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## Comparison of Conventional Cervical Cytology versus Visual Inspection with Acetic Acid (VIA) among HIV-Infected Women in Western Kenya

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### Abstract

**Objective**—To determine the accuracy of visual inspection with Acetic Acid (VIA) versus conventional Pap smear as a screening tool for cervical intraepithelial neoplasia (CIN)/cancer among HIV-infected women.

**Materials and Methods**—150 HIV-infected women attending the Moi Teaching and Referral Hospital HIV clinic in Eldoret underwent conventional Pap smear, VIA, colposcopy and biopsy. VIA and Pap smears were done by nurses while colposcopy and biopsy were done by a physician. Receiver Operating Characteristic (ROC) analysis was conducted to compare the accuracies between VIA and Pap smear in sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

**Results**—Among the study participants: VIA was abnormal in 55.3% (83/150, CI=47.0–63.5%); Pap smear showed atypical squamous cells of undetermined significance (ASCUS) or worse in 43.7% (59/135, CI=35.2–52.5%) and 10% (15/150) of the Pap smears were unsatisfactory. Of the abnormal Pap smears, 3% (2/59) had ASCUS, 7% (4/59) had ASC-high grade, 60% (35/59) had low-grade squamous intraepithelial lesions (SIL), 29% (17/59) had high grade SIL, and 2% (1/59) was suspicious for cervical cancer. Using cervical intraepithelial neoplasia (CIN) 2 or higher disease on biopsy as an end point, VIA has a sensitivity of 69.6% (CI=55.1–81.0%), specificity of 51.0% (CI=41.5–60.4%), PPV of 38.6% (CI=28.8–49.3%) and NPV of 79.1% (CI=67.8–87.2%). For conventional Pap smear, sensitivity was 52.5% (CI=42.1–71.5%), specificity 66.3% (CI=52.0–71.2%), PPV 39.7% (CI=27.6–51.8%), and NPV 76.8% (CI=67.0–85.6%).

**Conclusion**—VIA is comparable to Pap smear and acceptable for screening HIV-infected women in resource limited settings such as Western Kenya.

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## Keywords

Visual Inspection with Acetic Acid (VIA); Pap smear; Kenya; HIV

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## INTRODUCTION

Many of the developing countries with the highest cervical cancer burden also face an expanding HIV/AIDS epidemic. Kenya's adult HIV prevalence rate in 2007 was 7.1–8.5%, and more than half of these 1.4–1.8 million people are women<sup>1</sup>. It is well-known that women who are infected with HIV are at greater risk for developing CIN/cancer. The incidence of CIN is 4 to 5 times higher among HIV-infected women compared to their uninfected counterparts<sup>2,3</sup>. Women with low CD4 cell counts have the highest prevalence of Human Papillomavirus (HPV) infection, higher rates of persistent HPV infection, and more commonly harbor high-risk oncogenic HPV types that are associated with severe CIN and cervical cancer<sup>4–8</sup>. The natural progression of CIN is also affected; the average interval between diagnosis of CIS and invasive disease may be significantly shortened from 15.7 to 3.2 years<sup>9</sup>. Until recently, access to antiretroviral therapy was very limited for HIV-infected women in the developing world. As highly active antiretroviral therapy (HAART) has become more accessible, HIV-infected women in the developing world are living longer and are increasingly vulnerable to more common diseases, including cervical cancer. In contrast to other AIDS-defining malignancies (Kaposi's sarcoma, central nervous system lymphoma), introduction of HAART has not decreased the incidence of cervical cancer in HIV infected women<sup>10–12</sup>.

Screening for cervical cancer in the developing world has many inherent challenges, from a general lack of awareness to cultural aversion of reproductive health diseases. The many logistical prerequisites for a successful Pap smear based program have been difficult to implement in developing countries. It requires the preparation of high quality smears, well-trained and experienced personnel, internal and external control mechanisms, reaching a high percentage of the population, high return rates, and scheduled follow-up and treatment of abnormal lesions. HPV typing is currently beyond the capacity of many developing countries. In response to this challenge, more cost-effective methods of cervical cancer screening have been developed and tested. The most promising of these is visual inspection with acetic acid (VIA), which has been proven both sensitive and specific enough to decrease incidence and mortality in developing world settings<sup>13</sup>. However, few studies have specifically examined VIA as a screening tool in an exclusively HIV infected cohort. We sought to determine the accuracy of VIA versus conventional Pap smear as a screening tool for CIN/cancer among HIV infected women in Western Kenya with biopsy as the reference gold standard.

## METHODS

### Study Setting and Study Population

The study was approved by the Institutional Review Board of the Miriam Hospital, Providence, RI, USA as well as the Institutional Research and Ethics Committee of the Moi University school of Medicine (MUSOM) /Moi Teaching and Referral Hospital (MTRH) in Eldoret, Kenya. 150 participants from the waiting rooms of the HIV clinics in the Academic Model for the Prevention and Treatment of AIDS (AMPATH) building of the MUSOM/ MTRH campus were recruited. The inclusion criteria were: female, age 15–49 years, documented HIV infection, and generally healthy and with no debilitating disease. The exclusion criteria were: current pregnancy or pregnancy in the last 6 months, history of or treatment for cervical cancer, total hysterectomy, dilatation and curettage in the last 6

months, current mucopurulent discharge, active vaginal bleeding, and diagnosis of a sexually transmitted infection (STI) in the last 3 weeks. After an explanation of the study, clinical procedures involved, and the basics of cervical cancer and screening, the participants signed an informed consent document in either English or Swahili.

### Clinical Tests

Each participant completed a demographic questionnaire as well as a survey regarding the importance of cervical cancer and screening. Each participant underwent a VIA test and a conventional Pap smear done by nurses. A colposcopic examination, a colposcopic-guided punch biopsy, and an endocervical curettage were done by physicians. The criteria for VIA were taken from A Practice Manual on Visual Screening for Cervical Neoplasia. WHO 2003. The conventional Pap smears were collected from the endocervix and ectocervix simultaneously using a plastic cervibroom that was rotated 3 times. Both sides of the cervibroom were then smeared on a slide and immediately fixed. A single punch biopsy was taken from a visible abnormal lesion with the aid of a colposcope. If no abnormal lesion was visible, a single punch biopsy was taken from either the 6 or 12 o'clock position, whichever was more feasible. A single Kenyan pathologist read all the Pap smears and punch biopsies and made the histological diagnoses. The pathologist was blinded to the VIA results when interpreting pap smears, and was blinded to both the VIA and pap smear results when interpreting the biopsies. The blinding was accomplished using several measures. The pathologist interpreted biopsy slides only after he had interpreted and returned all prior pap slides to the study coordinator. Also, the pathologist was not allowed to keep a log of the slide diagnoses. Ten percent of the Pap smears and biopsy specimens were reviewed at Brown University by a single pathologist for quality control purposes. At the end of the procedures, participants were asked about their experience and the acceptability of the various screening methods.

### Statistical Analysis

The prevalence of CIN based on histological results and the rates of abnormal VIA and Pap smear screens among the study population were estimated with 95% confidence intervals. ROC analyses were performed to compare the accuracies of VIA and Pap smear in identifying CIN with histology (colposcopic-guided biopsy) results as the 'gold standard'. Pap smears were read based on the Bethesda Classification with a six-point scale classification: normal, ASCUS, ASC-H, LSIL, HSIL, and cancer. VIA was classified on a two-point scale: normal and abnormal. Histological classification (gold standard) was: normal, CIN 1, CIN 2, CIN 3, or cancer. Two 'gold standard' thresholds – CIN 1 or higher, and CIN 2 or higher – were considered to define CIN. ROC curves were plotted to compare the trade-off between sensitivity and specificity. The areas under the ROC curves (AUC's) were calculated to characterize the overall screening accuracies of Pap smear and VIA. The AUC's were compared using paired t-test, where the standard error was calculated using bootstrap for these paired outcomes (bootstrapped samples = 2,000).

Based on conventions and our estimated ROC, a decision threshold for Pap smear was chosen as LSIL or higher. Pap smear and VIA were compared in sensitivity, specificity, PPV, and NPV (Table 3). All analyses were done using the statistical software package STATA10 (STATA Corp LP, [www.stata.com](http://www.stata.com)). ROC curves were plotted and bootstrap p-values were computed by R (The R Foundation for Statistical Computing, version 2.10.1. <http://www.r-project.org/>).

### Ethics and Treatment

All women with CIN 2 or worse on histology were referred to the Moi Teaching and Referral Hospital for treatment, ranging from LEEP to radical hysterectomy. Of the 4

patients with cervical cancer, only 1 was not a surgical candidate and she was referred to Kampala and Nairobi for radiotherapy.

## RESULTS

The age range of the women was 20–45 years old with a mean age of 34 years. Most were on HAART (67.1%, 100/149). The mean CD4 count was over 400/mm<sup>3</sup> with a range from 10–1198/mm<sup>3</sup>. Very few had ever had a Pap smear and about a quarter had a previous sexually transmitted infection. Only 1.3% (2/150) ever smoked. (Table 1)

Screening results are summarized in Table 2. 15/150 (10%) of the Pap smears were unsatisfactory. Cervical cytology was abnormal (ASCUS or worse) in 59/135 (43.7%, CI=35.2–52.5%) women. Of the abnormal Pap smears, 2/59 (3%) had ASCUS, 4/59 (7%) had ASC-high grade, 35/59 (60%) had LSIL, 17/59 (29%) had HSIL, and 1/59 (2%) was suspicious for cervical cancer. VIA was successfully performed on all women. A total of 83/150 (55.3%, CI=47.0–63.5%) women had an abnormal VIA result. The histology report was abnormal in 92/150 (61.3%, CI=53.1–69.2%). Of the abnormal histology results, 46/92 (50%) were CIN 1, 20/92 (21.7%) were CIN 2, 22/92 (23.9%) were CIN 3, and 4/92 (4.3%) had microinvasive cervical cancer.

Screening ROC curves of VIA and Pap smear are shown in Figure 1. Using AUC as an overall measure of screening accuracies and using CIN 1 or higher as the gold standard threshold, the performance of Pap smear is slightly better than VIA, but the difference is not significant (Pap smear: AUC = 0.596, VIA: AUC = 0.571, p-value = 0.64). When using CIN 2 or higher as the gold standard threshold, the performance of Pap smear and VIA are more comparable (Pap smear: AUC = 0.606, VIA: AUC = 0.603, p-value = 0.93). Using CIN 2 or higher disease on biopsy as the gold standard threshold, the sensitivity of VIA was 69.6% (95% CI=55.1–81.0%), specificity 51.0% (95% CI=41.5–60.4%), PPV 38.6% (95% CI=28.8–49.3%), and NPV 79.1% (95% CI=67.8–87.2%). For conventional Pap smear (threshold of LSIL or worse); sensitivity was 52.5% (95% CI=42.1–71.5%), specificity 66.3% (95% CI=52.0–71.2%), PPV 39.7% (95% CI=27.6–51.8%), and NPV 76.8% (95% CI=67.0–85.6%) (Table 3). Because of the tradeoff between sensitivity and specificity, the significant differences in sensitivity and specificity between Pap smear and VIA do not necessarily imply that one screening method performed better than the other one. In terms of PPV, NPV, and overall misclassification rate, there was a good agreement between Pap smear and VIA.

Of the 10% of cases that were reviewed by the Brown pathologist for quality control, the correlation kappa was 0.67 for pap smears and 0.52 for histology. Where there were discrepancies in the readings of the pap smears and biopsies, both pathologists were made aware of the discrepancy and the reading reviewed again by the Kenyan pathologist. In all cases, the Kenyan pathologist agreed with Brown pathologist after review.

## DISCUSSION

Results from this study reveal a high prevalence of CIN/cancer (61%, 92/150 by histology) among HIV infected women in Western Kenya. This is consistent with many other studies of HIV infected women<sup>14</sup>. An effective cervical cancer screening program is crucial to reduce the morbidity and mortality among HIV infected women living with HIV/AIDS due to the positive effects HAART. Findings from this study also show that even with high CD4 counts on HAART (mean CD4 457/mm<sup>3</sup>) a significant proportion (31%, 46/150) of women had CIN 2 or worse cervical lesions. Before this study, there was no existing program to screen for cervical cancer at AMPATH clinics. These results (including 4 cases of cervical

cancer) underscore the need for an affordable, feasible, and accurate screening method to prevent late stage presentation of cervical cancer.

Conventional cytology is not widely used in resource-limited settings because it requires technical competence and has significant associated costs. HPV testing is currently too expensive for most resource-limited countries. Cervical cancer screening using VIA has proven to be a simple, feasible, and realistic method for higher population coverage<sup>15</sup>. However, multiple large scale studies of VIA (with or without magnification) have detected CIN with comparable results to Pap smears<sup>16</sup>. Most of these studies have been done in HIV negative or in women with unknown HIV status.

In this study, sensitivity, specificity, PPV, and NPV of VIA is 69.6%, 51.0%, 38.6% and 79.1% respectively, for CIN 2 or worse lesions. This falls within the range reported by other studies<sup>17</sup>. Akinwuntan et al reported that VIA among 250 HIV infected women had a sensitivity, specificity, PPV, and NPV of 76%, 83%, 34%, and 97%, respectively<sup>18</sup>. They concluded that VIA is a sensitive screening test for cervical cancer in HIV infected women although it is not ideal as a diagnostic test. From our study, the sensitivity, specificity, PPV, and NPV of conventional Pap smear is 52.5%, 66.3%, 39.7%, and 76.8% respectively, for CIN 2 or worse lesions. In the study of HIV infected women in Nigeria (Akinwuntan et.al)<sup>18</sup>, the results were 57%, 95%, 55%, and 95% respectively. These results were for any CIN lesion. Other studies have shown sensitivities ranging from 48–81% and specificities from 87 to 94%.

In this study, VIA had a higher sensitivity compared to conventional Pap smear (69.6% vs 52.5%) for CIN 2 or worse lesions, although specificity was lower (51.0% vs 66.3%). This is no different from other studies which reported similar patterns. Based on these results, we propose that VIA is a reasonable tool for population based cervical cancer screening among HIV infected women in Western Kenya. For the future, implementation of “see and treat” programs partnering VIA with cryotherapy in a single visit should be explored to decrease the high prevalence of CIN and the risk of cervical cancer among these women.

## Conclusion

There is a high prevalence of severe cervical neoplasia among HIV-infected Kenyan women despite good CD4 counts on HAART. In this study, VIA had higher sensitivity, lower specificity, and almost similar positive and negative predictive value as Pap smears prepared and read at MTRH. VIA is easy to learn, does not require laboratory infrastructure, results are immediate and allows for immediate colposcopy/biopsy or treatment, and supply costs are low. VIA is an acceptable, cost-effective population based screening method for cervical cancer screening among HIV-infected women in Western Kenya. Although VIA has limitations, it will allow for more widespread implementation of cervical cancer screening among the most vulnerable women at risk for cervical cancer in Western Kenya.

## Limitations

There are several limitations regarding the study. The results are generalizable only to resource-poor settings where the prevalence of HIV and the incidence of CIN/cancer are relatively high. Most of the women in the study were on antiretroviral therapy with good CD4 cell counts and results may vary for more immunocompromised women or women not on antiretroviral therapy. This study was unable to compare VIA and pap smears to other screening methods, such as high risk HPV testing. The pap smears were read by a pathologist, since there are no sub-specialist cytopathologists in Eldoret.



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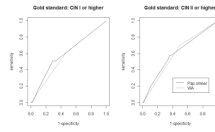
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**Figure 1.**  
Receiver operating characteristic curves for Pap smear and VIA.

**Table: 1**

Baseline demographic characteristics of the study population stratified by severity of CIN

	All (N=150)	CIN 2 or Higher (N=46)	Cervical Cancer (N=4)
Age (mean, range)	34.4 (20–45)	34.8 (25–45)	29.25 (26–32)
Most recent CD4 (mean, range)	438 (10–1198)	457 (61–1198)	414 (168–800)
Currently on HAART	67.1%	69.6%	100%
Currently using contraception	66.0%	73.9%	50%
Experience contact or post-coital bleeding	6.7%	10.8%	0%
Ever had a previous Pap smear	12.2%	8.7%	0%
Condom Use			
Always	55.6%	65.9%	66.7%
Sometimes	31.2%	25.0%	0%
Never	13.2%	9.1%	33.3%
# Sex partners in last 6 months (mean, range)	.89 (0–3)	.89 (0–3)	.75 (0–1)
Age at first sexual intercourse (mean, range)	17.7 (12–28)	17.9 (12–28)	17.5 (15–19)
Previous STI	23.5%	23.9%	0%
Marital Status			
Married	40.3%	41.3%	25%
Single	26.9%	28.3%	25%
Widow	22.8%	19.6%	50%
Other (divorced, separated)	10.1%	10.9%	0%
# of pregnancies (mean, range)	3.1 (0–10)	3.2 (1–8)	2 (1–3)
Ever Smoke	1.3%	0%	0%

**Table: 2**  
Comparison of Pap smear and VIA Results with Cervical Biopsy as gold standard

	Biopsy (gold standard)						Total
	Normal	CIN 1	CIN 2	CIN 3	Cancer		
<b>Pap smear</b>							
Normal	35	24	11	4	2	76 (56.3%)	
ASCUS	2	0	0	0	0	2 (1.5%)	
ASC-H	1	1	1	1	0	4 (3.0%)	
LSIL	9	13	4	9	0	35 (25.9%)	
HSIL	4	5	2	6	0	17 (12.6%)	
Cancer suspicious	1	0	0	0	0	1 (0.7%)	
<i>Unsatisfactory</i>	6	3	2	2	2	15 -	
<b>VIA</b>							
Normal	31	22	10	3	1	67 (44.7%)	
Abnormal	27	24	10	19	3	83 (55.3%)	
Total	58 (38.7%)	46 (30.7%)	20 (13.3%)	22 (14.7%)	4 (2.7%)	N=150	

