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# BMJ Open

## A Study of Cervical Cancer Screening Technologies in Human Immunodeficiency Virus-Infected Women Living in Rwanda

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Complete List of Authors:	Murenzi, Gad; Rwanda Military Hospital Dusingize, Jean; Regional Alliance for Sustainable Development , Research and Scientific Capacity Building Rurangwa, Theogene; Rwanda Military Hospital Sinayobye, Jean d'Amour; Regional Alliance for Sustainable Development, ; Women's Equity in Access to Care & Treatment (WE-ACTx), Munyaneza, Athanase; Rwanda Military Hospital Murangwa, Anthere; Rwanda Military Hospital Zawadi, Thierry; Rwanda Military Hospital Hebert, Tiffany; Yeshiva University Albert Einstein College of Medicine Mugenzi, Pacifique; Rwanda Military Hospital Adedimeji, Adebola; Yeshiva University Albert Einstein College of Medicine Mutesa, Leon; Rwanda Military Hospital; University of Rwanda College of Medicine and Health Sciences Anastos, Kathryn; Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, Castle, Philip; Yeshiva University Albert Einstein College of Medicine,
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Manuscripts

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3 **1 A Study of Cervical Cancer Screening Technologies in Human Immunodeficiency Virus-**  
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5 **2 Infected Women Living in Rwanda**  
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9 3 Gad Murenzi, MD\*<sup>1</sup>, Jean-Claude Dusingize, MD, MS<sup>1</sup>, Theogene Rurangwa, MD, MMed<sup>1</sup>,  
10  
11 4 Jean d'Amour Sinayobye, MD, MS<sup>1</sup>, Athanase Munyaneza, RN<sup>1</sup>, Anthere Murangwa, MS<sup>1</sup>,  
12  
13 5 Thierry Zawadi, MD<sup>1</sup>, Tiffany Hebert, MD<sup>2</sup>, Pacifique Mugenzi, MD<sup>1</sup>, MMed, Adebola  
14  
15 6 Adedimeji, PhD, MPH<sup>2</sup>, Leon Mutesa, MD, PhD<sup>1,3</sup>, Kathryn Anastos, MD<sup>2</sup>, Philip E. Castle,  
16  
17 7 PhD, MPH<sup>2,4</sup>  
18  
19  
20

21 8 <sup>1</sup>Rwanda Military Hospital, Kigali, Rwanda; <sup>2</sup>Albert Einstein College of Medicine, Bronx, NY,  
22  
23 9 USA; <sup>3</sup>University of Rwanda, Kigali, Rwanda; <sup>4</sup>Global Coalition Against Cervical Cancer,  
24  
25 10 Arlington, VA, USA.  
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29 11 \*Correspondence: [gadcollins@gmail.com](mailto:gadcollins@gmail.com), +250788589085  
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40 15 Prevent Cancer Foundation.  
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3 **17 Abstract**  
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5 **18** *Introduction.* The optimal method(s) for screening human immunodeficiency virus-infected  
6 **19** women, especially for those living in sub-Saharan Africa, for cervical precancer and early cancer  
7 **20** has yet to be established.  
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10 **21** *Methods and analysis.* A convenience sample of >5,000 Rwandan women, aged 30-54 years,  
11 **22** living with HIV infection will be enrolled into a cross-sectional study of cervical cancer  
12 **23** screening strategies. Eligible and consenting women will be enrolled into the study, complete a  
13 **24** short risk-factor questionnaire, and screened for high-risk human papillomavirus (hrHPV) using  
14 **25** the Xpert HPV assay (Cepheid, Sunnyvale, CA, USA), unaided visual inspection after acetic  
15 **26** acid (VIA), and aided VIA using the EVA system (Mobile ODT, Tel Aviv, Israel). Women  
16 **27** positive for hrHPV or by VIA will undergo colposcopy, which will include the collection of two  
17 **28** cervical specimens prior to undergoing a 4-quadrant microbiopsy protocol. The colposcopy-  
18 **29** collected specimens will be tested by dual immunocytochemical staining for p16<sup>INK4a</sup> and Ki-67  
19 **30** (CINtec® PLUS Cytology, Ventana, Tucson, AZ, USA) and for E6 or E7 for 8 hrHPV  
20 **31** genotypes (HPV16, 18, 31, 33, 35, 45, 52, and 58) using the next-generation AV Advantage  
21 **32** hrHPV E6/E7 test (Arbor Vita Corporation, Freemont, CA, USA). Women with local pathology  
22 **33** diagnosis of cervical intraepithelial neoplasia (CIN) grade 2 (CIN2) or more severe (CIN2+) or  
23 **34** pathology-review diagnosis of CIN grade 3 or more severe (CIN3+) will receive treatment.  
24 **35** Clinical performance and cost-effectiveness (e.g., sensitivity, specificity, and predictive values)  
25 **36** of different screening strategies and algorithms will be evaluated.  
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29 **37** *Ethics and dissemination.* The protocol has been approved by local and institutional review  
30 **38** boards for human subjects research. Results will be disseminated to the scientific community  
31 **39** through peer-reviewed publication and to the Rwandan stakeholders through an external  
32 **40** advisory panel.  
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3 42 **Strengths and Weaknesses**  
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6 43 • We will enroll a very large sample size of HIV-infected women living in Rwanda who  
7 44 otherwise would probably not get cervical cancer screening.  
8 45 • We will employ rigorous disease ascertainment protocols to minimize misclassification.  
9 46 • Some tests, such as the Xpert HPV and the AV Advantage HPV E6/E7 assays, will be  
10 47 done on site in Rwanda using technologies that could feasibly be deployed there.  
11 48 • A weakness of the study is that it will not be feasible to include cervical cytology as a  
12 49 comparator due to financial and logistical constraints.  
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- We will enroll a very large sample size of HIV-infected women living in Rwanda who otherwise would probably not get cervical cancer screening.
  - We will employ rigorous disease ascertainment protocols to minimize misclassification.
  - Some tests, such as the Xpert HPV and the AV Advantage HPV E6/E7 assays, will be done on site in Rwanda using technologies that could feasibly be deployed there.
  - A weakness of the study is that it will not be feasible to include cervical cytology as a comparator due to financial and logistical constraints.

## 51 **Introduction**

52 Invasive cervical cancer (ICC) remains a significant cause of morbidity and mortality globally.  
53 Approximately 530,000 cases of and 270,000 deaths due to ICC occur annually, making it the 4<sup>th</sup>  
54 most common malignancy and cause of cancer-related death in women globally.<sup>1</sup> In many high-  
55 income countries (HIC), cervical cancer rates have declined by 50% or more<sup>2</sup> due to the  
56 introduction of effective, high-coverage Pap testing (cervical cytology)-based screening  
57 programs that include timely follow-up of screen positives, treatment of women with precursor  
58 lesions, and management of cancers. Now, almost 90% of ICC and ICC-related deaths occur in  
59 low- and middle income countries (LMICs) due to a lack of resources and healthcare  
60 infrastructure needed to provide preventive services.

61 ICC and ICC-related mortality rates are particularly high in Sub-Saharan Africa, which also has  
62 the highest rates of HIV infection in the world. Now, over 12 million HIV-infected (HIV[+])  
63 women in Sub-Saharan Africa are living longer because of anti-retroviral therapy, thus  
64 increasing their likelihood of dying from ICC.<sup>3</sup> However, many of these women are already  
65 exposed to human papillomavirus (HPV), the viral cause of cervical cancer, and will not benefit  
66 from or be targeted for prophylactic HPV vaccination. Thus, cervical cancer screening is needed  
67 for the foreseeable future.

68 However, setting up effective cytology for cervical cancer screening is expensive and requires a  
69 complex clinical and lab infrastructure that generally does not exist in LMICs.<sup>4;5</sup> Moreover, it is  
70 now well understood that cytology has only a low- to moderate one-time sensitivity for precursor  
71 lesions and therefore must be done repeatedly over many years to reduce cancer risk. Alternative

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3 72 strategies to address the cervical cancer burden in LMICs, especially in SSA, must be developed  
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5 73 and validated.  
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9 74 Persistent cervical infections by high-risk HPV (hrHPV) types cause virtually all ICC and its  
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11 75 immediate precursor lesions, e.g. cervical intraepithelial neoplasia grade 3 (CIN3) and  
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13 76 adenocarcinoma *in situ* (AIS) everywhere in the world.<sup>6;7</sup> hrHPV causes most anal and vaginal  
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15 77 cancer and a significant proportion of vulvar, penile, and oropharyngeal cancers.<sup>8</sup> HPV16 is the  
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17 78 most important causal type, responsible for ~60% of ICC.<sup>9</sup> HPV18 is the next most important,  
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19 79 responsible for 10-15% of ICC, including 30-40% of adenocarcinoma of the cervix<sup>9</sup>, which is on  
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21 80 the rise in Western Countries.<sup>10;11</sup> Together, HPV16 and HPV18 account for ~70% of ICC, and  
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23 81 the same 15 hrHPV types account for ~99% of ICC everywhere in the world.<sup>9</sup>  
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28 82 There is now overwhelming evidence to suggest that testing for hrHPV is more sensitive, albeit  
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30 83 less specific, than high-quality cytology for identifying women with cervical precancer.<sup>12-16</sup> One-  
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32 84 time hrHPV testing can reduce the risk of ICC incidence by approximately 40% in 6.5 years  
33  
34 85 compared to cytology screening<sup>16</sup>, and ICC mortality by approximately 40% (approximately  
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36 86 50% overall) in 8 years compared to cytology.<sup>17</sup> Importantly, a negative hrHPV test provides  
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38 87 superior reassurance against CIN3+<sup>18</sup> and against ICC<sup>16;17</sup>, permitting safe extension of  
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40 88 screening intervals.  
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45 89 The World Health Organization released cervical cancer screening and treatment guidelines in  
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47 90 2013, recommending two evidence-based approaches to ICC screening<sup>19</sup>: (I) Use either hrHPV  
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49 91 testing or visual inspection after acetic acid (VIA), which involves the inspection of the cervix  
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51 92 with a speculum in place and following the application of dilute acetic acid to help identify  
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53 93 potential CIN by its characteristic white coloring in the presence of acetic acid (acetowhite), as  
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3 94 alternative initial screening tests instead of Pap, and (II) immediately treat those who screen  
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5 95 positive using the screening test, rather than require diagnostic verification through colposcopy  
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7 96 and biopsy. This approach is commonly called screen-and-treat (S&T), and is increasingly  
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10 97 thought to be more amenable to LMIC settings.

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13 98 However, hrHPV testing is also a much more effective screen than VIA<sup>17</sup>, which on a large-scale  
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15 99 appears to only down-stage cancer rather than prevent it.<sup>20</sup> Thus, the recent American Society for  
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17 100 Clinical Oncology (ASC) resource-stratified guidelines for secondary cervical cancer  
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19 101 prevention<sup>21;22</sup> emphasize that hrHPV testing is the preferred choice for screening, with VIA  
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21 102 only being used until hrHPV testing becomes available, and that HIV-infected women, because  
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23 103 of their higher risk, should be screened twice as frequently as the general (HIV-uninfected)  
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25 104 population.

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30 105 Recent data in HIV[+] women living in the U.S. suggest that hrHPV testing may have clinical  
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32 106 utility similar to that in HIV-negative (HIV[-]) women. Several observational studies have  
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34 107 shown that an extended screening interval is safe in HIV[+] women who test hrHPV and Pap  
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36 108 negative as it is for HIV[-] women.<sup>23;24</sup> In a study of women enrolled in Women's Interagency  
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38 109 Health Study (WIHS) in 2002, HIV[+] and HIV[-] women who tested hrHPV and Pap negative  
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40 110 were at a similarly low risk of cervical intraepithelial neoplasia (CIN) grade 2 (CIN2) or more  
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42 111 severe (CIN2+) histology over a 5-year follow-up.<sup>23</sup> In addition, no cases of histologically  
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44 112 confirmed CIN2+ were diagnosed in the follow-up of hrHPV- and Pap-negative HIV[+] women  
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46 113 aged 30-64 years who underwent routine three-year hrHPV and cytology cotesting at Kaiser  
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48 114 Permanente Northern California.<sup>24</sup> Thus, both studies found very high negative predictive values  
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50 115 (NPV) >99% in HIV[+] women who test hrHPV negative. However, how hrHPV testing can  
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3 116 best be used to screen HIV[+] women living in Sub-Saharan Africa to prevent cervical cancer  
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5 117 remains to be determined.  
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9 118 Recent studies<sup>25-28</sup> in HIV[+] women living in Sub-Saharan Africa have compared hrHPV, VIA,  
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11 119 and/or Pap for the detection of cervical precancer/cancer. The results can be summarized as  
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13 120 follows: 1) hrHPV detection was more sensitive but less specific than VIA and 2) surprisingly,  
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15 121 cytology was equally or more sensitive but less specific than VIA and 2) surprisingly, cytology  
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17 122 was equally or more sensitive but less specific (vs. the converse) than hrHPV testing. Results and  
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19 123 conclusions are varied, leaving unanswered the question of what screening strategy in HIV[+]  
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21 124 women living in Sub-Saharan Africa has the greatest effectiveness and cost effectiveness.  
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26 125 Regardless of the screening method, most screen-positive women who go to colposcopy or are  
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28 126 treated immediately without diagnostic verification do not have cervical precancer and cancer  
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30 127 (positive predictive value [PPV] for screening tests are typically 10%-20%). In places like Sub-  
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32 128 Saharan Africa that lack necessary infrastructure and personnel such as pathologists<sup>29</sup>, excessive  
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34 129 referral to colposcopy is problematic. Although WHO recommendations for S&T will hopefully  
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36 130 overcome this bottleneck and increase the number of women living in LMICs who get screened,  
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38 131 many countries may not adopt current S&T strategies because of concerns of low specificity and  
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40 132 overtreatment, resulting in increased costs, unnecessary patient discomfort and concern, and  
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42 133 wasting valuable healthcare resources that could otherwise be used to expand access to  
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44 134 screening. Thus, methods to increase the accuracy of screening by reducing the numbers of  
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46 135 women having colposcopy and biopsy or getting treated immediately in this context are highly  
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48 136 desirable as they will likely increase the uptake of cervical cancer screening.  
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3 137 In order to improve the specificity of screening tests, secondary tests (biomarkers) are used  
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5 138 following a screen-positive result, with women who test positive for the triage undergoing  
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7 139 further management (e.g., colposcopy or immediate treatment) and those who test negative  
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10 140 typically being deferred to further evaluation in 6-18 months to allow hrHPV infections to clear.  
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12 141 There are several very promising biomarkers that might be used to improve the specificity and  
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14 142 positive predictive value (PPV) of the screening tests.<sup>24</sup> Given that HIV-infected women are  
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16 143 more likely to test hrHPV positive<sup>30-34</sup>, it is important to validate a triage strategy of using a  
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18 144 secondary biomarker that sensitively and specifically rules-in women with cervical precancer  
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20 145 and cancer among the HIV-positive, hrHPV-positive women.

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25 146 We are therefore conducting a cervical cancer screening study of >5,000 Rwandan women, aged  
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27 147 30-54 years, living with HIV infection. We will evaluate different screening tests (hrHPV DNA  
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29 148 and VIA) and different triage tests and biomarkers for screen-positive women (E6/E7  
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31 149 oncoprotein detection, p16INK4a immunocytochemistry, and hrHPV viral methylation). Screen-  
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33 150 positive women undergo a rigorous colposcopic evaluation with multiple biopsies taken and the  
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35 151 biopsies will undergo pathology review, to minimize the misclassification of endpoints. The  
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37 152 primary objective of the study is to determine and compare clinical performance (Sensitivity  
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39 153 (Se), Specificity (Sp), PPV, and NPV) and cost-effectiveness for identifying HIV[+] women with  
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41 154 CIN3+ and CIN2+ of different cervical cancer screening and management algorithms.  
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## 46 155 **Methods and Analysis**

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50 156 *Study design and population:* We are recruiting those women receiving care in health centers  
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52 157 (HC) and various hospitals operated by the Ministry of Health or Rwanda Military Hospital  
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54 158 during 2016-18 (**Table 1**). Sites were selected in collaboration with Rwanda Biomedical Center  
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3 159 (RBC), which calculated estimated numbers of potentially eligible women using data from the  
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5 160 HIV database (OpenMRS-Open Medical Records System; <http://openmrs.org/>). Sites were also  
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7 161 selected from all provinces to ensure geographic representation. The total is the number of 30-54  
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9 162 year old women receiving care at all selected sites and our study population is an estimate of the  
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11 163 women who will actually be eligible according to all inclusion and exclusion criteria.

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15 164 Inclusion criteria include 1) living and receiving HIV care in Rwanda, 2) aged 30-54 years, 3)  
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17 165 confirmed HIV+, 4) no prior cervical cancer screening, 5) no history of ICC, and 6) willing, able  
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19 166 and competent to provide written, informed consent. Exclusion criteria, in addition to not  
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21 167 meeting the inclusion criteria, include 1) pregnant, 2) signs of abnormal, non-menstrual bleeding  
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23 168 suggestive of ICC, 3) without a cervix due to hysterectomy, and 4) not sufficiently healthy to  
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25 169 participate in a research study based on the judgment of the clinicians. Excluded women will be  
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27 170 advised to seek routine cervical cancer screening through government programs.

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32 171 Prior to initiation of enrollment at a specific clinic, the local HIV-care provider team identifies  
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34 172 potentially eligible women at their routine clinic visits and offer them enrollment. Women  
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36 173 indicating interest in the study are then registered by our research nurses using the eligibility  
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38 174 criteria checklist. We enroll women at one site until all the eligible women at that site are  
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40 175 screened for cervical cancer. Our team of at least two research nurses schedules 12 to 15 women  
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42 176 three to four days a week by calling them and confirming appointments over the telephone. We  
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44 177 have two teams in the field meaning that we run two cervical cancer screening clinics  
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46 178 simultaneously.

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52 179 *Enrollment Visit:* The study participant flow is summarized in **Figure 1**. During their  
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54 180 appointment for screening, women are educated on cervical cancer risk factors, mainly HPV

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3 181 infection, and why they are more at risk to develop cervical cancer than HIV-uninfected women.  
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5 182 They are also allowed to ask questions before they commit to participating in the study. Women  
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7 183 are asked to provide informed, written consent to participate in the study using a printed out  
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9 184 consent form. Those who provide consent complete a short nurse-administered questionnaire on  
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11 185 cervical cancer risk factors and sociodemographic characteristics using a data capture screen in  
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13 186 Microsoft Access.

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18 187 Enrolled women then undergo a pelvic exam, with VIA and a single cervical exfoliated (“Pap”)  
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20 188 specimen collected and placed into 20 ml PreservCyt (Hologic, Bedford, MA, USA) which is  
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22 189 then sent to the lab at RMH for hrHPV testing. Finally, a portable colposcope  
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24 190 (<http://www.mobileodt.com/>; MobleODT, Tel Aviv, Israel) is used for digital cervicography  
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26 191 (~VIA with magnification) and the image is captured and saved for quality control, research, and  
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28 192 to develop a digital library.

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33 193 *Colposcopy Visit:* Screen-positive women are called using a telephone as soon as the hrHPV  
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35 194 result is available and provided colposcopy. All women receiving colposcopy will have two  
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37 195 additional specimens collected, one into PreservCyt for the evaluation of other molecular  
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39 196 biomarkers (genotype-specific hrHPV viral methylation and load, and p16/Ki-67  
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41 197 immunocytochemistry CINtec® PLUS Cytology Kit [Roche, Tucson, AZ, USA]) and a second  
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43 198 using a dry swab for HPV16, 18, 31, 33, 35, 45, 52, and 58 E6/E7 oncoprotein detection by the  
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45 199 next generation lateral flow hrHPV oncoprotein test from Arbor Vita Corporation (Fremont, CA,  
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47 200 USA) included in this study as a triage for screen-positive women to identify those women who  
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49 201 are at higher risk of having CIN3+. The residual PreservCyt specimens from both the screening  
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51 202 and colposcopy visits will be stored at -20°C, creating a biobank in Rwanda for future  
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53 203 retrospective evaluations of promising new biomarkers and tests.  
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3 204 After specimen collection, a colposcopic evaluation of the cervix is done and a modified version  
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5 205 of the 4-quadrant microbiopsy procedure is performed.<sup>35</sup> Compared to the standard biopsy, the  
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7 206 microbiopsy protocol improves disease ascertainment and reduces biases by selecting on the  
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9 207 most obvious acetowhite lesions while removing less tissue (~13 mm<sup>2</sup> for 4 microbiopsies vs.  
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11 208 ~28mm<sup>2</sup> for 1 standard biopsy). Modifications to the standard 4-quadrant microbiopsy procedure  
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13 209 are: 1) endocervical curettage is taken only for those women whose squamocolumnar junction is  
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15 210 not entirely visible and the lesion extends into the endocervical canal; and 2) standard biopsies of  
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17 211 very large lesions can be taken to increase the likelihood that the most severe area is biopsied.  
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22 212 *Pathology:* Biopsies are processed in a single cassette so that a single slide has a section from all  
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24 213 biopsies taken. Biopsies read by a local pathologist at RMH and Dr. Hebert or another  
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26 214 pathologist at Montefiore Medical Center, Bronx, NY, USA. Women receiving a diagnosis of  
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28 215 CIN2+ by the local pathologist or CIN3+ diagnosis by consensus review will be referred for  
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30 216 treatment. A slide with biopsies also will undergo p16 immunohistochemistry (IHC) using the  
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32 217 CINtec® Histology Kit (Roche).  
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37 218 *Endpoints:* The primary scientific endpoint of the study will be histologically confirmed,  
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39 219 consensus CIN3+. The secondary, clinical endpoint will be histologically confirmed ≥+  
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41 220 diagnosed by the Rwandan pathologist. Additional endpoints using pathology review and p16  
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43 221 IHC will be used but not for evaluating the performance of p16 immunocytochemistry due to the  
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45 222 possibility of p16-related autocorrelation.  
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50 223 *Treatment:* Women diagnosed with CIN2+ will be referred for treatment. Those precancerous  
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52 224 lesions will be treated by ablation if they meet WHO criteria for cryotherapy.<sup>36</sup> Those who do  
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54 225 not meet those criteria will undergo an excision procedure (e.g., loop electrosurgical excision  
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3 226 procedure [LEEP] or cold-knife cone [CKC]) or, in the case of an ICC diagnosis, referred for  
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5 227 cancer management. Screen-positive women with <CIN2 will be advised to seek re-screening in  
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8 228 a year through the existing healthcare system.  
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11 229 *Data sources:* Data are collected from the following sources:  
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14 230 1. A nurse-administered questionnaire on sociodemographic characteristics and cervical  
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16 231 cancer risk factors including age at first sexual intercourse, number of sexual partners,  
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18 232 smoking, contraception, parity and socioeconomic status.  
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22 233 2. Pelvic exam, VIA, Mobile ODT and colposcopy data capture forms  
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25 234 3. Medical record data on HIV status (e.g.,  
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28 235 (<http://www.who.int/hiv/pub/guidelines/HIVstaging150307.pdf>), CD4 count, viral load,  
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30 236 antiretroviral therapy (ART) regimen(s)), care, and dates.  
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33 237 *Laboratory Testing:* The following laboratory tests will be performed:  
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36 238 Xpert HPV Testing—cervical Pap specimens in PreservCyt will be sent to the RMH laboratory  
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38 239 in Kigali, Rwanda for hrHPV DNA testing using the Xpert HPV test (Cepheid, Sunnyvale, CA,  
39  
40 240 USA).<sup>37-42</sup> The Xpert HPV Assay is a new, qualitative, real-time PCR assay for the detection of  
41  
42 241 hrHPV DNA. The Xpert HPV Assay includes simultaneous detection of 14 hrHPV types,  
43  
44 242 hydroxymethylbilane synthase (HMBS), and an internal Probe Check Control (PCC). The 14  
45  
46 243 targeted hrHPV types are detected in 5 fluorescent channels: 1) HPV16, 2) HPV18 and hrHPV  
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48 244 45 (HPV18/45), 3) HPV31, 33, 35, 52, and 58, 4) HPV51 and HPV59, and 5) HPV39, 56, 66,  
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51 245 and 68. HMBS (fluorescent channel 6) verifies specimen adequacy.  
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3 246 Specimens are mixed and a 1-mL pre-aliquot is removed using a disposable pipette and placed in  
4  
5 247 the testing cartridge per the manufacturer's instructions. Unsatisfactory results due to insufficient  
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7 248 cellular content are re-run. If the second test is also unsatisfactory, the final result will be  
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10 249 unsatisfactory but women are referred to colposcopy for safety.

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13 250 HPV Viral Methylation—To identify single hrHPV type infections, we will select single-channel  
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15 251 positives from the Xpert HPV assay. For those that are hrHPV positive for a channel other than  
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17 252 HPV16, which is detected singly, we will test them to identify the single type infections using a  
18  
19 253 standard protocol for PCR amplification using MY09/11 L1 consensus primers and hrHPV  
20  
21 254 genotype detection using dot-hybridization for 39 individual type-specific probes and a mixture  
22  
23 255 of probes for 10 other uncommon hrHPV types as previously described.<sup>43;44</sup> To isolate the DNA,  
24  
25 256 ThinPrep specimens (1.5 mL) will be pelleted, re-suspended in STM, digested with Proteinase K,  
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27 257 precipitated overnight in ammonium acetate ethanol at -20°C, washed, and suspended and stored  
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29  
30 258 in TE buffer.

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35 259 The isolated DNA will then undergo bisulfite conversion.<sup>45</sup> Following bisulfite conversion and  
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37 260 DNA purification and de-sulphonation, bisulfite-treated DNA will be used as template for Next-  
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39 261 Gen Sequencing (NGS) (HiSeq2000, Illumina, San Diego, CA) using barcoded-type specific  
40  
41 262 primers. Sequences for pads and barcodes are not found in the targeted genomic region. Use of  
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43 263 padding and barcodes enables reads to be identified by amplicon (forward or reverse) or by  
44  
45 264 sample during downstream bioinformatics analysis.<sup>46</sup>

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50 265 All PCR products for NGS are pooled (by assay) and a single DNA band containing multiple  
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52 266 amplicons from different samples (with unique barcodes) is isolated from a gel for NGS.<sup>46</sup>  
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54 267 Briefly, equal concentrations of each barcoded PCR product (based on PCR band intensity) are

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3 268 pooled and isolated. Upon confirmation of correct product size, all purified DNA pools are  
4  
5 269 combined and submitted for library preparation and paired-end 100 base pair Illumina  
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7 270 HiSeq2000 sequencing at the Einstein Genomics Core Facility.  
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10  
11 271 Methylation status will be determined in the lab of Dr. Robert Burk at Albert Einstein College of  
12  
13 272 Medicine (USA). Prior to determination of methylation status, de-multiplexing based on the  
14  
15 273 unique barcodes is performed using in-house generated scripts to obtain paired-end NGS reads of  
16  
17 274 each sample. Reads are aligned with hrHPV reference genome sequences by bowtie v0.12.9.<sup>47</sup>  
18  
19 275 Methylation status of each CpG site is then determined by bismark v0.7.7<sup>48</sup> using the default  
20  
21 276 quality score parameter set to Q30, and the formula of the methylation ratio of the number of C  
22  
23 277 read by the number of C+T read.  
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28 278 E6/E7 Oncoprotein Testing—Dry swab specimens collected at the time of colposcopy will be  
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30 279 tested for individual E6/E7 oncoproteins as previously described<sup>49,50</sup>, according to the  
31  
32 280 manufacturer's instructions, at the RMH laboratory in Kigali, Rwanda. The only deviation from  
33  
34 281 the previous methods is that 3 lateral flow strips will be used to detect 8 hrHPV types in this  
35  
36 282 study vs. 1 lateral flow strip used to detect 3 types in the study in China.  
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41 283 *Analyses:* We will evaluate combinations of the above mentioned screening strategies and tests  
42  
43 284 to estimate the clinical performance (e.g., Se, Sp, PPV, and NPV) for the detection of consensus  
44  
45 285 CIN3+ and community CIN2+. A log binomial model implemented with generalized estimating  
46  
47 286 equations will be used to take into account correlation between different tests from the same  
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49 287 subject. Note that these models are developed for the estimation and comparison of performance  
50  
51 288 for two tests, but the model can be extended to allow more than two tests by including more  
52  
53 289 indicator variables for test type.  
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290 Some analyses of biomarkers, such as viral methylation are restricted to hrHPV-positives.

291 Comparisons of hrHPV viral methylation to other triage biomarkers will be restricted to the  
292 subset that gets tested for viral methylation as described.

293 *Sample size calculations:* We based our sample size on the ability to detect modest but minimally  
294 important differences in Se of 15%. We conservatively assume that the population prevalence of  
295 CIN3+ is 2% based on our past study in Rwanda.<sup>33;51</sup> We propose to enroll and have complete  
296 follow-up of at least 5,000 HIV[+] women. A sample size of 5,000 HIV[+] women with  
297 completed follow-up of the screen positives will yield 100 cases of CIN3+, which has at least  
298 80% power ( $\alpha=5\%$ ) to crudely detect a 15% difference in Se between two screening strategies  
299 for a range of 10%-25% discordance. With this sample size of 5,000 women, 4,900 will not have  
300 CIN3+; we will also have at least 90% power ( $\alpha=5\%$ ) to detect a difference in Sp of 3% for  
301 discordance up to 40%. Finally, we will have 80% power ( $\alpha=5\%$ ) to crudely detect an 8%, 10%,  
302 or 11% difference in PPV if the reference PPV is 10%, 20%, or 30%, respectively.<sup>52</sup>

303 *Cost Effectiveness:* We will conduct assessments of the costs and cost-effectiveness of the  
304 different combinations of screening and triage tests, i.e., algorithms, as well as those of the entire  
305 community-based screening “system.” Costs measurement will be conducted using a micro-  
306 costing (ingredients) approach in which resource use throughout each step in the screening  
307 process will be tracked and unit costs for each of the resources will be applied to generate an  
308 average screening cost per woman to be compared against what the estimated costs are for a  
309 possible program based on hrHPV screening and VIA triage or VIA screening. For estimating  
310 costs of the screening system and scale-up of screening to 100,000 women in a month, analyses  
311 will distinguish financial costs, which reflect actual expenditures of the program, from economic

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3 312 costs, including the value of donated and shared resources to more fully assess opportunity costs.  
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5 313 Projections on budget impact and economic cost implications over time will be made under  
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7 314 varying assumptions of screening uptake, follow-up compliance, and scenarios of changing  
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9 315 disease burden.  
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13 316 Clinical outcomes will include true positive, true negative, false negative, and false positive test  
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15 317 results, number of colposcopies, incident cancer, and cancer death. Cost-effectiveness will be  
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17 318 measured as cost/CIN2+ detected, cost/CIN3+ detected, cost/invasive cancer prevented,  
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19 319 cost/cancer death prevented, cost/life-year saved, and cost/quality-adjusted life year (QALY)  
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21 320 saved; in addition, we will calculate harm/benefit ratios, using varying definitions of harms  
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23 321 (colposcopies, false positive results) to benefits (cancers prevented, deaths prevented, life years  
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25 322 and QALYs saved). Costs and effectiveness will be discounted at a 3% annual rate, with the rate  
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27 323 varied from 0-5% in sensitivity analysis. For assessment of value-of-information (VOI), we will  
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29 324 use net monetary benefits (NMB), defined as a function of the willingness-to-pay threshold  
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31 325 (WTP) for different costs and outcomes as:  $NMB = (WTP * Effectiveness) - Costs$ .  
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### 34 326 **Ethics and Dissemination** 35 36 37

38 327 *Ethics*: This study protocol was reviewed and approved by the Rwanda National Ethics  
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40 328 Committee (RNEC) as well as the Institutional Review Board for human subjects research at  
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42 329 Albert Einstein College of Medicine.  
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45 330 *Confidentiality measures and protection against potential risks*: The risks for those participating  
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47 331 in our study include:  
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3 332           • Collection of Pap specimens/cervical swabs involves a modest risk of bleeding  
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5           333           which is typically very limited when it occurs. Testing positive for any test may  
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7           334           cause psychological distress (anxiety).  
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10           335           • Colposcopy and excisional treatments induce vaginal bleeding and may incur pain,  
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12           336           infection, and short-term psychological distress (anxiety). A diagnosis of CIN2 or  
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14           337           more severe may cause psychological distress (anxiety). A diagnosis of invasive  
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16           338           cervical cancer may cause severe psychological distress.  
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20           339           • Questions in the questionnaire, regarding sexual behavior and other matters of a  
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22           340           personal nature, may cause anxiety and embarrassment. Participants are advised  
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24           341           that they are free not to answer specific questions.  
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28           342           • There is also the risk of psycho-social stress which could occur if there was  
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30           343           inadvertent disclosure of confidential medical or other personal information.  
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33 344 Protection against the risk of inadvertent disclosure of confidential information is addressed by  
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35 345 the standard procedures at the Rwandan study site, including: (i) storing completed paper copies  
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37 346 of questionnaires and other hard copy information (described above), identified by study number  
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39 347 only, in a filing system separate from the name-address file of participants in the study; and (ii)  
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41 348 only the designated local personnel have access to cross-reference the files; (iii) all paper files,  
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43 349 including consent forms, will be maintained in locked cabinets in locked rooms, with access  
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45 350 restricted to specific research personnel.  
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50 351 In addition, we will include the following security measures to protect the data:  
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- 54 352           • Controlled access to project data;  
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- 353 • A tracking system for data forms and activities;
- 354 • Date and time of stamping of all data records with electronic signatures;
- 355 • Audit trails to track all changes made to data records; and
- 356 • Data kept on password-protected computers and in locked rooms.

357 *Potential Patient Benefits:* There are potential direct benefits to study participants. HIV[+] women participating in the study are at very high risk of cervical cancer. They will be rigorously screened and evaluated, more effectively than the standard of care anywhere in the world. As a result, women with precancer who are at imminent risk of invasive cervical cancer will be diagnosed sooner and treated more effectively than women receiving routine care and thereby more likely averting the development of cervical cancer. Women with cervical cancer will be diagnosed earlier thereby reducing the morbidity and the risk of mortality caused by cervical cancer. Conversely, any pain, bleeding, or stress that might occur related to colposcopy or cervical swab are typically modest and well tolerated.

366 There are also substantial potential societal benefits related to the study due to its implications to improving cervical cancer screening practices and guidelines in HIV[+] women – changes in practice which might also benefit the study participants themselves, if and when these changes are enacted. There is a great need to identify more effective and practical methods for cervical cancer screening for HIV[+] women living in Africa, who are living longer than ever and are therefore at potentially greater risk of cervical cancer.

372 *Dissemination:* We plan to publish a series of scientific reports in peer-reviewed scientific journals. As building research capacity in Rwanda is a major goal of this research project, all investigators of the research team will be asked and supported to lead at least one analysis and one manuscript preparation, based on interests and expertise.

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3 376 In addition, an external advisory panel (EAP) composed of leaders from the Rwanda Ministry of  
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5 377 Health, University of Rwanda, and Rwanda medical community has been formed. The  
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7 378 responsibilities of the EAP include providing advice on the conduct of the project and  
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10 379 interpretation for and dissemination of the study results to Rwandan stakeholders. The latter is  
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12 380 important for the adoption of evidence-based best practices for cervical cancer screening as  
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14 381 warranted.  
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For peer review only

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3 383 Contributor Statement:  
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6 384 KA, PEC, JCD, AA, and JDS conceived the original concept of the study and the interventions.  
7  
8 385 GM, KA, JDS, and PEC drafted the protocol. PEC performed the sample size calculations, and  
9  
10 386 PEC and GM will lead analysis of the results. GM, JDS, and Athanase Munyaneza are  
11  
12 387 supporting patient recruitment. GM, TR, and Athanase Munyaneza are providing clinical care for  
13  
14 388 patients. Anthere Murangwa and LM oversee laboratory testing, and TZ and TH are responsible  
15  
16 389 for pathology. PM and LM oversee and administer the study activities at the clinical site in  
17  
18 390 Rwanda. All authors (GM, JCD, TR, JDS, Athanase Munyaneza, Anthere Murangwa, TZ, TH,  
19  
20 391 PM, AA, LM, KA, and PEC) contributed to the scientific design of the study and the protocol  
21  
22 392 development, are involved in the implementation of the project, and have read and approved the  
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24 393 final manuscript.  
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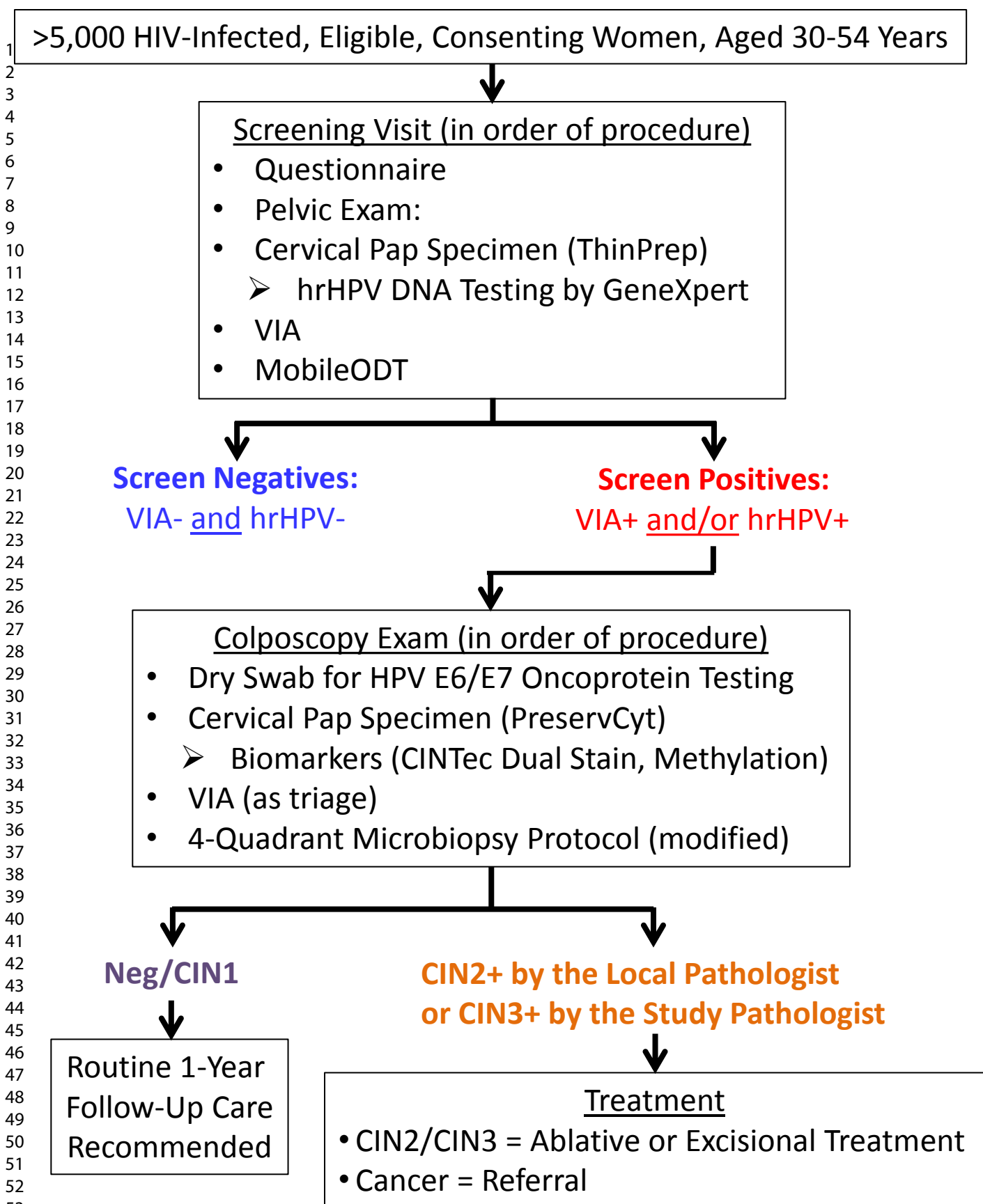
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547 **Table 1:** Recruitment sites and estimated population to be recruited from each site.

Province	Site	Type of site	Potential participants per site (approximate)
Kigali	Cor-unum HC	Public Health Center	1,405
Kigali	Kimironko HC	Public Health Center	1,227
Kigali	Rwampara HC	Public Referral Hospital	1,098
Kigali	Kicukiro HC	Public Health Center	1,054
Kigali	Kacyiru HC	Public Health Center	905
Kigali	Gikondo HC	Public Health Center	925
Kigali	Rwanda Military Hospital	Public Referral Hospital	300
Kigali	WEACTx for Hope	Private HIV Clinic	500
Kigali	Busanza HC	Public Health Center	100
Kigali	Nyarugunga HC	Public Health Center	100
West	Gisenyi DH	Public District Hospital	810
Total			8,424

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# BMJ Open

## A Study of Cervical Cancer Screening Technologies in Human Immunodeficiency Virus-Infected Women Living in Rwanda

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Keywords:	human papillomavirus (HPV), cervical cancer, HIV & AIDS < INFECTIOUS DISEASES, GYNAECOLOGY, cervical intraepithelial neoplasia

SCHOLARONE™  
Manuscripts

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3 **1 A Study of Cervical Cancer Screening Technologies in Human Immunodeficiency Virus-**  
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5 **2 Infected Women Living in Rwanda**  
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9 3 Gad Murenzi, MD\*<sup>1</sup>, Jean-Claude Dusingize, MD, MS<sup>1</sup>, Theogene Rurangwa, MD, MMed<sup>1</sup>,  
10  
11 4 Jean d'Amour Sinayobye, MD, MS<sup>1</sup>, Athanase Munyaneza, RN<sup>1</sup>, Anthere Murangwa, MS<sup>1</sup>,  
12  
13 5 Thierry Zawadi, MD<sup>1</sup>, Tiffany Hebert, MD<sup>2</sup>, Pacifique Mugenzi, MD<sup>1</sup>, MMed, Adebola  
14  
15 6 Adedimeji, PhD, MPH<sup>2</sup>, Leon Mutesa, MD, PhD<sup>1,3</sup>, Kathryn Anastos, MD<sup>2</sup>, Philip E. Castle,  
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17 7 PhD, MPH<sup>2,4</sup>  
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20

21 8 <sup>1</sup>Rwanda Military Hospital, Kigali, Rwanda; <sup>2</sup>Albert Einstein College of Medicine, Bronx, NY,  
22  
23 9 USA; <sup>3</sup>University of Rwanda, Kigali, Rwanda; <sup>4</sup>Global Coalition Against Cervical Cancer,  
24  
25 10 Arlington, VA, USA.  
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29 11 \*Correspondence: [gadcollins@gmail.com](mailto:gadcollins@gmail.com), +250788589085  
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32 12 Disclosures: This research study has received HPV tests for reduced or no cost from Cepheid,  
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34 13 Arbor Vita Corporation, and Roche.  
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40 15 Prevent Cancer Foundation.  
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3 17 **Abstract**  
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5 18 *Introduction.* The optimal method(s) for screening human immunodeficiency virus-infected  
6 19 women, especially for those living in sub-Saharan Africa, for cervical precancer and early cancer  
7 20 has yet to be established.  
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10 21 *Methods and analysis.* A convenience sample of >5,000 Rwandan women, aged 30-54 years,  
11 22 living with HIV infection will be enrolled into a cross-sectional study of cervical-cancer  
12 23 screening strategies. Eligible and consenting women will be enrolled into the study, complete a  
13 24 short risk-factor questionnaire, and screened for high-risk human papillomavirus (hrHPV) using  
14 25 the Xpert HPV assay (Cepheid, Sunnyvale, CA, USA), unaided visual inspection after acetic  
15 26 acid (VIA), and aided VIA using the EVA system (Mobile ODT, Tel Aviv, Israel). Women  
16 27 positive for hrHPV or by VIA will undergo colposcopy, which will include the collection of two  
17 28 cervical specimens prior to undergoing a 4-quadrant microbiopsy protocol. The colposcopy-  
18 29 collected specimens will be tested by dual immunocytochemical staining for p16<sup>INK4a</sup> and Ki-67  
19 30 (CINtec® PLUS Cytology, Ventana, Tucson, AZ, USA) and for E6 or E7 for 8 hrHPV  
20 31 genotypes (HPV16, 18, 31, 33, 35, 45, 52, and 58) using the next-generation AV Advantage  
21 32 hrHPV E6/E7 test (Arbor Vita Corporation, Freemont, CA, USA). Women with local pathology  
22 33 diagnosis of cervical intraepithelial neoplasia (CIN) grade 2 (CIN2) or more severe (CIN2+) or  
23 34 pathology-review diagnosis of CIN grade 3 or more severe (CIN3+) will receive treatment.  
24 35 Clinical performance and cost-effectiveness (e.g., sensitivity, specificity, and predictive values)  
25 36 of different screening strategies and algorithms will be evaluated.  
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29 37 *Ethics and dissemination.* The protocol has been approved by local and institutional review  
30 38 boards for human subjects research. Results will be disseminated to the scientific community  
31 39 through peer-reviewed publication and to the Rwandan stakeholders through an external  
32 40 advisory panel.  
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3 42 **Strengths and Weaknesses**  
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6 43 • We will enroll a very large sample size of HIV-infected women living in Rwanda who  
7 44 otherwise would probably not get cervical-cancer screening.  
8 45 • We will employ rigorous disease ascertainment protocols to minimize misclassification.  
9 46 • Some tests, such as the Xpert HPV and the AV Advantage HPV E6/E7 assays, will be  
10 47 done on site in Rwanda using technologies that could feasibly be deployed there.  
11 48 • A weakness of the study is that it will not be feasible to include cervical cytology as a  
12 49 comparator due to financial and logistical constraints.  
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- We will enroll a very large sample size of HIV-infected women living in Rwanda who otherwise would probably not get cervical-cancer screening.
  - We will employ rigorous disease ascertainment protocols to minimize misclassification.
  - Some tests, such as the Xpert HPV and the AV Advantage HPV E6/E7 assays, will be done on site in Rwanda using technologies that could feasibly be deployed there.
  - A weakness of the study is that it will not be feasible to include cervical cytology as a comparator due to financial and logistical constraints.

## 51 **Introduction**

52 Invasive cervical cancer (ICC) remains a significant cause of morbidity and mortality globally.  
53 Approximately 530,000 cases of and 270,000 deaths due to ICC occur annually, making it the 4<sup>th</sup>  
54 most common malignancy and cause of cancer-related death in women globally.<sup>1</sup> In many high-  
55 income countries (HIC), cervical cancer rates have declined by 50% or more<sup>2</sup> due to the  
56 introduction of effective, high-coverage Pap testing (cervical cytology)-based screening  
57 programs that include timely follow-up of screen positives, treatment of women with precursor  
58 lesions, and management of cancers. Now, almost 90% of ICC and ICC-related deaths occur in  
59 low- and middle income countries (LMICs) due to a lack of resources and healthcare  
60 infrastructure needed to provide preventive services.

61 ICC and ICC-related mortality rates are particularly high in Sub-Saharan Africa, which also has  
62 the highest rates of HIV infection in the world. Now, over 12 million HIV-infected (HIV[+])  
63 women in Sub-Saharan Africa are living longer because of anti-retroviral therapy, thus  
64 increasing their likelihood of dying from ICC.<sup>3</sup> However, many of these women are already  
65 exposed to human papillomavirus (HPV), the viral cause of cervical cancer, and will not benefit  
66 from or be targeted for prophylactic HPV vaccination. Thus, cervical-cancer screening is needed  
67 for the foreseeable future.

68 However, setting up effective cytology for cervical-cancer screening is expensive and requires a  
69 complex clinical and lab infrastructure that generally does not exist in LMICs.<sup>4;5</sup> Moreover, it is  
70 now well understood that cytology has only a low- to moderate one-time sensitivity for precursor  
71 lesions and therefore must be done repeatedly over many years to reduce cancer risk. Alternative

72 strategies to address the cervical cancer burden in LMICs, especially in SSA, must be developed  
73 and validated.

74 Persistent cervical infections by high-risk HPV (hrHPV) types cause virtually all ICC and its  
75 immediate precursor lesions, e.g. cervical intraepithelial neoplasia grade 3 (CIN3) and  
76 adenocarcinoma *in situ* (AIS) everywhere in the world.<sup>6;7</sup> hrHPV causes most anal and vaginal  
77 cancer and a significant proportion of vulvar, penile, and oropharyngeal cancers.<sup>8</sup> HPV16 is the  
78 most important causal type, responsible for ~60% of ICC.<sup>9</sup> HPV18 is the next most important,  
79 responsible for 10-15% of ICC, including 30-40% of adenocarcinoma of the cervix<sup>9</sup>, which is on  
80 the rise in Western Countries.<sup>10;11</sup> Together, HPV16 and HPV18 account for ~70% of ICC, and  
81 the same 15 hrHPV types account for ~99% of ICC everywhere in the world.<sup>9</sup>

82 There is now overwhelming evidence to suggest that testing for hrHPV is more sensitive, albeit  
83 less specific, than high-quality cytology for identifying women with cervical precancer.<sup>12-16</sup> One-  
84 time hrHPV testing can reduce the risk of ICC incidence by approximately 40% in 6.5 years  
85 compared to cytology screening<sup>16</sup>, and ICC mortality by approximately 40% (approximately  
86 50% overall) in 8 years compared to cytology.<sup>17</sup> Importantly, a negative hrHPV test provides  
87 superior reassurance against CIN3+<sup>18</sup> and against ICC<sup>16;17</sup>, permitting safe extension of  
88 screening intervals.

89 The World Health Organization released cervical-cancer screening and treatment guidelines in  
90 2013, recommending two evidence-based approaches to cervical-cancer screening<sup>19</sup>: (I) Use  
91 either hrHPV testing or visual inspection after acetic acid (VIA), which involves the inspection  
92 of the cervix with a speculum in place and following the application of dilute acetic acid to help  
93 identify potential CIN by its characteristic white coloring in the presence of acetic acid

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3 94 (acetowhite), as alternative initial screening tests instead of Pap, and (II) immediately treat those  
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5 95 who screen positive using the screening test, rather than require diagnostic verification through  
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8 96 colposcopy and biopsy. This approach is commonly called screen-and-treat (S&T), and is  
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10 97 increasingly thought to be more amenable to LMIC settings.

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13 98 However, hrHPV testing is also a much more effective screen than VIA<sup>17</sup>, which on a large-scale  
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15 99 appears to only down-stage cancer rather than prevent it.<sup>20</sup> Thus, the recent American Society for  
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18 100 Clinical Oncology (ASC) resource-stratified guidelines for secondary cervical cancer  
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20 101 prevention<sup>21;22</sup> emphasize that hrHPV testing is the preferred choice for screening, with VIA  
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22 102 only being used until hrHPV testing becomes available, and that HIV-infected women, because  
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25 103 of their higher risk, should be screened twice as frequently as the general (HIV-uninfected)  
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27 104 population.

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30 105 Recent data in HIV[+] women living in the U.S. suggest that hrHPV testing may have clinical  
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32 106 utility similar to that in HIV-negative (HIV[-]) women. Several observational studies have  
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35 107 shown that an extended screening interval is safe in HIV[+] women who test hrHPV and Pap  
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37 108 negative as it is for HIV[-] women.<sup>23;24</sup> In a study of women enrolled in Women's Interagency  
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39 109 Health Study (WIHS) in 2002, HIV[+] and HIV[-] women who tested hrHPV and Pap negative  
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42 110 were at a similarly low risk of cervical intraepithelial neoplasia (CIN) grade 2 (CIN2) or more  
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44 111 severe (CIN2+) histology over a 5-year follow-up.<sup>23</sup> In addition, no cases of histologically  
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46 112 confirmed CIN2+ were diagnosed in the follow-up of hrHPV- and Pap-negative HIV[+] women  
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49 113 aged 30-64 years who underwent routine three-year hrHPV and cytology cotesting at Kaiser  
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51 114 Permanente Northern California.<sup>24</sup> Thus, both studies found very high negative predictive values  
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53 115 (NPV) >99% in HIV[+] women who test hrHPV negative. However, how hrHPV testing can

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3 116 best be used to screen HIV[+] women living in Sub-Saharan Africa to prevent cervical cancer  
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5 117 remains to be determined.  
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9 118 Recent studies<sup>25-28</sup> in HIV[+] women living in Sub-Saharan Africa have compared hrHPV, VIA,  
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11 119 and/or Pap for the detection of cervical precancer/cancer. The results can be summarized as  
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13 120 follows: 1) hrHPV detection was more sensitive but less specific than VIA; 2) surprisingly,  
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15 121 cytology was equally or more sensitive but less specific than VIA; and 3) surprisingly, cytology  
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17 122 was equally or more sensitive but less specific (vs. the converse) than hrHPV testing. Results and  
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19 123 conclusions are varied, leaving unanswered the question of what screening strategy in HIV[+]  
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21 124 women living in Sub-Saharan Africa has the greatest effectiveness and cost effectiveness.  
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26 125 Regardless of the screening method, most screen-positive women who go to colposcopy or are  
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28 126 treated immediately without diagnostic verification do not have cervical precancer and cancer  
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30 127 (positive predictive value [PPV] for screening tests are typically 10%-20%). In places like Sub-  
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32 128 Saharan Africa that lack necessary infrastructure and personnel such as pathologists<sup>29</sup>, excessive  
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34 129 referral to colposcopy is problematic. Although WHO recommendations for S&T will hopefully  
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36 130 overcome this bottleneck and increase the number of women living in LMICs who get screened,  
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38 131 many countries may not adopt current S&T strategies because of concerns of low specificity and  
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40 132 overtreatment, resulting in increased costs, unnecessary patient discomfort and concern, and  
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42 133 wasting valuable healthcare resources that could otherwise be used to expand access to  
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44 134 screening. Thus, methods to increase the accuracy of screening by reducing the numbers of  
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46 135 women having colposcopy and biopsy or getting treated immediately in this context are highly  
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50 136 desirable as they will likely increase the uptake of cervical-cancer screening.  
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3 137 In order to improve the specificity of screening tests, secondary tests (biomarkers) are used  
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5 138 following a screen-positive result, with women who test positive for the triage undergoing  
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7 139 further management (e.g., colposcopy or immediate treatment) and those who test negative  
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10 140 typically being deferred to further evaluation in 6-18 months to allow hrHPV infections to clear.  
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12 141 There are several very promising biomarkers that might be used to improve the specificity and  
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14 142 positive predictive value (PPV) of the screening tests.<sup>24</sup> Given that HIV-infected women are  
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16 143 more likely to test hrHPV positive<sup>30-34</sup>, it is important to validate a triage strategy of using a  
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18 144 secondary biomarker that sensitively and specifically rules-in women with cervical precancer  
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20 145 and cancer among the HIV-positive, hrHPV-positive women.

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25 146 We are therefore conducting a cervical-cancer screening study of >5,000 Rwandan women, aged  
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27 147 30-54 years, living with HIV infection. We will evaluate different screening tests (hrHPV DNA  
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29 148 and VIA), those recommended by the WHO for cervical-cancer screening<sup>19</sup>, and different triage  
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31 149 tests and biomarkers for screen-positive women (E6/E7 oncoprotein detection, p16INK4a  
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33 150 immunocytochemistry, and hrHPV viral methylation). Screen-positive women undergo a  
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35 151 rigorous colposcopic evaluation with multiple biopsies taken and the biopsies will undergo  
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37 152 pathology review, to minimize the misclassification of endpoints. The primary objective of the  
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39 153 study is to determine and compare clinical performance (Sensitivity (Se), Specificity (Sp), PPV,  
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41 154 and NPV) and cost-effectiveness for identifying HIV[+] women with CIN3+ and CIN2+ of  
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43 155 different cervical-cancer screening and management algorithms.  
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## 49 156 **Methods and Analysis**

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52 157 *Study design and population:* We are recruiting those women receiving care for confirmed HIV  
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54 158 infection at health centers (HC) and various hospitals operated by the Ministry of Health or  
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3 159 Rwanda Military Hospital during 2016-18 (**Table 1**). Sites were selected in collaboration with  
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5 160 Rwanda Biomedical Center (RBC), which calculate estimated numbers of potentially eligible  
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8 161 women using data from the HIV database (OpenMRS-Open Medical Records System;  
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10 162 <http://openmrs.org/>). Sites were also selected from all provinces to ensure geographic  
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12 163 representation. The total of 8,424 is the estimated number of women eligible for the study  
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15 164 according to the inclusion/exclusion criteria (see below) at the beginning for the study. From  
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17 165 each site, a convenience sample of women were recruited to participate.

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20 166 *Inclusion criteria* include 1) living and receiving HIV care in Rwanda, 2) aged 30-54 years, 3)  
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22 167 confirmed HIV+ based on medical records, 4) no prior cervical-cancer screening, 5) no history of  
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24 168 ICC, and 6) willing, able and competent to provide written, informed consent. We extended age  
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27 169 range beyond that of age range (30-49 years) recommended by the WHO for cervical-cancer  
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29 170 screening<sup>19</sup> because there is limited evidence for the optimal upper age for cervical-cancer  
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31 171 screening of HIV-infected women. *Exclusion criteria*, in addition to not meeting the inclusion  
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33 172 criteria, include 1) pregnant, 2) signs of abnormal, non-menstrual bleeding suggestive of ICC, 3)  
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35 173 without a cervix due to hysterectomy, and 4) not sufficiently healthy to participate in a research  
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38 174 study based on the judgment of the clinicians. Excluded women are advised to seek routine  
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41 175 cervical-cancer screening through government programs.

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44 176 Prior to initiation of enrollment at a specific clinic, the local HIV-care provider team identifies  
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46 177 potentially eligible women at their routine clinic visits and offers them enrollment. Women  
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48 178 indicating interest in the study are then registered by our research nurses using the eligibility  
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50 179 criteria checklist. Women at one site are enrolled until all the eligible and willing women at that  
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53 180 site are screened for cervical cancer. The study team of at least two research nurses schedules 12  
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56 181 to 15 women three to four days a week by calling them and confirming appointments over the  
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3 182 telephone. Two teams of nurses are in the field, meaning that two cervical-cancer screening  
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5 183 clinics can be run simultaneously.  
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9 184 *Enrollment Visit:* Enrollment visits, including pelvic exams with VIA and specimen collection,  
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11 185 are done entirely by a team of two study nurses. During their enrollment visit, women are  
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13 186 educated on cervical cancer risk factors, mainly HPV infection, and why they are more at risk to  
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15 187 develop cervical cancer than HIV-uninfected women. They are also allowed to ask questions  
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17 188 before they commit to participating in the study. Women are asked to provide informed, written  
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19 189 consent to participate in the study using a printed out consent form. Those who provide consent  
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21 190 complete a short nurse-administered questionnaire (**Appendix I**) on cervical cancer risk factors  
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23 191 and sociodemographic characteristics using a data capture screen in Microsoft Access. The  
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25 192 questionnaire collects information on basic sociodemographics, factors associated with acquiring  
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27 193 HPV (e.g., marital status and recent and lifetime number of sexual partners), factors associated  
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29 194 with increased risk of progression of hrHPV infection to precancer and cancer (e.g., smoking and  
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31 195 other tobacco use, parity, and oral and other contraceptive use), and other infections common in  
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33 196 Rwanda such as malaria<sup>35</sup> and tuberculosis<sup>36</sup> that have been previously reported to be associated  
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35 197 with precancer among hrHPV-infected women. The questionnaire was not pretested.  
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42 198 Enrolled women then undergo a pelvic exam, with VIA and a single cervical exfoliated (“Pap”)  
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44 199 specimen collected and placed into 20 ml PreservCyt (Hologic, Bedford, MA, USA) which is  
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46 200 then sent to the lab at RMH for hrHPV testing. Finally, a portable colposcope  
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48 201 (<http://www.mobileodt.com/>; MobleODT, Tel Aviv, Israel) is used for digital cervicography  
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50 202 (~VIA with magnification) and the image is captured and saved for quality control, research, and  
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52 203 to develop a digital library.  
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3 204 *Colposcopy Visit:* Screen-positive women (women who test hrHPV and/or VIA positive) are  
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5 205 called using a telephone as soon as the hrHPV result is available and invited to return for  
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7 206 colposcopy within one month. All women receiving colposcopy will have two additional  
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9 207 specimens collected, one into PreservCyt for the evaluation of other molecular biomarkers  
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11 208 (genotype-specific hrHPV viral methylation and load, and p16/Ki-67 immunocytochemistry  
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13 209 CINtec® PLUS Cytology Kit [Roche, Tucson, AZ, USA]) and a second using a dry swab for  
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15 210 HPV16, 18, 31, 33, 35, 45, 52, and 58 E6/E7 oncoprotein detection by the next generation lateral  
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17 211 flow hrHPV oncoprotein test from Arbor Vita Corporation (Fremont, CA, USA) included in this  
18  
19 212 study as a triage for screen-positive women to identify those women who are at higher risk of  
20  
21 213 having CIN3+. The residual PreservCyt specimens from both the screening and colposcopy visits  
22  
23 214 will be stored at -20°C, creating a biobank in Rwanda for future retrospective evaluations of  
24  
25 215 promising new biomarkers and tests.

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31 216 After specimen collection, a colposcopic evaluation of the cervix is done and a modified version  
32  
33 217 of the 4-quadrant microbiopsy procedure is performed.<sup>37</sup> Compared to the standard biopsy, the  
34  
35 218 microbiopsy protocol improves disease ascertainment and reduces biases by selecting on the  
36  
37 219 most obvious acetowhite lesions while removing less tissue (~13 mm<sup>2</sup> for 4 microbiopsies vs.  
38  
39 220 ~28mm<sup>2</sup> for 1 standard biopsy). Modifications to the standard 4-quadrant microbiopsy procedure  
40  
41 221 are: 1) endocervical curettage is taken only for those women whose squamocolumnar junction is  
42  
43 222 not entirely visible and the lesion extends into the endocervical canal; and 2) standard biopsies of  
44  
45 223 very large lesions can be taken to increase the likelihood that the most severe area is biopsied.

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51 224 *Pathology:* Biopsies are processed in a single cassette so that a single slide has a section from all  
52  
53 225 biopsies taken. Biopsies read by a local pathologist at RMH and Dr. Hebert or another  
54  
55 226 pathologist at Montefiore Medical Center, Bronx, NY, USA. Women receiving a diagnosis of

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3 227 CIN2+ by the Rwandan pathologist (T.Z.) or, as a safety precaution, CIN3+ diagnosis by  
4  
5 228 Montefiore pathologist (T.H.) will receive treatment<sup>38</sup>: 1) CIN2, CIN3, or AIS will be referred to  
6  
7 229 study doctors to undergo an excision procedure (e.g., loop electrosurgical excision procedure  
8  
9  
10 230 [LEEP] or cold-knife cone [CKC]) and 2) ICC will be referred to RMH Hospital for care.  
11  
12 231 Women with <CIN2 will be advised to seek re-screening in a year.

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14  
15 232 A slide with biopsies also will undergo p16 immunohistochemistry (IHC) using the CINtec®  
16  
17 233 Histology Kit (Roche) for research purposes only.

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21 234 *Endpoints*: The primary scientific endpoint of the study will be histologically confirmed,  
22  
23 235 consensus CIN2+ i.e., both pathologists diagnosed CIN2+ (without adjudication). The  
24  
25 236 secondary, clinical endpoint will be histologically confirmed CIN2+ diagnosed by the Rwandan  
26  
27 237 pathologist. Additional endpoints using pathology review and p16 IHC will be used but not for  
28  
29 238 evaluating the performance of p16 immunocytochemistry due to the possibility of p16-related  
30  
31 239 autocorrelation.

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36 240 *Treatment*: Women diagnosed with CIN2+ will be referred for treatment. Those precancerous  
37  
38 241 lesions will be treated by ablation if they meet WHO criteria for cryotherapy.<sup>39</sup> Those who do  
39  
40 242 not meet those criteria will undergo an excision procedure (e.g., loop electrosurgical excision  
41  
42 243 procedure [LEEP] or cold-knife cone [CKC]) or, in the case of an ICC diagnosis, referred for  
43  
44 244 cancer management. Screen-positive women with <CIN2 will be advised to seek re-screening in  
45  
46 245 a year through the existing healthcare system.

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51 246 *Data sources*: Data are collected from the following sources:  
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3 247 1. A nurse-administered questionnaire on sociodemographic characteristics and cervical  
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5 248 cancer risk factors including age at first sexual intercourse, number of sexual partners,  
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7 249 smoking, contraception, parity and socioeconomic status.  
8  
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10
- 11 250 2. Pelvic exam, VIA, Mobile ODT and colposcopy data capture forms  
12  
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- 14 251 3. Medical record data on HIV status (e.g.,  
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16 252 (<http://www.who.int/hiv/pub/guidelines/HIVstaging150307.pdf>), CD4 count, viral load,  
17  
18 253 antiretroviral therapy (ART) regimen(s)), care, and dates.  
19  
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21

22 254 *Laboratory Testing:* The following laboratory tests will be performed:  
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25 255 Xpert HPV Testing—cervical Pap specimens in PreservCyt will be sent to the RMH laboratory  
26  
27 256 in Kigali, Rwanda for hrHPV DNA testing using the Xpert HPV test (Cepheid, Sunnyvale, CA,  
28  
29 257 USA).<sup>40-45</sup> The Xpert HPV Assay is a new, qualitative, real-time PCR assay for the detection of  
30  
31 258 hrHPV DNA. The Xpert HPV Assay includes simultaneous detection of 14 hrHPV types,  
32  
33 259 hydroxymethylbilane synthase (HMBS), and an internal Probe Check Control (PCC). The 14  
34  
35 260 targeted hrHPV types are detected in 5 fluorescent channels: 1) HPV16, 2) HPV18 and hrHPV  
36  
37 261 45 (HPV18/45), 3) HPV31, 33, 35, 52, and 58, 4) HPV51 and HPV59, and 5) HPV39, 56, 66,  
38  
39 262 and 68. HMBS (fluorescent channel 6) verifies specimen adequacy.  
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45 263 Specimens are mixed and a 1-mL pre-aliquot is removed using a disposable pipette and placed in  
46  
47 264 the testing cartridge per the manufacturer's instructions. Unsatisfactory results due to insufficient  
48  
49 265 cellular content are re-run. If the second test is also unsatisfactory, the final result will be  
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51 266 unsatisfactory but women are referred to colposcopy for safety.  
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3 267 HPV Viral Methylation—To identify single hrHPV type infections, we will select single-channel  
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5 268 positives from the Xpert HPV assay. For those that are hrHPV positive for a channel other than  
6  
7 269 HPV16, which is detected singly, we will test them to identify the single type infections using a  
8  
9  
10 270 standard protocol for PCR amplification using MY09/11 L1 consensus primers and hrHPV  
11  
12 271 genotype detection using dot-hybridization for 39 individual type-specific probes and a mixture  
13  
14 272 of probes for 10 other uncommon hrHPV types as previously described.<sup>46;47</sup> To isolate the DNA,  
15  
16 273 ThinPrep specimens (1.5 mL) will be pelleted, re-suspended in STM, digested with Proteinase K,  
17  
18 274 precipitated overnight in ammonium acetate ethanol at -20°C, washed, and suspended and stored  
19  
20  
21 275 in TE buffer.

22  
23  
24 276 The isolated DNA will then undergo bisulfite conversion.<sup>48</sup> Following bisulfite conversion and  
25  
26 277 DNA purification and de-sulphonation, bisulfite-treated DNA will be used as template for Next-  
27  
28 278 Gen Sequencing (NGS) (HiSeq2000, Illumina, San Diego, CA) using barcoded-type specific  
29  
30 279 primers. Sequences for pads and barcodes are not found in the targeted genomic region. Use of  
31  
32 280 padding and barcodes enables reads to be identified by amplicon (forward or reverse) or by  
33  
34 281 sample during downstream bioinformatics analysis.<sup>49</sup>

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39 282 All PCR products for NGS are pooled (by assay) and a single DNA band containing multiple  
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41 283 amplicons from different samples (with unique barcodes) is isolated from a gel for NGS.<sup>49</sup>  
42  
43 284 Briefly, equal concentrations of each barcoded PCR product (based on PCR band intensity) are  
44  
45 285 pooled and isolated. Upon confirmation of correct product size, all purified DNA pools are  
46  
47 286 combined and submitted for library preparation and paired-end 100 base pair Illumina  
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49 287 HiSeq2000 sequencing at the Einstein Genomics Core Facility.  
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3 288 Methylation status will be determined in the lab of Dr. Robert Burk at Albert Einstein College of  
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5 289 Medicine (USA). Prior to determination of methylation status, de-multiplexing based on the  
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7  
8 290 unique barcodes is performed using in-house generated scripts to obtain paired-end NGS reads of  
9  
10 291 each sample. Reads are aligned with hrHPV reference genome sequences by bowtie v0.12.9.<sup>50</sup>  
11  
12 292 Methylation status of each CpG site is then determined by bismark v0.7.7<sup>51</sup> using the default  
13  
14 293 quality score parameter set to Q30, and the formula of the methylation ratio of the number of C  
15  
16 294 read by the number of C+T read.

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20 295 E6/E7 Oncoprotein Testing—Dry swab specimens collected at the time of colposcopy will be  
21  
22 296 tested for individual E6/E7 oncoproteins as previously described<sup>52;53</sup>, according to the  
23  
24 297 manufacturer's instructions, at the RMH laboratory in Kigali, Rwanda. The E6/E7 oncoprotein  
25  
26 298 test uses three lateral flow strips to detect 8 hrHPV types whereas the E6 oncoprotein test used a  
27  
28 299 single lateral flow strip to detect 3 hrHPV types.

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33 300 *Analyses:* We will evaluate combinations of the above mentioned screening strategies and tests  
34  
35 301 to estimate the clinical performance (e.g., Se, Sp, PPV, and NPV) for the detection of consensus  
36  
37 302 CIN3+ and community CIN2+. A log binomial model implemented with generalized estimating  
38  
39 303 equations will be used to take into account correlation between different tests from the same  
40  
41 304 subject. Note that these models are developed for the estimation and comparison of performance  
42  
43 305 for two tests, but the model can be extended to allow more than two tests by including more  
44  
45 306 indicator variables for test type.

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50 307 Some analyses of biomarkers, such as viral methylation are restricted to hrHPV-positives.  
51  
52 308 Comparisons of hrHPV viral methylation to other triage biomarkers will be restricted to the  
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54 309 subset that gets tested for viral methylation as described.

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3 310 *Sample size calculations:* We based our sample size on the ability to detect modest but minimally  
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5 311 important differences in Se of 15%. We conservatively assume that the population prevalence of  
6  
7 312 CIN3+ is 2% based on our past study in Rwanda.<sup>33,35</sup> We propose to enroll and have complete  
8  
9 313 follow-up of at least 5,000 HIV[+] women. A sample size of 5,000 HIV[+] women with  
10  
11 314 completed follow-up of the screen positives will yield 100 cases of CIN3+, which has at least  
12  
13 315 80% power ( $\alpha=5\%$ ) to crudely detect a 15% difference in Se between two screening strategies  
14  
15 316 for a range of 10%-25% discordance. With this sample size of 5,000 women, 4,900 will not have  
16  
17 317 CIN3+; we will also have at least 90% power ( $\alpha=5\%$ ) to detect a difference in Sp of 3% for  
18  
19 318 discordance up to 40%. Finally, we will have 80% power ( $\alpha=5\%$ ) to crudely detect an 8%, 10%,  
20  
21 319 or 11% difference in PPV if the reference PPV is 10%, 20%, or 30%, respectively.<sup>54</sup>  
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27 320 *Cost Effectiveness:* We will conduct assessments of the costs and cost-effectiveness of the  
28  
29 321 different combinations of screening and triage tests, i.e., algorithms, as well as those of the entire  
30  
31 322 community-based screening “system.” Costs measurement will be conducted using a micro-  
32  
33 323 costing (ingredients) approach in which resource use throughout each step in the screening  
34  
35 324 process will be tracked and unit costs for each of the resources will be applied to generate an  
36  
37 325 average screening cost per woman to be compared against what the estimated costs are for a  
38  
39 326 possible program based on hrHPV screening and VIA triage or VIA screening. For estimating  
40  
41 327 costs of the screening system and scale-up of screening to 100,000 women in a month, analyses  
42  
43 328 will distinguish financial costs, which reflect actual expenditures of the program, from economic  
44  
45 329 costs, including the value of donated and shared resources to more fully assess opportunity costs.  
46  
47 330 Projections on budget impact and economic cost implications over time will be made under  
48  
49 331 varying assumptions of screening uptake, follow-up compliance, and scenarios of changing  
50  
51 332 disease burden.  
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3 333 Clinical outcomes will include true positive, true negative, false negative, and false positive test  
4  
5 334 results, number of colposcopies, incident cancer, and cancer death. Cost-effectiveness will be  
6  
7 335 measured as cost/CIN2+ detected, cost/CIN3+ detected, cost/invasive cancer prevented,  
8  
9 336 cost/cancer death prevented, cost/life-year saved, and cost/quality-adjusted life year (QALY)  
10  
11 337 saved; in addition, we will calculate harm/benefit ratios, using varying definitions of harms  
12  
13 338 (colposcopies, false positive results) to benefits (cancers prevented, deaths prevented, life years  
14  
15 339 and QALYs saved). Costs and effectiveness will be discounted at a 3% annual rate, with the rate  
16  
17 340 varied from 0-5% in sensitivity analysis. For assessment of value-of-information (VOI), we will  
18  
19 341 use net monetary benefits (NMB), defined as a function of the willingness-to-pay threshold  
20  
21 342 (WTP) for different costs and outcomes as:  $NMB = (WTP * Effectiveness) - Costs$ .

### 27 343 **Patient and Public Involvement**

- 28  
29  
30 344 • *How was the development of the research question and outcome measures informed by*  
31  
32 345 *patients' priorities, experience, and preferences?* There was no patient engagement in the  
33  
34 346 development of the study.
- 35  
36  
37  
38 347 • *How did you involve patients in the design of this study?* There was no patient  
39  
40 348 engagement in the design of the study.
- 41  
42  
43  
44 349 • *Were patients involved in the recruitment to and conduct of the study?* There was no  
45  
46 350 patient involvement in the recruitment and conduct of the study.
- 47  
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49  
50 351 • *How will the results be disseminated to study participants?* Participants will receive their  
51  
52 352 results directly since it is related to their care.
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3 353 • *For randomized controlled trials, was the burden of the intervention assessed by patients*  
4  
5 354 *themselves?* Not applicable

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9 355 • *Patient advisers should also be thanked in the contributorship*  
10  
11 356 *statement/acknowledgements.* Not applicable

12  
13  
14 357 **Ethics and Dissemination**

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16  
17 358 *Ethics:* This study protocol was reviewed and approved by the Rwanda National Ethics  
18  
19 359 Committee (RNEC) as well as the Institutional Review Board for human subjects research at  
20  
21 360 Albert Einstein College of Medicine.

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23 361 *Confidentiality measures and protection against potential risks:* The risks for those participating  
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25 362 in our study include:

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31 363 • Collection of Pap specimens/cervical swabs involves a modest risk of bleeding  
32  
33 364 which is typically very limited when it occurs. Testing positive for any test may  
34  
35 365 cause psychological distress (anxiety).
- 36  
37  
38 366 • Colposcopy and excisional treatments induce vaginal bleeding and may incur pain,  
39  
40 367 infection, and short-term psychological distress (anxiety). A diagnosis of CIN2 or  
41  
42 368 more severe may cause psychological distress (anxiety). A diagnosis of invasive  
43  
44 369 cervical cancer may cause severe psychological distress.
- 45  
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47  
48 370 • Questions in the questionnaire, regarding sexual behavior and other matters of a  
49  
50 371 personal nature, may cause anxiety and embarrassment. Participants are advised  
51  
52 372 that they are free not to answer specific questions.
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3 373           • There is also the risk of psycho-social stress which could occur if there was  
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5 374           inadvertent disclosure of confidential medical or other personal information.  
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8 375 Protection against the risk of inadvertent disclosure of confidential information is addressed by  
9  
10 376 the standard procedures at the Rwandan study site, including: (i) storing completed paper copies  
11  
12 377 of questionnaires and other hard copy information (described above), identified by study number  
13  
14 378 only, in a filing system separate from the name-address file of participants in the study; and (ii)  
15  
16 379 only the designated local personnel have access to cross-reference the files; (iii) all paper files,  
17  
18 380 including consent forms, will be maintained in locked cabinets in locked rooms, with access  
19  
20 381 restricted to specific research personnel.  
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25 382 In addition, we will include the following security measures to protect the data:  
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- 28  
29 383           • Controlled access to project data;  
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31 384           • A tracking system for data forms and activities;  
32  
33 385           • Date and time of stamping of all data records with electronic signatures;  
34  
35 386           • Audit trails to track all changes made to data records; and  
36  
37 387           • Data kept on password-protected computers and in locked rooms.  
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41  
42 388 *Potential Patient Benefits:* There are potential direct benefits to study participants. HIV[+]  
43  
44 389 women participating in the study are at very high risk of cervical cancer. They will be rigorously  
45  
46 390 screened and evaluated, more effectively than the standard of care anywhere in the world. As a  
47  
48 391 result, women with precancer who are at imminent risk of invasive cervical cancer will be  
49  
50 392 diagnosed sooner and treated more effectively than women receiving routine care and thereby  
51  
52 393 more likely averting the development of cervical cancer. Women with cervical cancer will be  
53  
54 394 diagnosed earlier thereby reducing the morbidity and the risk of mortality caused by cervical  
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3 395 cancer. Conversely, any pain, bleeding, or stress that might occur related to colposcopy or  
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5 396 cervical swab are typically modest and well tolerated.  
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8 397 There are also substantial potential societal benefits related to the study due to its implications to  
9  
10 398 improving cervical-cancer screening practices and guidelines in HIV[+] women – changes in  
11  
12 399 practice which might also benefit the study participants themselves, if and when these changes  
13  
14 400 are enacted. There is a great need to identify more effective and practical methods for cervical-  
15  
16 401 cancer screening for HIV[+] women living in Africa, who are living longer than ever and are  
17  
18 402 therefore at potentially greater risk of cervical cancer.  
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22 403 *Dissemination:* We plan to publish a series of scientific reports in peer-reviewed scientific  
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24 404 journals. As building research capacity in Rwanda is a major goal of this research project, all  
25  
26 405 investigators of the research team will be asked and supported to lead at least one analysis and  
27  
28 406 one manuscript preparation, based on interests and expertise.  
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32 407 In addition, an external advisory panel (EAP) composed of leaders from the Rwanda Ministry of  
33  
34 408 Health, University of Rwanda, and Rwanda medical community has been formed. The  
35  
36 409 responsibilities of the EAP include providing advice on the conduct of the project and  
37  
38 410 interpretation for and dissemination of the study results to Rwandan stakeholders. The latter is  
39  
40 411 important for the adoption of evidence-based best practices for cervical-cancer screening as  
41  
42 412 warranted.  
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### 47 413 **Limitations**

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50 414 There are several limitations to the study that bear mentioning. First, cervical cytology was not  
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52 415 included in the study. There is limited cervical cytology services available locally and of  
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54 416 unknown quality and it is unlikely that cytology will be widely available in Rwanda, making its  
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3 417 inclusion as a comparator test of limited value. Moreover, there are significant costs and  
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5 418 logistical challenges in shipping PreservCyt specimens to and having cytology slides made and  
6  
7 419 read in the U.S. Second, we did not conduct biopsies in screen-negative women, which would  
8  
9 420 have allowed us to estimate absolute clinical performance. The burden of sending screen-  
10  
11 421 negative women to colposcopy was deemed too great and it was impractical to send a sufficient  
12  
13 422 numbers of screen-negative women to colposcopy to accurately estimate the false-negative  
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15 423 disease (CIN3+) fraction. Thus, only relative clinical performance of the screening tests can be  
16  
17 424 estimated from this study.  
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3 425 Contributor Statement:  
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5

6 426 KA, PEC, JCD, AA, and JDS conceived the original concept of the study and the interventions.  
7 427 GM, KA, JDS, and PEC drafted the protocol. PEC performed the sample size calculations, and  
8 428 PEC and GM will lead analysis of the results. GM, JDS, and Athanase Munyaneza are  
9 429 supporting patient recruitment. GM, TR, and Athanase Munyaneza are providing clinical care for  
10 430 patients. Anthere Murangwa and LM oversee laboratory testing, and TZ and TH are responsible  
11 431 for pathology. PM and LM oversee and administer the study activities at the clinical site in  
12 432 Rwanda. All authors (GM, JCD, TR, JDS, Athanase Munyaneza, Anthere Murangwa, TZ, TH,  
13 433 PM, AA, LM, KA, and PEC) contributed to the scientific design of the study and the protocol  
14 434 development, are involved in the implementation of the project, and have read and approved the  
15  
16  
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18 435 final manuscript.  
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594 **Table 1:** Recruitment sites and estimated eligible population.

Province	Site	Type of site	Potential participants per site (approximate)
Kigali	Cor-unum HC	Public Health Center	1,405
Kigali	Kimironko HC	Public Health Center	1,227
Kigali	Rwampara HC	Public Referral Hospital	1,098
Kigali	Kicukiro HC	Public Health Center	1,054
Kigali	Kacyiru HC	Public Health Center	905
Kigali	Gikondo HC	Public Health Center	925
Kigali	Rwanda Military Hospital	Public Referral Hospital	300
Kigali	WEACTx for Hope	Private HIV Clinic	500
Kigali	Busanza HC	Public Health Center	100
Kigali	Nyarugunga HC	Public Health Center	100
West	Gisenyi DH	Public District Hospital	810
Total			8,424

595

## Appendix I. Study Questionnaire

### A. Socio-demographics

1. What is your date of birth? D D / M M / Y Y Y Y (enter all 0 if not remembered) (If date of birth or year of birth is given, skip to Question 3)

2. What is your age? \_\_\_ \_\_\_ (Years)

3. What is your marital status?

Married/Cohabiting

Divorced

Widowed

Separated

Single

Choose not to answer

4. Do you live in Kigali?

Yes

No {Skip to Question A5}

Choose not to answer {Skip to Question A5}

5. In what area of Kigali City Province do you live?

Nyarugenge

Kicukiro

Gasabo urban (e.g., Kacyiru, Kimironko, Kinyinya, etc.)

Gasabo rural (e.g., Nduba, Jabana, Gikomero, etc.)

Do not know

Choose not to answer

6. What is your household income per month in FRW?

< 5,000

5,000 – 9,999

10,000 – 34,999

35,000 – 59,999

- 1  
2  
3  60,000 – 84,999  
4  85,000 – 109,999  
5  110,000 – 134,999  
6  135,000 – 269,999  
7  270,000 or more

8  
9  
10  
11  
12 **7. How many people live in your household?** \_\_\_ \_\_\_ {Enter 00 if refuse to answer}

13  
14 **8. What is your occupation?**

- 15  
16  Employed by government, another institution, or company  
17  Self-employed (Small and medium enterprises)  
18  Self-employed (High income earnings)  
19  Farming (peasants)  
20  Unemployed/Does not work  
21  Other (specify) \_\_\_\_\_  
22  Choose not to answer {Skip to Question B1}

23  
24  
25  
26  
27  
28 {The following questions are sensitive and personal in nature. Your answers will be kept  
29 confidential. You may choose not to answer certain questions. Answering any question is  
30 voluntary}

31  
32  
33 **B. Sexual Behaviors**

34  
35 **1. How old were you when you first had sex?** \_\_\_ \_\_\_ (Years) {Enter 00 if refuse to  
36 answer}

37  
38 **2. How many sexual partners have you had in your lifetime?**

- 39  
40  No Partners (Ineligible)  
41  1 Partner  
42  2-3 Partners  
43  4-5 Partners  
44  6-9 Partners  
45  10 or more partners  
46  Choose not to answer

47  
48  
49  
50  
51  
52 **3. How many sexual partners have you had in the last 6 months?**

- 53  
54  No Partners

- 1  
2  
3  1 Partner  
4  
5  2 or more Partners  
6  
7  Choose not to answer

8  
9 **C. Parity**

- 10  
11 **1. What age did you have your first child? \_\_\_ (Years) {Enter 00 if refuse to**  
12 **answer, Enter 99 if never pregnant} (if 00 or 99, skip to Question D1)**  
13  
14 **2. How many live births have you had in your lifetime? \_\_\_**  
15  
16 **3. Have you given birth in the last year?**

- 17  
18  Yes  
19  
20  No  
21  
22  Choose not to answer

23  
24 **D. Tobacco Use**

- 25  
26 **1. Have you ever smoked cigarettes?**

- 27  
28  Yes  
29  
30  No (skip to Question D3)  
31  
32  Choose not to answer (skip to Question D3)

- 33  
34 **2. Do you currently smoke cigarettes?**

- 35  
36  Yes  
37  
38  No  
39  
40  Choose not to answer

- 41  
42 **3. Have you ever chewed/used tobacco orally (Ubugoro)?**

- 43  
44  Yes  
45  
46  No (skip to Question D5)  
47  
48  Choose not to answer (skip to Question D5)

- 49  
50 **4. Are you currently chewing/using tobacco orally (Ubugoro)?**

- 51  
52  Yes  
53  
54  No  
55  
56  Choose not to answer

1  
2  
3 **5. Have you ever chewed/used tobacco orally (Tobacco leaves-Igikamba)?**  
4

- 5  Yes  
6  
7  No (skip to Question E1)  
8  
9  Choose not to answer (skip to Question E1)

10  
11 **6. Are you currently chewing/using tobacco orally (Tobacco leaves-Igikamba)?**  
12

- 13  Yes  
14  
15  No  
16  
17  Choose not to answer

18 **E. Contraceptive Use**  
19

20  
21 **1. Have you ever used oral contraceptives?**  
22

- 23  Yes  
24  
25  No (Skip to Question E3)  
26  
27  Choose not to answer (Skip to Question E3)

28 **2. Do you currently use oral contraceptives?**  
29

- 30  Yes  
31  
32  No  
33  
34  Choose not to answer

35  
36 **3. Have you ever used Depo Provera (contraceptive)?**  
37

- 38  Yes  
39  
40  No (Skip to Question E5)  
41  
42  Choose not to answer (Skip to Question E5)

43 **4. Do you currently use Depo Provera (contraceptive)?**  
44

- 45  Yes  
46  
47  No  
48  
49  Choose not to answer

50  
51 **5. Have you ever used Jadell (contraceptive)?**  
52

- 53  Yes  
54  
55  No (Skip to Question E7)  
56  
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Choose not to answer (Skip to Question E7)

**6. Do you currently use Jadell (contraceptive)?**

Yes

No

Choose not to answer

**7. Have you ever used IUD (contraceptive)?**

Yes

No (Skip to Question E9)

Choose not to answer (Skip to Question E9)

**8. Do you currently use IUD (contraceptive)?**

Yes

No

Choose not to answer

**9. Do use condoms as a contraceptive?**

Yes

No (skip to Question F1)

Choose not to answer (skip to Question F1)

**10. Have you used condoms as a contraceptive in the last 6 months?**

Yes

No

Choose not to answer

**F. Infections**

**1. Have you ever had Malaria**

Yes

No (skip to Question F8)

Choose not to answer (skip to Question F8)

**2. How many times have you had Malaria? \_\_\_**

1  
2  
3 **3. What was the year that you last had Malaria? Y Y Y Y**

4  
5 **4. Have you had your Malaria treated using drugs?**

6  
7  Yes

8  No

9  Choose not to answer

10  
11 **5. If Yes, how was it treated?**

12  Treated only with traditional medicine

13  Treated only with drugs (e.g. Coartem)

14  Treated with both (traditional and modern)

15  Not treated

16  Choose not to answer

17  
18  
19 **6. Was your last episode of Malaria treated using drugs?**

20  Yes

21  No

22  Choose not to answer

23  
24 **7. If Yes, how was it treated?**

25  Treated only with traditional medicine

26  Treated only with drugs (e.g. Coartem)

27  Treated with both (traditional and modern)

28  Not treated

29  Choose not to answer

30  
31 **8. Have you ever had Tuberculosis (TB)?**

32  Yes

33  No (skip to end)

34  Choose not to answer (skip to end)

35  
36 **9. If Yes, how many times have you had TB in your lifetime? \_\_\_**

37  
38 **10. What year did you last have Tuberculosis (TB)? Y Y Y Y**

39  
40  
41  
42 **END OF QUESTIONNAIRE**



# BMJ Open

## A Study of Cervical Cancer Screening Technologies in Human Immunodeficiency Virus-Infected Women Living in Rwanda

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-020432.R2
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Date Submitted by the Author:	22-May-2018
Complete List of Authors:	Murenzi, Gad; Rwanda Military Hospital Dusingize, Jean; Regional Alliance for Sustainable Development , Research and Scientific Capacity Building Rurangwa, Theogene; Rwanda Military Hospital Sinayobye, Jean d'Amour; Regional Alliance for Sustainable Development, ; Women's Equity in Access to Care & Treatment (WE-ACTx), Munyaneza, Athanase; Rwanda Military Hospital Murangwa, Anthere; Rwanda Military Hospital Zawadi, Thierry; Rwanda Military Hospital Hebert, Tiffany; Yeshiva University Albert Einstein College of Medicine Mugenzi, Pacifique; Rwanda Military Hospital Adedimeji, Adebola; Yeshiva University Albert Einstein College of Medicine Mutesa, Leon; Rwanda Military Hospital; University of Rwanda College of Medicine and Health Sciences Anastos, Kathryn; Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, Castle, Philip; Yeshiva University Albert Einstein College of Medicine,
<b>Primary Subject Heading</b>:	Global health
Secondary Subject Heading:	Obstetrics and gynaecology, Infectious diseases, HIV/AIDS, Epidemiology, Diagnostics
Keywords:	human papillomavirus (HPV), cervical cancer, HIV & AIDS < INFECTIOUS DISEASES, GYNAECOLOGY, cervical intraepithelial neoplasia

SCHOLARONE™  
Manuscripts

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3 **1 A Study of Cervical Cancer Screening Technologies in Human Immunodeficiency Virus-**  
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5 **2 Infected Women Living in Rwanda**  
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9 3 Gad Murenzi, MD\*<sup>1</sup>, Jean-Claude Dusingize, MD, MS<sup>1</sup>, Theogene Rurangwa, MD, MMed<sup>1</sup>,  
10  
11 4 Jean d'Amour Sinayobye, MD, MS<sup>1</sup>, Athanase Munyaneza, RN<sup>1</sup>, Anthere Murangwa, MS<sup>1</sup>,  
12  
13 5 Thierry Zawadi, MD<sup>1</sup>, Tiffany Hebert, MD<sup>2</sup>, Pacifique Mugenzi, MD<sup>1</sup>, MMed, Adebola  
14  
15 6 Adedimeji, PhD, MPH<sup>2</sup>, Leon Mutesa, MD, PhD<sup>1,3</sup>, Kathryn Anastos, MD<sup>2</sup>, Philip E. Castle,  
16  
17 7 PhD, MPH<sup>2</sup>  
18  
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20

21 8 <sup>1</sup>Rwanda Military Hospital, Kigali, Rwanda; <sup>2</sup>Albert Einstein College of Medicine, Bronx, NY,  
22  
23 9 USA; <sup>3</sup>University of Rwanda, Kigali, Rwanda  
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27 10 \*Correspondence: [gadcollins@gmail.com](mailto:gadcollins@gmail.com), +250788589085  
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31  
32 12 Arbor Vita Corporation, and Roche.  
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38 14 Prevent Cancer Foundation.  
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3 16 **Abstract**  
4

5 17 *Introduction.* The optimal method(s) for screening human immunodeficiency virus-infected  
6 18 women, especially for those living in sub-Saharan Africa, for cervical precancer and early cancer  
7 19 has yet to be established.

8  
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10 20 *Methods and analysis.* A convenience sample of >5,000 Rwandan women, ages 30-54 years and  
11 21 living with HIV infection, are being consented and enrolled into a cross-sectional study of  
12 22 cervical-cancer screening strategies. Participants are completing an administered short risk-factor  
13 23 questionnaire and being screened for high-risk human papillomavirus (hrHPV) using the Xpert  
14 24 HPV assay (Cepheid, Sunnyvale, CA, USA), unaided visual inspection after acetic acid (VIA),  
15 25 and aided VIA using the EVA system (Mobile ODT, Tel Aviv, Israel). Women positive for  
16 26 hrHPV and/or by VIA undergo colposcopy, which includes the collection of two cervical  
17 27 specimens prior to undergoing a 4-quadrant microbiopsy protocol. The colposcopy-collected  
18 28 specimens are being tested by dual immunocytochemical staining for p16<sup>INK4a</sup> and Ki-67  
19 29 (CINtec® PLUS Cytology, Ventana, Tucson, AZ, USA) and for E6 or E7 for 8 hrHPV  
20 30 genotypes (HPV16, 18, 31, 33, 35, 45, 52, and 58) using the next-generation AV Advantage  
21 31 hrHPV E6/E7 test (Arbor Vita Corporation, Freemont, CA, USA). Women with local pathology  
22 32 diagnosis of cervical intraepithelial neoplasia (CIN) grade 2 (CIN2) or more severe (CIN2+) or  
23 33 pathology-review diagnosis of CIN grade 3 or more severe (CIN3+) receive treatment. Clinical  
24 34 performance and cost-effectiveness (e.g., sensitivity, specificity, and predictive values) of  
25 35 different screening strategies and algorithms will be evaluated.

26  
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29 36 *Ethics and dissemination.* The protocol was approved by local and institutional review boards for  
30 37 human subjects research. At the completion of the study, results will be disseminated to the  
31 38 scientific community through peer-reviewed publication and to the Rwandan stakeholders  
32 39 through an external advisory panel.  
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3 41 **Strengths and Weaknesses**  
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- 5  
6 42 • We are enrolling a very large sample size of HIV-infected women living in Rwanda who  
7 43 otherwise would probably not get cervical-cancer screening.  
8 44 • We are employing rigorous disease ascertainment protocols to minimize  
9 45 misclassification.  
10 46 • Some tests, such as the Xpert HPV and the AV Advantage HPV E6/E7 assays, are being  
11 47 done on site in Rwanda using technologies that could feasibly be deployed there.  
12 48 • A weakness of the study is that cervical cytology is not being included as a comparator  
13 49 due to financial and logistical constraints.  
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For peer review only

## 51 **Introduction**

52 Invasive cervical cancer (ICC) remains a significant cause of morbidity and mortality globally.  
53 Approximately 530,000 cases of and 270,000 deaths due to ICC occur annually, making it the 4<sup>th</sup>  
54 most common malignancy and cause of cancer-related death in women globally.<sup>1</sup> In many high-  
55 income countries (HIC), ICC rates have declined by 50% or more<sup>2</sup> due to the introduction of  
56 effective, high-coverage Pap testing (cervical cytology)-based screening programs that include  
57 timely follow-up of screen positives, treatment of women with precursor lesions, and  
58 management of cancers. Now, almost 90% of ICC and ICC-related deaths occur in low- and  
59 middle income countries (LMICs) due to a lack of resources and healthcare infrastructure needed  
60 to provide preventive services.

61 ICC and ICC-related mortality rates are particularly high in Sub-Saharan Africa, which also has  
62 the highest rates of HIV infection in the world. Now, over 12 million HIV-infected (HIV[+])  
63 women in Sub-Saharan Africa are living longer because of anti-retroviral therapy, thus  
64 increasing their likelihood of dying from ICC.<sup>3</sup> However, many of these women are already  
65 exposed to human papillomavirus (HPV), the viral cause of ICC, and will not benefit from or be  
66 targeted for prophylactic HPV vaccination. Thus, cervical-cancer screening is needed for the  
67 foreseeable future.

68 However, setting up effective cytology for cervical-cancer screening is expensive and requires a  
69 complex clinical and lab infrastructure that generally does not exist in LMICs.<sup>4;5</sup> Moreover, it is  
70 now well understood that cytology has only a low- to moderate one-time sensitivity for precursor  
71 lesions and therefore must be done repeatedly over many years to reduce cancer risk. Alternative

72 strategies to address the ICC burden in LMICs, especially in SSA, must be developed and  
73 validated.

74 Persistent cervical infections by high-risk HPV (hrHPV) types cause virtually all ICC and its  
75 immediate precursor lesions, e.g. cervical intraepithelial neoplasia grade 3 (CIN3) and  
76 adenocarcinoma *in situ* (AIS) everywhere in the world.<sup>6;7</sup> hrHPV causes most anal and vaginal  
77 cancer and a significant proportion of vulvar, penile, and oropharyngeal cancers.<sup>8</sup> HPV16 is the  
78 most important causal type, responsible for ~60% of ICC.<sup>9</sup> HPV18 is the next most important,  
79 responsible for 10-15% of ICC, including 30-40% of adenocarcinoma of the cervix<sup>9</sup>, which is on  
80 the rise in Western Countries.<sup>10;11</sup> Together, HPV16 and HPV18 account for ~70% of ICC, and  
81 the same 15 hrHPV types account for ~99% of ICC everywhere in the world.<sup>9</sup>

82 There is now overwhelming evidence to suggest that testing for hrHPV is more sensitive, albeit  
83 less specific, than high-quality cytology for identifying women with cervical precancer.<sup>12-16</sup> One-  
84 time hrHPV testing can reduce the risk of ICC incidence by approximately 40% in 6.5 years  
85 compared to cytology screening<sup>16</sup>, and ICC mortality by approximately 40% (approximately  
86 50% overall) in 8 years compared to cytology.<sup>17</sup> Importantly, a negative hrHPV test provides  
87 superior reassurance against CIN3+<sup>18</sup> and against ICC<sup>16;17</sup>, permitting safe extension of  
88 screening intervals.

89 The World Health Organization released cervical-cancer screening and treatment guidelines in  
90 2013, recommending two evidence-based approaches to cervical-cancer screening<sup>19</sup>: (I) Use  
91 either hrHPV testing or visual inspection after acetic acid (VIA), which involves the inspection  
92 of the cervix with a speculum in place and following the application of dilute acetic acid to help  
93 identify potential CIN by its characteristic white coloring in the presence of acetic acid

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3 94 (acetowhite), as alternative initial screening tests instead of Pap, and (II) immediately treat those  
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5 95 who screen positive using the screening test, rather than require diagnostic verification through  
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8 96 colposcopy and biopsy. This approach is commonly called screen-and-treat (S&T), and is  
9  
10 97 increasingly thought to be more amenable to LMIC settings.

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13 98 However, hrHPV testing is also a much more effective screen than VIA<sup>17</sup>, which on a large-scale  
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15 99 appears to only down-stage cancer rather than prevent it.<sup>20</sup> Thus, the recent American Society for  
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18 100 Clinical Oncology (ASC) resource-stratified guidelines for secondary cervical-cancer  
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20 101 prevention<sup>21;22</sup> emphasize that hrHPV testing is the preferred choice for screening, with VIA  
21  
22 102 only being used until hrHPV testing becomes available, and that HIV-infected women, because  
23  
24  
25 103 of their higher risk, should be screened twice as frequently as the general (HIV-uninfected)  
26  
27 104 population.

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30 105 Recent data in HIV[+] women living in the U.S. suggest that hrHPV testing may have clinical  
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32 106 utility similar to that in HIV-negative (HIV[-]) women. Several observational studies have  
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34  
35 107 shown that an extended screening interval is safe in HIV[+] women who test hrHPV and Pap  
36  
37 108 negative as it is for HIV[-] women.<sup>23;24</sup> In a study of women enrolled in Women's Interagency  
38  
39 109 Health Study (WIHS) in 2002, HIV[+] and HIV[-] women who tested hrHPV and Pap negative  
40  
41  
42 110 were at a similarly low risk of cervical intraepithelial neoplasia (CIN) grade 2 (CIN2) or more  
43  
44 111 severe (CIN2+) histology over a 5-year follow-up.<sup>23</sup> In addition, no cases of histologically  
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46 112 confirmed CIN2+ were diagnosed in the follow-up of hrHPV- and Pap-negative HIV[+] women  
47  
48  
49 113 aged 30-64 years who underwent routine three-year hrHPV and cytology cotesting at Kaiser  
50  
51 114 Permanente Northern California.<sup>24</sup> Thus, both studies found very high negative predictive values  
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53 115 (NPV) >99% in HIV[+] women who test hrHPV negative. However, how hrHPV testing can

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3 116 best be used to screen HIV[+] women living in Sub-Saharan Africa to prevent ICC remains to be  
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5 117 determined.

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8 118 Recent studies<sup>25-28</sup> in HIV[+] women living in Sub-Saharan Africa have compared hrHPV, VIA,  
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10  
11 119 and/or Pap for the detection of cervical precancer/cancer. The results can be summarized as  
12  
13 120 follows: 1) hrHPV detection was more sensitive but less specific than VIA; 2) surprisingly,  
14  
15 121 cytology was equally or more sensitive but less specific than VIA; and 3) surprisingly, cytology  
16  
17 122 was equally or more sensitive but less specific (vs. the converse) than hrHPV testing. Results and  
18  
19 123 conclusions are varied, leaving unanswered the question of what screening strategy in HIV[+]  
20  
21 124 women living in Sub-Saharan Africa has the greatest effectiveness and cost effectiveness.

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25 125 Regardless of the screening method, most screen-positive women who go to colposcopy or are  
26  
27 126 treated immediately without diagnostic verification do not have cervical precancer and cancer  
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29 127 (positive predictive value [PPV] for screening tests are typically 10%-20%). In places like Sub-  
30  
31 128 Saharan Africa that lack necessary infrastructure and personnel such as pathologists<sup>29</sup>, excessive  
32  
33 129 referral to colposcopy is problematic. Although WHO recommendations for S&T will hopefully  
34  
35 130 overcome this bottleneck and increase the number of women living in LMICs who get screened,  
36  
37 131 many countries may not adopt current S&T strategies because of concerns of low specificity and  
38  
39 132 overtreatment, resulting in increased costs, unnecessary patient discomfort and concern, and  
40  
41 133 wasting valuable healthcare resources that could otherwise be used to expand access to  
42  
43 134 screening. Thus, methods to increase the accuracy of screening by reducing the numbers of  
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45 135 women having colposcopy and biopsy or getting treated immediately in this context are highly  
46  
47 136 desirable as they will likely increase the uptake of cervical-cancer screening.



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3 137 In order to improve the specificity of screening tests, secondary tests (biomarkers) are used  
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5 138 following a screen-positive result, with women who test positive for the triage undergoing  
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7 139 further management (e.g., colposcopy or immediate treatment) and those who test negative  
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9  
10 140 typically being deferred to further evaluation in 6-18 months to allow hrHPV infections to clear.  
11  
12 141 There are several very promising biomarkers that might be used to improve the specificity and  
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14 142 positive predictive value (PPV) of the screening tests.<sup>24</sup> Given that HIV-infected women are  
15  
16 143 more likely to test hrHPV positive<sup>30-34</sup>, it is important to validate a triage strategy of using a  
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18 144 secondary biomarker that sensitively and specifically rules-in women with cervical precancer  
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20 145 and cancer among the HIV-positive, hrHPV-positive women.

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25 146 We are therefore conducting a cervical-cancer screening study of >5,000 Rwandan women, ages  
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27 147 30-54 years, living with HIV infection. We are evaluating different screening tests (hrHPV DNA  
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29 148 and VIA), those recommended by the WHO for cervical-cancer screening<sup>19</sup>, and different triage  
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31 149 tests and biomarkers for the management of screen-positive women (E6/E7 oncoprotein  
32  
33 150 detection, p16INK4a immunocytochemistry, and hrHPV viral methylation). Screen-positive  
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35 151 women undergo a rigorous colposcopic evaluation with multiple biopsies taken and the biopsies  
36  
37 152 will undergo pathology review, to minimize the misclassification of endpoints. The primary  
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39 153 objective of the study is to determine and compare clinical performance (Sensitivity (Se),  
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41 154 Specificity (Sp), PPV, and NPV) and cost-effectiveness for identifying HIV[+] women with  
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43 155 CIN3+ and CIN2+ of different cervical-cancer screening and management algorithms.  
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## 49 156 **Methods and Analysis**

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52 157 *Study design and population:* We are recruiting those women receiving care for confirmed HIV  
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54 158 infection at health centers (HC) and various hospitals operated by the Ministry of Health or  
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3 159 Rwanda Military Hospital during 2016-18 (**Table 1**). Sites were selected in collaboration with  
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5 160 Rwanda Biomedical Center (RBC), which calculate estimated numbers of potentially eligible  
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7 161 women using data from their HIV database (OpenMRS-Open Medical Records System;  
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10 162 <http://openmrs.org/>). Sites were also selected from all provinces to ensure geographic  
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12 163 representation. The total of 8,424 was the estimated number of women eligible for the study  
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14 164 according to the inclusion/exclusion criteria (see below) at the beginning for the study in 2016.  
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16 165 From each site, a convenience sample of women were/are being recruited to participate.  
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20 166 *Inclusion criteria* include 1) living and receiving HIV care in Rwanda, 2) ages 30-54 years, 3)  
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22 167 confirmed HIV+ based on medical records, 4) no prior cervical-cancer screening, 5) no history of  
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24 168 ICC, and 6) willing, able and competent to provide written, informed consent. We extended age  
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26 169 range beyond that of age range (30-49 years) recommended by the WHO for cervical-cancer  
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28 170 screening<sup>19</sup> because there is limited evidence for the optimal upper age for cervical-cancer  
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30 171 screening of HIV-infected women. *Exclusion criteria*, in addition to not meeting the inclusion  
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32 172 criteria, include 1) pregnant, 2) signs of abnormal, non-menstrual bleeding suggestive of ICC, 3)  
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34 173 without a cervix due to hysterectomy, and 4) not sufficiently healthy to participate in a research  
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36 174 study based on the judgment of the clinicians. Excluded women are being advised to seek routine  
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38 175 cervical-cancer screening through government programs.  
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44 176 Prior to initiation of enrollment at a specific clinic, the local HIV-care provider team identifies  
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46 177 potentially eligible women at their routine clinic visits and offers them enrollment. Women  
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48 178 indicating interest in the study then are being registered by our research nurses using the  
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50 179 eligibility criteria checklist. Women at one site are being enrolled until all the eligible and  
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52 180 willing women at that site are screened. The study team of at least two research nurses schedules  
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54 181 12 to 15 women three to four days a week by calling them and confirming appointments over the  
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3 182 telephone. Two teams of nurses are in the field, meaning that two cervical-cancer screening  
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5 183 clinics can be run simultaneously.  
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9 184 *Enrollment Visit:* The study participant flow is summarized in **Figure 1**. Enrollment visits,  
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11 185 including pelvic exams with VIA and specimen collection, are being done entirely by a team of  
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13 186 two study nurses. During their enrollment visit, women are being educated on cervical-cancer  
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15 187 risk factors, mainly HPV infection, and why they are more at risk to develop ICC than HIV-  
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17 188 uninfected women. They also are being allowed to ask questions before they commit to  
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19 189 participating in the study. Women are then being asked to provide informed, written consent to  
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21 190 participate in the study using a printed out consent form. Those who provide consent complete a  
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23 191 short nurse-administered questionnaire (**Appendix I**) on cervical cancer risk factors and  
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25 192 sociodemographic characteristics using a data capture screen in Microsoft Access. The  
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27 193 questionnaire collects information on basic sociodemographics, factors associated with acquiring  
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29 194 HPV (e.g., marital status and recent and lifetime number of sexual partners), factors associated  
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31 195 with increased risk of progression of hrHPV infection to precancer and cancer (e.g., smoking and  
32  
33 196 other tobacco use, parity, and oral and other contraceptive use), and other infections common in  
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35 197 Rwanda such as malaria<sup>35</sup> and tuberculosis<sup>36</sup> that have been previously reported to be associated  
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37 198 with precancer among hrHPV-infected women. The questionnaire was not pretested.  
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44 199 Enrolled women then undergo a pelvic exam, with VIA and a single cervical exfoliated (“Pap”)  
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46 200 specimen collected and placed into 20 ml PreservCyt (Hologic, Bedford, MA, USA) which is  
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48 201 then being sent to the lab at RMH for hrHPV testing. Finally, a portable colposcope  
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50 202 (<http://www.mobileodt.com/>; MobleODT, Tel Aviv, Israel) is being used for digital  
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52 203 cervicography (comparable to VIA with magnification) and the image is being captured and  
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54 204 saved for quality control, research, and to develop a digital library.  
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3 205 *Colposcopy Visit:* Screen-positive women (women who test hrHPV and/or VIA positive) are  
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5 206 being called using a telephone as soon as the hrHPV result is available and being invited to  
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7 207 return for colposcopy within one month. All screen-positive women receiving colposcopy will  
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9 208 are having two additional specimens collected, one into PreservCyt for the evaluation of other  
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11 209 molecular biomarkers (genotype-specific hrHPV viral methylation and load, and p16/Ki-67  
12  
13 210 immunocytochemistry CINtec® PLUS Cytology Kit [Roche, Tucson, AZ, USA]) and a second  
14  
15 211 as a dry swab for HPV16, 18, 31, 33, 35, 45, 52, and 58 E6/E7 oncoprotein detection by the next  
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17 212 generation lateral flow hrHPV oncoprotein test from Arbor Vita Corporation (Fremont, CA,  
18  
19 213 USA) being included in this study as a triage for screen-positive women to identify those women  
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21 214 who are at higher risk of having CIN3+. The residual PreservCyt specimens from both the  
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23 215 screening and colposcopy visits are being stored at -20°C, creating a biobank in Rwanda for  
24  
25 216 future retrospective evaluations of promising new biomarkers and tests.

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31 217 After specimen collection, a colposcopic evaluation of the cervix is being done with a modified  
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33 218 version of the 4-quadrant microbiopsy procedure being performed.<sup>37</sup> Compared to the standard  
34  
35 219 biopsy, the microbiopsy protocol improves disease ascertainment and reduces biases related to  
36  
37 220 selecting the most visually obvious acetowhite lesions while removing less tissue (~13 mm<sup>2</sup> for 4  
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39 221 microbiopsies vs. ~28mm<sup>2</sup> for 1 standard biopsy). Modifications to the standard 4-quadrant  
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41 222 microbiopsy procedure are: 1) endocervical curettage is being taken only for those women whose  
42  
43 223 squamocolumnar junction is not entirely visible and the lesion extends into the endocervical  
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45 224 canal; and 2) standard-size biopsies of very large lesions are being taken to increase the  
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47 225 likelihood that the most severe area is being biopsied.

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53 226 *Pathology:* Biopsies are being processed in a single cassette so that a single slide has a section  
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55 227 from all biopsies taken. Biopsies are being read by a local pathologist at RMH and Dr. Hebert or

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3 228 another pathologist at Montefiore Medical Center, Bronx, NY, USA. Women receiving a  
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5 229 diagnosis of CIN2+ by the Rwandan pathologist (T.Z.) or, as a safety precaution, CIN3+  
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7 230 diagnosis by Montefiore pathologist (T.H.) are receiving treatment<sup>38</sup>: 1) CIN2, CIN3, or AIS are  
8  
9 231 being referred to study doctors to undergo an excision procedure (e.g., loop electrosurgical  
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11 232 excision procedure [LEEP] or cold-knife cone [CKC]) and 2) ICC are being referred to RMH  
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13 233 Hospital for care. Women with <CIN2 are being advised to seek re-screening in a year.

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18 234 A slide with biopsies also are undergoing p16 immunohistochemistry (IHC) using the CINtec®  
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20 235 Histology Kit (Roche) for research purposes only.

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23 236 *Endpoints*: The primary scientific endpoints of the study are histologically confirmed, consensus  
24  
25 237 CIN2+ i.e., both pathologists diagnose CIN2+ (without adjudication) or CIN3+ by the study  
26  
27 238 pathologist. The secondary, clinical endpoint is histologically confirmed CIN2+ diagnosed by  
28  
29 239 the Rwandan pathologist. Additional endpoints using pathology review and p16 IHC are being  
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31 240 used but not for evaluating the performance of p16 immunocytochemistry due to the possibility  
32  
33 241 of p16-related autocorrelation.

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38 242 *Treatment*: Women diagnosed with CIN2+ are being referred for treatment. Those precancerous  
39  
40 243 lesions are being treated by ablation if they meet WHO criteria for cryotherapy.<sup>39</sup> Those who do  
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42 244 not meet those criteria undergo an excision procedure (e.g., loop electrosurgical excision  
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44 245 procedure [LEEP] or cold-knife cone [CKC]) or, in the case of an ICC diagnosis, referred for  
45  
46 246 cancer management. Screen-positive women with <CIN2 are being advised to seek re-screening  
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48 247 in a year through the existing healthcare system.

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53 248 *Data sources*: Data are being collected from the following sources:  
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3 249 1. A nurse-administered questionnaire on sociodemographic characteristics and cervical  
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5 250 cancer risk factors including age at first sexual intercourse, number of sexual partners,  
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7 251 smoking, contraception, parity and socioeconomic status.  
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10  
11 252 2. Pelvic exam, VIA, Mobile ODT and colposcopy data capture forms  
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13  
14 253 3. Medical record data on HIV status (e.g.,  
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16 254 (<http://www.who.int/hiv/pub/guidelines/HIVstaging150307.pdf>), CD4 count, viral load,  
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18 255 antiretroviral therapy (ART) regimen(s)), care, and dates.  
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22 256 *Laboratory Testing:* The following laboratory tests are being performed:  
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25 257 Xpert HPV Testing—cervical Pap specimens in PreservCyt are being sent to the RMH  
26  
27 258 laboratory in Kigali, Rwanda for hrHPV DNA testing using the Xpert HPV test (Cepheid,  
28  
29 259 Sunnyvale, CA, USA).<sup>40-45</sup> The Xpert HPV Assay is a new, qualitative, real-time PCR assay for  
30  
31 260 the detection of hrHPV DNA. The Xpert HPV Assay includes simultaneous detection of 14  
32  
33 261 hrHPV types, hydroxymethylbilane synthase (HMBS), and an internal Probe Check Control  
34  
35 262 (PCC). The 14 targeted hrHPV types are detected in 5 fluorescent channels: 1) HPV16, 2)  
36  
37 263 HPV18 and hrHPV 45 (HPV18/45), 3) HPV31, 33, 35, 52, and 58, 4) HPV51 and HPV59, and  
38  
39 264 5) HPV39, 56, 66, and 68. HMBS (fluorescent channel 6) verifies specimen adequacy.  
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44 265 Specimens are being mixed and a 1-mL pre-aliquot is being removed using a disposable pipette  
45  
46 266 and placed in the testing cartridge per the manufacturer's instructions. Unsatisfactory results due  
47  
48 267 to insufficient cellular content are being re-tested. If the second test is also unsatisfactory, the  
49  
50 268 final result are being recorded as unsatisfactory but women with unsatisfactory results are being  
51  
52 269 referred to colposcopy for safety.  
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3 270 HPV Viral Methylation—We will conduct a retrospective analysis of HPV viral methylation and  
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5 271 its association with CIN2+. To identify single hrHPV type infections, we will select single-  
6  
7 272 channel positives from the Xpert HPV assay. For those that are hrHPV positive for a channel  
8  
9 273 other than HPV16, which is detected singly, we will test them to identify the single type  
10  
11 274 infections using a standard protocol for PCR amplification using MY09/11 L1 consensus primers  
12  
13 275 and hrHPV genotype detection using dot-hybridization for 39 individual type-specific probes and  
14  
15 276 a mixture of probes for 10 other uncommon hrHPV types as previously described.<sup>46;47</sup> To isolate  
16  
17 277 the DNA, ThinPrep specimens (1.5 mL) will be pelleted, re-suspended in STM, digested with  
18  
19 278 Proteinase K, precipitated overnight in ammonium acetate ethanol at -20°C, washed, and  
20  
21 279 suspended and stored in TE buffer.

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27 280 The isolated DNA then will undergo bisulfite conversion.<sup>48</sup> Following bisulfite conversion and  
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29 281 DNA purification and de-sulphonation, bisulfite-treated DNA will be used as template for Next-  
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31 282 Gen Sequencing (NGS) (HiSeq2000, Illumina, San Diego, CA) using barcoded-type specific  
32  
33 283 primers. Sequences for pads and barcodes are not found in the targeted genomic region. Use of  
34  
35 284 padding and barcodes will enable reads to be identified by amplicon (forward or reverse) or by  
36  
37 285 sample during downstream bioinformatics analysis.<sup>49</sup>

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42 286 All PCR products for NGS will be pooled (by assay) and a single DNA band containing multiple  
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44 287 amplicons from different samples (with unique barcodes) will be isolated from a gel for NGS.<sup>49</sup>  
45  
46 288 Briefly, equal concentrations of each barcoded PCR product (based on PCR band intensity) will  
47  
48 289 be pooled and isolated. Upon confirmation of correct product size, all purified DNA pools will  
49  
50 290 be combined and submitted for library preparation and paired-end 100 base pair Illumina  
51  
52 291 HiSeq2000 sequencing at the Einstein Genomics Core Facility.

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3 292 Methylation status are being determined in the lab of Dr. Robert Burk at Albert Einstein College  
4  
5 293 of Medicine (USA). Prior to determination of methylation status, de-multiplexing based on the  
6  
7 294 unique barcodes is being performed using in-house generated scripts to obtain paired-end NGS  
8  
9  
10 295 reads of each sample. Reads are being aligned with hrHPV reference genome sequences by  
11  
12 296 bowtie v0.12.9.<sup>50</sup> Methylation status of each CpG site is then determined by bismark v0.7.7<sup>51</sup>  
13  
14 297 using the default quality score parameter set to Q30, and the formula of the methylation ratio of  
15  
16 298 the number of C read by the number of C+T read.  
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20 299 E6/E7 Oncoprotein Testing—Dry swab specimens, collected at the time of colposcopy, are being  
21  
22 300 tested for individual E6/E7 oncoproteins as previously described<sup>52;53</sup>, according to the  
23  
24 301 manufacturer's instructions, at the RMH laboratory in Kigali, Rwanda. The E6/E7 oncoprotein  
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26 302 test uses three lateral flow strips to detect 8 hrHPV types whereas the E6 oncoprotein test used a  
27  
28 303 single lateral flow strip to detect 3 hrHPV types.  
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32  
33 304 *Analyses:* We will evaluate combinations of the above mentioned screening strategies and tests  
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35 305 to estimate the clinical performance (e.g., Se, Sp, PPV, and NPV) for the detection of consensus  
36  
37 306 CIN3+ and community CIN2+. A log binomial model using generalized estimating equations  
38  
39 307 will be used to take into account correlation between different tests from the same subject. Note  
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41 308 while these models will be developed for the estimation and comparison of performance for two  
42  
43 309 tests, the model can be extended to allow more than two tests by including more indicator  
44  
45 310 variables for test type.  
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50 311 Some analyses of biomarkers, such as viral methylation will be restricted to hrHPV-positives.  
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52 312 Comparisons of hrHPV viral methylation to other triage biomarkers will be restricted to the  
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54 313 subset that gets tested for viral methylation as described.  
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3 314 *Sample size calculations:* We are basing our sample size on the ability to detect modest but  
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5 315 minimally important differences in Se of 15%. We conservatively assume that the population  
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7 316 prevalence of CIN3+ is 2% based on our past study in Rwanda.<sup>33,35</sup> We will enroll and have  
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9  
10 317 complete follow-up of at least 5,000 HIV[+] women. A sample size of 5,000 HIV[+] women  
11  
12 318 with completed follow-up of the screen positives will yield 100 cases of CIN3+, which will have  
13  
14 319 at least 80% power ( $\alpha=5\%$ ) to crudely detect a 15% difference in Se between two screening  
15  
16 320 strategies for a range of 10%-25% discordance. With this sample size of 5,000 women, 4,900  
17  
18 321 will not have CIN3+; we will also have at least 90% power ( $\alpha=5\%$ ) to detect a difference in Sp  
19  
20 322 of 3% for discordance up to 40%. Finally, we will have 80% power ( $\alpha=5\%$ ) to crudely detect an  
21  
22 323 8%, 10%, or 11% difference in PPV if the reference PPV is 10%, 20%, or 30%, respectively.<sup>54</sup>  
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27 324 *Cost Effectiveness:* We will conduct assessments of the costs and cost-effectiveness of the  
28  
29 325 different combinations of screening and triage tests, i.e., algorithms, as well as those of the entire  
30  
31 326 community-based screening “system.” Costs measurement will be conducted using a micro-  
32  
33 327 costing (ingredients) approach in which resource use throughout each step in the screening  
34  
35 328 process will be tracked and unit costs for each of the resources will be applied to generate an  
36  
37 329 average screening cost per woman to be compared against what the estimated costs are for a  
38  
39 330 possible program based on hrHPV screening and VIA triage or VIA screening. For estimating  
40  
41 331 costs of the screening system and scale-up of screening to 100,000 women in a month, analyses  
42  
43 332 will distinguish financial costs, which reflect actual expenditures of the program, from economic  
44  
45 333 costs, including the value of donated and shared resources to more fully assess opportunity costs.  
46  
47 334 Projections on budget impact and economic cost implications over time will be made under  
48  
49 335 varying assumptions of screening uptake, follow-up compliance, and scenarios of changing  
50  
51 336 disease burden.  
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3 337 Clinical outcomes will include true positive, true negative, false negative, and false positive test  
4  
5 338 results, number of colposcopies, incident cancer, and cancer death. Cost-effectiveness will be  
6  
7 339 measured as cost/CIN2+ detected, cost/CIN3+ detected, cost/invasive cancer prevented,  
8  
9 340 cost/cancer death prevented, cost/life-year saved, and cost/quality-adjusted life year (QALY)  
10  
11 341 saved; in addition, we will calculate harm/benefit ratios, using varying definitions of harms  
12  
13 342 (colposcopies, false positive results) to benefits (cancers prevented, deaths prevented, life years  
14  
15 343 and QALYs saved). Costs and effectiveness will be discounted at a 3% annual rate, with the rate  
16  
17 344 varied from 0-5% in sensitivity analysis. For assessment of value-of-information (VOI), we will  
18  
19 345 use net monetary benefits (NMB), defined as a function of the willingness-to-pay threshold  
20  
21 346 (WTP) for different costs and outcomes as:  $NMB = (WTP * Effectiveness) - Costs$ .

### 27 347 **Patient and Public Involvement**

28  
29  
30 348 There was no patient engagement in the development or design of the study, recruitment, and the  
31  
32 349 conduct of the study. Participants are receiving their results directly since it is related to their  
33  
34 350 care. As this was not a randomized controlled trial, the burden of the intervention was not  
35  
36 351 assessed by patients themselves. There were no patient advisors to acknowledge.

### 40 352 **Ethics and Dissemination**

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43 353 *Ethics:* This study protocol was reviewed and approved by the Rwanda National Ethics

44  
45 354 Committee (RNEC) as well as the Institutional Review Board for human subjects research at  
46  
47 355 Albert Einstein College of Medicine.

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51 356 *Confidentiality measures and protection against potential risks:* The risks for those participating  
52  
53 357 in our study include:

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2  
3 358 • Collection of Pap specimens/cervical swabs involves a modest risk of bleeding  
4  
5 359 which is typically very limited when it occurs. Testing positive for any test may  
6  
7 360 cause psychological distress (anxiety).  
8  
9  
10 361 • Colposcopy and excisional treatments induce vaginal bleeding and may incur pain,  
11  
12 362 infection, and short-term psychological distress (anxiety). A diagnosis of CIN2 or  
13  
14 363 more severe may cause psychological distress (anxiety). A diagnosis of ICC may  
15  
16 364 cause severe psychological distress.  
17  
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19  
20 365 • Questions in the questionnaire, regarding sexual behavior and other matters of a  
21  
22 366 personal nature, may cause anxiety and embarrassment. Participants are advised  
23  
24 367 that they are free not to answer specific questions.  
25  
26  
27  
28 368 • There is also the risk of psycho-social stress which could occur if there was  
29  
30 369 inadvertent disclosure of confidential medical or other personal information.  
31  
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33 370 Protection against the risk of inadvertent disclosure of confidential information is being  
34  
35 371 addressed by the standard procedures at the Rwandan study site, including: (i) storing completed  
36  
37 372 paper copies of questionnaires and other hard copy information (described above), identified by  
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39 373 study number only, in a filing system separate from the name-address file of participants in the  
40  
41 374 study; and (ii) only the designated local personnel have access to cross-reference the files; (iii)  
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43 375 all paper files, including consent forms, are being maintained in locked cabinets in locked rooms,  
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45 376 with access restricted to specific research personnel.  
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50 377 In addition, we will include the following security measures to protect the data:  
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- 54 378 • Controlled access to project data;  
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- 379 • A tracking system for data forms and activities;
- 380 • Date and time of stamping of all data records with electronic signatures;
- 381 • Audit trails to track all changes made to data records; and
- 382 • Data kept on password-protected computers and in locked rooms.

383 *Potential Patient Benefits:* There are potential direct benefits to study participants. HIV[+] women participating in the study are at very high risk of ICC. They are being rigorously screened and evaluated, more effectively than the standard of care anywhere in the world. As a result of the study, women with precancer who are at imminent risk of ICC are being diagnosed sooner and treated more effectively than women receiving routine care and thereby more likely averting the development of ICC. Women with cervical cancer are being diagnosed earlier thereby reducing the morbidity and the risk of mortality caused by ICC. Conversely, any pain, bleeding, or stress that might occur related to colposcopy or cervical swab are typically modest and well tolerated.

392 There are also substantial potential societal benefits related to the study due to its implications to improving cervical-cancer screening practices and guidelines in HIV[+] women – changes in practice which might also benefit the study participants themselves, if and when these changes are enacted. There is a great need to identify more effective and practical methods for cervical-cancer screening for HIV[+] women living in Africa, who are living longer than ever and are therefore at potentially greater risk of ICC.

398 *Dissemination:* We plan to publish a series of scientific reports in peer-reviewed scientific journals. As building research capacity in Rwanda is a major goal of this research project, all investigators of the research team are being asked and supported to lead at least one analysis and one manuscript preparation, based on interests and expertise.

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3 402 In addition, an external advisory panel (EAP) composed of leaders from the Rwanda Ministry of  
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5 403 Health, University of Rwanda, and Rwanda medical community has been formed. The  
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7 404 responsibilities of the EAP include providing advice on the conduct of the project and  
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9  
10 405 interpretation for and dissemination of the study results to Rwandan stakeholders. The latter is  
11  
12 406 important for the adoption of evidence-based best practices for cervical-cancer screening as  
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14  
15 407 warranted.

### 16 17 18 408 **Limitations**

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20  
21 409 There are several limitations to the study that bear mentioning. First, cervical cytology was not  
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23 410 included in the study. There is limited cervical cytology services available locally and of  
24  
25 411 unknown quality and it is unlikely that cytology are being widely available in Rwanda, making  
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27  
28 412 its inclusion as a comparator test of limited value. Moreover, there are significant costs and  
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30 413 logistical challenges in shipping PreservCyt specimens to and having cytology slides made and  
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32 414 read in the U.S. Second, we did not conduct biopsies in screen-negative women, which would  
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34  
35 415 have allowed us to estimate absolute clinical performance. The burden of sending screen-  
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37 416 negative women to colposcopy was deemed too great and it was impractical to send a sufficient  
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39 417 numbers of screen-negative women to colposcopy to accurately estimate the false-negative  
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41  
42 418 disease (CIN3+) fraction. Thus, only relative clinical performance of the screening tests can be  
43  
44 419 estimated from this study.

## 420 Contributor Statement:

421 KA, PEC, JCD, AA, and JDS conceived the original concept of the study and the interventions.  
422 GM, KA, JDS, and PEC drafted the protocol. PEC performed the sample size calculations, and  
423 PEC and GM will lead analysis of the results. GM, JDS, and Athanase Munyaneza are  
424 supporting patient recruitment. GM, TR, and Athanase Munyaneza are providing clinical care for  
425 patients. Anthere Murangwa and LM oversee laboratory testing, and TZ and TH are responsible  
426 for pathology. PM and LM oversee and administer the study activities at the clinical site in  
427 Rwanda. All authors (GM, JCD, TR, JDS, Athanase Munyaneza, Anthere Murangwa, TZ, TH,  
428 PM, AA, LM, KA, and PEC) contributed to the scientific design of the study and the protocol  
429 development, are involved in the implementation of the project, and have read and approved the  
430 final manuscript.

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3 432 Figure Legends:  
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7 434 Figure 1. Study Design  
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For peer review only

594 **Table 1:** Recruitment sites and estimated eligible population.

Province	Site	Type of site	Potential participants per site (approximate)
Kigali	Cor-unum HC	Public Health Center	1,405
Kigali	Kimironko HC	Public Health Center	1,227
Kigali	Rwampara HC	Public Referral Hospital	1,098
Kigali	Kicukiro HC	Public Health Center	1,054
Kigali	Kacyiru HC	Public Health Center	905
Kigali	Gikondo HC	Public Health Center	925
Kigali	Rwanda Military Hospital	Public Referral Hospital	300
Kigali	WEACTx for Hope	Private HIV Clinic	500
Kigali	Busanza HC	Public Health Center	100
Kigali	Nyarugunga HC	Public Health Center	100
West	Gisenyi DH	Public District Hospital	810
Total			8,424

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Figure 1. Study diagram

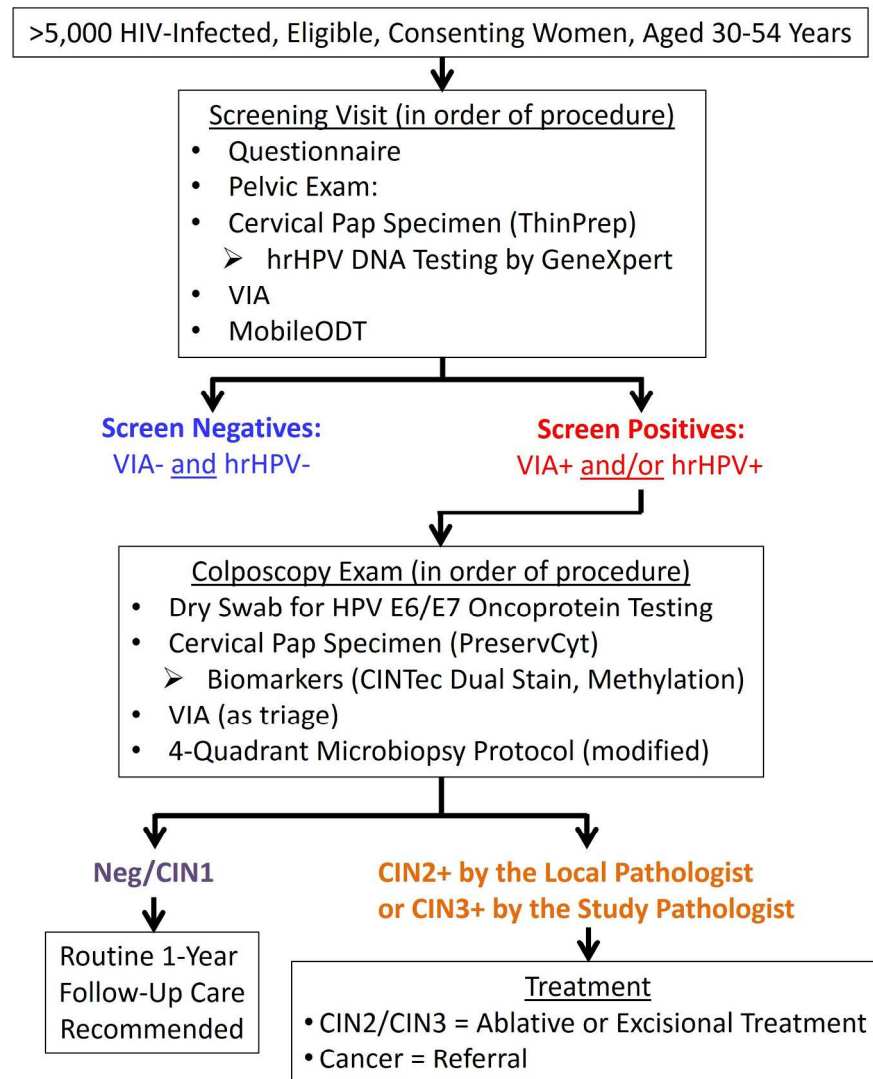


Figure 1. Study Design

190x254mm (300 x 300 DPI)

## Appendix I. Study Questionnaire

### A. Socio-demographics

1. What is your date of birth? D D / M M / Y Y Y Y (enter all 0 if not remembered) (If date of birth or year of birth is given, skip to Question 3)

2. What is your age? \_\_\_ \_\_\_ (Years)

3. What is your marital status?

Married/Cohabiting

Divorced

Widowed

Separated

Single

Choose not to answer

4. Do you live in Kigali?

Yes

No {Skip to Question A5}

Choose not to answer {Skip to Question A5}

5. In what area of Kigali City Province do you live?

Nyarugenge

Kicukiro

Gasabo urban (e.g., Kacyiru, Kimironko, Kinyinya, etc.)

Gasabo rural (e.g., Nduba, Jabana, Gikomero, etc.)

Do not know

Choose not to answer

6. What is your household income per month in FRW?

< 5,000

5,000 – 9,999

10,000 – 34,999

35,000 – 59,999

- 1  
2  
3  60,000 – 84,999  
4  
5  85,000 – 109,999  
6  
7  110,000 – 134,999  
8  
9  135,000 – 269,999  
10  
11  270,000 or more

12 **7. How many people live in your household?** \_\_\_ \_\_\_ {Enter 00 if refuse to answer}

13  
14 **8. What is your occupation?**

- 15  
16  Employed by government, another institution, or company  
17  
18  Self-employed (Small and medium enterprises)  
19  
20  Self-employed (High income earnings)  
21  
22  Farming (peasants)  
23  
24  Unemployed/Does not work  
25  
26  Other (specify) \_\_\_\_\_  
27  
28  Choose not to answer {Skip to Question B1}

29 {The following questions are sensitive and personal in nature. Your answers will be kept  
30 confidential. You may choose not to answer certain questions. Answering any question is  
31 voluntary}

32  
33 **B. Sexual Behaviors**

34  
35 **1. How old were you when you first had sex?** \_\_\_ \_\_\_ (Years) {Enter 00 if refuse to  
36 answer}

37  
38 **2. How many sexual partners have you had in your lifetime?**

- 39  
40  No Partners (Ineligible)  
41  
42  1 Partner  
43  
44  2-3 Partners  
45  
46  4-5 Partners  
47  
48  6-9 Partners  
49  
50  10 or more partners  
51  
52  Choose not to answer

53 **3. How many sexual partners have you had in the last 6 months?**

- 54  
55  No Partners  
56  
57  
58  
59  
60



- 1  
2  
3  1 Partner  
4  
5  2 or more Partners  
6  
7  Choose not to answer

8  
9 **C. Parity**

- 10  
11 **1. What age did you have your first child? \_\_\_ (Years) {Enter 00 if refuse to**  
12 **answer, Enter 99 if never pregnant} (if 00 or 99, skip to Question D1)**  
13  
14 **2. How many live births have you had in your lifetime? \_\_\_**  
15  
16 **3. Have you given birth in the last year?**

- 17  
18  Yes  
19  
20  No  
21  
22  Choose not to answer

23  
24 **D. Tobacco Use**

- 25  
26 **1. Have you ever smoked cigarettes?**

- 27  
28  Yes  
29  
30  No (skip to Question D3)  
31  
32  Choose not to answer (skip to Question D3)

- 33  
34 **2. Do you currently smoke cigarettes?**

- 35  
36  Yes  
37  
38  No  
39  
40  Choose not to answer

- 41  
42 **3. Have you ever chewed/used tobacco orally (Ubugoro)?**

- 43  
44  Yes  
45  
46  No (skip to Question D5)  
47  
48  Choose not to answer (skip to Question D5)

- 49  
50 **4. Are you currently chewing/using tobacco orally (Ubugoro)?**

- 51  
52  Yes  
53  
54  No  
55  
56  Choose not to answer

1  
2  
3 **5. Have you ever chewed/used tobacco orally (Tobacco leaves-Igikamba)?**  
4

- 5  Yes  
6  
7  No (skip to Question E1)  
8  
9  Choose not to answer (skip to Question E1)

10  
11 **6. Are you currently chewing/using tobacco orally (Tobacco leaves-Igikamba)?**  
12

- 13  Yes  
14  
15  No  
16  
17  Choose not to answer

18 **E. Contraceptive Use**  
19

20  
21 **1. Have you ever used oral contraceptives?**  
22

- 23  Yes  
24  
25  No (Skip to Question E3)  
26  
27  Choose not to answer (Skip to Question E3)

28 **2. Do you currently use oral contraceptives?**  
29

- 30  Yes  
31  
32  No  
33  
34  Choose not to answer

35  
36 **3. Have you ever used Depo Provera (contraceptive)?**  
37

- 38  Yes  
39  
40  No (Skip to Question E5)  
41  
42  Choose not to answer (Skip to Question E5)

43 **4. Do you currently use Depo Provera (contraceptive)?**  
44

- 45  Yes  
46  
47  No  
48  
49  Choose not to answer

50  
51 **5. Have you ever used Jadell (contraceptive)?**  
52

- 53  Yes  
54  
55  No (Skip to Question E7)  
56  
57  
58  
59  
60

Choose not to answer (Skip to Question E7)

**6. Do you currently use Jadell (contraceptive)?**

Yes

No

Choose not to answer

**7. Have you ever used IUD (contraceptive)?**

Yes

No (Skip to Question E9)

Choose not to answer (Skip to Question E9)

**8. Do you currently use IUD (contraceptive)?**

Yes

No

Choose not to answer

**9. Do use condoms as a contraceptive?**

Yes

No (skip to Question F1)

Choose not to answer (skip to Question F1)

**10. Have you used condoms as a contraceptive in the last 6 months?**

Yes

No

Choose not to answer

**F. Infections**

**1. Have you ever had Malaria**

Yes

No (skip to Question F8)

Choose not to answer (skip to Question F8)

**2. How many times have you had Malaria? \_\_\_**

1  
2  
3 **3. What was the year that you last had Malaria? Y Y Y Y**  
4

5 **4. Have you had your Malaria treated using drugs?**  
6

7  Yes

8  No

9  Choose not to answer  
10

11 **5. If Yes, how was it treated?**  
12

13  Treated only with traditional medicine

14  Treated only with drugs (e.g. Coartem)

15  Treated with both (traditional and modern)

16  Not treated

17  Choose not to answer  
18  
19  
20  
21  
22

23 **6. Was your last episode of Malaria treated using drugs?**  
24

25  Yes

26  No

27  Choose not to answer  
28  
29  
30

31 **7. If Yes, how was it treated?**  
32

33  Treated only with traditional medicine

34  Treated only with drugs (e.g. Coartem)

35  Treated with both (traditional and modern)

36  Not treated

37  Choose not to answer  
38  
39  
40  
41

42 **8. Have you ever had Tuberculosis (TB)?**  
43

44  Yes

45  No (skip to end)

46  Choose not to answer (skip to end)  
47  
48

49 **9. If Yes, how many times have you had TB in your lifetime? \_ \_**  
50

51 **10. What year did you last have Tuberculosis (TB)? Y Y Y Y**  
52

53 **END OF QUESTIONNAIRE**  
54  
55  
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# BMJ Open

## A Protocol for the Study of Cervical-Cancer Screening Technologies in Human Immunodeficiency Virus-Infected Women Living in Rwanda

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-020432.R3
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Complete List of Authors:	Murenzi, Gad; Rwanda Military Hospital Dusingize, Jean; Regional Alliance for Sustainable Development , Research and Scientific Capacity Building Rurangwa, Theogene; Rwanda Military Hospital Sinayobye, Jean d'Amour; Regional Alliance for Sustainable Development, ; Women's Equity in Access to Care & Treatment (WE-ACTx), Munyaneza, Athanase; Rwanda Military Hospital Murangwa, Anthere; Rwanda Military Hospital Zawadi, Thierry; Rwanda Military Hospital Hebert, Tiffany; Yeshiva University Albert Einstein College of Medicine Mugenzi, Pacifique; Rwanda Military Hospital Adedimeji, Adebola; Yeshiva University Albert Einstein College of Medicine Mutesa, Leon; Rwanda Military Hospital; University of Rwanda College of Medicine and Health Sciences Anastos, Kathryn; Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, Castle, Philip; Yeshiva University Albert Einstein College of Medicine,
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Keywords:	human papillomavirus (HPV), cervical cancer, HIV & AIDS < INFECTIOUS DISEASES, GYNAECOLOGY, cervical intraepithelial neoplasia

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Manuscripts

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2  
3 **1 A Protocol for the Study of Cervical-Cancer Screening Technologies in Human**  
4  
5 **2 Immunodeficiency Virus-Infected Women Living in Rwanda**  
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9 3 Gad Murenzi, MD\*<sup>1</sup>, Jean-Claude Dusingize, MD, MS<sup>1</sup>, Theogene Rurangwa, MD, MMed<sup>1</sup>,  
10  
11 4 Jean d'Amour Sinayobye, MD, MS<sup>1</sup>, Athanase Munyaneza, RN<sup>1</sup>, Anthere Murangwa, MS<sup>1</sup>,  
12  
13 5 Thierry Zawadi, MD<sup>1</sup>, Tiffany Hebert, MD<sup>2</sup>, Pacifique Mugenzi, MD<sup>1</sup>, MMed, Adebola  
14  
15 6 Adedimeji, PhD, MPH<sup>2</sup>, Leon Mutesa, MD, PhD<sup>1,3</sup>, Kathryn Anastos, MD<sup>2</sup>, Philip E. Castle,  
16  
17 7 PhD, MPH<sup>2</sup>  
18  
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20

21 8 <sup>1</sup>Rwanda Military Hospital, Kigali, Rwanda; <sup>2</sup>Albert Einstein College of Medicine, Bronx, NY,  
22  
23 9 USA; <sup>3</sup>University of Rwanda, Kigali, Rwanda  
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27 10 \*Correspondence: [gadcollins@gmail.com](mailto:gadcollins@gmail.com), +250788589085  
28  
29

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31  
32 12 Arbor Vita Corporation, and Roche.  
33  
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35  
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37  
38 14 Prevent Cancer Foundation.  
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3 **16 Abstract**  
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5 **17** *Introduction.* The optimal method(s) for screening human immunodeficiency virus-infected  
6 **18** women, especially for those living in sub-Saharan Africa, for cervical precancer and early cancer  
7 **19** has yet to be established.

8  
9  
10 **20** *Methods and analysis.* A convenience sample of >5,000 Rwandan women, ages 30-54 years and  
11 **21** living with HIV infection, are being consented and enrolled into a cross-sectional study of  
12 **22** cervical-cancer screening strategies. Participants are completing an administered short risk-factor  
13 **23** questionnaire and being screened for high-risk human papillomavirus (hrHPV) using the Xpert  
14 **24** HPV assay (Cepheid, Sunnyvale, CA, USA), unaided visual inspection after acetic acid (VIA),  
15 **25** and aided VIA using the EVA system (Mobile ODT, Tel Aviv, Israel). Women positive for  
16 **26** hrHPV and/or by VIA undergo colposcopy, which includes the collection of two cervical  
17 **27** specimens prior to undergoing a 4-quadrant microbiopsy protocol. The colposcopy-collected  
18 **28** specimens are being tested by dual immunocytochemical staining for p16<sup>INK4a</sup> and Ki-67  
19 **29** (CINtec® PLUS Cytology, Ventana, Tucson, AZ, USA) and for E6 or E7 for 8 hrHPV  
20 **30** genotypes (HPV16, 18, 31, 33, 35, 45, 52, and 58) using the next-generation AV Advantage  
21 **31** hrHPV E6/E7 test (Arbor Vita Corporation, Freemont, CA, USA). Women with local pathology  
22 **32** diagnosis of cervical intraepithelial neoplasia (CIN) grade 2 (CIN2) or more severe (CIN2+) or  
23 **33** pathology-review diagnosis of CIN grade 3 or more severe (CIN3+) receive treatment. Clinical  
24 **34** performance and cost-effectiveness (e.g., sensitivity, specificity, and predictive values) of  
25 **35** different screening strategies and algorithms will be evaluated.

26  
27  
28  
29 **36** *Ethics and dissemination.* The protocol was approved by local and institutional review boards for  
30 **37** human subjects research. At the completion of the study, results will be disseminated to the  
31 **38** scientific community through peer-reviewed publication and to the Rwandan stakeholders  
32 **39** through an external advisory panel.

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3 41 **Strengths and Weaknesses**  
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- 5  
6 42 • We are enrolling a very large sample size of HIV-infected women living in Rwanda who  
7 43 otherwise would probably not get cervical-cancer screening.  
8 44 • We are employing rigorous disease ascertainment protocols to minimize  
9 45 misclassification.  
10 46 • Some tests, such as the Xpert HPV and the AV Advantage HPV E6/E7 assays, are being  
11 47 done on site in Rwanda using technologies that could feasibly be deployed there.  
12 48 • A weakness of the study is that cervical cytology is not being included as a comparator  
13 49 due to financial and logistical constraints.  
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For peer review only



## 51 **Introduction**

52 Invasive cervical cancer (ICC) remains a significant cause of morbidity and mortality globally.  
53 Approximately 530,000 cases of and 270,000 deaths due to ICC occur annually, making it the 4<sup>th</sup>  
54 most common malignancy and cause of cancer-related death in women globally.<sup>1</sup> In many high-  
55 income countries (HIC), ICC rates have declined by 50% or more<sup>2</sup> due to the introduction of  
56 effective, high-coverage Pap testing (cervical cytology)-based screening programs that include  
57 timely follow-up of screen positives, treatment of women with precursor lesions, and  
58 management of cancers. Now, almost 90% of ICC and ICC-related deaths occur in low- and  
59 middle income countries (LMICs) due to a lack of resources and healthcare infrastructure needed  
60 to provide preventive services.

61 ICC and ICC-related mortality rates are particularly high in Sub-Saharan Africa, which also has  
62 the highest rates of HIV infection in the world. Now, over 12 million HIV-infected (HIV[+])  
63 women in Sub-Saharan Africa are living longer because of anti-retroviral therapy, thus  
64 increasing their likelihood of dying from ICC.<sup>3</sup> However, many of these women are already  
65 exposed to human papillomavirus (HPV), the viral cause of ICC, and will not benefit from or be  
66 targeted for prophylactic HPV vaccination. Thus, cervical-cancer screening will be needed for  
67 the foreseeable future.

68 However, setting up effective cytology for cervical-cancer screening is expensive and requires a  
69 complex clinical and lab infrastructure that generally does not exist in LMICs.<sup>4;5</sup> Moreover, it is  
70 now well understood that cytology has only a low- to moderate one-time sensitivity for precursor  
71 lesions and therefore must be done repeatedly over many years to reduce cancer risk. Alternative

1  
2  
3 72 strategies to address the ICC burden in LMICs, especially in SSA, must be developed and  
4  
5 73 validated.  
6  
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8  
9 74 Persistent cervical infections by high-risk HPV (hrHPV) types cause virtually all ICC and its  
10  
11 75 immediate precursor lesions, e.g. cervical intraepithelial neoplasia grade 3 (CIN3) and  
12  
13 76 adenocarcinoma *in situ* (AIS) everywhere in the world.<sup>6;7</sup> hrHPV causes most anal and vaginal  
14  
15 77 cancer and a significant proportion of vulvar, penile, and oropharyngeal cancers.<sup>8</sup> HPV16 is the  
16  
17 78 most important causal type, responsible for ~60% of ICC.<sup>9</sup> HPV18 is the next most important,  
18  
19 79 responsible for 10-15% of ICC, including 30-40% of adenocarcinoma of the cervix<sup>9</sup>, which is on  
20  
21 80 the rise in Western Countries.<sup>10;11</sup> Together, HPV16 and HPV18 account for ~70% of ICC, and  
22  
23 81 the same 15 hrHPV types account for ~99% of ICC everywhere in the world.<sup>9</sup>  
24  
25  
26  
27

28 82 There is now overwhelming evidence to suggest that testing for hrHPV is more sensitive, albeit  
29  
30 83 less specific, than high-quality cytology for identifying women with cervical precancer.<sup>12-16</sup> One-  
31  
32 84 time hrHPV testing can reduce the risk of ICC incidence by approximately 40% in 6.5 years  
33  
34 85 compared to cytology screening<sup>16</sup>, and ICC mortality by approximately 40% (approximately  
35  
36 86 50% overall) in 8 years compared to cytology.<sup>17</sup> Importantly, a negative hrHPV test provides  
37  
38 87 superior reassurance against CIN3+<sup>18</sup> and against ICC<sup>16;17</sup>, permitting safe extension of  
39  
40 88 screening intervals.  
41  
42  
43  
44

45 89 The World Health Organization released cervical-cancer screening and treatment guidelines in  
46  
47 90 2013, recommending two evidence-based approaches to cervical-cancer screening<sup>19</sup>: (I) Use  
48  
49 91 either hrHPV testing or visual inspection after acetic acid (VIA), which involves the inspection  
50  
51 92 of the cervix with a speculum in place and following the application of dilute acetic acid to help  
52  
53 93 identify potential CIN by its characteristic white coloring in the presence of acetic acid  
54  
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3 94 (acetowhite), as alternative initial screening tests instead of Pap, and (II) immediately treat those  
4  
5 95 who screen positive using the screening test, rather than require diagnostic verification through  
6  
7  
8 96 colposcopy and biopsy. This approach is commonly called screen-and-treat (S&T), and is  
9  
10 97 increasingly thought to be more amenable to LMIC settings.

11  
12  
13 98 However, hrHPV testing is also a much more effective screen than VIA<sup>17</sup>, which on a large-scale  
14  
15 99 appears to only down-stage cancer rather than prevent it.<sup>20</sup> Thus, the recent American Society for  
16  
17  
18 100 Clinical Oncology (ASC) resource-stratified guidelines for secondary cervical-cancer  
19  
20 101 prevention<sup>21;22</sup> emphasize that hrHPV testing is the preferred choice for screening, with VIA  
21  
22 102 only being used until hrHPV testing becomes available, and that HIV-infected women, because  
23  
24  
25 103 of their higher risk, should be screened twice as frequently as the general (HIV-uninfected)  
26  
27 104 population.

28  
29  
30 105 Recent data in HIV[+] women living in the U.S. suggest that hrHPV testing may have clinical  
31  
32 106 utility similar to that in HIV-negative (HIV[-]) women. Several observational studies have  
33  
34  
35 107 shown that an extended screening interval is safe in HIV[+] women who test hrHPV and Pap  
36  
37 108 negative as it is for HIV[-] women.<sup>23;24</sup> In a study of women enrolled in Women's Interagency  
38  
39  
40 109 Health Study (WIHS) in 2002, HIV[+] and HIV[-] women who tested hrHPV and Pap negative  
41  
42 110 were at a similarly low risk of cervical intraepithelial neoplasia (CIN) grade 2 (CIN2) or more  
43  
44 111 severe (CIN2+) histology over a 5-year follow-up.<sup>23</sup> In addition, no cases of histologically  
45  
46 112 confirmed CIN2+ were diagnosed in the follow-up of hrHPV- and Pap-negative HIV[+] women  
47  
48  
49 113 aged 30-64 years who underwent routine three-year hrHPV and cytology cotesting at Kaiser  
50  
51 114 Permanente Northern California.<sup>24</sup> Thus, both studies found very high negative predictive values  
52  
53 115 (NPV) >99% in HIV[+] women who test hrHPV negative. However, how hrHPV testing can

1  
2  
3 116 best be used to screen HIV[+] women living in Sub-Saharan Africa to prevent ICC remains to be  
4  
5 117 determined.

6  
7  
8 118 Recent studies<sup>25-28</sup> in HIV[+] women living in Sub-Saharan Africa have compared hrHPV, VIA,  
9  
10  
11 119 and/or Pap for the detection of cervical precancer/cancer. The results can be summarized as  
12  
13 120 follows: 1) hrHPV detection was more sensitive but less specific than VIA; 2) surprisingly,  
14  
15 121 cytology was equally or more sensitive but less specific than VIA; and 3) surprisingly, cytology  
16  
17 122 was equally or more sensitive but less specific (vs. the converse) than hrHPV testing. Results and  
18  
19 123 conclusions are varied, leaving unanswered the question of what screening strategy in HIV[+]  
20  
21 124 women living in Sub-Saharan Africa has the greatest effectiveness and cost effectiveness.

22  
23  
24  
25 125 Regardless of the screening method, most screen-positive women who go to colposcopy or are  
26  
27 126 treated immediately without diagnostic verification do not have cervical precancer and cancer  
28  
29 127 (positive predictive value [PPV] for screening tests are typically 10%-20%). In places like Sub-  
30  
31 128 Saharan Africa that lack necessary infrastructure and personnel such as pathologists<sup>29</sup>, excessive  
32  
33 129 referral to colposcopy is problematic. Although WHO recommendations for S&T will hopefully  
34  
35 130 overcome this bottleneck and increase the number of women living in LMICs who get screened,  
36  
37 131 many countries may not adopt current S&T strategies because of concerns of low specificity and  
38  
39 132 overtreatment, resulting in increased costs, unnecessary patient discomfort and concern, and  
40  
41 133 wasting valuable healthcare resources that could otherwise be used to expand access to  
42  
43 134 screening. Thus, methods to increase the accuracy of screening by reducing the numbers of  
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45 135 women having colposcopy and biopsy or getting treated immediately in this context are highly  
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49 136 desirable as they will likely increase the uptake of cervical-cancer screening.  
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3 137 In order to improve the specificity of screening tests, secondary tests (biomarkers) are used  
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5 138 following a screen-positive result, with women who test positive for the triage undergoing  
6  
7 139 further management (e.g., colposcopy or immediate treatment) and those who test negative  
8  
9  
10 140 typically being deferred to further evaluation in 6-18 months to allow hrHPV infections to clear.  
11  
12 141 There are several very promising biomarkers that might be used to improve the specificity and  
13  
14 142 positive predictive value (PPV) of the screening tests.<sup>24</sup> Given that HIV-infected women are  
15  
16 143 more likely to test hrHPV positive<sup>30-34</sup>, it is important to validate a triage strategy of using a  
17  
18 144 secondary biomarker that sensitively and specifically rules-in women with cervical precancer  
19  
20 145 and cancer among the HIV-positive, hrHPV-positive women.

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25 146 We are therefore conducting a cervical-cancer screening study of >5,000 Rwandan women, ages  
26  
27 147 30-54 years, living with HIV infection. We are evaluating different screening tests (hrHPV DNA  
28  
29 148 and VIA), those recommended by the WHO for cervical-cancer screening<sup>19</sup>, and different triage  
30  
31 149 tests and biomarkers for the management of screen-positive women (E6/E7 oncoprotein  
32  
33 150 detection, p16INK4a immunocytochemistry, and hrHPV viral methylation). Screen-positive  
34  
35 151 women undergo a rigorous colposcopic evaluation with multiple biopsies taken and the biopsies  
36  
37 152 will undergo pathology review, to minimize the misclassification of endpoints. The primary  
38  
39 153 objective of the study is to determine and compare clinical performance (Sensitivity (Se),  
40  
41 154 Specificity (Sp), PPV, and NPV) and cost-effectiveness for identifying HIV[+] women with  
42  
43 155 CIN3+ and CIN2+ of different cervical-cancer screening and management algorithms.  
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## 49 156 **Methods and Analysis**

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52 157 *Study design and population:* We are recruiting those women receiving care for confirmed HIV  
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54 158 infection at health centers (HC) and various hospitals operated by the Ministry of Health or  
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3 159 Rwanda Military Hospital during 2016-18 (**Table 1**). Sites were selected in collaboration with  
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5 160 Rwanda Biomedical Center (RBC), which calculate estimated numbers of potentially eligible  
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7 161 women using data from their HIV database (OpenMRS-Open Medical Records System;  
8  
9  
10 162 <http://openmrs.org/>). Sites were also selected from all provinces to ensure geographic  
11  
12 163 representation. The total of 8,424 was the estimated number of women eligible for the study  
13  
14 164 according to the inclusion/exclusion criteria (see below) at the beginning for the study in 2016.  
15  
16 165 From each site, a convenience sample of women are being recruited to participate.  
17  
18  
19

20 166 *Inclusion criteria* include 1) living and receiving HIV care in Rwanda, 2) ages 30-54 years, 3)  
21  
22 167 confirmed HIV+ based on medical records, 4) no prior cervical-cancer screening, 5) no history of  
23  
24 168 ICC, and 6) willing, able and competent to provide written, informed consent. We are extending  
25  
26 169 age range beyond that of the age range (30-49 years) recommended by the WHO for cervical-  
27  
28 170 cancer screening<sup>19</sup> because there is limited evidence for the optimal upper age for cervical-cancer  
29  
30 171 screening of HIV-infected women. *Exclusion criteria*, in addition to not meeting the inclusion  
31  
32 172 criteria, include 1) pregnant, 2) signs of abnormal, non-menstrual bleeding suggestive of ICC, 3)  
33  
34 173 without a cervix due to hysterectomy, and 4) not sufficiently healthy to participate in a research  
35  
36 174 study based on the judgment of the clinicians. Excluded women are being advised to seek routine  
37  
38 175 cervical-cancer screening through government programs.  
39  
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41  
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44 176 Prior to initiation of enrollment at a specific clinic, the local HIV-care provider team identifies  
45  
46 177 potentially eligible women at their routine clinic visits and offers them enrollment. Women  
47  
48 178 indicating interest in the study then are then registered by our research nurses using the eligibility  
49  
50 179 criteria checklist. All eligible and willing women at that site enroll and receive screening before  
51  
52 180 moving to another site. The study team of at least two research nurses schedules 12 to 15 women  
53  
54 181 three to four days a week by calling them and confirming appointments over the telephone. Two  
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2  
3 182 teams of nurses are in the field, meaning that two cervical-cancer screening clinics can be run  
4  
5 183 simultaneously.

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8  
9 184 *Enrollment Visit:* The study participant flow is summarized in **Figure 1**. Enrollment visits,  
10  
11 185 including pelvic exams with VIA and specimen collection, are being done entirely by a team of  
12  
13 186 two study nurses. During their enrollment visit, women are being educated on cervical-cancer  
14  
15 187 risk factors, mainly HPV infection, and why they are more at risk to develop ICC than HIV-  
16  
17 188 uninfected women. They also are being allowed to ask questions before they commit to  
18  
19 189 participating in the study. Women are then being asked to provide informed, written consent to  
20  
21 190 participate in the study using a printed out consent form. Those who provide consent complete a  
22  
23 191 short nurse-administered questionnaire (**Appendix I**) on cervical cancer risk factors and  
24  
25 192 sociodemographic characteristics using a data capture screen in Microsoft Access. The  
26  
27 193 questionnaire collects information on basic sociodemographics, factors associated with acquiring  
28  
29 194 HPV (e.g., marital status and recent and lifetime number of sexual partners), factors associated  
30  
31 195 with increased risk of progression of hrHPV infection to precancer and cancer (e.g., smoking and  
32  
33 196 other tobacco use, parity, and oral and other contraceptive use), and other infections common in  
34  
35 197 Rwanda such as malaria<sup>35</sup> and tuberculosis<sup>36</sup> that have been previously reported to be associated  
36  
37 198 with precancer among hrHPV-infected women. The questionnaire was not pretested.

38  
39  
40  
41 199 Enrolled women then undergo a pelvic exam, with VIA and a single cervical exfoliated (“Pap”)  
42  
43 200 specimen collected and placed into 20 ml PreservCyt (Hologic, Bedford, MA, USA) which is  
44  
45 201 then being sent to the lab at RMH for hrHPV testing. Finally, a portable colposcope  
46  
47 202 (<http://www.mobileodt.com/>; MobleODT, Tel Aviv, Israel) is being used for digital  
48  
49 203 cervicography (comparable to VIA with magnification) and the image is being captured and  
50  
51 204 saved for quality control, research, and to develop a digital library.

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3 205 *Colposcopy Visit:* Screen-positive women (women who test hrHPV and/or VIA positive) are  
4  
5 206 being called using a telephone as soon as the hrHPV result is available and being invited to  
6  
7 207 return for colposcopy within one month. All screen-positive women receiving colposcopy will  
8  
9 208 are having two additional specimens collected, one into PreservCyt for the evaluation of other  
10  
11 209 molecular biomarkers (genotype-specific hrHPV viral methylation and load, and p16/Ki-67  
12  
13 210 immunocytochemistry CINtec® PLUS Cytology Kit [Roche, Tucson, AZ, USA]) and a second  
14  
15 211 as a dry swab for HPV16, 18, 31, 33, 35, 45, 52, and 58 E6/E7 oncoprotein detection by the next  
16  
17 212 generation lateral flow hrHPV oncoprotein test from Arbor Vita Corporation (Fremont, CA,  
18  
19 213 USA) being included in this study as a triage for screen-positive women to identify those women  
20  
21 214 who are at higher risk of having CIN3+. The residual PreservCyt specimens from both the  
22  
23 215 screening and colposcopy visits are being stored at -20°C, creating a biobank in Rwanda for  
24  
25 216 future retrospective evaluations of promising new biomarkers and tests.

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31 217 After specimen collection, a colposcopic evaluation of the cervix is being done with a modified  
32  
33 218 version of the 4-quadrant microbiopsy procedure being performed.<sup>37</sup> Compared to the standard  
34  
35 219 biopsy, the microbiopsy protocol improves disease ascertainment and reduces biases related to  
36  
37 220 selecting the most visually obvious acetowhite lesions while removing less tissue (~13 mm<sup>2</sup> for 4  
38  
39 221 microbiopsies vs. ~28mm<sup>2</sup> for 1 standard biopsy). Modifications to the standard 4-quadrant  
40  
41 222 microbiopsy procedure are: 1) endocervical curettage is being taken only for those women whose  
42  
43 223 squamocolumnar junction is not entirely visible and the lesion extends into the endocervical  
44  
45 224 canal; and 2) standard-size biopsies of very large lesions are being taken to increase the  
46  
47 225 likelihood that the most severe area is being biopsied.

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53 226 *Pathology:* Biopsies are being processed in a single cassette so that a single slide has a section  
54  
55 227 from all biopsies taken. Biopsies are being read by a local pathologist at RMH and Dr. Hebert or



1  
2  
3 228 another pathologist at Montefiore Medical Center, Bronx, NY, USA. Women receiving a  
4  
5 229 diagnosis of CIN2+ by the Rwandan pathologist (T.Z.) or, as a safety precaution, CIN3+  
6  
7 230 diagnosis by Montefiore pathologist (T.H.) are receiving treatment<sup>38</sup>: 1) CIN2, CIN3, or AIS are  
8  
9 231 being referred to study doctors to undergo an excision procedure (e.g., loop electrosurgical  
10  
11 232 excision procedure [LEEP] or cold-knife cone [CKC]) and 2) ICC are being referred to RMH  
12  
13 233 Hospital for care. Women with <CIN2 are being advised to seek re-screening in a year.

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17  
18 234 A slide with biopsies also are undergoing p16 immunohistochemistry (IHC) using the CINtec®  
19  
20 235 Histology Kit (Roche) for research purposes only.

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22  
23 236 *Endpoints*: The primary scientific endpoints of the study are histologically confirmed, consensus  
24  
25 237 CIN2+ i.e., both pathologists diagnose CIN2+ (without adjudication) or CIN3+ by the study  
26  
27 238 pathologist. The secondary, clinical endpoint is histologically confirmed CIN2+ diagnosed by  
28  
29 239 the Rwandan pathologist. Additional endpoints using pathology review and p16 IHC are being  
30  
31 240 used but not for evaluating the performance of p16 immunocytochemistry due to the possibility  
32  
33 241 of p16-related autocorrelation.

34  
35  
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37  
38 242 *Treatment*: Women diagnosed with CIN2+ are being referred for treatment. Those precancerous  
39  
40 243 lesions are being treated by ablation if they meet WHO criteria for cryotherapy.<sup>39</sup> Those who do  
41  
42 244 not meet those criteria undergo an excision procedure (e.g., loop electrosurgical excision  
43  
44 245 procedure [LEEP] or cold-knife cone [CKC]) or, in the case of an ICC diagnosis, referred for  
45  
46 246 cancer management. Screen-positive women with <CIN2 are being advised to seek re-screening  
47  
48 247 in a year through the existing healthcare system.

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53 248 *Data sources*: Data are being collected from the following sources:  
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3 249 1. A nurse-administered questionnaire on sociodemographic characteristics and cervical  
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5 250 cancer risk factors including age at first sexual intercourse, number of sexual partners,  
6  
7 251 smoking, contraception, parity and socioeconomic status.  
8  
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10  
11 252 2. Pelvic exam, VIA, Mobile ODT and colposcopy data capture forms  
12  
13  
14 253 3. Medical record data on HIV status (e.g.,  
15  
16 254 (<http://www.who.int/hiv/pub/guidelines/HIVstaging150307.pdf>), CD4 count, viral load,  
17  
18 255 antiretroviral therapy (ART) regimen(s)), care, and dates.  
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21

22 256 *Laboratory Testing:* The following laboratory tests are being performed:  
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24

25 257 Xpert HPV Testing—cervical Pap specimens in PreservCyt are being sent to the RMH  
26  
27 258 laboratory in Kigali, Rwanda for hrHPV DNA testing using the Xpert HPV test (Cepheid,  
28  
29 259 Sunnyvale, CA, USA).<sup>40-45</sup> The Xpert HPV Assay is a new, qualitative, real-time PCR assay for  
30  
31 260 the detection of hrHPV DNA. The Xpert HPV Assay includes simultaneous detection of 14  
32  
33 261 hrHPV types, hydroxymethylbilane synthase (HMBS), and an internal Probe Check Control  
34  
35 262 (PCC). The 14 targeted hrHPV types are detected in 5 fluorescent channels: 1) HPV16, 2)  
36  
37 263 HPV18 and hrHPV 45 (HPV18/45), 3) HPV31, 33, 35, 52, and 58, 4) HPV51 and HPV59, and  
38  
39 264 5) HPV39, 56, 66, and 68. HMBS (fluorescent channel 6) verifies specimen adequacy.  
40  
41  
42  
43  
44 265 Specimens are being mixed and a 1-mL pre-aliquot is being removed using a disposable pipette  
45  
46 266 and placed in the testing cartridge per the manufacturer's instructions. Unsatisfactory results due  
47  
48 267 to insufficient cellular content are being re-tested. If the second test is also unsatisfactory, the  
49  
50 268 final result are being recorded as unsatisfactory but women with unsatisfactory results are being  
51  
52 269 referred to colposcopy for safety.  
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3 270 HPV Viral Methylation—We will conduct a retrospective analysis of HPV viral methylation and  
4  
5 271 its association with CIN2+. To identify single hrHPV type infections, we will select single-  
6  
7 272 channel positives from the Xpert HPV assay. For those that are hrHPV positive for a channel  
8  
9 273 other than HPV16, which is detected singly, we will test them to identify the single type  
10  
11 274 infections using a standard protocol for PCR amplification using MY09/11 L1 consensus primers  
12  
13 275 and hrHPV genotype detection using dot-hybridization for 39 individual type-specific probes and  
14  
15 276 a mixture of probes for 10 other uncommon hrHPV types as previously described.<sup>46;47</sup> To isolate  
16  
17 277 the DNA, ThinPrep specimens (1.5 mL) will be pelleted, re-suspended in STM, digested with  
18  
19 278 Proteinase K, precipitated overnight in ammonium acetate ethanol at -20°C, washed, and  
20  
21 279 suspended and stored in TE buffer.

22  
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26  
27 280 The isolated DNA then will undergo bisulfite conversion.<sup>48</sup> Following bisulfite conversion and  
28  
29 281 DNA purification and de-sulphonation, bisulfite-treated DNA will be used as template for Next-  
30  
31 282 Gen Sequencing (NGS) (HiSeq2000, Illumina, San Diego, CA) using barcoded-type specific  
32  
33 283 primers. Sequences for pads and barcodes are not found in the targeted genomic region. Use of  
34  
35 284 padding and barcodes will enable reads to be identified by amplicon (forward or reverse) or by  
36  
37 285 sample during downstream bioinformatics analysis.<sup>49</sup>

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42 286 All PCR products for NGS will be pooled (by assay) and a single DNA band containing multiple  
43  
44 287 amplicons from different samples (with unique barcodes) will be isolated from a gel for NGS.<sup>49</sup>  
45  
46 288 Briefly, equal concentrations of each barcoded PCR product (based on PCR band intensity) will  
47  
48 289 be pooled and isolated. Upon confirmation of correct product size, all purified DNA pools will  
49  
50 290 be combined and submitted for library preparation and paired-end 100 base pair Illumina  
51  
52 291 HiSeq2000 sequencing at the Einstein Genomics Core Facility.

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3 292 Methylation status are being determined in the lab of Dr. Robert Burk at Albert Einstein College  
4  
5 293 of Medicine (USA). Prior to determination of methylation status, de-multiplexing based on the  
6  
7 294 unique barcodes is being performed using in-house generated scripts to obtain paired-end NGS  
8  
9  
10 295 reads of each sample. Reads are being aligned with hrHPV reference genome sequences by  
11  
12 296 bowtie v0.12.9.<sup>50</sup> Methylation status of each CpG site is then determined by bismark v0.7.7<sup>51</sup>  
13  
14 297 using the default quality score parameter set to Q30, and the formula of the methylation ratio of  
15  
16  
17 298 the number of C read by the number of C+T read.

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20 299 E6/E7 Oncoprotein Testing—Dry swab specimens, collected at the time of colposcopy, are being  
21  
22 300 tested for individual E6/E7 oncoproteins as previously described<sup>52;53</sup>, according to the  
23  
24 301 manufacturer's instructions, at the RMH laboratory in Kigali, Rwanda. The E6/E7 oncoprotein  
25  
26  
27 302 test uses three lateral flow strips to detect 8 hrHPV types whereas the E6 oncoprotein test used a  
28  
29 303 single lateral flow strip to detect 3 hrHPV types.

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33 304 *Analyses:* We will evaluate combinations of the above mentioned screening strategies and tests  
34  
35 305 to estimate the clinical performance (e.g., Se, Sp, PPV, and NPV) for the detection of consensus  
36  
37 306 CIN3+ and community CIN2+. A log binomial model using generalized estimating equations  
38  
39 307 will be used to take into account correlation between different tests from the same subject. Note  
40  
41 308 while these models will be developed for the estimation and comparison of performance for two  
42  
43  
44 309 tests, the model can be extended to allow more than two tests by including more indicator  
45  
46 310 variables for test type.

47  
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50 311 Some analyses of biomarkers, such as viral methylation will be restricted to hrHPV-positives.  
51  
52 312 Comparisons of hrHPV viral methylation to other triage biomarkers will be restricted to the  
53  
54 313 subset that gets tested for viral methylation as described.

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3 314 *Sample size calculations:* We are basing our sample size on the ability to detect modest but  
4  
5 315 minimally important differences in Se of 15%. We conservatively assume that the population  
6  
7 316 prevalence of CIN3+ is 2% based on our past study in Rwanda.<sup>33,35</sup> We will enroll and have  
8  
9  
10 317 complete follow-up of at least 5,000 HIV[+] women. A sample size of 5,000 HIV[+] women  
11  
12 318 with completed follow-up of the screen positives will yield 100 cases of CIN3+, which will have  
13  
14 319 at least 80% power ( $\alpha=5\%$ ) to crudely detect a 15% difference in Se between two screening  
15  
16 320 strategies for a range of 10%-25% discordance. With this sample size of 5,000 women, 4,900  
17  
18 321 will not have CIN3+; we will also have at least 90% power ( $\alpha=5\%$ ) to detect a difference in Sp  
19  
20 322 of 3% for discordance up to 40%. Finally, we will have 80% power ( $\alpha=5\%$ ) to crudely detect an  
21  
22 323 8%, 10%, or 11% difference in PPV if the reference PPV is 10%, 20%, or 30%, respectively.<sup>54</sup>  
23  
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27 324 *Cost Effectiveness:* We will conduct assessments of the costs and cost-effectiveness of the  
28  
29 325 different combinations of screening and triage tests, i.e., algorithms, as well as those of the entire  
30  
31 326 community-based screening “system.” Costs measurement will be conducted using a micro-  
32  
33 327 costing (ingredients) approach in which resource use throughout each step in the screening  
34  
35 328 process will be tracked and unit costs for each of the resources will be applied to generate an  
36  
37 329 average screening cost per woman to be compared against what the estimated costs are for a  
38  
39 330 possible program based on hrHPV screening and VIA triage or VIA screening. For estimating  
40  
41 331 costs of the screening system and scale-up of screening to 100,000 women in a month, analyses  
42  
43 332 will distinguish financial costs, which reflect actual expenditures of the program, from economic  
44  
45 333 costs, including the value of donated and shared resources to more fully assess opportunity costs.  
46  
47 334 Projections on budget impact and economic cost implications over time will be made under  
48  
49 335 varying assumptions of screening uptake, follow-up compliance, and scenarios of changing  
50  
51 336 disease burden.  
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3 337 Clinical outcomes will include true positive, true negative, false negative, and false positive test  
4  
5 338 results, number of colposcopies, incident cancer, and cancer death. Cost-effectiveness will be  
6  
7 339 measured as cost/CIN2+ detected, cost/CIN3+ detected, cost/invasive cancer prevented,  
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9 340 cost/cancer death prevented, cost/life-year saved, and cost/quality-adjusted life year (QALY)  
10  
11 341 saved; in addition, we will calculate harm/benefit ratios, using varying definitions of harms  
12  
13 342 (colposcopies, false positive results) to benefits (cancers prevented, deaths prevented, life years  
14  
15 343 and QALYs saved). Costs and effectiveness will be discounted at a 3% annual rate, with the rate  
16  
17 344 varied from 0-5% in sensitivity analysis. For assessment of value-of-information (VOI), we will  
18  
19 345 use net monetary benefits (NMB), defined as a function of the willingness-to-pay threshold  
20  
21 346 (WTP) for different costs and outcomes as:  $NMB = (WTP * Effectiveness) - Costs$ .

### 27 347 **Patient and Public Involvement**

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29  
30 348 There was no patient engagement in the development or design of the study, recruitment, and the  
31  
32 349 conduct of the study. Participants are receiving their results directly since it is related to their  
33  
34 350 care. As this was not a randomized controlled trial, the burden of the intervention was not  
35  
36 351 assessed by patients themselves. There were no patient advisors to acknowledge.

### 40 352 **Ethics and Dissemination**

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44 353 *Ethics*: This study protocol was reviewed and approved by the Rwanda National Ethics

45  
46 354 Committee (RNEC) as well as the Institutional Review Board for human subjects research at  
47  
48 355 Albert Einstein College of Medicine.

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51 356 *Confidentiality measures and protection against potential risks*: The risks for those participating  
52  
53 357 in our study include:

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3 358           • Collection of Pap specimens/cervical swabs involves a modest risk of bleeding  
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5           359           which is typically very limited when it occurs. Testing positive for any test may  
6  
7           360           cause psychological distress (anxiety).  
8  
9  
10           361           • Colposcopy and excisional treatments induce vaginal bleeding and may incur pain,  
11  
12           362           infection, and short-term psychological distress (anxiety). A diagnosis of CIN2 or  
13  
14           363           more severe may cause psychological distress (anxiety). A diagnosis of ICC may  
15  
16           364           cause severe psychological distress.  
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20           365           • Questions in the questionnaire, regarding sexual behavior and other matters of a  
21  
22           366           personal nature, may cause anxiety and embarrassment. Participants are advised  
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24           367           that they are free not to answer specific questions.  
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26  
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28           368           • There is also the risk of psycho-social stress which could occur if there was  
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30           369           inadvertent disclosure of confidential medical or other personal information.  
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33 370 Protection against the risk of inadvertent disclosure of confidential information is being  
34  
35 371 addressed by the standard procedures at the Rwandan study site, including: (i) storing completed  
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37 372 paper copies of questionnaires and other hard copy information (described above), identified by  
38  
39 373 study number only, in a filing system separate from the name-address file of participants in the  
40  
41 374 study; and (ii) only the designated local personnel have access to cross-reference the files; (iii)  
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43 375 all paper files, including consent forms, are being maintained in locked cabinets in locked rooms,  
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45 376 with access restricted to specific research personnel.  
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50 377 In addition, we will include the following security measures to protect the data:  
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- 54 378           • Controlled access to project data;  
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- 379 • A tracking system for data forms and activities;
- 380 • Date and time of stamping of all data records with electronic signatures;
- 381 • Audit trails to track all changes made to data records; and
- 382 • Data kept on password-protected computers and in locked rooms.

383 *Potential Patient Benefits:* There are potential direct benefits to study participants. HIV[+] women participating in the study are at very high risk of ICC. They are being rigorously screened and evaluated, more effectively than the standard of care anywhere in the world. As a result of the study, women with precancer who are at imminent risk of ICC are being diagnosed sooner and treated more effectively than women receiving routine care and thereby more likely averting the development of ICC. Women with cervical cancer are being diagnosed earlier thereby reducing the morbidity and the risk of mortality caused by ICC. Conversely, any pain, bleeding, or stress that might occur related to colposcopy or cervical swab are typically modest and well tolerated.

392 There are also substantial potential societal benefits related to the study due to its implications to improving cervical-cancer screening practices and guidelines in HIV[+] women – changes in practice which might also benefit the study participants themselves, if and when these changes are enacted. There is a great need to identify more effective and practical methods for cervical-cancer screening for HIV[+] women living in Africa, who are living longer than ever and are therefore at potentially greater risk of ICC.

398 *Dissemination:* We plan to publish a series of scientific reports in peer-reviewed scientific journals. As building research capacity in Rwanda is a major goal of this research project, all investigators of the research team are being asked and supported to lead at least one analysis and one manuscript preparation, based on interests and expertise.



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2  
3 402 In addition, an external advisory panel (EAP) composed of leaders from the Rwanda Ministry of  
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5 403 Health, University of Rwanda, and Rwanda medical community has been formed. The  
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7 404 responsibilities of the EAP include providing advice on the conduct of the project and  
8  
9 405 interpretation for and dissemination of the study results to Rwandan stakeholders. The latter is  
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11 406 important for the adoption of evidence-based best practices for cervical-cancer screening as  
12  
13 407 warranted.  
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### 18 408 **Limitations**

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20  
21 409 There are several limitations to the study that bear mentioning. First, cervical cytology is not  
22  
23 410 being included in the study. There is limited cervical cytology services available locally and of  
24  
25 411 unknown quality and it is unlikely that cytology will be widely available in Rwanda, making its  
26  
27 412 inclusion as a comparator test of limited value. Moreover, there are significant costs and  
28  
29 413 logistical challenges in shipping PreservCyt specimens to and having cytology slides made and  
30  
31 414 read in the U.S. Second, we are not conducting colposcopy and taking biopsies in screen-  
32  
33 415 negative women, which would have allowed us to estimate absolute clinical performance. The  
34  
35 416 burden of sending screen-negative women to colposcopy is deemed too great and it is impractical  
36  
37 417 to send a sufficient numbers of screen-negative women to colposcopy to accurately estimate the  
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39 418 false-negative disease (CIN3+) fraction. Thus, only relative clinical performance of the screening  
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41 419 tests will be estimated from this study.  
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## 420 Contributor Statement:

421 KA, PEC, JCD, AA, and JDS conceived the original concept of the study and the interventions.  
422 GM, KA, JDS, and PEC drafted the protocol. PEC performed the sample size calculations, and  
423 PEC and GM will lead analysis of the results. GM, JDS, and Athanase Munyaneza are  
424 supporting patient recruitment. GM, TR, and Athanase Munyaneza are providing clinical care for  
425 patients. Anthere Murangwa and LM oversee laboratory testing, and TZ and TH are responsible  
426 for pathology. PM and LM oversee and administer the study activities at the clinical site in  
427 Rwanda. All authors (GM, JCD, TR, JDS, Athanase Munyaneza, Anthere Murangwa, TZ, TH,  
428 PM, AA, LM, KA, and PEC) contributed to the scientific design of the study and the protocol  
429 development, are involved in the implementation of the project, and have read and approved the  
430 final manuscript.

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3 432 Figure Legends:  
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7 434 Figure 1. Study Design  
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594 **Table 1:** Recruitment sites and estimated eligible population.

Province	Site	Type of site	Potential participants per site (approximate)
Kigali	Cor-unum HC	Public Health Center	1,405
Kigali	Kimironko HC	Public Health Center	1,227
Kigali	Rwampara HC	Public Referral Hospital	1,098
Kigali	Kicukiro HC	Public Health Center	1,054
Kigali	Kacyiru HC	Public Health Center	905
Kigali	Gikondo HC	Public Health Center	925
Kigali	Rwanda Military Hospital	Public Referral Hospital	300
Kigali	WEACTx for Hope	Private HIV Clinic	500
Kigali	Busanza HC	Public Health Center	100
Kigali	Nyarugunga HC	Public Health Center	100
West	Gisenyi DH	Public District Hospital	810
Total			8,424

595

Figure 1. Study diagram

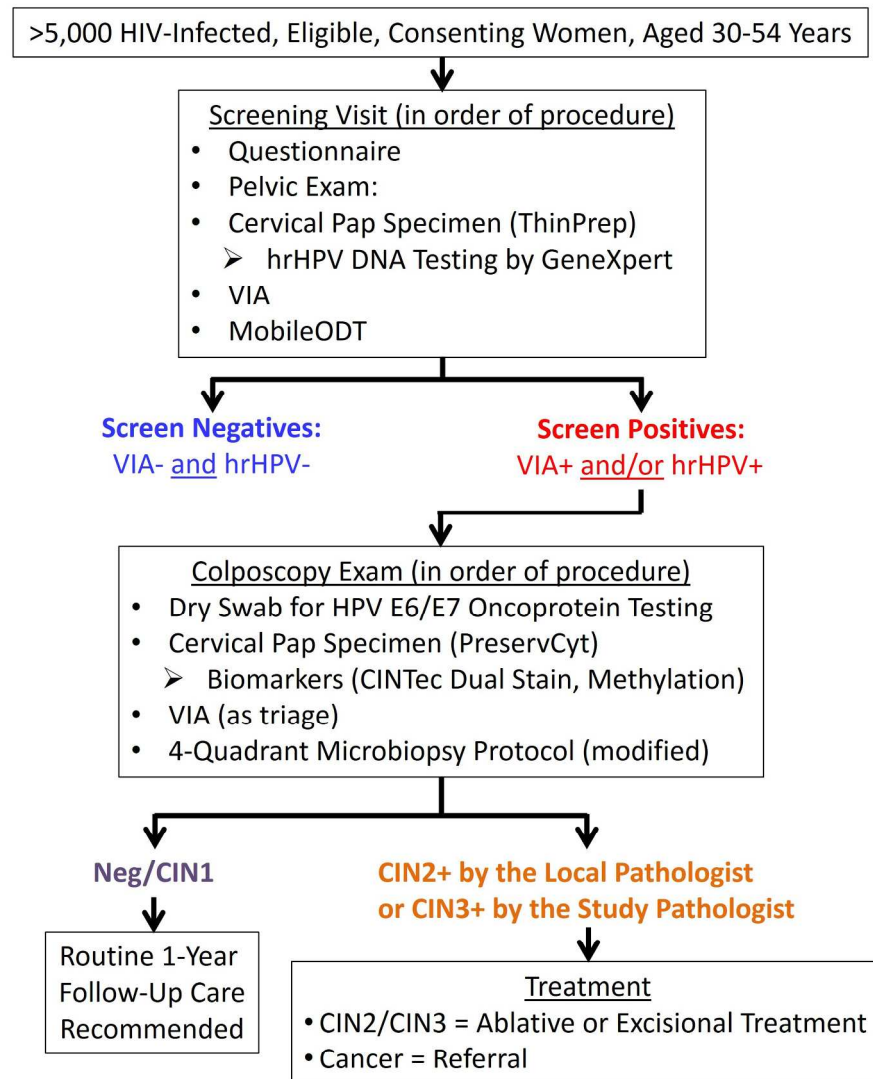


Figure 1. Study Design

190x254mm (300 x 300 DPI)

## Appendix I. Study Questionnaire

### A. Socio-demographics

1. What is your date of birth? D D / M M / Y Y Y Y (enter all 0 if not remembered) (If date of birth or year of birth is given, skip to Question 3)

2. What is your age? \_\_\_ \_\_\_ (Years)

3. What is your marital status?

Married/Cohabiting

Divorced

Widowed

Separated

Single

Choose not to answer

4. Do you live in Kigali?

Yes

No {Skip to Question A5}

Choose not to answer {Skip to Question A5}

5. In what area of Kigali City Province do you live?

Nyarugenge

Kicukiro

Gasabo urban (e.g., Kacyiru, Kimironko, Kinyinya, etc.)

Gasabo rural (e.g., Nduba, Jabana, Gikomero, etc.)

Do not know

Choose not to answer

6. What is your household income per month in FRW?

< 5,000

5,000 – 9,999

10,000 – 34,999

35,000 – 59,999

- 1  
2  
3  60,000 – 84,999  
4  
5  85,000 – 109,999  
6  
7  110,000 – 134,999  
8  
9  135,000 – 269,999  
10  
11  270,000 or more

12 **7. How many people live in your household?** \_\_\_ \_\_\_ {Enter 00 if refuse to answer}

13  
14 **8. What is your occupation?**

- 15  
16  Employed by government, another institution, or company  
17  
18  Self-employed (Small and medium enterprises)  
19  
20  Self-employed (High income earnings)  
21  
22  Farming (peasants)  
23  
24  Unemployed/Does not work  
25  
26  Other (specify) \_\_\_\_\_  
27  
28  Choose not to answer {Skip to Question B1}

29 {The following questions are sensitive and personal in nature. Your answers will be kept  
30 confidential. You may choose not to answer certain questions. Answering any question is  
31 voluntary}

32  
33 **B. Sexual Behaviors**

34  
35 **1. How old were you when you first had sex?** \_\_\_ \_\_\_ (Years) {Enter 00 if refuse to  
36 answer}

37  
38 **2. How many sexual partners have you had in your lifetime?**

- 39  
40  No Partners (Ineligible)  
41  
42  1 Partner  
43  
44  2-3 Partners  
45  
46  4-5 Partners  
47  
48  6-9 Partners  
49  
50  10 or more partners  
51  
52  Choose not to answer

53 **3. How many sexual partners have you had in the last 6 months?**

- 54  No Partners  
55  
56  
57  
58  
59  
60

- 1  
2  
3  1 Partner  
4  
5  2 or more Partners  
6  
7  Choose not to answer

8  
9 **C. Parity**

- 10  
11 **1. What age did you have your first child? \_\_\_ (Years) {Enter 00 if refuse to**  
12 **answer, Enter 99 if never pregnant} (if 00 or 99, skip to Question D1)**  
13  
14 **2. How many live births have you had in your lifetime? \_\_\_**  
15  
16 **3. Have you given birth in the last year?**

- 17  
18  Yes  
19  
20  No  
21  
22  Choose not to answer

23  
24 **D. Tobacco Use**

- 25  
26 **1. Have you ever smoked cigarettes?**

- 27  
28  Yes  
29  
30  No (skip to Question D3)  
31  
32  Choose not to answer (skip to Question D3)

- 33  
34 **2. Do you currently smoke cigarettes?**

- 35  
36  Yes  
37  
38  No  
39  
40  Choose not to answer

- 41  
42 **3. Have you ever chewed/used tobacco orally (Ubugoro)?**

- 43  
44  Yes  
45  
46  No (skip to Question D5)  
47  
48  Choose not to answer (skip to Question D5)

- 49  
50 **4. Are you currently chewing/using tobacco orally (Ubugoro)?**

- 51  
52  Yes  
53  
54  No  
55  
56  Choose not to answer

1  
2  
3 **5. Have you ever chewed/used tobacco orally (Tobacco leaves-Igikamba)?**  
4

- 5  Yes  
6  
7  No (skip to Question E1)  
8  
9  Choose not to answer (skip to Question E1)  
10

11 **6. Are you currently chewing/using tobacco orally (Tobacco leaves-Igikamba)?**  
12

- 13  Yes  
14  
15  No  
16  
17  Choose not to answer  
18

19 **E. Contraceptive Use**

20  
21 **1. Have you ever used oral contraceptives?**  
22

- 23  Yes  
24  
25  No (Skip to Question E3)  
26  
27  Choose not to answer (Skip to Question E3)  
28

29 **2. Do you currently use oral contraceptives?**  
30

- 31  Yes  
32  
33  No  
34  
35  Choose not to answer  
36

37 **3. Have you ever used Depo Provera (contraceptive)?**  
38

- 39  Yes  
40  
41  No (Skip to Question E5)  
42  
43  Choose not to answer (Skip to Question E5)  
44

45 **4. Do you currently use Depo Provera (contraceptive)?**  
46

- 47  Yes  
48  
49  No  
50  
51  Choose not to answer  
52

53 **5. Have you ever used Jadell (contraceptive)?**  
54

- 55  Yes  
56  
57  No (Skip to Question E7)  
58  
59  
60

Choose not to answer (Skip to Question E7)

**6. Do you currently use Jadell (contraceptive)?**

Yes

No

Choose not to answer

**7. Have you ever used IUD (contraceptive)?**

Yes

No (Skip to Question E9)

Choose not to answer (Skip to Question E9)

**8. Do you currently use IUD (contraceptive)?**

Yes

No

Choose not to answer

**9. Do use condoms as a contraceptive?**

Yes

No (skip to Question F1)

Choose not to answer (skip to Question F1)

**10. Have you used condoms as a contraceptive in the last 6 months?**

Yes

No

Choose not to answer

**F. Infections**

**1. Have you ever had Malaria**

Yes

No (skip to Question F8)

Choose not to answer (skip to Question F8)

**2. How many times have you had Malaria? \_\_\_**

1  
2  
3 **3. What was the year that you last had Malaria? Y Y Y Y**

4  
5 **4. Have you had your Malaria treated using drugs?**

6  
7  Yes

8  No

9  Choose not to answer

10  
11 **5. If Yes, how was it treated?**

12  Treated only with traditional medicine

13  Treated only with drugs (e.g. Coartem)

14  Treated with both (traditional and modern)

15  Not treated

16  Choose not to answer

17  
18  
19 **6. Was your last episode of Malaria treated using drugs?**

20  Yes

21  No

22  Choose not to answer

23  
24 **7. If Yes, how was it treated?**

25  Treated only with traditional medicine

26  Treated only with drugs (e.g. Coartem)

27  Treated with both (traditional and modern)

28  Not treated

29  Choose not to answer

30  
31 **8. Have you ever had Tuberculosis (TB)?**

32  Yes

33  No (skip to end)

34  Choose not to answer (skip to end)

35  
36 **9. If Yes, how many times have you had TB in your lifetime? \_ \_**

37  
38 **10. What year did you last have Tuberculosis (TB)? Y Y Y Y**

39  
40  
41  
42 **END OF QUESTIONNAIRE**