypothetical scenario where biopsies were taken only from visible lesions (n=106), sensitivity increased for all tests and specificity remained at similar levels (online supplemental appendix S11d). For hrHPV, sensitivity was 85.7% (95% CI 73.3% to 92.9%), specificity was 62.7% (95% CI 57.3% to 67.8%), and DOR was 10.1 (95% CI 4.4 to 23.2). Sensitivity of Gynocular increased to 93.9% (95% CI 83.5% to 97.9%), specificity to 81.5% (95% CI 76.9% to 85.3%) and DOR to 67.5 (95% CI 20.3 to 22.4). The sensitivity for VIA increased to 44.9% (95% CI 31.9% to 58.7%), specificity to 93.5% (95% CI 90.3% to 95.7%) and DOR to 11.8 (95% CI 5.75 to 24.1).

Two tests in combination

When we examined combinations of two tests with positive results, we found the specificities improved to above 90% for all test combinations but found a higher proportion of false negatives than when using single screening tests. Among combinations of tests, hrHPV followed by Gynocular yielded the most favourable balance of sensitivity 42.6% (95% CI 33.4% to 52.3%) and specificity 90.0% (95% CI 85.3% to 92.7%) (table 3, online supplemental appendix S12). Other analyses of test combinations are reported in online supplemental appendix S13.

Subgroup analyses

In a subgroup analysis, we found no clear differences in sensitivity and specificity according to age, menopause, education, contraception, parity, *T. vaginalis* result, ART status, HIV RNA viral load, CD4 cell count and previous treatment for precancerous disease (online supplemental appendix S14), and we did not detect effect modification by patient characteristics on the association between diagnostic test and disease status (online supplemental appendix S15).

DISCUSSION

We found a high prevalence of CIN2+precancerous lesions and hrHPV in WLHIV, almost all of whom were on ART. Stand-alone hrHPV, Gynocular and VIA testing missed almost a quarter of precancerous disease. Among visual screening tests, the Gynocular performed better than VIA. Combining tests did not improve test accuracy measures. In a sensitivity analysis in which only CIN2+ detected from visible lesions was used as the reference standard, all accuracy measures improved.

The study has several strengths. First, we tested a novel magnification device (Gynocular) among WLHIV with limited access to conventional colposcopy. Second, the index tests and reference standards were relevant to the context and performed by local experts. Third, we optimised the study methods with several strategies. Local and international experts contributed to protocol development and training staff. A data safety and monitoring board provided oversight.[9] All women received the reference standard, preventing partial verification biases. We reduced detection bias by obtaining 2–4 biopsies from each woman and considering the presence of disease at two time points 6 months apart. We used objective measures of HIV severity and concurrent *T. vaginalis* to examine associations between coexisting conditions and test performance.[15 16] We safeguarded blinding of screening tests and the reference standard. Furthermore, p16 immunostaining was used to determine HSIL objectively.[11 17] Because screening results often include indeterminate and missing results, we included a sensitivity analysis to understand the impact of these on test accuracy.

We acknowledge the limitations of our study methods. First, we used an index test (Gynocular) to guide biopsy samples for the reference standard. However, partial verification bias was avoided because all women received multiple biopsies irrespective of whether a lesion was seen.[18 19] Second, the COVID-19 pandemic interrupted follow-up, and only 104 (28%) women had a second reference test by the time the study had to close. We found five additional cases of CIN2+ among these, presumably missed at baseline. Were we able to complete follow-up on all women, disease prevalence may have been higher, affecting the predictive values of the tests performed at baseline.[20] Third, while we considered 6 months a short enough interval for the second reference standard test to detect missed disease, a 12-week time frame has also been used in previous studies.[7] Fourth, we used GeneXpert as the hrHPV testing platform, but an additional laboratory-based method would have enhanced quality control.

Table 3 Diagnostic accuracy of tests in combination and their precision

Test 1	Test 2	N	tp	fp	tn	fn	Sensitivity *95% CI*	Specificity *95% CI*	PPV *95% CI*	NPV *95% CI*	PLR *95% CI*	NLR *95% CI*
Gynocular and VIA												
Gynocular Youden	VIA	371	22	13	257	79	0.22 *0.15 to 0.31*	0.95 *0.92 to 0.97*	0.63 *0.46 to 0.77*	0.77 *0.72 to 0.81*	0.77 *0.72 to 0.81*	4.52 *2.37 to 8.64*
Gynocular max. sens	VIA	371	23	20	250	78	0.23 *0.16 to 0.32*	0.93 *0.89 to 0.95*	0.54 *0.39 to 0.68*	0.76 *0.71 to 0.81*	0.76 *0.71 to 0.81*	3.07 *1.77 to 5.35*
Gynocular max. spec	VIA	371	19	6	264	82	0.19 *0.12 to 0.28*	0.98 *0.95 to 0.99*	0.76 *0.57 to 0.89*	0.76 *0.72 to 0.81*	8.47 *3.48 to 20.59*	0.83 *0.76 to 0.91*
HPV and Gynocular												
HPV	Gynocular Youden	369	43	28	240	58	0.43 *0.33 to 0.52*	0.90 *0.85 to 0.93*	0.61 *0.49 to 0.71*	0.81 *0.76 to 0.85*	4.08 *2.69 to 6.19*	0.64 *0.54 to 0.76*
HPV16*	Gynocular Youden	369	17	5	263	84	0.17 *0.11 to 0.25*	0.98 *0.96 to 0.99*	0.77 *0.57 to 0.90*	0.76 *0.71 to 0.8*	9.02 *3.41 to 23.81*	0.85 *0.78 to 0.93*
HPV18/45*	Gynocular Youden	369	8	8	260	93	0.08 *0.04 to 0.15*	0.97 *0.94 to 0.99*	0.50 *0.28 to 0.72*	0.74 *0.69 to 0.78*	2.65 *1.02 to 6.88*	0.95 *0.89 to 1.01*
HPVother*	Gynocular Youden	369	31	18	250	70	0.30 *0.23 to 0.40*	0.93 *0.90 to 0.96*	0.63 *0.49 to 0.75*	0.78 *0.73 to 0.82*	4.57 *2.68 to 7.79*	0.74 *0.65 to 0.85*
HPV16 HPV18/45*	Gynocular Youden	369	22	13	255	79	0.22 *0.15 to 0.31*	0.95 *0.92 to 0.97*	0.63 *0.46 to 0.77*	0.76 *0.72 to 0.81*	4.49 *2.35 to 8.57*	0.82 *0.74 to 0.91*
HPV16 HPVother*	Gynocular Youden	369	41	22	246	60	0.41 *0.32 to 0.50*	0.92 *0.88 to 0.95*	0.65 *0.53 to 0.76*	0.80 *0.76 to 0.85*	4.95 *3.11 to 7.87*	0.65 *0.55 to 0.76*
HPV18/45† HPVother*	Gynocular Youden	369	33	24	244	68	0.33 *0.24 to 0.42*	0.91 *0.87 to 0.94*	0.58 *0.45 to 0.70*	0.78 *0.73 to 0.82*	3.65 *2.27 to 5.86*	0.734 *0.64 to 0.85*
HPV and VIA												
HPV	VIA	369	22	12	256	79	0.22 *0.15 to 0.31*	0.96 *0.92 to 0.97*	0.65 *0.48 to 0.79*	0.76 *0.72 to 0.81*	4.87 *2.50 to 9.46*	0.82 *0.74 to 0.91*
HPV16*	VIA	369	10	2	266	91	0.10 *0.06 to 0.17*	0.99 *0.97 to 0.10*	0.83 *0.55 to 0.95*	0.75 *0.70 to 0.79*	13.27 *2.96 to 59.51*	0.92 *0.85 to 0.97*
HPV18/45*	VIA	369	5	1	267	96	0.05 *0.02 to 0.11*	0.10 *0.98 to 0.10*	0.83 *0.44 to 0.97*	0.74 *0.69 to 0.78*	13.27 *1.57 to 112.18*	0.95 *0.91 to 0.10*
HPVother*	VIA	369	13	10	258	88	0.13 *0.08 to 0.21*	0.96 *0.93 to 0.98*	0.57 *0.37 to 0.74*	0.75 *0.70 to 0.79*	3.45 *1.56 to 7.62*	0.91 *0.84 to 0.98*
HPV16 HPV18/45*	VIA	369	13	3	265	88	0.13 *0.08 to 0.21*	0.99 *0.97 to 0.10*	0.81 *0.57 to 0.93*	0.75 *0.70 to 0.79*	11.50 *3.35 to 39.51*	0.88 *0.82 to 0.95*
HPV16 HPVother*	VIA	369	20	11	257	81	0.20 *0.13 to 0.29*	0.96 *0.93 to 0.98*	0.65 *0.47 to 0.79*	0.76 *0.71 to 0.80*	4.82 *2.40 to 9.71*	0.84 *0.76 to 0.92*

Continued

Table 3 Continued

Test 1	Test 2	N	tp	fp	tn	fn	Sensitivity 95% CI	Specificity 95% CI	PPV 95% CI	NPV 95% CI	PLR 95% CI	NLR 95% CI
HPV18/45 HPVother*	VIA	369	15	11	257	86	0.15 *0.09 to 0.23*	0.96 *0.93 to 0.98*	0.58 *0.39 to 0.75*	0.75 *0.71 to 0.79*	3.62 *1.72 to 7.61*	0.89 *0.82 to 0.97*

Data are n=number of participants.
Test accuracies are reported with the point estimate and the 95% CIs in italics.
*Sensitivity analysis.
†n missing HPV tests and missing histopathology.
AUC, area under the curve; DOR, diagnostic OR; fn, false negative; fp, false positive; HPV16, human papillomavirus subtype 16; HPV18/45, human papillomavirus subtypes 18 and 45; HPVother, human papillomavirus other high-risk subtypes pooled –31, 33, 35, 39, 51, 52, 56, 58, 59, 66 and 68; hrHPV, high-risk human papillomavirus; Max sens, using threshold that maximises sensitivity; Max.spec, using threshold that maximises specificity; NLR, negative likelihood ratio; NPV, negative predictive value; PLR, positive likelihood ratio; PPV, positive predictive value; Tn, true negative; Tp, true positive; VIA, visual inspection of the uterine cervix after application of 3%–5% acetic.

Fifth, the study assessed VIA, but many sites in SSA use an amended method, including cervicography.[21 22] The results of this study are, therefore, not applicable to the Cervical Cancer Prevention Programme in Zambia.

The sensitivity of testing for hrHPV was lower in our study than in many others.[6 7] In contrast to many previous studies, we took four biopsies from women with no visible lesions and repeated testing 6 months later to avoid partial verification bias when only acetowhite lesions are sampled. We found the sensitivity of hrHPV was 65.3% (95% CI 59.4% to 70.7%) when biopsies were obtained from all women and 85.7% (95% CI 73.3% to 92.9%) if only biopsies from visible lesions were considered. Kelly *et al*'s systematic review of cervical cancer screening strategies among WLHIV in studies published up to July 2022 found that the sensitivity of VIA was overestimated in studies with a risk of partial verification bias.[7] They did not, however, do a subgroup analysis stratified by the risk of verification bias for hrHPV testing. Studies in which the reference standard is obtained only from visible lesions during colposcopy[22 23] have higher estimates of sensitivity and specificity than when all women have biopsies.[6 24 25] We also found a prevalence of precancer among WLHIV that was higher than in another Zambian study, in which CIN2+ prevalence was 16% among 200 women screened at the University Teaching Hospital in 2016.[8] A systematic review evaluating diagnostic accuracy of cervical cancer screening strategies among WLHIV found a pooled prevalence of 12% (range 2%–26%),[9] with higher prevalence in tertiary settings where referral for abnormal cervical smear or positive HPV test suggested a high risk for CIN2+. Our reference standard methods, taking 2–4 biopsies at two time points, might have detected more CIN2+ cases than in studies taking one biopsy from the most severe cervical lesion[26 27] or a maximum of two biopsies.[6 7] Wentzensen *et al*[20] found that sensitivities for detecting CIN2+ increased from 61% (95% CI 55% to 67%) in a single biopsy to 86% (95% CI 80% to 90%) with two biopsies to 96% (95% CI 91% to 99%) with three biopsies.[27] In contrast to previous studies that calculated combined test accuracy using the denominator of women testing positive from the first test, we considered all women in our denominator so as not to miss any disease in the target population. This better emulates a real-life situation highlighting that combining tests does not improve accuracy when the sensitivity of the primary screening test is low. In different contexts, the choice of screening tests that prioritise sensitivity or specificity may vary depending on the resources and infrastructure available.[28 29] For example, if there is already a system in place to ensure women receive timely follow-up and treatment, providers can prioritise a test with lower sensitivity and higher specificity, to avoid unnecessary treatments. However, if this infrastructure is not available, a test that prioritises high sensitivity and enables a point-of-care strategy to link screening and treatment, may be preferred to ensure that fewer women with the potential to develop cervical cancer are missed. Ideally, a screening sequence should aim for a sensitivity

of 90%–95% and specificity of 85% to detect CIN3+ during one screening interval.[30] Although p16-positivity indicates a higher cancer potential than CIN2+alone, our results cannot be directly compared with this target. Larger test accuracy studies among WLHIV which minimise bias, would strengthen estimates of accuracy and enable improve the healthcare for women.

In our study, hrHPV testing, Gynocular colposcopy and VIA performed poorly as stand-alone screening tests among WLHIV, and 22.9% of cases were not detected by any test. Combining two tests did improve specificity but not overall accuracy when all women (and all disease) were considered in the denominator. Our findings have implications for research and cervical cancer screening policies among WLHIV if test accuracy in this high-risk population has been overestimated. According to our sensitivity analysis, the assumption that taking biopsies from visible lesions on colposcopy is an acceptable reference standard might need reassessment. WHO recommends 3–5 years screening intervals for WLHIV, based on the assumption that suboptimal screening tests at sufficiently frequent intervals will still prevent cancer because of the long precancerous phase. However, if accuracy measures informing modelling studies are overestimated, these screening intervals might be too long. Larger studies, among WLHIV, in countries with the highest disease burden and using methods that reduce verification bias are urgently required. Our robust descriptive study results can be used in future modelling studies and randomised controlled trials of screening effectiveness, both of which are needed to determine improved strategies for cervical cancer screening among WLHIV.

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Short title: Accuracy of screening tests in WLHIV: a paired prospective study in Zambia
Appendix

Appendix: Accuracy of screening tests for cervical pre-cancer in women living with HIV in low-resource settings: a paired prospective study in Lusaka, Zambia

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S1. Supplementary material 1: STARD checklist

Section & Topic	No	Item	Line
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy [such as sensitivity, specificity, predictive values, or AUC]	3
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions [for specific guidance, see STARD for Abstracts]	
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	109-117
	4	Study objectives and hypotheses	116-117
METHODS			
Study design	5	Whether data collection was planned before the index test and reference standard were performed [prospective study] or after [retrospective study]	127
Participants	6	Eligibility criteria	129-131
	7	On what basis potentially eligible participants were identified [such as symptoms, results from previous tests, inclusion in registry]	128
	8	Where and when potentially eligible participants were identified [setting, location and dates]	128
	9	Whether participants formed a consecutive, random or convenience series	128
Test methods	10a	Index test, in sufficient detail to allow replication	164-177 and supplement
	10b	Reference standard, in sufficient detail to allow replication	143-162
	11	Rationale for choosing the reference standard [if alternatives exist]	143-162
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	178-189
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	178-189
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	163-177
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	153
Analysis	14	Methods for estimating or comparing measures of diagnostic accuracy	189-222
	15	How indeterminate index test or reference standard results were handled	219
	16	How missing data on the index test and reference standard were handled	219
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	18	Intended sample size and how it was determined	191 -194 and supplement
RESULTS			
Participants	19	Flow of participants, using a diagram	230-236

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	21a	Distribution of severity of disease in those with the target condition	250-256
	21b	Distribution of alternative diagnoses in those without the target condition	252-253 and supplement
	22	Time interval and any clinical interventions between index test and reference standard	236
Test results	23	Cross tabulation of the index test results [or their distribution] by the results of the reference standard	Table 3
	24	Estimates of diagnostic accuracy and their precision [such as 95% confidence intervals]	258-270 table 3 and Figure 4
	25	Any adverse events from performing the index test or the reference standard	233
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	313-323
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S2. Supplementary material 2: Description of study site, procedures, training, treatment and evaluation of histology

Study site: The study population includes women who are enrolled in the ART program at Kanyama General Hospital in Lusaka, Zambia. It has a catchment population of 262 715. The ART program has registered 52,658 people living with HIV, of which 40,650 have commenced treatment. In addition to ART services, the hospital has also been providing cervical cancer screening since October 2006 The ART and cervical cancer screening clinics are adjacent. The cervical screening clinic is a collaboration with CIDRZ and the Cervical Cancer Prevention Program in Zambia [CCPPZ]. The clinic is well established in the application of cancer screening based on a "screen-and-treat" approach, where women undergo visual screening with acetic acid, followed by immediate treatment where possible. Since inception in 2006, the clinic has screened over 23,900 women for cervical cancer out of which 40% of these women are WLHIV. Clinic nurses are trained to offer cervical cancer screening and treatment with cryotherapy or thermal coagulation and to refer suspicious cases for LEEP and further treatment. The referral site is the University Teaching Hospital, the tertiary level hospital in Lusaka, Zambia in Lusaka. Close to 10% of women enrolled are treated with cryotherapy or thermal coagulation. Participants with suspected cervical cancer are referred to the University Teaching Hospital.

Procedures: The VIA examination included insertion of a speculum, visualisation of the vagina, vulva and cervix, assessment with the naked eye after application of normal saline, and further assessment after application of 5% acetic acid for one minute. The nurse recorded the findings as normal, abnormal, or suspicious of cancer. As per local guidelines, VIA nurses categorised indeterminate findings as abnormal.

The HPV sample was collected during the first speculum examination that participants received, and before the VIA examination. The hrHPV testing was done using a single-use cervical cytobroom provided by GeneXpert™ and placed into ThinPrep PreservCyt [Cepheid, Sunnyvale, CA] immediately after collection. ined Nurses were trained to insert the central bristles of the cervical cytobroom into the endocervical canal and to allow the shorter bristles to remain in full contact with the ectocervix, rotating the broom five times in a clockwise direction to collect cells from the transformation zone. The broom was then rinsed into the PreservCyt® solution without delay and separated into the vial without touching surrounding sites.

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The nurse performing colposcopy and obtaining biopsies received training from a gynaecologist based at the International Agency for Research on Cancer [IARC] and a local senior gynaecologist. The Gynocular™ examination included a speculum examination and visualisation of the vagina, vulva and cervix, assessment of the cervix at low and high magnification [>6x], examination of cervical vessel patterns using the red-free mode [or green filter], application of 5% acetic acid for one minute, and cervical assessment following application of Lugol's iodine.

The Swede score describes the Gynocular™ examination by scoring the following domains: vessels, margins or surface, acetic acid uptake, iodine staining, and lesion size. A score of zero and two is given to each domain based on the severity of the findings, and summed to a total score between zero [best] and ten [worst]. Ablative treatment was offered if the lesion boundaries were fully visible, covered less than 75% of the ectocervix, did not extend into the endocervical canal, and was covered by the cryotherapy/ thermoablation tip. For larger lesions, women were offered loop electrosurgical excision procedure.

The histological specimens were assessed by two independent laboratories in Zambia and South Africa. The laboratory in South Africa is one of the leading pathology laboratories operating throughout Africa, and provides diagnostic and monitoring pathology services in many countries in the region including South Africa, Botswana, Ghana, Kenya, Mozambique, Nigeria, Swaziland, Tanzania, Uganda, Zambia, and Zimbabwe. The pathologists reviewing the specimens were certified under the Health Professions Council of South Africa [HSCSA]. The pathologist we sought in Zambia was referred to us as a leading local expert in cervical pathology. He is registered as a certified pathologist under the Health Professionals Council of Zambia.
All biopsies we prepared by the SA Laboratory. The slides were sent to Zambia and reviewed by the second pathologist independently. The two pathologists then reviewed all slides together, via teleconference to reach consensus on each sample. If consensus could not be reached a third pathologists would be invited to give provide their opinion.

Training: We trained our study team on the study protocol, data-entry, and clinical procedures twice prior to study commencement and once as a refresher training during the study period. Clinical training sessions were led by a local expert gynaecologist as well as IARC trainers. We taught all gynaecological procedures on mannequins first and then on women at the cervical cancer screening clinic at Kanyama hospital under supervision. Cepheid company representatives in Lusaka set up and calibrated the GeneXpert™ device on an annual basis. They provided two training sessions on obtaining and processing hrHPV and T. Vaginalis swabs; one prior to study implementation and the other as a refresher training one year later.

Treatment: All women with VIA-positive findings, or CIN2+ on histology, were offered treatment with cryotherapy, thermoablation, or loop electrosurgical excision procedure [LEEP] as clinically indicated. Women with histopathologically confirmed cervical cancer were referred to the University Teaching Hospital in Lusaka for treatment.

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S3. Supplementary material 3: The Gynocular [Gynius Plus AB, Sweden]

This photograph was taken at the Centre for Infectious Disease Research Zambia headquarters on 27 April 2020

S4. Supplementary material 4: Swede score definition

Swede score	0	1	2
Aceto uptake	**Zero or transparent**	**Shady milky [not transparent not opaque]**	**Distinct, opaque white**
Margins/surface	**Diffuse**	**Sharp but irregular, jagged, "geographical" satellites**	**Sharp and even, difference in surface level including "cuffing"**
Vessels	**Fine, regular**	**Absent**	**Coarse or atypical**
Lesion size	**<5mm**	**5-15mm or 2 quadrants**	**>15mm or 3-4 quadrants or endocervically undefined**
Iodine staining	**Brown**	**Faintly or patchy yellow**	**Distinct yellow**
			TOTAL SCORE

Source: Bowring J, Strander B, Young M, Evans H, Walker P. The Swede score: evaluation of a scoring system designed to improve the predictive value of colposcopy. J Low Genit Tract Dis. 2010 Oct;14[4]:301-5. doi: 10.1097/LGT.0b013e3181d77756. PMID: 20885156.

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S5. Supplementary material 5: Sample-size calculation

This screening-test accuracy study requires 350 participants to estimate the sensitivity and specificity of Gynocular™, HR-HPV, and VIA for CIN2+ lesions with the precision detailed in **Table S5**. Screening accuracy measures will be estimated with 95% Wilson confidence intervals with no formal hypothesis testing between modalities.

We planned to enrol 450 women to obtain data from at least 350 patients for statistical analyses. We expected the prevalence of CIN2+ in WLHIV in Zambia to be 16–20%.1-3 We expected disease prevalence to be lower than in previous years. Increases in the number of women receiving ART, and commencing treatment at higher CD4 cell counts,4 may lead to a decline in HPV prevalence.5 Higher rates of voluntary male circumcision in male partners may also contribute to lower levels of HPV infection.[5] We also estimated that there may be up to 10% loss to follow-up and up to 10% of tests that are not analysable or interpretable. We implemented rigorous data collection methods, such as patient demographics, clinical history, and test results, to avoid missing data.

Table S5: Sensitivity and specificity table, N=350. The expected 95% Wilson confidence interval [%] for sensitivity and specificity with varying prevalence.

		Expected 95% confidence intervals for sensitivity [%]									
		Expected 95% confidence intervals for specificity [%]									
		50	55	60	65	70	75	80	85	90	95
Expected prevalence [%]	14	37.5–64.4 44.6–55.8	41.3–68.1 49.5–60.7	45.2–71.8 54.5–65.5	51.3–77.1 59.6–70.3	55.5–80.5 64.7–75.0	61.9–85.4 69.9–79.6	66.4–88.5 75.2–84.2	73.3–92.9 80.6–88.6	78.2–95.6 86.1–92.9	86.3–98.9 91.9–97.0
	16	37.3–62.7 44.3–55.7	42.4–67.6 49.4–60.7	47.6–72.4 54.2–65.3	51.2–75.5 59.4–70.2	56.7–80.1 64.6–75.0	62.3–84.5 69.9–79.8	68.2–88.7 75.0–84.1	74.3–92.6 80.5–88.7	78.5–95.0 86.2–93.0	85.4–98.2 91.8–96.9
	18	38.8–62.7 44.4–55.9	43.3–67.2 49.3–60.7	48.0–71.5 54.2–65.4	52.8–75.7 59.5–70.4	57.6–79.8 64.5–75.0	62.7–83.7 69.6–79.6	67.8–87.5 75.1–84.3	75.0–92.3 80.4–88.7	80.7–95.6 85.9–92.9	86.9–98.4 92.0–97.1
	20	38.6–61.4 44.2–55.8	44.1–66.8 49.1–60.7	48.3–70.7 54.2–65.6	54.0–75.8 59.2–70.3	58.5–79.5 64.4–75.1	64.5–84.2 69.6–79.7	69.2–87.7 74.9–84.3	75.7–92.1 80.3–88.7	80.8–95.1 85.9–93.0	88.1–98.5 91.8–97.0
	22	39.7–61.5 44.3–56.1	43.5–65.2 49.0–60.7	48.6–70.0 54.2–65.7	53.8–74.7 59.0–70.3	59.2–79.2 64.3–75.1	64.6–83.6 69.6–79.8	70.3–87.8 74.7–84.2	74.7–90.9 80.3–88.7	80.8–94.6 86.0–93.1	87.4–98.0 91.6–96.9

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S6. Supplementary material 6: Baseline characteristics of all women aged between 18 and 65 years seen at Kanyama clinic over the recruitment period of the study

Characteristic	N = 10,718
Highest educational level	
None	546 [6.2%]
Finished primary	3,524 [40%]
Started secondary	44 [0.5%]
Finished secondary	4,491 [51%]
College/University	156 [1.8%]
Unknown	1,957
Marital status	
In a relationship/Married	5,864 [66%]
Separated/Divorced	1,256 [14%]
Single	902 [10%]
Widowed	923 [10%]
Unknown	1,773
Age	
Median [IQR]	35 [28, 43]
Income [Kwacha/month]	
Greater than K3000	54 [2.1%]
K1000 - K1499	678 [27%]
K1500 - K1999	231 [9.0%]
K2000 - K2999	201 [7.9%]
K500 - K999	935 [37%]
Less than K500	454 [18%]
Unknown	8,165
Gravidity	
Median [IQR]	1.00 [1.00, 3.00]
Unknown	9,358
Parity	
Median [IQR]	0.00 [0.00, 1.00]
Unknown	9,358
Contraception use	
Yes	2,596*
Unknown	8,122
HIV RINA Viral load	
< 50 copies/ml	5,503 [73%]
[50,1000]	1,386 [18%]
[1000,10000]	226 [3.0%]
10000 and more	456 [6.0%]
Unknown	3,147

*percentage not provided as it would be misleading, some of the women categorized with contraception status unknow are likely not using contraception, however it was not possible to differentiate the number from the raw data source. Categories have been aligned as much as possible with baseline characteristics reported in our study. HIV RNA, human immunodeficiency virus ribonucleic acid; hrHPV, high-risk human papillomavirus; IQR, inter-quartile range; n, number of participants; VIA, visual inspection of the uterine cervix after application of 3–5% acetic; CIN2+, Cervical intra-epithelial neoplasia grade two and above; <CIN2, Cervical intra-epithelial neoplasia grade one and below; copies/mL: copies per millilitre; cells/mm3: cell per cubic millimetre.

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S7. Supplementary material 7: Measures taken to link women to treatment

From March to December 2021, nurses made calls to five to ten women daily, while a peer educator visited two to three homes each day. Women were offered appointments three times on average. To make treatment more accessible, additional travel funds were provided. We also hired a Gynaecologist who was available daily at the clinic to perform LEEPs on scheduled patients and any participant who agreed to receive treatment on the spot.

S8. Supplementary material 8: Distribution of alternative diagnoses in those without the target condition

	Negative		Positive		
	Normal tissue [n=129]	CIN1 [n=141]	CIN2 [n=44]	CIN3 [n=56]	Cancer [n=1]
T. vaginalis					
Negative	106 [82.2%]	117 [83.0%]	34 [77.3%]	45 [80.4%]	0 [0%]
Positive	23 [17.8%]	24 [17.0%]	10 [22.7%]	11 [19.6%]	1 [100%]
HPV					
Negative	96 [74.4%]	79 [56.0%]	24 [54.5%]	9 [16.1%]	0 [0%]
Positive	32 [24.8%]	61 [43.3%]	20 [45.5%]	47 [83.9%]	1 [100%]
Undetermined	1 [0.8%]	1 [0.7%]	0 [0%]	0 [0%]	0 [0%]

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S9. Supplementary material 9: Estimates of diagnostic accuracy and their precision

Test	n	tp	fp	tn	fn	Sensitivity % 95% CI	Specificity % 95% CI	PPV % 95% CI	NPV % 95% CI	PLR 95% CI	NLR 95% CI	DOR 95% CI	AUC
Primary analysis													
Gynocular [Youden]	371	52	54	216	49	51.5 41.9 - 61.0	80.0 74.8 - 84.3	49.1 39.7 - 58.4	81.5 76.4 - 85.7	2.6 1.9 - 3.5	0.6 0.5, 0.7	4.2 2.6 - 6.9	0.69 0.63 - 0.75
hrHPV	369*	68	93	175	33	67.3 57.7 - 75.7	65.3 59.4 - 70.7	41.6 34.3 - 49.3	84.1 78.6 - 88.5	1.9 1.6 - 2.4	0.5 0.4 - 0.7	3.9 2.4 - 6.3	
VIA	371	23	20	250	78	22.8 15.7 - 31.9	92.6 88.8 - 95.2	51.2 36.8 - 65.4	76.2 71.3 - 80.5	3.1 1.8 - 5.3	0.8 0.7 - 0.9	3.7 1.9 -7.1	
Additional analyses													
Gynocular [max.spec]	371	30	16	254	71	29.7 21.7 - 39.2	94.1 90.6 - 96.3	65.2 50.8 - 77.3	78.2 73.3 - 82.3	1.0 1.0 - 1.1	0.9 0.3 - 3.2	6.7 3.46 - 13.0	0.69 0.63 - 0.75
HPV16 HPVother†	369*	65	76	192	36	64.4 54.6 - 73.0	71.6 66.0 - 76.7	46.1 38.1 - 54.3	84.2 78.9 - 88.4	2.3 1.8 - 2.9	0.5 0.4 - 0.7	4.6 2.8 - 7.4	
HPVother†	369*	53	62	206	48	52.5 42.8 - 61.9	76.9 71.5 - 81.5	46.1 37.3 - 55.2	81.1 75.8 - 85.4	2.3 1.7 - 3.0	0.6 0.5 - 0.8	3.7 2.3 - 5.9	
HPV16†	369*	20	19	249	81	19.8 13.2 - 28.6	92.9 89.2 - 95.4	51.3 36.2 - 66.1	75.5 70.5 - 79.8	2.8 1.6 - 5.0	0.9 0.8 - 1.0	3.236 1.6 - 6.4	
HPV18/45 HPVother†	369*	56	79	189	45	55.4 45.7 - 64.8	70.5 64.8 - 75.7	41.5 33.5 - 49.9	80.8 75.2 - 85.3	1.8 1.5 - 2.4	0.6 0.5 - 0.8	3.0 1.9 - 4.8	
HPV16 HPV18/45†	369*	28	40	228	73	27.7 19.9 - 37.1	85.1 80.3 - 88.8	41.2 30.3 - 53.0	75.7 70.6 - 80.2	1.9 1.2 - 2.8	0.9 0.7 - 1.0	2.2 1.3 - 3.8	
HPV18/45†	369*	11	21	247	90	10.9 6.2 - 18.5	92.2 88.3 - 94.8	34.4 20.4 - 51.7	73.3 68.3 - 77.7	1.4 0.7 - 2.8	1.0 0.9 - 1.0	1.4 0.7 - 3.1	
Gynocular [max.sens]	371	98	261	9	3	97.0 91.6 - 99.0	3.3 1.8 - 6.2	27.3 22.9 - 32.1	75.0 46.8 - 91.1	1.0 1.0 - 1.1	0.9 0.2 - 3.2	1.1 0.3 - 4.2	0.69 0.6 - 0.8

Data are n= number of participants. Tp= true positive. Fp= false positive. Tn= true negative. Fn= false negative. Test accuracies are reported with the point estimate and the 95% confidence intervals below in italics. PPV= positive predictive value. NPV= negative predictive value. PLR= positive likelihood ratio. NLR= negative likelihood ratio. DOR= diagnostic odds ratio. AUC= area under the receiver operating curve. hrHPV= high-risk human papillomavirus. VIA= visual inspection of the uterine cervix after application of 3–5% acetic. Max.spec= using threshold that maximises specificity. Max sens= using threshold that maximises sensitivity. HPV16= human papillomavirus subtype 16. HPV18/45= human papillomavirus subtypes 18 and 45. HPVother = human papillomavirus other high-risk subtypes pooled -31, 33, 35, 39, 51, 52, 56, 58, 59, 66 and 68. 95% CI= ninety-five percent confidence interval. *= n missing HPV tests and missing histopathology. † sensitivity analysis

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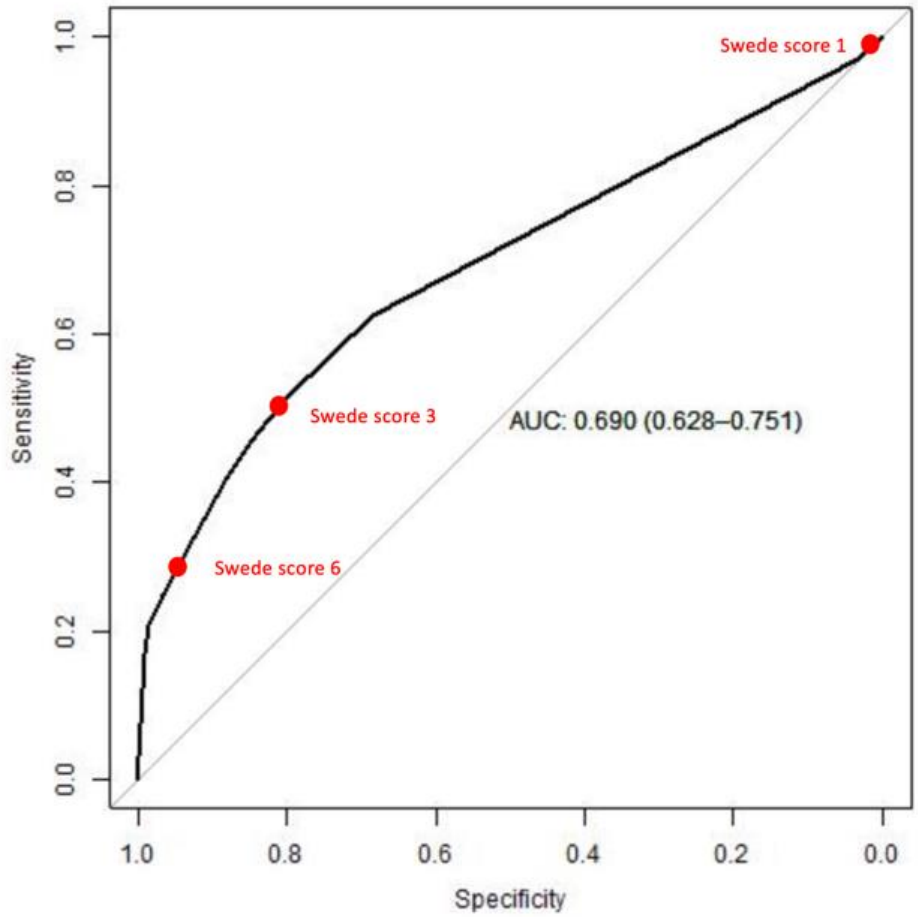
S10. Supplementary material 10: Area under the receiver operating curve for Gynocular



AUC= area under the curve. Swede score 6 = score of 6 when using the Swede score to determine lesion severity. Swede score 6 was associated with the maximum specificity achievable using the Gynocular and Swede score. Swede score 3 = score of 3 when using the Swede score to determine lesion severity. Swede score 3 achieved the Youden threshold, which maximises sensitivity and specificity. Swede score 1 = score of 1 when using the Swede score to determine lesion severity. Swede score 1 was associated with the maximum sensitivity achievable using the Gynocular and Swede score

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S11. Supplementary material 11: Sensitivity analyses

A. Training effect

Test accuracy in first 10% of patients

	n	tp	fp	tn	fn	Sensitivity % 95% CI	Specificity % 95% CI	PPV % 95% CI	NPV % 95% CI	PLR 95% CI	NLR 95% CI	DOR 95% CI
HPV	36	6	8	21	1	0.85 [0.49, 0.97]	0.72 [0.54, 0.85]	0.43 [0.21, 0.67]	0.96 [0.78, 0.99]	3.11 [1.60, 6.03]	0.20 [0.032, 1.228]	15.70 [1.6 152]
VIA	37	1	4	26	6	0.14 [0.03, 0.51]	0.86 [0.70, 0.95]	0.20 [0.04, 0.62]	0.81 [0.65, 0.91]	1.07 [0.14, 8.17]	0.99 [0.709, 1.38]	1.08 [0.10, 11.52]
Gyncoluar* [youden]	37	3	6	24	4	0.42 [0.16, 0.75]	0.80 [0.63, 0.91]	0.33 [0.12, 0.65]	0.86 [0.69, 0.94]	2.14 [0.70, 6.54]	0.71 [0.367, 1.39]	3.00 [0.52, 17.16]

*AUC: 0.517 [0.24, 0.793], Tp, true positive; fp, false positive; tn, true negative; fn, false negative; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; DOR, diagnostic odds ratio; AUC, area under the receiver operating curve; hrHPV, high-risk human papillomavirus; VIA , visual inspection of the uterine cervix after application of 3–5% acetic, HPV, human papillomavirus test, 95% CI, ninety-five percent confidence interval, n number of women

Test accuracy in women recruited later in the study

	n	tp	fp	tn	fn	Sensitivity % 95% CI	Specificity % 95% CI	PPV % 95% CI	NPV % 95% CI	PLR 95% CI	NLR 95% CI	DOR 95% CI
HPV	333	62	85	154	32	0.66 [0.56, 0.76]	0.64 [0.58, 0.70]	0.42 [0.35, 0.50]	0.83 [0.77, 0.88]	1.86 [1.48, 2.32]	0.528 [0.39, 0.71]	3.51 [2.13, 5.80]
VIA	334	22	16	224	72	0.23 [0.16, 0.33]	0.93 [0.89, 0.96]	0.58 [0.42, 0.72]	0.76 [0.71, 0.80]	3.51 [1.93, 6.39]	0.82 [0.73, 0.92]	4.28 [2.13, 8.59]
Gyncoluar* [youden]	334	49	48	192	45	0.52 [0.42, 0.62]	0.80 [0.75, 0.85]	0.51 [0.41, 0.60]	0.81 [0.76, 0.86]	2.61 [1.90, 3.59]	0.60 [0.48, 0.75]	4.36 [2.61, 7.28]

Tp, true positive; fp, false positive; tn, true negative; fn, false negative; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; DOR, diagnostic odds ratio; AUC, area under the receiver operating curve; hrHPV, high-risk human papillomavirus; VIA , visual inspection of the uterine cervix after application of 3–5% acetic, HPV, human papillomavirus test, 95% CI, ninety-five percent confidence interval, n number of women
AUC: 0.705 [0.642, 0.767]

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B. COVID_19

In this sensitivity analysis, we aimed to show the impact of the COVID pandemic on our study. The sensitivity analysis also answers the question: does testing at two time-points (baseline and 6months) change the results compared with testing at just baseline. Keeping in mind that the second testing period (6 months), aimed to identify missed cases of disease at baseline.

Women who did not stop due to the COVID 19 pandemic

	n	tp	fp	tn	fn	Sensitivity % 95% CI	Specificity % 95% CI	PPV % 95% CI	NPV % 95% CI	PLR 95% CI	NLR 95% CI	DOR 95% CI
HPV	110	18	27	54	11	0.62 [0.44, 0.77]	0.667 [0.56, 0.76]	0.40 [0.27, 0.55]	0.83 [0.72, 0.90]	1.86 [1.22, 2.83]	0.57 [0.35, 0.93]	3.27 [1.36, 7.90]
VIA	111	7	6	76	22	0.24 [0.12, 0.42]	0.93 [0.85, 0.97]	0.54 [0.29, 0.77]	0.78 [0.68, 0.85]	3.30 [1.21, 9.01]	0.82 [0.66, 1.01]	4.03 [1.23, 13.24]
Gyncoluar* [youden]	111	17	17	65	12	0.59 [0.41, 0.75]	0.79 [0.69, 0.87]	0.50 [0.34, 0.66]	0.84 [0.75, 0.91]	2.83 [1.68, 4.77]	0.52 [0.33, 0.82]	5.42 [2.18, 13.48]

*AUC: 0.726 [0.613, 0.839] Tp, true positive; fp, false positive; tn, true negative; fn, false negative; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; DOR, diagnostic odds ratio; AUC, area under the receiver operating curve; hrHPV, high-risk human papillomavirus; VIA , visual inspection of the uterine cervix after application of 3–5% acetic, HPV, human papillomavirus test, 95% CI, ninety-five percent confidence interval, n number of women. NB: n=110 includes the total number of women who received followup before study stopped due to COVID and women who had to stop due to pregnancy.

Women who stopped the study on March 28 2020 due to COVID 19 pandemic

	n	tp	fp	tn	fn	Sensitivity % 95% CI	Specificity % 95% CI	PPV % 95% CI	NPV % 95% CI	PLR 95% CI	NLR 95% CI	DOR 95% CI
HPV	259	50	66	121	22	0.69 [0.58, 0.79]	0.65 [0.58, 0.71]	0.43 [0.35, 0.52]	0.84 [0.78, 0.90]	1.97 [1.53, 2.52]	0.47 [0.33, 0.68]	4.17 [2.32, 7.47]
VIA	260	16	14	174	56	0.22 [0.14, 0.33]	0.92 [0.88, 0.96]	0.53 [0.36, 0.70]	0.76 [0.70, 0.81]	2.98 [1.54, 5.80]	0.84 [0.74, 0.96]	3.55 [1.63, 7.73]
Gyncoluar* [youden]	260	35	37	151	37	0.48 [0.37, 0.59]	0.80 [0.74, 0.85]	0.48 [0.37, 0.60]	0.80 [0.74, 0.85]	2.47 [1.70, 3.59]	0.64 [0.51, 0.81]	3.86 [2.15, 6.93]

*AUC: 0.677 [0.603, 0.75] Tp, true positive; fp, false positive; tn, true negative; fn, false negative; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; DOR, diagnostic odds ratio; AUC, area under the receiver operating curve; hrHPV, high-risk human papillomavirus; VIA , visual inspection of the uterine cervix after application of 3–5% acetic, HPV, human papillomavirus test, 95% CI, ninety-five percent confidence interval, n number of women

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C. Missing data

In this analysis, we examine the effect of missing data on our results. We had only two tests missing for the HPV analysis.

Missing tests excluded [as per main analysis Table 3]

	n	tp	fp	tn	fn	Sensitivity % 95% CI	Specificity % 95% CI	PPV % 95% CI	NPV % 95% CI	PLR 95% CI	NLR 95% CI	DOR 95% CI
HPV	369	68	93	175	33	0.67 [0.58, 0.76]	0.65 [0.59, 0.71]	0.42 [0.35, 0.50]	0.84 [0.79, 0.88]	1.94 [1.57, 2.40]	0.50 [0.37, 0.67]	3.88 [2.39, 6.30]
VIA	371	23	20	250	78	0.23 [0.16, 0.32]	0.93 [0.89, 0.95]	0.55 [0.39, 0.68]	0.76 [0.71, 0.81]	3.07 [1.77, 5.35]	0.83 [0.75, 0.93]	3.69 [1.92, 7.07]
Gyncoluar* [youden]	371	52	54	216	49	0.52 [0.42, 0.61]	0.80 [0.75, 0.84]	0.49 [0.40, 0.58]	0.82 [0.76, 0.86]	2.57 [1.90, 3.49]	0.61 [0.49, 0.75]	4.25 [2.60, 6.94]

AUC: 0.69 [0.63, 0.75], Tp, true positive; fp, false positive; tn, true negative; fn, false negative; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; DOR, diagnostic odds ratio; AUC, area under the receiver operating curve; hrHPV, high-risk human papillomavirus; VIA , visual inspection of the uterine cervix after application of 3–5% acetic, HPV, human papillomavirus test, 95% CI, ninety-five percent confidence interval, n number of women

Missing tests considered positive **results would be the same if we considered the missing data as perfectly matching the reference standard.*

	n	tp	fp	tn	fn	Sensitivity % 95% CI	Specificity % 95% CI	PPV % 95% CI	NPV % 95% CI	PLR 95% CI	NLR 95% CI	DOR 95% CI
HPV	371	68	95	175	33	0.67 [0.58, 0.76]	0.65 [0.59, 0.70]	0.41 [0.34, 0.49]	0.841 [0.78, 0.89]	1.913 [1.55, 2.36]	0.504 [0.38, 0.68]	3.796 [2.38, 6.17]
VIA	371	23	20	250	78	0.23 [0.16, 0.32]	0.926 [0.89, 0.95]	0.535 [0.39, 0.68]	0.762 [0.71, 0.81]	3.074 [1.77, 5.35]	0.834 [0.75, 0.93]	3.686 [1.92, 7.07]
Gyncoluar* [youden]	371	52	54	216	49	0.515 [0.42, 0.61]	0.8 [0.75, 0.84]	0.491 [0.40, 0.58]	0.815 [0.76, 0.86]	2.574 [1.90, 3.49]	0.606 [0.49, 0.75]	4.245 [2.60, 6.94]

AUC: 0.69 [0.628, 0.751] Tp, true positive; fp, false positive; tn, true negative; fn, false negative; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; DOR, diagnostic odds ratio; AUC, area under the receiver operating curve; hrHPV, high-risk human papillomavirus; VIA , visual inspection of the uterine cervix after application of 3–5% acetic, HPV, human papillomavirus test, 95% CI, ninety-five percent confidence interval, n number of women

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Missing tests considered negative

	n	tp	fp	tn	fn	Sensitivity % 95% CI	Specificity % 95% CI	PPV % 95% CI	NPV % 95% CI	PLR 95% CI	NLR 95% CI	DOR 95% CI
HPV	371	68	93	177	33	0.67 [0.58, 0.76]	0.65 [0.59, 0.70]	0.41 [0.34, 0.49]	0.841 [0.78, 0.89]	1.913 [1.55, 2.36]	0.504 [0.38, 0.68]	3.796 [2.38, 6.17]
VIA	371	23	20	250	78	0.23 [0.16, 0.32]	0.926 [0.89, 0.95]	0.535 [0.39, 0.68]	0.762 [0.71, 0.81]	3.074 [1.77, 5.35]	0.834 [0.75, 0.93]	3.686 [1.92, 7.07]
Gyncoluar* [youden]	371	52	54	216	49	0.515 [0.42, 0.61]	0.8 [0.75, 0.84]	0.491 [0.40, 0.58]	0.815 [0.76, 0.86]	2.574 [1.90, 3.49]	0.606 [0.49, 0.75]	4.245 [2.60, 6.94]

*AUC: 0.69 [0.628, 0.751] Tp, true positive; fp, false positive; tn, true negative; fn, false negative; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; DOR, diagnostic odds ratio; AUC, area under the receiver operating curve; hrHPV, high-risk human papillomavirus; VIA , visual inspection of the uterine cervix after application of 3–5% acetic, HPV, human papillomavirus test, 95% CI, ninety-five percent confidence interval, n number of women

D. Biopsy of visible lesions only

	n	tp	fp	tn	fn	Sensitivity % 95% CI	Specificity % 95% CI	PPV % 95% CI	NPV % 95% CI	PLR 95% CI	NLR 95% CI	DOR 95% CI
HPV	371	42	120	202	7	0.86 [0.73, 0.93]	0.63 [0.57, 0.68]	0.30 [0.20, 0.33]	0.97 [0.93, 0.98]	2.30 [1.92, 2.76]	0.23 [0.11, 0.45]	10.10 [4.40, 23.20]
VIA	373	22	21	303	27	0.45 [0.32, 0.59]	0.94 [0.90, 0.96]	0.51 [0.37, 0.65]	0.92 [0.88, 0.94]	6.93 [4.13, 11.62]	0.60 [0.46, 0.76]	11.76 [5.75, 24.05]
Gyncoluar* [youden]	373	46	60	264	3	0.94 [0.84, 0.98]	0.82 [0.77, 0.85]	0.43 [0.34, 0.53]	0.99 [0.97, 0.99]	5.07 [3.99, 6.44]	0.08 [0.03, 0.23]	67.47 [20.30, 224.24]

*AUC: 0.936 [0.904, 0.968] Tp, true positive; fp, false positive; tn, true negative; fn, false negative; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; DOR, diagnostic odds ratio; AUC, area under the receiver operating curve; hrHPV, high-risk human papillomavirus; VIA , visual inspection of the uterine cervix after application of 3–5% acetic, HPV, human papillomavirus test, 95% CI, ninety-five percent confidence interval, n number of women

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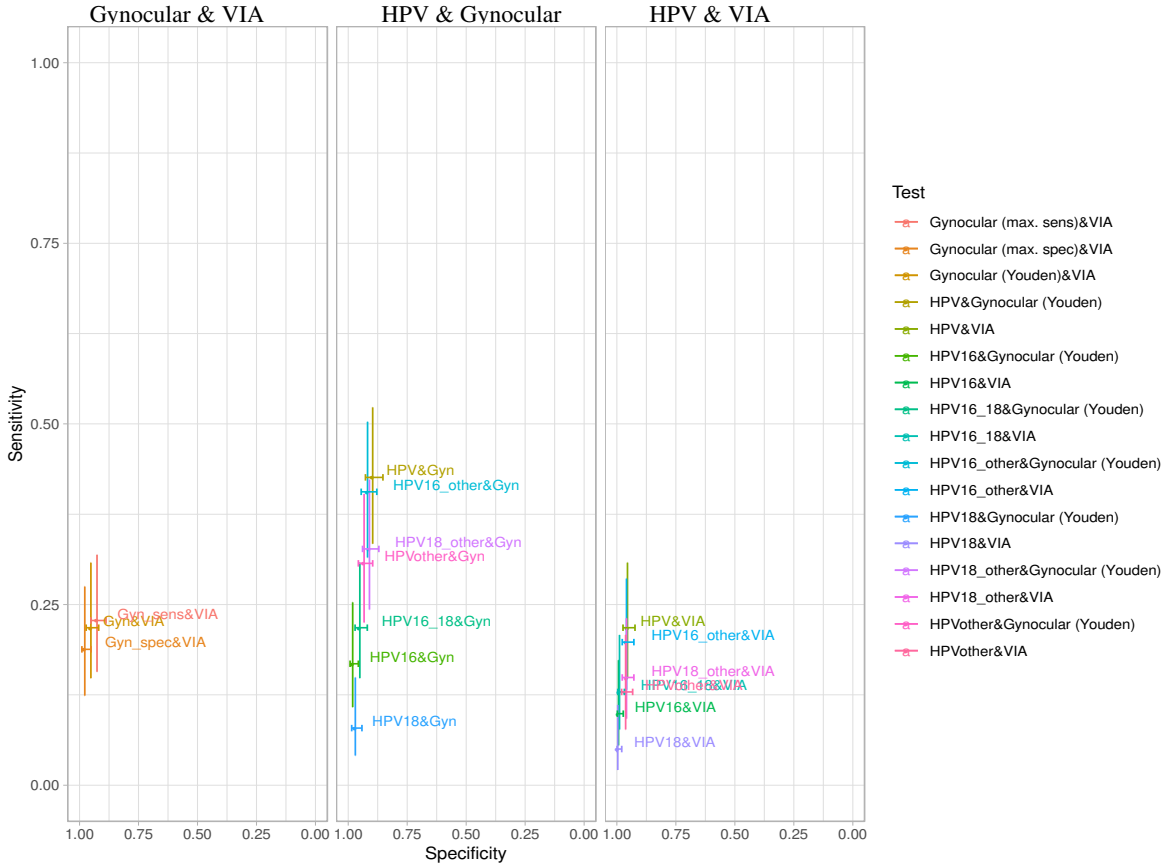
S12. Supplementary material 12: Sensitivity and specificity of combination test screening strategies for prevalent CIN2



Max.spec= using a threshold that maximises specificity. Max.sens= using a threshold that maximises sensitivity. HPV16= human papillomavirus subtype 16. HPV18= human papillomavirus subtypes 18 and 45. HPVother= human papillomavirus other high-risk subtypes pooled -31, 33, 35, 39, 51, 52, 56, 58, 59, 66, and 68. hrHPV= high-risk human papillomavirus. VIA= visual inspection of the uterine cervix after application of 3–5% acetic.

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S13. Supplementary material 13: Calculation of combined tests – alternate calculation
Only participants who test positive to the first test are included in the denominator in these analyses – this correlates to the clinical scenario when you assess accuracy of a test at a referral site for example a colposcopy clinic.

A. Sequential combination of two tests

Test 1	Test 2	n	TP	FP	TN	FN	Sens. [CI]	Spec. [CI]	PPV [CI]	NPV [CI]	PLR [CI]	NLR [CI]
HPV [pos.]	Gynocular [Youden]	161 [neg.:93; pos.:68]	43	28	65	25	0.632 [0.514, 0.737]	0.699 [0.599, 0.783]	0.606 [0.489, 0.711]	0.722 [0.622, 0.804]	2.1 [1.467, 3.007]	0.526 [0.375, 0.738]
	Gynocular [max. spec]	161 [neg.:93; pos.:68]	27	10	83	41	0.397 [0.289, 0.516]	0.892 [0.813, 0.941]	0.73 [0.57, 0.846]	0.669 [0.583, 0.746]	3.693 [1.919, 7.107]	0.676 [0.55, 0.83]
	VIA	161 [neg.:93; pos.:68]	22	12	81	46	0.324 [0.224, 0.442]	0.871 [0.788, 0.925]	0.647 [0.479, 0.785]	0.638 [0.551, 0.716]	2.507 [1.335, 4.708]	0.777 [0.647, 0.932]
	HPV16	161 [neg.:93; pos.:68]	20	19	74	48	0.294 [0.199, 0.411]	0.796 [0.703, 0.865]	0.513 [0.362, 0.661]	0.607 [0.518, 0.689]	1.44 [0.835, 2.482]	0.887 [0.737, 1.067]
	HPV16 18	161 [neg.:93; pos.:68]	28	40	53	40	0.412 [0.303, 0.53]	0.57 [0.468, 0.666]	0.412 [0.303, 0.53]	0.57 [0.468, 0.666]	0.957 [0.663, 1.383]	1.032 [0.791, 1.347]
	HPV16 other	161 [neg.:93; pos.:68]	65	76	17	3	0.956 [0.878, 0.985]	0.183 [0.117, 0.273]	0.461 [0.381, 0.543]	0.85 [0.64, 0.948]	1.17 [1.049, 1.304]	0.241 [0.074, 0.791]
	HPV18	161 [neg.:93; pos.:68]	11	21	72	57	0.162 [0.093, 0.267]	0.774 [0.679, 0.847]	0.344 [0.204, 0.517]	0.558 [0.472, 0.641]	0.716 [0.371, 1.385]	1.083 [0.931, 1.26]
	HPV18 other	161 [neg.:93; pos.:68]	56	79	14	12	0.824 [0.716, 0.896]	0.151 [0.092, 0.237]	0.415 [0.335, 0.499]	0.538 [0.355, 0.712]	0.969 [0.843, 1.114]	1.172 [0.579, 2.372]
	HPVother	161 [neg.:93; pos.:68]	53	62	31	15	0.779 [0.667, 0.862]	0.333 [0.246, 0.434]	0.461 [0.373, 0.552]	0.674 [0.53, 0.791]	1.169 [0.965, 1.416]	0.662 [0.389, 1.126]
HPV16 [pos.]	Gynocular [Youden]	39 [neg.:19; pos.:20]	17	5	14	3	0.85 [0.64, 0.948]	0.737 [0.512, 0.882]	0.773 [0.566, 0.899]	0.824 [0.59, 0.938]	3.23 [1.489, 7.008]	0.204 [0.069, 0.598]

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Test 1	Test 2	n	TP	FP	TN	FN	Sens. [CI]	Spec. [CI]	PPV [CI]	NPV [CI]	PLR [CI]	NLR [CI]
	Gynocular [max. spec]	39 [neg.:19; pos.:20]	11	2	17	9	0.55 [0.342, 0.742]	0.895 [0.686, 0.971]	0.846 [0.578, 0.957]	0.654 [0.462, 0.806]	5.225 [1.328, 20.553]	0.503 [0.302, 0.836]
	VIA	39 [neg.:19; pos.:20]	10	2	17	10	0.5 [0.299, 0.701]	0.895 [0.686, 0.971]	0.833 [0.552, 0.953]	0.63 [0.442, 0.785]	4.75 [1.192, 18.923]	0.559 [0.351, 0.889]
HPV16 18 [pos.]	Gynocular [Youden]	68 [neg.:40; pos.:28]	22	13	27	6	0.786 [0.605, 0.898]	0.675 [0.52, 0.799]	0.629 [0.463, 0.768]	0.818 [0.656, 0.914]	2.418 [1.486, 3.933]	0.317 [0.151, 0.666]
	Gynocular [max. spec]	68 [neg.:40; pos.:28]	14	4	36	14	0.5 [0.326, 0.674]	0.9 [0.769, 0.96]	0.778 [0.548, 0.91]	0.72 [0.583, 0.825]	5 [1.838, 13.602]	0.556 [0.378, 0.816]
	VIA	68 [neg.:40; pos.:28]	13	3	37	15	0.464 [0.026, 0.199]	0.925 [0.358, 0.705]	0.812 [0.066, 0.43]	0.712 [0.183, 0.423]	6.19 [0.051, 0.515]	0.579 [1.21, 2.465]
HPV16 other [pos.]	Gynocular [Youden]	141 [neg.:76; pos.:65]	41	22	54	24	0.631 [0.509, 0.738]	0.711 [0.6, 0.8]	0.651 [0.528, 0.757]	0.692 [0.583, 0.784]	2.179 [1.463, 3.245]	0.52 [0.367, 0.736]
	Gynocular [max. spec]	141 [neg.:76; pos.:65]	25	8	68	40	0.385 [0.276, 0.506]	0.895 [0.806, 0.946]	0.758 [0.59, 0.872]	0.63 [0.536, 0.715]	3.654 [1.771, 7.537]	0.688 [0.559, 0.846]
	VIA	141 [neg.:76; pos.:65]	20	11	65	45	0.308 [0.209, 0.428]	0.855 [0.759, 0.917]	0.645 [0.469, 0.789]	0.591 [0.497, 0.678]	2.126 [1.102, 4.101]	0.809 [0.672, 0.976]
HPV18 [pos.]	Gynocular [Youden]	32 [neg.:21; pos.:11]	8	8	13	3	0.727 [0.434, 0.903]	0.619 [0.409, 0.792]	0.5 [0.28, 0.72]	0.812 [0.57, 0.934]	1.909 [0.992, 3.673]	0.441 [0.159, 1.224]
	Gynocular [max. spec]	32 [neg.:21; pos.:11]	5	2	19	6	0.455 [0.027, 0.289]	0.905 [0.28, 0.787]	0.714 [0.082, 0.641]	0.76 [0.115, 0.434]	4.773 [0.048, 0.91]	0.603 [0.95, 2.895]
	VIA	32 [neg.:21; pos.:11]	5	1	20	6	0.455 [0.008, 0.227]	0.952 [0.28, 0.787]	0.833 [0.03, 0.564]	0.769 [0.11, 0.421]	9.545 [0.014, 0.789]	0.573 [1.01, 3.02]
HPV18 other [pos.]	Gynocular [Youden]	135 [neg.:79; pos.:56]	33	24	55	23	0.589 [0.459, 0.708]	0.696 [0.588, 0.787]	0.579 [0.45, 0.698]	0.705 [0.596, 0.795]	1.94 [1.301, 2.891]	0.59 [0.417, 0.834]

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Test 1	Test 2	n	TP	FP	TN	FN	Sens. [CI]	Spec. [CI]	PPV [CI]	NPV [CI]	PLR [CI]	NLR [CI]
	Gynocular [max. spec]	135 [neg.:79; pos.:56]	20	9	70	36	0.357 [0.245, 0.488]	0.886 [0.797, 0.939]	0.69 [0.508, 0.827]	0.66 [0.566, 0.744]	3.135 [1.544, 6.366]	0.726 [0.588, 0.896]
	VIA	135 [neg.:79; pos.:56]	15	11	68	41	0.268 [0.17, 0.396]	0.861 [0.768, 0.92]	0.577 [0.389, 0.745]	0.624 [0.53, 0.709]	1.924 [0.957, 3.869]	0.851 [0.709, 1.02]
HPVother [pos.]	Gynocular [Youden]	115 [neg.:62; pos.:53]	31	18	44	22	0.585 [0.451, 0.707]	0.71 [0.587, 0.808]	0.633 [0.493, 0.753]	0.667 [0.547, 0.768]	2.015 [1.284, 3.161]	0.585 [0.409, 0.836]
	Gynocular [max. spec]	115 [neg.:62; pos.:53]	18	7	55	35	0.34 [0.227, 0.474]	0.887 [0.785, 0.944]	0.72 [0.524, 0.857]	0.611 [0.508, 0.705]	3.008 [1.362, 6.643]	0.744 [0.602, 0.921]
	VIA	115 [neg.:62; pos.:53]	13	10	52	40	0.245 [0.149, 0.376]	0.839 [0.728, 0.91]	0.565 [0.368, 0.744]	0.565 [0.463, 0.662]	1.521 [0.727, 3.182]	0.9 [0.745, 1.086]
Gynocular [max. sens] [pos.]	HPV	358 [neg.:260; pos.:98]	67	91	169	31	0.684 [0.586, 0.767]	0.65 [0.59, 0.705]	0.424 [0.35, 0.502]	0.845 [0.788, 0.889]	1.953 [1.578, 2.418]	0.487 [0.359, 0.66]
	HPV16	358 [neg.:260; pos.:98]	20	18	242	78	0.204 [0.136, 0.294]	0.931 [0.893, 0.956]	0.526 [0.373, 0.675]	0.756 [0.706, 0.8]	2.948 [1.629, 5.333]	0.855 [0.769, 0.95]
	HPV16 18	358 [neg.:260; pos.:98]	28	39	221	70	0.286 [0.206, 0.382]	0.85 [0.802, 0.888]	0.418 0.307, 0.537]	0.759 [0.707, 0.805]	1.905 [1.244, 2.917]	0.84 [0.734, 0.962]
	HPV16_other	358 [neg.:260; pos.:98]	64	74	186	34	0.653 [0.555, 0.74]	0.715 [0.658, 0.767]	0.464 [0.383, 0.547]	0.845 [0.792, 0.887]	2.295 [1.804, 2.919]	0.485 [0.366, 0.643]
	HPV18	358 [neg.:260; pos.:98]	11	21	239	87	0.112 [0.064, 0.19]	0.919 [0.88, 0.947]	0.344 [0.204, 0.517]	0.733 [0.683, 0.778]	1.39 [0.696, 2.775]	0.966 [0.892, 1.045]
	HPV18 other	358 [neg.:260; pos.:98]	55	78	182	43	0.561 [0.463, 0.655]	0.7 [0.642, 0.752]	0.414 [0.333, 0.499]	0.809 [0.752, 0.855]	1.871 [1.449, 2.415]	0.627 [0.494, 0.795]
	HPVother	358 [neg.:260; pos.:98]	52	61	199	46	0.531 [0.433, 0.626]	0.765 [0.71, 0.813]	0.46 [0.371, 0.552]	0.812 [0.759, 0.856]	2.262 [1.696, 3.016]	0.613 [0.492, 0.765]

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Test 1	Test 2	n	TP	FP	TN	FN	Sens. [CI]	Spec. [CI]	PPV [CI]	NPV [CI]	PLR [CI]	NLR [CI]
Gynocular [max. spec] [neg.]	HPV	323 [neg.:252; pos.:71]	41	83	169	30	0.577 [0.462, 0.685]	0.671 [0.61, 0.726]	0.331 [0.254, 0.417]	0.849 [0.793, 0.892]	1.753 [1.344, 2.287]	0.63 [0.474, 0.838]
	HPV16	323 [neg.:252; pos.:71]	9	17	235	62	0.127 [0.068, 0.224]	0.933 [0.895, 0.957]	0.346 [0.194, 0.538]	0.791 [0.741, 0.834]	1.879 [0.875, 4.033]	0.936 0.852, 1.029]
	HPV16 18	323 [neg.:252; pos.:71]	14	36	216	57	0.197 [0.121, 0.304]	0.857 [0.809, 0.895]	0.28 [0.175, 0.417]	0.791 [0.739, 0.835]	1.38 [0.79, 2.412]	0.937 [0.826, 1.062]
	HPV16 other	323 [neg.:252; pos.:71]	40	68	184	31	0.563 [0.448, 0.673]	0.73 [0.672, 0.781]	0.37 [0.285, 0.464]	0.856 [0.803, 0.897]	2.088 [1.565, 2.786]	0.598 [0.454, 0.787]
	HPV18	323 [neg.:252; pos.:71]	6	19	233	65	0.085 [0.039, 0.172]	0.925 [0.885, 0.951]	0.24 [0.115, 0.434]	0.782 [0.732, 0.825]	1.121 [0.465, 2.7]	0.99 [0.915, 1.072]
	HPV18 other	323 [neg.:252; pos.:71]	36	70	182	35	0.507 [0.393, 0.62]	0.722 [0.664, 0.774]	0.34 [0.256, 0.434]	0.839 [0.784, 0.882]	1.825 [1.347, 2.473]	0.683 [0.533, 0.875]
	HPVother	323 [neg.:252; pos.:71]	35	55	197	36	0.493 [0.38, 0.607]	0.782 [0.727, 0.828]	0.389 [0.295, 0.492]	0.845 [0.794, 0.886]	2.259 [1.62, 3.148]	0.649 [0.511, 0.823]
Gynocular [Youden] [neg.]	HPV	263 [neg.:214; pos.:49]	25	65	149	24	0.51 [0.375, 0.644]	0.696 [0.632, 0.754]	0.278 [0.196, 0.378]	0.861 [0.802, 0.905]	1.68 [1.194, 2.363]	0.703 [0.522, 0.949]
	HPV16	263 [neg.:214; pos.:49]	3	14	200	46	0.061 [0.021, 0.165]	0.935 [0.893, 0.961]	0.176 [0.062, 0.41]	0.813 [0.76, 0.857]	0.936 [0.28, 3.131]	1.004 [0.927, 1.088]
	HPV16_18	263 [neg.:214; pos.:49]	6	27	187	43	0.122 [0.057, 0.242]	0.874 [0.823, 0.912]	0.182 [0.086, 0.344]	0.813 [0.758, 0.858]	0.971 [0.424, 2.222]	1.004 [0.894, 1.128]
	HPV16 other	263 [neg.:214; pos.:49]	24	54	160	25	0.49 [0.356, 0.625]	0.748 [0.685, 0.801]	0.308 [0.216, 0.417]	0.865 [0.808, 0.907]	1.941 [1.344, 2.802]	0.682 [0.513, 0.908]
	HPV18	263 [neg.:214; pos.:49]	3	13	201	46	0.061 [0.021, 0.165]	0.939 [0.899, 0.964]	0.188 [0.066, 0.43]	0.814 [0.761, 0.857]	1.008 [0.299, 3.402]	0.999 [0.923, 1.082]

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	HPV18 other	263 [neg.:214; pos.:49]	23	55	159	26	0.469 [0.337, 0.606]	0.743 [0.681, 0.797]	0.295 [0.205, 0.404]	0.859 [0.802, 0.902]	1.826 [1.255, 2.657]	0.714 [0.543, 0.94]
	HPVother	263 [neg.:214; pos.:49]	22	44	170	27	0.449 [0.319, 0.587]	0.794 [0.735, 0.843]	0.333 [0.232, 0.453]	0.863 [0.808, 0.904]	2.184 [1.454, 3.28]	0.694 [0.534, 0.901]
Gynocular [Youden] [pos.]	HPV	106 [neg.:54; pos.:52]	43	28	26	9	0.827 [0.703, 0.906]	0.481 [0.354, 0.611]	0.606 [0.489, 0.711]	0.743 [0.579, 0.858]	1.595 [1.199, 2.122]	0.359 [0.187, 0.692]
	HPV16	106 [neg.:54; pos.:52]	17	5	49	35	0.327 [0.215, 0.462]	0.907 [0.801, 0.96]	0.773 [0.566, 0.899]	0.583 [0.477, 0.683]	3.531 [1.405, 8.873]	0.742 [0.603, 0.913]
	HPV16 18	106 [neg.:54; pos.:52]	22	13	41	30	0.423 [0.299, 0.558]	0.759 [0.631, 0.854]	0.629 [0.463, 0.768]	0.577 [0.462, 0.685]	1.757 [0.994, 3.108]	0.76 [0.576, 1.002]
	HPV16 other	106 [neg.:54; pos.:52]	41	22	32	11	0.788 [0.66, 0.878]	0.593 [0.46, 0.713]	0.651 [0.528, 0.757]	0.744 [0.598, 0.851]	1.935 [1.362, 2.749]	0.357 [0.202, 0.631]
	HPV18	106 [neg.:54; pos.:52]	8	8	46	44	0.154 [0.08, 0.275]	0.852 [0.734, 0.923]	0.5 [0.28, 0.72]	0.511 [0.41, 0.612]	1.038 [0.421, 2.562]	0.993 [0.846, 1.166]
	HPV18 other	106 [neg.:54; pos.:52]	33	24	30	19	0.635 [0.499, 0.752]	0.556 [0.424, 0.68]	0.579 [0.45, 0.698]	0.612 [0.472, 0.736]	1.428 [0.994, 2.052]	0.658 [0.428, 1.011]
	HPVother	106 [neg.:54; pos.:52]	31	18	36	21	0.596 [0.461, 0.718]	0.667 [0.534, 0.778]	0.633 [0.493, 0.753]	0.632 [0.502, 0.745]	1.788 [1.154, 2.773]	0.606 [0.414, 0.886]
VIA [neg.]	Gynocular [Youden]	328 [neg.:250; pos.:78]	30	41	209	48	0.385 [0.284, 0.496]	0.836 [0.785, 0.877]	0.423 [0.315, 0.538]	0.813 [0.761, 0.856]	2.345 [1.578, 3.486]	0.736 [0.612, 0.885]
	Gynocular [max. spec]	328 [neg.:250; pos.:78]	11	10	240	67	0.141 [0.081, 0.235]	0.96 [0.928, 0.978]	0.524 [0.324, 0.717]	0.782 [0.732, 0.824]	3.526 [1.556, 7.987]	0.895 [0.815, 0.982]
	HPV	326 [neg.:248; pos.:78]	46	81	167	32	0.59 [0.479, 0.692]	0.673 [0.613, 0.729]	0.362 [0.284, 0.449]	0.839 [0.782, 0.884]	1.806 [1.396, 2.335]	0.609 [0.461, 0.806]

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Test 1	Test 2	n	TP	FP	TN	FN	Sens. [CI]	Spec. [CI]	PPV [CI]	NPV [CI]	PLR [CI]	NLR [CI]
	HPV16	326 [neg.:248; pos.:78]	10	17	231	68	0.128 [0.071, 0.22]	0.931 [0.893, 0.957]	0.37 [0.215, 0.558]	0.773 [0.722, 0.816]	1.87 [0.894, 3.914]	0.936 [0.854, 1.026]
	HPV16_18	326 [neg.:248; pos.:78]	15	37	211	63	0.192 [0.12, 0.293]	0.851 [0.801, 0.89]	0.288 [0.183, 0.423]	0.77 [0.717, 0.816]	1.289 [0.749, 2.219]	0.949 [0.842, 1.071]
	HPV16 other	326 [neg.:248; pos.:78]	45	65	183	33	0.577 [0.466, 0.68]	0.738 [0.68, 0.789]	0.409 [0.322, 0.503]	0.847 [0.793, 0.889]	2.201 [1.66, 2.919]	0.573 [0.438, 0.751]
	HPV18	326 [neg.:248; pos.:78]	6	20	228	72	0.077 [0.036, 0.158]	0.919 [0.879, 0.947]	0.231 [0.11, 0.421]	0.76 [0.709, 0.805]	0.954 [0.397, 2.291]	1.004 [0.933, 1.081]
	HPV18_other	326 [neg.:248; pos.:78]	41	68	180	37	0.526 [0.416, 0.633]	0.726 [0.667, 0.778]	0.376 [0.291, 0.47]	0.829 [0.774, 0.874]	1.917 [1.431, 2.568]	0.654 [0.511, 0.836]
	HPVother	326 [neg.:248; pos.:78]	40	52	196	38	0.513 [0.404, 0.621]	0.79 [0.735, 0.836]	0.435 [0.338, 0.537]	0.838 [0.785, 0.879]	2.446 [1.768, 3.383]	0.616 [0.487, 0.781]
VIA [pos.]	Gynocular [Youden]	43 [neg.:20; pos.:23]	22	13	7	1	0.957 [0.79, 0.992]	0.35 [0.181, 0.567]	0.629 [0.463, 0.768]	0.875 [0.529, 0.978]	1.472 [1.055, 2.053]	0.124 [0.017, 0.925]
	Gynocular [max. spec]	43 [neg.:20; pos.:23]	19	6	14	4	0.826 [0.629, 0.93]	0.7 [0.481, 0.855]	0.76 [0.566, 0.885]	0.778 [0.548, 0.91]	2.754 [1.374, 5.519]	0.248 [0.097, 0.633]
	HPV	43 [neg.:20; pos.:23]	22	12	8	1	0.957 [0.79, 0.992]	0.4 [0.219, 0.613]	0.647 [0.479, 0.785]	0.889 [0.565, 0.98]	1.594 [1.103, 2.304]	0.109 [0.015, 0.796]
	HPV16	43 [neg.:20; pos.:23]	10	2	18	13	0.435 [0.256, 0.632]	0.9 [0.699, 0.972]	0.833 [0.552, 0.953]	0.581 [0.408, 0.736]	4.348 [1.078, 17.542]	0.628 [0.426, 0.925]
	HPV16 18	43 [neg.:20; pos.:23]	13	3	17	10	0.565 [0.368, 0.744]	0.85 [0.64, 0.948]	0.812 [0.57, 0.934]	0.63 [0.442, 0.785]	3.768 [1.25, 11.356]	0.512 [0.31, 0.844]
	HPV16 other	43 [neg.:20; pos.:23]	20	11	9	3	0.87 [0.679, 0.955]	0.45 [0.258, 0.658]	0.645 [0.469, 0.789]	0.75 [0.468, 0.911]	1.581 [1.032, 2.423]	0.29 [0.091, 0.926]

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Test 1	Test 2	n	TP	FP	TN	FN	Sens. [CI]	Spec. [CI]	PPV [CI]	NPV [CI]	PLR [CI]	NLR [CI]
	HPV18	43 [neg.:20; pos.:23]	5	1	19	18	0.217 [0.097, 0.419]	0.95 [0.764, 0.991]	0.833 [0.436, 0.97]	0.514 [0.359, 0.666]	4.348 [0.553, 34.171]	0.824 [0.65, 1.045]
	HPV18 other	43 [neg.:20; pos.:23]	15	11	9	8	0.652 [0.449, 0.812]	0.45 [0.258, 0.658]	0.577 [0.389, 0.745]	0.529 [0.31, 0.738]	1.186 [0.722, 1.948]	0.773 [0.369, 1.62]
	HPVother	43 [neg.:20; pos.:23]	13	10	10	10	0.565 [0.368, 0.744]	0.5 [0.299, 0.701]	0.565 [0.368, 0.744]	0.5 [0.299, 0.701]	1.13 [0.642, 1.991]	0.87 [0.459, 1.649]

Tp, true positive; fp, false positive; tn, true negative; fn, false negative; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; DOR, diagnostic odds ratio; AUC, area under the receiver operating curve; hrHPV, high-risk human papillomavirus; VIA , visual inspection of the uterine cervix after application of 3–5% acetic, HPV, human papillomavirus test, 95% CI, ninety-five percent confidence interval, n number of women

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S14. Supplementary material 14: Subgroup analyses

A. HPV test

Primary measures					
Data	Group	Number of women [0: <CIN2; 1: CIN2+]	Sens [CI]	Spec [CI]	ratio-sens
Age	[0,25]	33 [0:22; 1:11]			
	[25,35]	122 [0:87; 1:35]	0.686 [0.52, 0.814]	0.575 [0.47, 0.673]	ref
	[35,45]	133 [0:98; 1:35]	0.657 [0.492, 0.792]	0.724 [0.629, 0.803]	0.96 [0.69, 1.33]
	[45,70]	75 [0:57; 1:18]	0.667 [0.437, 0.837]	0.719 [0.592, 0.819]	0.97 [0.65, 1.44]
Menopause	No	317 [0:227; 1:90]	0.689 [0.587, 0.775]	0.648 [0.583, 0.707]	ref
	Yes	52 [0:41; 1:11]	0.545 [0.28, 0.787]	0.683 [0.53, 0.804]	0.79 [0.45, 1.38]
Education	Did not finished secondary	303 [0:217; 1:86]	0.674 [0.57, 0.764]	0.664 [0.598, 0.723]	ref
	Finished secondary	53 [0:41; 1:12]	0.667 [0.391, 0.862]	0.585 [0.434, 0.722]	0.99 [0.65, 1.51]
	More than secondary	13 [0:10; 1:3]			
Contraception method	Condoms method	36 [0:30; 1:6]			
	long acting reversible contraception	86 [0:61; 1:25]	0.64 [0.445, 0.798]	0.689 [0.564, 0.791]	ref
	Oral hormonal	16 [0:13; 1:3]			
	Withdrawal & others	4 [0:3; 1:1]			
	none	227 [0:161; 1:66]	0.682 [0.562, 0.782]	0.64 [0.563, 0.71]	1.07 [0.76, 1.49]
Parity categories	0	11 [0:8; 1:3]			
	1-3	181 [0:122; 1:59]	0.746 [0.622, 0.839]	0.615 [0.526, 0.696]	ref
	>3	177 [0:138; 1:39]	0.59 [0.434, 0.729]	0.674 [0.592, 0.746]	0.79 [0.59, 1.07]
Trichomoniasis result	Negative	300 [0:221; 1:79]	0.671 [0.561, 0.764]	0.656 [0.591, 0.716]	ref
	Positive	69 [0:47; 1:22]	0.682 [0.473, 0.836]	0.638 [0.495, 0.76]	1.02 [0.73, 1.41]
	Undetermined	0 [:]			
On ART	No	1 [0:1]			
	Yes	368 [0:267; 1:101]	0.673 [0.577, 0.757]	0.652 [0.593, 0.706]	ref
CD4 count [cells/mm3]	<200	12 [0:5; 1:7]			
	[200,350]	44 [0:32; 1:12]	0.75 [0.468, 0.911]	0.562 [0.393, 0.718]	ref
	[350,500]	95 [0:70; 1:25]	0.6 [0.407, 0.766]	0.629 [0.511, 0.732]	0.8 [0.51, 1.26]
	500 and more	217 [0:160; 1:57]	0.649 [0.519, 0.76]	0.675 [0.599, 0.743]	0.87 [0.59, 1.26]
HIV RNA load [copies/mL]	< 1000 copies/ml	340 [0:251; 1:89]	0.64 [0.537, 0.732]	0.665 [0.605, 0.721]	ref
	1000 and more	29 [0:17; 1:12]			
History of treatment for precancer	No	362 [0:263; 1:99]	0.667 [0.569, 0.752]	0.65 [0.591, 0.705]	ref
	Yes	7 [0:5; 1:2]			

tp, true positive; fp, false positive; tn, true negative; fn, false negative; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; DOR, diagnostic odds ratio; AUC, area under the receiver operating curve; hrHPV, high-risk human papillomavirus; VIA , visual inspection of the uterine cervix after application of 3–5% acetic, HPV, human papillomavirus test, 95% CI, ninety-five percent confidence interval, ART= antiretroviral therapy. CD4= cluster of differentiation 4. HIV RNA= human immunodeficiency virus ribonucleic acid, CIN2+= Cervical intra-epithelial neoplasia grade two and above. <CIN2= Cervical intra-epithelial neoplasia grade one and below. copies/mL= copies per millilitre. cells/mm3= cell per cubic millimetre.

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Figures

B. VIA

Primary measures					
Data	Group	Number of women [0: <CIN2; 1: CIN2+]	Sens [CI]	Spec [CI]	ratio-sens
Age	[0,25]	33 [0:22; 1:11]			
	[25,35]	123 [0:88; 1:35]	0.286 [0.163, 0.451]	0.932 [0.859, 0.968]	ref
	[35,45]	133 [0:98; 1:35]	0.2 [0.1, 0.359]	0.949 [0.886, 0.978]	0.7 [0.3, 1.63]
	[45,70]	75 [0:57; 1:18]	0.333 [0.163, 0.563]	0.93 [0.833, 0.972]	1.17 [0.5, 2.7]
Menopause	No	319 [0:229; 1:90]	0.244 [0.167, 0.342]	0.921 [0.879, 0.95]	ref
	Yes	52 [0:41; 1:11]	0.091 [0.016, 0.377]	0.951 [0.839, 0.987]	0.37 [0.06, 2.5]
Education	Did not finished secondary	305 [0:219; 1:86]	0.209 [0.137, 0.307]	0.918 [0.874, 0.947]	ref
	Finished secondary	53 [0:41; 1:12]	0.417 [0.193, 0.68]	0.951 [0.839, 0.987]	1.99 [0.91, 4.37]
	More than secondary	13 [0:10; 1:3]			
Contraception method	Condoms method	36 [0:30; 1:6]			
	long-acting reversible contraception	88 [0:63; 1:25]	0.12 [0.042, 0.3]	0.952 [0.869, 0.984]	ref
	Oral hormonal	16 [0:13; 1:3]			
	Withdrawal & others	4 [0:3; 1:1]			
	none	227 [0:161; 1:66]	0.273 [0.18, 0.39]	0.907 [0.852, 0.943]	2.27 [0.73, 7.05]
Parity categories	0	11 [0:8; 1:3]			
	1-3	183 [0:124; 1:59]	0.254 [0.161, 0.378]	0.911 [0.848, 0.95]	ref
	>3	177 [0:138; 1:39]	0.205 [0.108, 0.355]	0.942 [0.89, 0.97]	0.81 [0.38, 1.72]
Trichomoniasis result	Negative	302 [0:223; 1:79]	0.228 [0.149, 0.332]	0.919 [0.876, 0.948]	ref
	Positive	69 [0:47; 1:22]	0.227 [0.101, 0.434]	0.957 [0.858, 0.988]	1 [0.42, 2.38]
	Undetermined	0 [:]			
On ART	No	1 [0:1]			
	Yes	370 [0:269; 1:101]	0.228 [0.157, 0.319]	0.926 [0.888, 0.951]	ref
CD4 count [cells/mm3]	<200	12 [0:5; 1:7]			
	[200,350]	44 [0:32; 1:12]	0.25 [0.089, 0.532]	0.844 [0.682, 0.931]	ref
	[350,500]	95 [0:70; 1:25]	0.16 [0.064, 0.347]	0.943 [0.862, 0.978]	0.64 [0.17, 2.42]
	500 and more	219 [0:162; 1:57]	0.193 [0.111, 0.313]	0.932 [0.883, 0.962]	0.77 [0.25, 2.35]
HIV RNA load [copies/mL]	< 1000 copies/ml	342 [0:253; 1:89]	0.213 [0.141, 0.31]	0.933 [0.895, 0.958]	ref
	1000 and more	29 [0:17; 1:12]			
History of treatment for precancer	No	364 [0:265; 1:99]	0.222 [0.152, 0.314]	0.925 [0.886, 0.951]	ref
	Yes	7 [0:5; 1:2]			

tp, true positive; fp, false positive; tn, true negative; fn, false negative; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; DOR, diagnostic odds ratio; AUC, area under the receiver operating curve; hrHPV, high-risk human papillomavirus; VIA , visual inspection of the uterine cervix after application of 3–5% acetic, HPV, human papillomavirus test, 95% CI, ninety-five percent confidence interval, ART= antiretroviral therapy. CD4= cluster of differentiation 4. HIV RNA= human immunodeficiency virus ribonucleic acid, CIN2+= Cervical intra-epithelial neoplasia grade two and above. <CIN2= Cervical intra-epithelial neoplasia grade one and below. copies/mL= copies per millilitre. cells/mm3= cell per cubic millimetre.

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C. Gynocular

Primary measures					
Data	Group	Number of women [0: <CIN2; 1: CIN2+]	Sens [CI]	Spec [CI]	ratio-sens
Age	[0,25]	33 [0:22; 1:11]			
	[25,35]	123 [0:88; 1:35]	0.514 [0.356, 0.67]	0.784 [0.687, 0.857]	ref
	[35,45]	133 [0:98; 1:35]	0.486 [0.33, 0.644]	0.786 [0.695, 0.855]	0.94 [0.59, 1.51]
	[45,70]	75 [0:57; 1:18]	0.611 [0.386, 0.797]	0.86 [0.747, 0.927]	1.19 [0.73, 1.94]
Menopause	No	319 [0:229; 1:90]	0.522 [0.42, 0.622]	0.799 [0.742, 0.846]	ref
	Yes	52 [0:41; 1:11]	0.455 [0.213, 0.72]	0.805 [0.66, 0.898]	0.87 [0.44, 1.71]
Education	Did not finished secondary	305 [0:219; 1:86]	0.535 [0.43, 0.637]	0.813 [0.756, 0.859]	ref
	Finished secondary	53 [0:41; 1:12]	0.5 [0.254, 0.746]	0.732 [0.581, 0.843]	0.93 [0.51, 1.7]
	More than secondary	13 [0:10; 1:3]			
Contraception method	Condoms method	36 [0:30; 1:6]			
	long acting reversible contraception	88 [0:63; 1:25]	0.44 [0.267, 0.629]	0.841 [0.732, 0.911]	ref
	Oral hormonal	16 [0:13; 1:3]			
	Withdrawal & others	4 [0:3; 1:1]			
	none	227 [0:161; 1:66]	0.5 [0.383, 0.617]	0.776 [0.706, 0.834]	1.14 [0.69, 1.88]
Parity categories	0	11 [0:8; 1:3]			
	1-3	183 [0:124; 1:59]	0.508 [0.384, 0.632]	0.79 [0.71, 0.853]	ref
	>3	177 [0:138; 1:39]	0.564 [0.41, 0.707]	0.812 [0.738, 0.868]	1.11 [0.76, 1.61]
Trichomoniasis result	Negative	302 [0:223; 1:79]	0.506 [0.398, 0.614]	0.807 [0.75, 0.854]	ref
	Positive	69 [0:47; 1:22]	0.545 [0.347, 0.731]	0.766 [0.628, 0.864]	1.08 [0.69, 1.67]
	Undetermined	0 [:]			
On ART	No	1 [0:1]			
	Yes	370 [0:269; 1:101]	0.515 [0.419, 0.61]	0.799 [0.747, 0.843]	ref
CD4 count [cells/mm3]	<200	12 [0:5; 1:7]			
	[200,350]	44 [0:32; 1:12]	0.667 [0.391, 0.862]	0.75 [0.579, 0.867]	ref
	[350,500]	95 [0:70; 1:25]	0.4 [0.234, 0.593]	0.757 [0.645, 0.842]	0.6 [0.32, 1.12]
	500 and more	219 [0:162; 1:57]	0.491 [0.366, 0.617]	0.827 [0.762, 0.878]	0.74 [0.46, 1.19]
HIV RNA load [copies/mL]	< 1000 copies/ml	342 [0:253; 1:89]	0.494 [0.393, 0.596]	0.822 [0.77, 0.864]	ref
	1000 and more	29 [0:17; 1:12]			
History of treatment for precancer	No	364 [0:265; 1:99]	0.505 [0.408, 0.601]	0.796 [0.744, 0.84]	ref
	Yes	7 [0:5; 1:2]			

tp, true positive; fp, false positive; tn, true negative; fn, false negative; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; DOR, diagnostic odds ratio; AUC, area under the receiver operating curve; hrHPV, high-risk human papillomavirus; VIA , visual inspection of the uterine cervix after application of 3–5% acetic, HPV, human papillomavirus test, 95% CI, ninety-five percent confidence interval, ART= antiretroviral therapy. CD4= cluster of differentiation 4. HIV RNA= human immunodeficiency virus ribonucleic acid, CIN2+= Cervical intra-epithelial neoplasia grade two and above. <CIN2= Cervical intra-epithelial neoplasia grade one and below. copies/mL= copies per millilitre. cells/mm3= cell per cubic millimetre.

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S15. Supplementary material 15: Investigation of interaction between patient characteristics on the association between diagnostic test and disease status

A. Interaction between patient characteristics and HPV

	Crude			
	N	Odds Ratios	CI	Pval
HPV x Age [per 10 years]	363	1.363	[0.783, 2.4]	0.28
HPV x CD4 count [cells/mm3] [per 1000 cells]	368	0.299	[0.047, 1.976]	0.2
HPV x Contraception use	369	1.036	[0.379, 2.884]	0.95
HPV x Education	369	0.759	[0.208, 3.029]	0.68
HPV x History of treatment for precancer				
HPV x HIV RNA load [copies/mL]	369	2.761	[0.37, 58.118]	0.39
HPV x Menopause	369	0.635	[0.148, 2.829]	0.54
HPV x Parity	369	0.842	[0.656, 1.08]	0.18
HPV x Trichomoniasis result	369	0.972	[0.297, 3.376]	0.96

HPV, human papillomavirus test, 95% CI, ninety-five percent confidence interval, CD4= cluster of differentiation 4. HIV RNA= human immunodeficiency virus ribonucleic acid, copies/mL= copies per millilitre. cells/mm3= cell per cubic millimetre.

* We do not present adjusted odds ratios in this table because there were no p values <0.05, which was the criterion for inclusion in the multivariable analysis.

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B. Interaction between patient characteristics and VIA

	Crude				Adjusted				
	N	Odds Ratios	CI	Pval	N	Odds Ratios	CI	Pval	Adj. model
VIA x Age [per 10 years]	364	1.642	[0.744, 3.728]	0.22					
VIA x CD4 count [cells/mm3] [per 1000 cells]	370	0.376	[0.03, 3.991]	0.43					
VIA x Contraception use	371	0.950	[0.205, 4.395]	0.95					
VIA x Education	371	4.144	[0.671, 35.443]	0.14	363	3.941	[0.629, 34.062]	0.16	out1 ~ education_level.factor3 + via + parity_nr + education_level.factor3:via
VIA x History of treatment for precancer									
VIA x HIV RNA load [copies/mL]	371	0.619	[0.096, 4.367]	0.62					
VIA x Menopause	371	0.514	[0.021, 6.462]	0.61					
VIA x Parity	371	1.009	[0.715, 1.409]	0.96					
VIA x Trichomoniasis result	371	1.969	[0.331, 16.305]	0.48	363	4.767	[0.6, 101.918]	0.19	out1 ~ STI_BL + via + parity_nr + hiv_rna_cat_subgrp + STI_BL:via

HPV, human papillomavirus test, 95% CI, ninety-five percent confidence interval, CD4= cluster of differentiation 4. HIV RNA= human immunodeficiency virus ribonucleic acid, copies/mL= copies per millilitre. cells/mm3= cell per cubic millimetre

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Figures

C. Interaction between patient characteristics and Gynocular [using Youden cut-off]

	Crude				Adjusted				
	N	Odds Ratios	CI	Pval	N	Odds Ratios	CI	Pval	Adj. model
Gynocular x Age [per 10 years]	364	1.224	[0.701, 2.145]	0.48					
Gynocular x CD4 count [cells/mm3] [per 1000 cells]	370	1.508	[0.213, 11.086]	0.68					
Gynocular x Contraception use	371	1.729	[0.618, 4.911]	0.3					
Gynocular x Education	371	0.390	[0.101, 1.465]	0.16					
Gynocular x History of treatment for precancer									
Gynocular x HIV RNA load [copies/mL]	371	0.393	[0.08, 2.121]	0.26					
Gynocular x Menopause	371	0.791	[0.171, 3.656]	0.76					
Gynocular x Parity	371	1.212	[0.942, 1.566]	0.14	363	1.237	[0.961, 1.603]	0.1	out1 ~ parity nr + swede cat1 + parity_nr:swede_cat1
Gynocular x Trichomoniasis result	371	0.915	[0.276, 3.14]	0.89					

HPV, human papillomavirus test, 95% CI, ninety-five percent confidence interval, CD4= cluster of differentiation 4. HIV RNA= human immunodeficiency virus ribonucleic acid, copies/mL= copies per millilitre. cells/mm3= cell per cubic millimetre