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1 **Title**

2 **Comparison of *CareHPV* and Hybrid Capture 2 Assays for Detection of**  
3 **High-Risk HPV DNA in Cervical Samples from HIV-1-Infected African**  
4 **Women**

5

6 **Running title**

7 **Comparison of *CareHPV* with HC2**

8

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25 **ABSTRACT**

26 **The *careHPV* and HC2 assays were compared for high-risk HPV (HR-HPV) DNA**  
27 **detection in cervical samples from 149 HIV-1-infected African women. HR-HPV DNA**  
28 **detection rate was 37.6% and 34.9% by *careHPV* and HC2, respectively. Agreement**  
29 **between the two tests was 94.6% (95% CI, 89.7%-97.7%) with a Kappa value of 0.88,**  
30 **(95% CI, 0.81-0.96) indicating an excellent agreement. *CareHPV* may be considered as**  
31 **suitable as HC2 for cervical cancer screening among HIV-infected African women.**

32

33

34 Cervical cancer is the third most common cancer in women worldwide, with more than  
35 500,000 annual cases, and the fourth most common cause of cancer death in women, with  
36 about 275,000 annual deaths. However, more than 85% of cases and deaths occur in  
37 developing countries, cervical cancer being the commonest cancer and the leading cause of  
38 cancer death in African women (Globocan 2008, <http://globocan.iarc.fr>). The high mortality  
39 rate observed in Africa is mainly due to the absence of cervical cancer screening, resulting in  
40 diagnosis of advanced and often untreatable disease (1).

41 Virtually, all cases of cervical cancer result from persistent infection with carcinogenic  
42 genotypes of human papillomavirus (HPV) (2). It is now well established that detection of  
43 these high-risk HPV (HR-HPV) genotypes in cervical samples allows to identify women at  
44 risk of precancerous or cancerous cervical lesions, and HR-HPV DNA testing has been  
45 proposed as a primary screening test for cervical cancer prevention (3, 4).

46 Incidence of HR-HPV infection and of high-grade cervical lesions is significantly increased in  
47 women infected with HIV-1 (5-7). Therefore, a screening strategy based on HR-HPV testing  
48 in African women infected with HIV-1 may play an important role in cervical cancer  
49 prevention.

50 The Hybrid Capture 2 (HC2) assay (Qiagen Corporation, Gaithersburg, MD) is a Food and  
51 Drug Administration (FDA)-approved test for cervical cancer screening. This assay is based  
52 on HR-HPV detection using a cocktail of RNA probes targeting 13 HR-HPV types, namely  
53 HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56,  
54 HPV58, HPV59, and HPV68. The *careHPV* assay (Qiagen) is a new signal-amplification  
55 assay adapted from HC2. This assay, which is designed to be simpler and more rapid to use,  
56 and more affordable than HC2 in resource-poor settings, targets 14 HR-HPV types, HPV66  
57 being included in the probe cocktail in addition to the 13 HR-HPV types targeted by the HC2  
58 assay (8, 9). There has been no published evaluation of the direct comparison between the two  
59 assays.

60 We compared the *careHPV* assay with the HC2 assay in a subset of women enrolled in the  
61 HARP (HPV in Africa Research Partnership) study, which is conducted in two Sub-Saharan  
62 African countries, South Africa and Burkina Faso, with the aim to evaluate cervical cancer  
63 screening and treatment approaches for the prevention of cervical neoplasia in HIV-1 infected  
64 African women. Over 1200 consenting HIV-1 seropositive women aged 25-50, of whom two-  
65 thirds were on ART, were enrolled in the HARP study between November 2011 and October  
66 2012 and followed up at 6 monthly intervals for 18 months. The study was approved by the  
67 research ethics committees of the University of the Witwatersrand in South Africa, the  
68 Ministry of Health in Burkina Faso, and the London School of Hygiene & Tropical Medicine.  
69 The comparison was done on samples collected from 149 unselected consecutive HARP study  
70 participants (75 in Johannesburg, South Africa and 74 in Ouagadougou, Burkina Faso)  
71 attending their regular research clinic appointment 12 months after enrolment, between  
72 February and April 2013. At baseline visit, 68 (46%) women were 25-34 years old and 81  
73 (54%) were 35-50 years old, and 48 (32%) had a CD4+ T cell count  $\leq 350$  cells/ $\mu$ l.

74 Two cervical samples were consecutively taken for each woman. The first sample was  
75 collected using the *careHPV* sample collection device consisting of a *careBrush* and a vial of  
76 *careHPV* collection medium. The second sample was collected using the Digene cervical  
77 sampler consisting of a cervical brush and Specimen Transport Medium. *CareHPV* tests were  
78 performed in the respective sites by medical scientists specifically trained by a Qiagen's  
79 scientist and the HC2 tests were performed in Montpellier, France, on samples stored at -80°C  
80 and shipped in dry ice. The assays were performed according to the Manufacturer's  
81 instructions. The HC2 assay was considered positive when the relative light unit/cutoff  
82 (RLU/CO) ratio was  $\geq 1$ . The positive or negative result of the *careHPV* assay was displayed  
83 by the *careHPV* test controller without additional specification of the luminescent signal  
84 intensity. Samples for which a discrepant result between the two assays was observed were  
85 tested for HPV detection and typing using the INNO-LiPA HPV genotyping Extra assay  
86 (Innogenetics, Courtaboeuf, France). In case of non-typable HPV as identified by the INNO-  
87 LiPA HPV genotyping Extra assay, genotyping was performed by sequencing as previously  
88 described (10).

89 The HR-HPV prevalence was 37.6% (95% CI, 29.8%-45.9%) by *careHPV* and 34.9% (95%  
90 CI, 27.3%-43.1%) by HC2. In South Africa, prevalence of HR-HPV was 37.3% by *careHPV*  
91 and 33.3% by HC2, whereas in Burkina Faso, this prevalence was 37.8% by *careHPV* and  
92 36.5% by HC2. The overall agreement between tests was 94.6% (141/149, 95% CI, 89.7%-  
93 97.7%) (Table 1). Agreement was 96.0% (72/75; 95% CI, 88.8%-99.2%) in South Africa and  
94 93.2% (69/74; 95% CI, 84.9%-97.8%) in Burkina Faso. The Kappa test value of 0.88 (95%  
95 CI, 0.81-0.96) indicated an excellent agreement. The results obtained for the discrepant  
96 samples are shown in Table 2. All the discrepant samples were positive for HPV detection by  
97 the INNO-LiPA HPV genotyping Extra assay. Among the six samples positive by *careHPV*  
98 and negative by HC2, five were positive for HR-HPV types targeted by HC2 probes and one

99 was positive for HPV25, a non-HR-HPV type. Among the two samples negative by *careHPV*  
100 and positive by HC2 one was positive for the HR-type HPV51 and the other was only positive  
101 for the low-risk type HPV6.

102 Taken together these results indicate an excellent agreement between the *careHPV* and HC2  
103 assays. The few cases of discrepancy observed may be due to amounts of HR-HPV DNA at  
104 the limit of detectability or to cross-reactivity with non-HR-HPV types (11). Moreover, the  
105 fact that the two assays were not performed on the same sample but on consecutive samples  
106 collected in the assay-specific collection medium may have been a cause of discrepancy,  
107 independently from the performances of the assays themselves. Results from this study  
108 indicate that *careHPV* may be considered as suitable as HC2 for cervical cancer screening  
109 among HIV-infected women in resource-constrained settings.

110

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176

177 TABLE 1: Agreement between the *careHPV* and HC2 assays among 149 HIV-positive  
178 women from Burkina Faso and South Africa.

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<i>careHPV</i>		
<b>HC2</b>	<b>Positive</b>	<b>Negative</b>
<b>Positive</b>	50 (33.6%)	2 (1.3%)
<b>Negative</b>	6 (4.0%)	91 (61.1%)

$P < 0.0001$  (McNemar's test)

186 TABLE 2. Results obtained for 8 samples with discrepant results between *careHPV* and HC2.

187

Sample No.	Country <sup>a</sup>	CareHPV	HC2		
			HC2	RLU/CO	Genotyping
S253	SA	Positive	Negative	0.50	HPV11, HPV16, HPV18
S295	SA	Positive	Negative	0.16	HPV68
S604	SA	Positive	Negative	0.43	HPV52, HPV68, HPV73
B231	BF	Positive	Negative	0.27	HPV35
B292	BF	Positive	Negative	0.26	HPV52
B304	BF	Positive	Negative	0.19	HPV25 <sup>b</sup>
B331	BF	Negative	Positive	5.07	HPV6
B393	BF	Negative	Positive	10.38	HPV51, HPV69/71 <sup>c</sup> , HPV70

188 <sup>a</sup> SA, South Africa; BF, Burkina Faso

189 <sup>b</sup>Identified by sequencing

190 <sup>c</sup>No discrimination between HPV69 and HPV71 by the INNO-LiPA HPV genotyping Extra

191 assay.