

# **Triage of HPV positivity in a high HIV prevalence setting: A prospective cohort study comparing visual triage methods and HPV genotype restriction in Botswana**

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## **ABSTRACT**

**Objective:** Guidelines for effective triage following positive primary high-risk human papillomavirus (HPV) screening in low- and middle-income countries with high human immunodeficiency virus (HIV)-prevalence have not previously been established. In the present study, we evaluated the performance of three triage methods for positive HPV results in women living with HIV (WLHIV) and without HIV in Botswana.

**Methods:** We conducted baseline enrollment of a prospective cohort study from February 2021 to August 2022 in South-East District, Botswana. Non-pregnant women aged 25 or older with an intact cervix and no prior diagnosis of cervical cancer were systematically consented for enrollment, with enrichment of the cohort for WLHIV. Those who consented completed a questionnaire and then collected vaginal self-samples for HPV testing. Primary HPV testing for 15 individual genotypes was conducted using Atila AmpFire® HPV assay. Those with positive HPV results returned for a triage visit where all underwent visual inspection with acetic acid (VIA), colposcopy, and biopsy. Triage strategies with VIA, colposcopy and 8-type HPV genotype restriction (16/18/31/33/35/45/52/58), separately and in combination, were compared using histopathology as the gold standard in diagnosing cervical intraepithelial neoplasia (CIN) 2 or worse (CIN2+).

**Results:** Among 2969 women enrolled, 1480 (50%) tested HPV positive. The cohort included 1478 (50%) WLHIV; 99% were virologically suppressed after a mean of 8 years on antiretroviral therapy. In total, 1269 (86%) women had histopathology data for analysis. Among WLHIV who tested positive for HPV, 131 (19%) of 688 had CIN2+ compared with 71 (12%) of 581 in women without HIV. Screening by 8-type HPV genotype restriction was more sensitive as triage to detect CIN2+ in WLHIV 87.79% (95% CI: 80.92–92.85) and women without HIV 85.92% (95% CI: 75.62–93.03) when compared with VIA (WLHIV 62.31% [95% CI: 53.39–70.65], women without HIV 44.29% [95% CI: 32.41–56.66]) and colposcopy (WLHIV 70.77% [95% CI: 62.15–78.41], women without HIV 45.71% [95% CI: 33.74–58.06]). However, 8-type HPV genotype restriction had low specificity in WLHIV of 30.88% (95% CI: 27.06–34.90) and women without HIV 37.06% (95% CI: 32.85–41.41). These results were similar when CIN3+ was used as the outcome. When combining 8-type HPV genotype restriction with VIA as the triage strategy, there was improved specificity to detect CIN2+ in WLHIV of 81.65% (95% CI: 78.18–84.79) but dramatically reduced sensitivity of 56.15% (95% CI: 47.18–64.84).

**Conclusions:** Eight-type HPV genotype restriction is a promising component of effective triage for HPV positivity. However, novel triage strategies in LMICs with high HIV prevalence may be needed to avoid the trade-off between sensitivity and specificity with currently available options.

**Clinical trials registration:** This study is registered on Clinicaltrials.gov no. NCT04242823, <https://clinicaltrials.gov/ct2/show/NCT04242823>.

## 1 INTRODUCTION

Cervical cancer is the leading cause of cancer-related death among women in countries with the highest burden of human immunodeficiency virus (HIV).<sup>1</sup> HIV confers a higher risk for developing cervical precancer and cancer due to its biological interaction with human papillomavirus (HPV), which causes over 99% of cervical cancers.<sup>2–4</sup> Women living with HIV (WLHIV) are more likely to have persistent HPV infection, infection with multiple HPV types, and reactivation of dormant disease, which contributes to their elevated risk for cervical cancer.<sup>2–4</sup> In addition, poor access to high-performance cervical screening is common in countries most affected by HIV.<sup>5</sup> Visual inspection with acetic acid (VIA) is the most common method of screening in low- and middle-income countries (LMICs),<sup>6</sup> yet is only 56% sensitive in detecting cervical disease in WLHIV.<sup>7</sup>

Primary HPV screening offers the potential to rapidly increase access to high-performance cervical screening globally, but it has low specificity and requires subsequent triage.<sup>8,9</sup> Global guidelines recommend VIA triage of HPV positivity where other alternatives do not exist, which is the case for most LMICs.<sup>10,11</sup> VIA triage of primary HPV testing has poor sensitivity for detection of cervical dysplasia, and has been shown to essentially eliminate the benefit of primary HPV testing in both women with and without HIV.<sup>7,12,13</sup>

Alternative HPV triage strategies for LMICs are urgently needed. One such strategy that has been explored is limiting further triage or management to women positive for only the eight HPV genotypes most associated with cervical cancer (HPV 16,18,31,33,35,45,52,58).<sup>14</sup> Improved triage with an objective laboratory-based method such as this could improve the effectiveness of cervical screening programs in LMICs. In this study, we evaluated the performance of HPV triage with 8-type HPV genotype restriction compared to both VIA (the current recommendation in Botswana) and colposcopy (the international standard) in women with and without HIV in Botswana. We hypothesized that 8-type HPV genotype restriction would have improved diagnostic accuracy in identifying cervical dysplasia compared to VIA and colposcopy in both women with and without HIV.

## **2 MATERIALS AND METHODS**

We conducted a prospective cohort study of women in South-East District, Botswana with baseline enrollment from February 2021 to August 2022. Enrollment was offered to any eligible woman seeking care at a health facility, accompanying someone seeking care at a health facility or working at or near the health facility. Inclusion criteria included: age 25 years or older with an intact cervix, no prior diagnosis of cervical cancer, not pregnant and able to give informed consent. Enrollment was aimed to be as inclusive as possible in order to reflect the Botswana Ministry of Health and Wellness' goal of offering cervical screening at any point of contact of women with the health system. However, to better evaluate triage strategies among WLHIV, the cohort was enriched to include 50% WLHIV.

Research assistants and facility-based cervical screening nurses provided informational talks on cervical screening to women in waiting areas. Interested and eligible women underwent informed consent and completed a questionnaire gathering data on demographics, HIV, and cervical screening history. Laboratory data not known by the participant was obtained from the electronic medical record. Participants were then instructed on HPV vaginal self-sampling and went to the health facility bathroom to collect their sample. Samples were transported to the Botswana Harvard Partnership Reference Laboratory in Gaborone and tested for 15 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68) using the AmpFire® HPV Assay (Atila BioSystems, Mountain View, California, USA) on high through-put PCR platforms already used in Botswana.

Participants who tested HPV positive were recalled for visual triage and biopsy. At the triage visit, VIA was performed by one of three trained nurses who recorded her visual impression as normal, lesion eligible for ablation (low-grade impression), or lesion requires referral for LEEP (high-grade impression). Blinded to the VIA impression, one of three gynecologists then performed colposcopy and recorded lesion appearance and overall impression as low- or high-grade. All participants had a biopsy collected by the colposcopist; if there was a visible lesion, a punch biopsy or LEEP was performed according to standard practice in Botswana's see-and-treat program. If no lesion was visible, a small endocervical sample was collected. Pathology specimens were processed by the Botswana National Health Laboratory. Histopathology data

was reported according to the cervical intraepithelial neoplasia (CIN) classification system<sup>15</sup> and was categorized by severity. Invasive cervical cancer (ICC) included CIN3 with invasive features, squamous cell carcinoma and adenocarcinoma. CIN3 or worse (CIN3+) included CIN3 and ICC. CIN2 or worse (CIN2+) included CIN2 and CIN3+. Participants with CIN2+ on endocervical curettage or cervical punch biopsy were recalled for an excision procedure. Women with ICC were referred for definitive treatment.

## 2.1 Data analysis

The goal of an HPV test-and-triage screening algorithm is to maintain sensitivity while maximizing specificity, thus detecting the most cases of CIN2+ while minimizing overtreatment. Our sample was powered to detect an improvement in diagnostic accuracy of 15% from the current best triage strategy available in Botswana (61% for colposcopy in our prior study) to 76%. In WLHIV, we assumed an HPV positivity of 29% and CIN2+ prevalence among those with HPV to be 35%.<sup>13</sup> Assuming a two-sided  $\alpha = 0.05$ , power = 80%, and lost to follow-up of 10%, a sample size of 3000 was required.

The primary outcome of the study was the performance of triage of HPV positivity with 8-type HPV genotype restriction compared to both VIA and colposcopy in detecting CIN2+. Descriptive statistics were presented as mean with standard deviation or proportion. Continuous variables were compared with a *t* or Wilcoxon rank sum test and categorical variables with a chi-square or Fisher exact test. SAS version 9.4 (SAS Institute, Cary, North Carolina, USA) was used for analyses.

The institutional review boards of the Botswana Ministry of Health and Wellness (13/18/1), the University of Botswana (URB/IRB/1543), Beth Israel Deaconess Medical Center (2019P001130) and the South-East District Health Management Team approved this study. The study is registered on Clinicaltrials.gov (NCT04242823).

## 3 RESULTS

Participants were recruited from February 2021 to August 2022, and follow-up visual evaluation visits were completed by February 2023. Of the 3000 women enrolled, 1494 (50%) were WLHIV and 1506 (50%) were without HIV. Details of HPV positivity, ineligibility after consent, withdrawals, loss to follow-up, and availability of histopathology results for analysis are presented in Figure 1. In the final analysis 2959 women were included: 1478 (50%) WLHIV and 1481 (50%) women without HIV.

Baseline characteristics of participants stratified by HIV status are shown in Table 1. The average age was 42 years (range: 25–77 years). Most women (2121, 72%) were never married and had 1 to 3 children (2055, 64%). WLHIV reported more lifetime sexual partners and were more likely to smoke than women without HIV (both  $P < 0.001$ ). WLHIV had higher rates of prior cervical screening (1180, 80%) compared to women without HIV (807, 54%;  $P < 0.001$ ). WLHIV had an average duration of diagnosis of 10 years. Among WLHIV, all but one participant was on ART and mean duration of treatment was 8 years. Most WLHIV (1161, 79%) had normal CD4 counts ( $>500$  cells per  $\mu\text{L}$ ); only 26 (2%) had a CD4 count of  $<200$  cells per  $\mu\text{L}$ . Nearly all WLHIV (1463, 99%) were virally suppressed.

**TABLE 1.** Demographics of characteristics of 2959 participants who underwent HPV testing in South-East District, Botswana stratified by HIV status.

Characteristic	All <i>n</i> = 2959	HIV positive <i>n</i> = 1478	HIV negative <i>n</i> = 1481	<i>P</i> value
Age, mean years $\pm$ standard deviation	42 $\pm$ 11	43 $\pm$ 9	41 $\pm$ 11	<0.001
Range	25–77	25–76	25–77	
Education				
≤Primary	561 (19)	314 (21)	247 (17)	0.002
≥Secondary	2397 (81)	1163 (79)	1234 (83)	
Employed	1672 (57)	837 (57)	835 (56)	0.89
Marital status				
Single	2121 (72)	1106 (75)	1015 (69)	<0.001
Married	673 (23)	284 (19)	389 (26)	
Divorced/separated	38 (1)	20 (1)	18 (1)	
Widowed	127 (4)	68 (5)	59 (4)	
Gravidity				0.01
0	193 (7)	845 (6)	108 (7)	
1–3	1896 (64)	926 (63)	970 (66)	
≥4	870 (29)	467 (32)	403 (27)	
Parity				0.03
0	225 (8)	102 (7)	123 (8)	
1–3	2055 (69)	1009 (68)	1046 (71)	
≥4	679 (23)	367 (25)	312 (21)	
Premenopausal	2207 (75)	1070 (72)	1137 (77)	0.01
Desire more children				
Yes	916 (42)	398 (37)	518 (46)	<0.001
No	1144 (52)	614 (57)	530 (47)	
Unsure	147 (7)	58 (5)	89 (8)	
Age of sexual debut, years $\pm$ standard deviation	19 $\pm$ 3	19 $\pm$ 3	19 $\pm$ 3	0.02
Lifetime sexual partners <sup>a</sup>				
0	4 (0.1)	0 (0)	4 (0.3)	<0.001
1–5	1943 (66)	893 (61)	1050 (71)	
≥6	994 (34)	576 (39)	418 (28)	
Smoking	171 (6)	113 (8)	58 (4)	<0.001
Health facility where recruited				
Hospital	1121 (38)	500 (34)	621 (42)	<0.001
Clinic	1838 (62)	978 (66)	860 (58)	
History of cervical cancer screening	1987 (68)	1180 (80)	807 (54)	<0.001

History of cervical excisional procedure	25 (1)	17 (1)	8 (0.5)	0.07
Duration of HIV diagnosis <sup>b</sup> , years $\pm$ standard deviation	—	10 $\pm$ 6	—	—
Currently on ART	—	1477 (99.9)	—	—
Length of time on ART, years $\pm$ standard deviation	—	8 $\pm$ 5	—	—
CD4 count (per $\mu$ L)	—	—	—	—
<200	—	26 (2)	—	—
200–500	—	291 (20)	—	—
>500	—	1161 (79)	—	—
Detectable viral load <sup>a</sup>	—	14 (1)	—	—
High-risk HPV positive detected	1480 (50)	823 (56)	657 (44)	<0.001

*Note:* All table entries are number of study subjects (%) unless otherwise noted.

Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; HPV, human papilloma virus; VIA, visual inspection with acetic acid.

<sup>a</sup> Missing data: one participant missing education, 18 missing number of sexual partners, one missing viral load.

<sup>b</sup> All rows here and below only relevant to women living with HIV.

**TABLE 2.** Prevalence of high-grade cervical dysplasia by HPV infection with any high-risk type, HPV coinfection and individual HPV genotypes, stratified by HIV status.

HPV type	Number undergoing colposcopy ( <i>n</i> )	Women with HIV			Women without HIV		
		Number undergoing colposcopy ( <i>n</i> )	CIN2+ <sup>a</sup> <i>n</i> (%)	CIN3+ <sup>b</sup> <i>n</i> (%)	Number undergoing colposcopy ( <i>n</i> )	CIN2+ <sup>a</sup> <i>n</i> (%)	CIN3+ <sup>b</sup> <i>n</i> (%)
None	123	45	1 (2)	1 (2)	78	1 (1)	0 (0)
Any high-risk HPV	1269	688	131 (19)	96 (14)	581	71 (12)	45 (8)
>1 high-risk HPV	565	356	88 (25)	66 (19)	209	38 (18)	28 (13)
33	145	77	28 (36)	17 (22)	68	12 (18)	9 (13)
18	143	90	27 (30)	22 (24)	53	8 (15)	6 (11)
16	189	103	31 (30)	26 (25)	86	12 (14)	7 (8)
58	134	97	25 (26)	21 (22)	37	10 (27)	8 (22)
31	101	62	15 (24)	12 (19)	39	6 (15)	5 (13)
53	168	102	24 (24)	19 (19)	66	10 (15)	6 (9)
45	117	63	15 (24)	12 (19)	54	5 (9)	4 (7)
66	79	50	12 (24)	10 (20)	29	2 (7)	1 (3)
35	210	134	31 (23)	25 (19)	76	15 (20)	9 (12)
59	127	75	17 (23)	12 (16)	52	6 (12)	4 (8)
52	182	106	22 (21)	13 (12)	76	20 (26)	11 (14)
56	124	75	15 (20)	8 (11)	49	9 (18)	5 (10)
51	152	81	14 (17)	9 (11)	71	6 (8)	4 (6)
39	144	75	13 (17)	6 (8)	69	7 (10)	3 (4)
68	201	123	18 (15)	13 (11)	78	6 (8)	4 (5)

Abbreviations: HIV, human immunodeficiency virus; HPV, human papilloma virus.

<sup>a</sup> CIN2+ includes cervical intraepithelial neoplasia (CIN) 2, CIN3, CIN3 with microinvasion, adenocarcinoma in situ, squamous cell carcinoma, adenocarcinoma.

<sup>b</sup> CIN3+ includes CIN3, CIN3 with microinvasion, adenocarcinoma in-situ, squamous cell carcinoma, adenocarcinoma.

WLHIV were more likely to have detectable high-risk HPV (823, 56%) than women without HIV (657, 44%;  $P < 0.001$ ). Of the 1480 women who tested positive for any high-risk HPV type, 1269 (88% [84%] of WLHIV; 581 [88%] of women without HIV) attended the subsequent visual assessment visit and had histopathology available for analysis. Among WLHIV with any high-risk HPV type, 131 (19%) had CIN2+ and 96 (14%) had CIN3+. The prevalence of both CIN2+ and CIN3+ were lower among women without HIV with any high-risk HPV type; 71 (12%) had CIN2+ and 45 (8%) had CIN3+. The HPV genotypes most commonly associated with CIN2+ and CIN3+ stratified by HIV status are shown in Table 2.

The performance of HPV triage strategies to detect CIN2+ stratified by HIV status is shown in Table 3. Triage with 8-type HPV genotype restriction maintained high sensitivity in the detection of CIN2+ in both WLHIV (87.79%, 95% CI: 80.92–92.85) and without HIV (85.92%, 95% CI: 75.62–93.03), though had a relatively low specificity of 30.88% (95% CI: 27.06–34.90) and 37.06% (95% CI: 32.85–41.41), respectively. Visual triage performed notably better in women with than without HIV. Colposcopy using a low-grade impression had a sensitivity of 70.77% (95% CI: 62.15–78.41) in WLHIV but only 45.71% (95% CI: 33.74–58.06) in women without HIV. VIA had low sensitivity, which was better in WLHIV (62.31% [95% CI: 53.39–70.65]) than in women without HIV (44.29% [95% CI: 32.41–56.66]).

Visual triage had a better specificity for detection of CIN2+ than 8-type HPV genotype restriction and thus we evaluated the performance of 8-type HPV genotyping followed by an additional triage step with VIA to assess its performance. The sensitivity of this strategy was the lowest of all other triage strategies, at 56.15% (95% CI: 47.18–64.84) in WLHIV and 35.71% (95% CI: 24.61–48.07) in women without HIV, though the specificity was notably higher at 81.65% (95% CI: 78.18–84.79) in WLHIV and 35.71% (95% CI: 24.61–48.07) in women without HIV.

The overall diagnostic accuracy for detection of CIN2+ was higher for visual triage methods (including 8-type HPV genotype restriction followed by VIA) compared to 8-type HPV genotype restriction in both women with and without HIV, which is accounted for by the higher specificity of visual triage methods compared to 8-type HPV genotype restriction, as shown in Table 3.

The performance of triage strategies for any positive high-risk HPV result in detecting CIN3+ was similar to the performance for detecting CIN2+ and is shown in Table 4.

There was notable improvement in the performance of visual triage methods to detect CIN2+ in WLHIV under the age of 50 (Table 5). The sensitivity of VIA at a low-grade impression threshold improved to 69.15% (95% CI: 58.78–78.27) in WLHIV under age 50, compared with 62.31% (95% CI: 53.39–70.65) in all WLHIV. Similarly, the sensitivity of colposcopy at a low-grade impression threshold improved to 79.79% (95% CI: 70.25–87.37) in WLHIV under age 50, compared with 70.77% (95% CI: 62.15–78.41) in all WLHIV.

**TABLE 3.** Performance of triage strategies in detecting CIN2+ among women who tested positive for high-risk HPV and underwent visual triage and biopsy, stratified by HIV status.

Triage strategies	Biopsy result		Triage test characteristics				
	CIN2+ (n)	<CIN2 (n)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Diagnostic Accuracy (95% CI)
HIV positive (n = 688)							
8-type HPV genotype restriction							
16/18/31/33/35/45/52/58 negative	16	172	—	—	—	—	—
16/18/31/33/35/45/52/58 positive	115	385	87.798 (80.921–92.853)	30.881 (27.06–34.905)	23.00 (19.38–26.947)	91.49 (86.557–95.06)	41.72 (38.00–45.50)
Colposcopy at 2 cutoff thresholds <sup>a</sup>							
Normal	38	377	—	—	—	—	—
≥low-grade impression	92	179	70.771 (62.15–78.41)	67.818 (63.754–71.682)	33.954 (28.33–39.9240)	90.841 (87.658–93.44)	68.37 (64.74–71.83)
≥high-grade impression	53	73	40.771 (32.24–49.7350)	86.877 (83.784–89.5790)	42.06 (33.33–51.18)	86.25 (83.12–88.999)	78.13 (74.85–81.17)
VIA at 2 cutoff thresholds <sup>a</sup>							
Normal	49	405	—	—	—	—	—
≥low-grade impression	81	151	62.31 (53.39–70.651)	72.843 (68.949–76.507)	34.915 (28.799–41.43)	89.21 (85.986–91.912)	70.85 (67.29–74.22)
≥high-grade impression	58	70	44.625 (35.906–53.584)	87.41 (84.36–90.05)	45.31 (36.507–54.35)	87.10 (84.03–89.7790.0)	79.30 (76.07–82.27)
8-type HPV genotype followed by VIA <sup>a</sup>							
HPV 8-type negative OR HPV 8-type positive AND VIA normal	57	454	—	—	—	—	—
HPV 8-type positive AND VIA ≥low-grade impression	73	102	56.15 (47.18–64.845)	81.652 (78.18–84.795)	41.712 (34.32–49.39)	88.859 (85.796–91.44)	76.82 (73.48–79.93)
HIV negative (n = 581)							
8-type HPV genotype restriction							
16/18/31/33/35/45/52/58 negative	10	189	—	—	—	—	—
16/18/31/33/35/45/52/58 positive	61	321	85.926 (75.626–93.03)	37.06 (32.853–41.41)	15.976 (12.44–20.03)	94.975 (90.951–97.568)	43.03 (38.96–47.17)
Colposcopy impression at 2 cutoff thresholds <sup>a</sup>							
Normal	38	363	—	—	—	—	—
≥low-grade impression	32	145	45.716 (33.744–58.06)	71.46 (67.31–75.35)	18.08 (12.713–24.555)	90.521 (87.23–93.21)	68.34 (64.37–72.12)

≥high-grade impression	18	49	25.716 (16.01–37.568)	90.34 (87.45–92.783)	26.877 (16.767–39.10)	89.8290.0 (86.877–92.31)	82.53 (79.18–85.54)
VIA at 2 cutoff thresholds <sup>a</sup>							
Normal	39	385	—	—	—	—	—
≥low-grade impression	31	125	44.29 (32.41–56.667)	75.49 (721.52–79.16)	19.8720 (13.924–27.00)	90.801 (87.648–93.38)	71.72 (67.87–75.36)
≥high-grade impression	20	65	28.579 (18.40–40.621)	87.25 (84.05–90.02)	23.534 (15.00–33.974)	89.9090 (86.907–92.41)	80.17 (76.69–83.34)
8-type HPV genotype followed by VIA <sup>a</sup>							
HPV 8-type negative	45	433	—	—	—	—	—
OR							
HPV 8-type positive AND VIA normal							
HPV 8-type positive AND VIA ≥low-grade impression	25	77	35.716 (24.615–48.07)	84.905 (81.502–87.908)	24.515 (16.537–34.02)	90.591 (87.618–93.05)	78.97 (75.42–82.21)

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; HPV, human papilloma virus; NPV, negative predictive value; PPV, positive predictive value; VIA, visual inspection with acetic acid.

<sup>a</sup> Unable to conduct VIA in 2 HIV+ participants and 1 HIV– participant. Unable to conduct colposcopy in 2 HIV+ participants and 3 HIV– participants.

**TABLE 4.** Performance of triage strategies in detecting CIN3+ among women who tested positive for high-risk HPV and underwent visual triage and biopsy, stratified by HIV status.

Triage strategies	Biopsy result		Triage test characteristics								
	CIN3+ (n)	<CIN3 (n)	Sensitivity (95% CI)		Specificity (95% CI)		PPV (95% CI)		NPV (95% CI)		Diagnostic accuracy (95% CI)
HIV positive (n = 688)											
8-type HPV genotype restriction											
16/18/31/33/35/45/52/58 negative	8	180	—		—		—		—		
16/18/31/33/35/45/52/58 positive	88	412	91.672	(84.24–96.33)	30.41	(26.727–34.29)	17.608	(14.36–21.23)	95.746	(91.792–98.15)	38.95 (35.29–42.71)
Colposcopy at 2 cutoff thresholds <sup>a</sup>											
Normal	23	392	—		—		—		—		
≥low-grade impression	73	198	76.04	(66.25–84.17)	66.44	(62.47–70.25)	26.947	(21.752–32.643)	94.46	(91.802–96.45)	67.78 (64.14–71.27)
≥high-grade impression	48	78	50.0	(39.6240–60.38)	86.787	(83.784–89.41)	38.10	(29.5930.0–47.17)	91.43	(88.809–93.614)	81.63 (78.53–84.46)
VIA at 2 cutoff thresholds <sup>a</sup>											
Normal	30	424	—		—		—		—		
≥low-grade impression	66	166	68.759	(58.489–77.828)	71.862	(68.05–75.46)	28.45	(22.743–34.725)	93.39	(90.701–95.506)	71.43 (67.89–74.78)
≥high-grade impression	50	78	52.08	(41.642–62.39)	86.787	(83.784–89.41)	39.06	(30.561–48.08)	91.762	(89.16–93.904)	81.92 (78.84–84.73)
8-type HPV genotype followed by VIA <sup>a</sup>											
HPV 8-type negative	37	474	—		—		—		—		
OR											
HPV 8-type positive AND VIA normal											
HPV 8-type positive AND VIA ≥low-grade impression	59	116	61.46	(50.971–71.22)	80.34	(76.907–83.47)	33.714	(26.767–41.24)	92.763	(90.16–94.855)	77.70 (74.39–80.76)
HIV negative (n = 581)											
8-type HPV genotype restriction											
16/18/31/33/35/45/52/58 negative	2	197	—		—		—		—		
16/18/31/33/35/45/52/58 positive	43	339	95.566	(84.855–99.46)	36.757	(32.663–40.991)	11.26	(8.27–14.865)	98.999	(96.42–99.88100)	41.31 (37.27–45.43)
Colposcopy impression at 2 cutoff thresholds <sup>a</sup>											

Normal	22	379	—	—	—	—	
≥low-grade impression	22	155	50.00 (34.565–65.44)	70.971 (66.927–74.795)	12.43 (7.968–18.21)	94.515 (91.812–96.537)	69.38 (65.44–73.11)
≥high-grade impression	13	54	29.5530 (16.767–45.20)	89.8990 (87.01–92.31)	19.40 (10.761–30.891)	93.934 (91.502–95.846)	85.29 (82.14–88.08)
VIA at 2 cutoff thresholds <sup>a</sup>							
Normal	23	401	—	—	—	—	
≥low-grade impression	21	135	47.738 (32.46–63.31)	74.815 (70.911–78.44)	13.46 (8.539–19.8420)	94.585 (91.972–96.53)	72.76 (68.94–76.34)
≥high-grade impression	12	73	27.27 (14.965–42.793)	86.38 (83.18–89.17)	14.12 (7.518–23.36)	93.544 (91.00–95.546)	81.90 (78.52–84.95)
8-type HPV genotype followed by VIA <sup>a</sup>							
HPV 8-type negative	25	453	—	—	—	—	
OR							
HPV 8-type positive AND VIA normal							
HPV 8-type positive AND VIA ≥low-grade impression	19	83	43.18 (28.35–58.979)	84.515 (81.17–87.47)	18.639 (11.602–27.558)	94.775 (92.38–96.597)	81.38 (77.97–84.47)

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; HPV, human papilloma virus; NPV, negative predictive value; PPV, positive predictive value; VIA, visual inspection with acetic acid.

<sup>a</sup> Unable to conduct VIA in 2 HIV+ participants and 1 HIV- participant. Unable to conduct colposcopy in 2 HIV+ participants and 3 HIV- participants.

**TABLE 5.** Performance of triage strategies in detecting CIN2+ among women <50 years old who tested positive for high-risk HPV and underwent visual triage and biopsy, stratified by HIV status.

Triage strategies	Biopsy result		Triage test characteristics				
	CIN2+ (n)	<CIN2 (n)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Diagnostic accuracy (95% CI)
HIV positive (n = 523)							
8-type HPV genotype restriction							
16/18/31/33/35/45/52/58 negative	11	134	—	—	—	—	—
16/18/31/33/35/45/52/58 positive	84	294	88.42 (80.23–94.08)	31.31 (26.94–35.94)	22.22 (18.13–26.75)	92.41 (86.83–96.15)	41.68 (37.42–46.04)
Colposcopy at 2 cutoff thresholds <sup>a</sup>							
Normal	19	275	—	—	—	—	—
≥low-grade impression	75	152	79.79 (70.25–87.37)	64.40 (59.66–68.95)	33.04 (26.96–39.57)	93.54 (90.09–96.06)	67.18 (62.96–71.20)
≥high-grade impression	43	59	45.74 (35.42–56.34)	86.18 (82.54–89.31)	42.16 (32.44–52.34)	87.83 (84.31–90.80)	78.89 (75.13–82.31)
VIA at 2 cutoff thresholds <sup>a</sup>							
Normal	29	301	—	—	—	—	—
≥low-grade impression	65	126	69.15 (58.78–78.27)	70.49 (65.92–74.78)	34.03 (27.35–41.22)	91.21 (87.62–94.04)	70.25 (66.12–74.15)
≥high-grade impression	48	54	51.06 (40.54–61.52)	87.35 (83.82–90.36)	47.06 (37.10–57.20)	89.02 (85.63–91.85)	80.81 (77.16–84.10)
8-type HPV genotype followed by VIA <sup>a</sup>							
HPV 8-type negative OR HPV 8-type positive AND VIA normal	35	340	—	—	—	—	—
HPV 8-type positive AND VIA ≥low-grade impression	59	87	62.77 (52.18–72.52)	79.63 (75.49–83.35)	40.41 (32.38–48.84)	90.67 (87.26–93.41)	76.58 (72.71–80.16)
HIV negative (n = 460)							

8-type HPV genotype restriction							
16/18/31/33/35/45/52/58 negative	7	146	—	—	—	—	
16/18/31/33/35/45/52/58 positive	50	257	87.72 (76.32–94.92)	36.23 (31.53–41.13)	16.29 (12.34–20.90)	95.42 (90.80–98.14)	42.61 (38.04–47.27)
Colposcopy impression at 2 cutoff thresholds <sup>a</sup>							
Normal	29	278	—	—	—	—	
≥low-grade impression	27	123	48.21 (34.66–61.97)	69.33 (64.56–73.81)	18.00 (12.21–25.10)	90.55 (86.72–93.58)	66.74 (62.21–71.05)
≥high-grade impression	18	49	25.71 (16.01–37.56)	90.35 (87.45–92.78)	26.87 (16.76–39.10)	89.82 (86.87–92.31)	82.53 (79.18–85.54)
VIA at 2 cutoff thresholds <sup>a</sup>							
Normal	31	293	—	—	—	—	
≥low-grade impression	25	110	44.64 (31.34–58.53)	72.70 (68.07–77.00)	18.52 (12.36–26.11)	90.43 (86.69–93.41)	69.28 (64.84–73.47)
≥high-grade impression	16	59	28.57 (17.30–42.21)	85.36 (81.53–88.66)	21.33 (12.71–32.32)	89.58 (86.09–92.45)	78.43 (74.38–82.11)
8-type HPV genotype followed by VIA <sup>a</sup>							
HPV 8-type negative OR HPV 8-type positive AND VIA normal	35	334	—	—	—	—	
HPV 8-type positive AND VIA ≥low-grade impression	21	69	37.50 (24.92–51.45)	82.88 (78.84–86.43)	23.33 (15.06–33.43)	90.51 (87.06–93.30)	77.34 (73.23–81.09)

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; HPV, human papilloma virus; NPV, negative predictive value; PPV, positive predictive value; VIA, visual inspection with acetic acid.

<sup>a</sup> Unable to conduct VIA in 2 HIV+ participants and 1 HIV- participant. Unable to conduct colposcopy in 2 HIV+ participants and 3 HIV- participants.

## 4 DISCUSSION

This study explored the effectiveness of triage strategies following primary HPV screening in women with and without HIV in Botswana. Our findings support 8-type HPV genotype restriction as the most sensitive triage strategy in detecting disease among the available clinical alternatives. Although overall diagnostic accuracy of 8-type HPV genotype restriction was lower than visual triage alternatives, the trade-off in loss of sensitivity with visual triage would result in an abundance of missed opportunities to identify and diagnose women with high grade cervical lesions that could be intervened on. The challenge remaining with 8-type HPV genotype restriction is determining the appropriate next step after a positive result; whether a national program algorithm recommends biopsy for diagnosis versus immediate treatment requires consideration of the performance parameters balanced with cost, available resources, and risk of loss to follow-up.

Our finding that 8-type HPV genotype restriction maintained high sensitivity, but had relatively low specificity, is generally in accord with prior studies of the performance of HPV genotype restriction in detecting CIN2+ in WLHIV. Kelly et al. demonstrated that 8-type HPV genotype restriction had higher sensitivity (77%) than visual triage methods, and, in contrast to our findings, also maintained a specificity of 74%.<sup>14</sup> Kahesa et al. reported high sensitivity of 8-type HPV genotype restriction in women with and without HIV (90% and 81%, respectively) but as in our study the specificity was low, with positive predictive values of only 30% and 20%, respectively.<sup>16</sup> Botha et al. recently reported only 14% reduction in sensitivity of 7-type HPV genotype restriction to detect CIN2+ in WLHIV, using the same types as 8-type HPV genotype restriction except HPV35.<sup>17</sup>

Our findings regarding the low sensitivity of visual triage methods are in-line with our prior findings<sup>13</sup> as well as a recent study from Papua New Guinea that reported a similar reduction in the sensitivity of HPV primary screening from 92% to 46% when triaged with VIA.<sup>12</sup> Similarly, 8-type HPV genotype restriction used as an intermediate step before VIA triage-to-treat had very low sensitivity, and while this strategy would reduce the number of women who require pelvic examination, it would also result in much lower detection of disease.

The major strength of this study was the large size of a mixed cohort of both women with and without HIV, which allowed for comparison of the triage strategies across groups. Additionally, we had a relatively low rate of loss to follow-up compared with similar studies in other settings.<sup>18</sup> We used self-sampling, which ideally can be replicated in a population-level screening program, as aligned with the Botswana Ministry of Health and Wellness' strategic plan. Prior research in Botswana demonstrated high acceptability of HPV vaginal self-sampling, and excellent concordance of HPV results between self-collected and provider-collected samples (92%, Cohen's  $\kappa$  0.80).<sup>19</sup>

The present study had several limitations. First, most WLHIV in Botswana have extremely well-controlled HIV, which may limit the generalizability of our findings to WLHIV with lower CD4<sup>+</sup> cell counts and shorter duration of ART. At the same time, the current study findings may not be fully representative of the potential benefit of universal ART on cervical disease, as the treat-all policy for HIV was only introduced in Botswana in 2016. Second, we did not document visibility of the squamocolumnar junction, and this factor is known to impact the effectiveness of visual triage methods. Third, while the goal of this study was to evaluate real-world pathology services currently available, some histopathology results were not

resulted when this analysis was conducted, and there was no formal quality assurance incorporated into our study design.

Our study provides concrete triage performance metrics to optimize guidelines for southern Africa, where the excess burden of cervical cancer is related to both the high burden of HIV and poor access to high-quality screening.<sup>10</sup> The findings support use of high-performance cervical screening with primary HPV testing, while also highlighting the challenges associated with available triage methods in LMICs. The differential results by HIV status demonstrate the need to consider HIV prevalence in LMICs when designing population-level screening to ensure it is effective and inclusive.

## **5 CONCLUSIONS**

Eight-type HPV genotype restriction is a promising component of effective triage for positive HPV results. However, novel triage strategies in LMICs with high HIV prevalence are needed to avoid the trade-off between sensitivity and specificity with all currently available triage options.

## **AUTHOR CONTRIBUTIONS**

Rebecca Luckett: Conception, planning, carrying out, analyzing and writing up the study. Doreen Ramogola-Masire: Conception, planning, carrying out, analyzing and writing up the study. Annika Gompers: Analyzing, writing up the study. Natasha Moraka: Carrying out, writing up the study. Sikhulile Moyo: Planning, carrying out, writing up the study. Leatile Sedabadi, Leabaneng Tawe, Thanolo Kashamba, Kelebogile Gaborone, Anikie Mathoma: Carrying out, writing up the study. Farzad Noubary: Analyzing, writing up the study. Maduke Kula: Carrying out the study, writing up the study. Surbhi Grover, Greta Dreyer: Analyzing, writing up the study. Joseph Makhema: Carrying out, analyzing and writing up the study. Roger Shapiro, Michele R. Hacker: Planning, carrying out, analyzing and writing up the study.

## **ACKNOWLEDGMENTS**

We would like to thank all study participants. We also appreciate the ongoing support from the Botswana Ministry of Health and Wellness, the South-East District Health Management Team, and the administrative leadership at Bamalete Lutheran Hospital. We are very grateful for the support of Chief Kgosi Mosadi, for her support of this study in her district and her commitment to improving the health and wellness of her constituency.

## **FUNDING INFORMATION**

Funding for this study was provided by the Young Investigator Award from the Department of Obstetrics and Gynecology at Beth Israel Deaconess Medical Center, the National Cancer Institute, National Institutes of Health Award 1K08CA271949, and National Institutes of Health Fogarty International Center K43 TW012350-01. The funders required external peer review for scientific quality. The funders had no role in the conduct of the study, data analysis or manuscript preparation.

## **CONFLICT OF INTEREST STATEMENT**

The authors declare that they have no conflicts of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## REFERENCES

1. Arbyn M, Weiderpass E, Bruni L, et al. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. *Lancet Glob Health*. 2020; 8(2): e191-e203.
2. Sun XW, Kuhn L, Ellerbrock TV, Chiasson MA, Bush TJ, Wright TC. Human papillomavirus infection in women infected with the human immunodeficiency virus. *N Engl J Med*. 1997; 337: 1343-1910.
3. Heard I, Cubie HA, Mesher D, Sasieni P, for the MACH-1 Study Group. Characteristics of HPV infection over time in European women who are HIV-1 positive. *BJOG*. 2013; 120(1): 41-49.
4. Denny LA, Franceschi S, de Sanjose S, Heard I, Moscicki AB, Palefsky J. Human papillomavirus, human immunodeficiency virus and immunosuppression. *Vaccine*. 2012; 30: F168-F174.
5. Stelzle D, Tanaka LF, Lee KK, et al. Estimates of the global burden of cervical cancer associated with HIV. *Lancet Global Health*. 2021; 9(2): E161-E169.
6. Bruni L, Serrano B, Roura E, et al. Cervical cancer screening programmes and age-specific coverage estimates for 202 countries and territories worldwide: a review and synthetic analysis. *Lancet Glob Health*. 2022; 10(8): e1115-e1127.
7. Kelly H, Jaafar I, Chung M, et al. Diagnostic accuracy of cervical cancer screening strategies for high-grade cervical intraepithelial neoplasia (CIN2+/CIN3+) among women living with HIV: a systematic review and meta-analysis. *eClinicalMedicine*. 2022; 53:101645.
8. Shastri S, Temin S, Almonte M, et al. Secondary prevention of cervical cancer: ASCO resource-stratified guideline update. *JCO Global Oncol*. 2022; 8:e2200217.
9. Wright TC, Stoler MH, Behrens CM, Sharma A, Zhang G, Wright TL. Primary cervical cancer screening with human papillomavirus: end of study results from the ATHENA study using HPV as the first-line screening test. *Gynecol Oncol*. 2015; 136(2): 189-197.
10. World Health Organization. WHO guideline for screening and treatment of cervical pre-cancer lesions for cervical cancer prevention, second edition. 2021. Accessed 04 April 2023. <https://www.who.int/publications/i/item/9789240030824>
11. Baena A, Mesher D, Salgado Y, et al. Performance of visual inspection of the cervix with acetic acid (VIA) for triage of HPV screen-positive women: results from the ESTAMPA study. *Int J Cancer*. 2022; 152: 1-12. doi:10.1002/ijc.34384
12. Toliman PJ, Kaldor JM, Badman SG, et al. Performance of clinical screening algorithms comprising point-of-care HPV-DNA testing using self-collected vaginal specimens, and visual inspection of the cervix with acetic acid, for the detection of underlying high-grade squamous intraepithelial lesions in Papua New Guinea. *Papillomavirus Res*. 2018; 6: 70-76.
13. Luckett R, Mogowa N, Li HJ, et al. Performance of two-stage cervical cancer screening strategies utilizing primary hrHPV testing for women living with HIV. *Obstet Gynecol*. 2019; 134(4): 840-849.
14. Kelly HA, Chikandiwa A, Sawadogo B, et al. Diagnostic accuracy of cervical cancer screening and screening-triage strategies among women living with HIV-1 in Burkina Faso and South Africa: a cohort study. *PLoS Med*. 2021; 18(3):e1003528.

15. World Health Organization. Comprehensive Cervical Cancer Control: A Guide to Essential Practice. 2nd ed. WHO Press; 2014.
16. Kahesa C, Thomsen LT, Linde DS, et al. Comparison of human papillomavirus-based cervical cancer screening strategies in Tanzania among women with and without HIV. *Int J Cancer*. 2023; 152: 686-696.
17. Botha MH, Van der Merwe FH, Snyman LC, Dreyer GJ, Visser C, Dreyer G. Utility of extended HPV genotyping as primary cervical screen in an unscreened population with high HIV co-infection. *J Lower Gen Tract Dis*. 2023; 27: 212-216.
18. Mwenda V, Bor JP, Nyangasi M, et al. Integrating human papillomavirus testing as a point-of-care service using GeneXpert platforms: findings and lessons from a Kenyan pilot study (2019-2020). *PloS One*. 2023; 18(5):e0286202.
19. Elliot T, Kohler R, Monare B, et al. Performance of vaginal self-sampling for HPV testing among women living with HIV in Botswana. *J STD AIDS*. 2019; 30(12): 1169-1176.