JCM Accepts, published online ahead of print on 9 October 2013
J. Clin. Microbiol. doi:10.1128/JCM.02144-13

Title

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

Running title

Comparison of *Care*HPV with HC2

Authors:

Jean Ngou,[a] Mahlape P. Magooa,[b] Clare Gilham,[c] Florencia Djigma,[d] Marie-Noelle Didelot,[a] Helen Kelly,[c] Albert Yonli,[d] Bernard Sawadogo,[e] David A. Lewis,[bf] Sinead Delany-Moretlwe,[g] Philippe Mayaud,[c] and Michel Segondy[a] for the HARP Study Group

Affiliations:

INSERM U1058 and University Hospital (CHU), Montpellier, France[a], Centre for HIV and STIs, National Institute for Communicable Diseases, National Health Laboratory Service, Johannesburg, South Africa[b], London School of Hygiene and Tropical Medicine, London, UK[c], Centre de Recherche Biomoléculaire Pietro Annigoni, Ouagadougou, Burkina Faso[d], Centre de Recherche Internationale en Santé, University of Ouagadougou, Burkina Faso[e], Department of Internal Medicine, Faculty of Health Sciences, University of the Witwatersrand[f], and Reproductive Health & HIV Institute, University of the Witwatersrand, Johannesburg, South Africa[g]

Corresponding author:

Michel Segondy: m-segondy@chu-montpellier.fr

ABSTRACT

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

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

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

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

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

We compared the *care*HPV assay with the HC2 assay in a subset of women enrolled in the HARP (HPV in Africa Research Partnership) study, which is conducted in two Sub-Saharan African countries, South Africa and Burkina Faso, with the aim to evaluate cervical cancer screening and treatment approaches for the prevention of cervical neoplasia in HIV-1 infected African women. Over 1200 consenting HIV-1 seropositive women aged 25-50, of whom two-thirds were on ART, were enrolled in the HARP study between November 2011 and October 2012 and followed up at 6 monthly intervals for 18 months. The study was approved by the research ethics committees of the University of the Witwatersrand in South Africa, the Ministry of Health in Burkina Faso, and the London School of Hygiene & Tropical Medicine.

The comparison was done on samples collected from 149 unselected consecutive HARP study participants (75 in Johannesburg, South Africa and 74 in Ouagadougou, Burkina Faso) attending their regular research clinic appointment 12 months after enrolment, between February and April 2013. At baseline visit, 68 (46%) women were 25-34 years old and 81 (54%) were 35-50 years old, and 48 (32%) had a CD4+ T cell count $\leq$ 350 cells/µl.

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

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

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

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

Acknowledgements:

The *care*HPV and HC2 kits were obtained through the Qiagen Corporation donation program.

The research leading to these results has received funding from the European Commission (EC) 7th Framework Programme under grant agreement No. HEALTH-2010-F2-265396.

Contributing members of the HARP study group included: A. Chikandiwa, E. Cutler, S. Delany-Moretlwe, D. A. Lewis, M.P. Magooa, V. Maseko, P. Michelow, B. Muzah, T. Omar, A. Puren (Johannesburg, South Africa); F. Djigma, J. Drabo, O. Goumbri-Lompo, N. Meda, B. Sawadogo, J. Simporé, A. Yonli, S Zan (Ouagadougou, Burkina Faso); V. Costes, M.N. Didelot, S. Doutre, N. Leventoux, N. Nagot, J. Ngou, M. Segondy (Montpellier, France); and A. Devine, C. Gilham, L. Gibson, H. Kelly, R. Legood, P. Mayaud, H.A. Weiss (London, UK).

The HARP Study Group also wishes to thank its International Scientific Advisory Group (ISAG) constituting of Prof. C. Lacey (Chair, University of York, UK), Prof. Y. Qiao

(Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China), Prof. M. Chirenje (University of Harare, Zimbabwe) and Prof. S. de Sanjosé (Institut Catala d'Oncologia, Barcelona, Spain).

References

1. **Denny L, Anorlu R.** 2012. Cervical cancer in Africa. Cancer Epidemiol Biomarkers Prev. **21**:1434-1438.
2. **Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, Snijders PJ, Meijer CJ, International Agency for Research on Cancer Multicenter Cervical Cancer Study Group.** 2003. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med.**348**:518-527.
3. **Cuzick J, Clavel C, Petry KU, Meijer CJ, Hoyer H, Ratnam S, Szarewski A, Birembaut P, Kulasingam S, Sasieni P, Iftner T.** 2006. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. Int J Cancer.**119**:1095-1101.
4. **Ronco G, Giorgi-Rossi P, Carozzi F, Confortini M, Dalla Palma P, Del Mistro A, Ghiringhello B, Girlando S, Gillio-Tos A, De Marco L, Naldoni C, Pierotti P, Rizzolo R, Schincaglia P, Zorzi M, Zappa M, Segnan N, Cuzick J.** 2010. New Technologies for Cervical Cancer screening (NTCC) Working Group. Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomised controlled trial. Lancet Oncol.**11**:249-257.
5. **Ahdieh L, Klein RS, Burk R, Cu-Uvin S, Schuman P, Duerr A, Safaeian M, Astemborski J, Daniel R, Shah K.** 2001. Prevalence, incidence, and type-specific persistence of human papillomavirus in human immunodeficiency virus (HIV)-positive and HIV-negative women. J Infect Dis.**184**:682-690.
6. **Schuman P, Ohmit SE, Klein RS, Duerr A, Cu-Uvin S, Jamieson DJ, Anderson J, Shah KV, HIV Epidemiology Research Study (HERS) Group.** 2003. Longitudinal

study of cervical squamous intraepithelial lesions in human immunodeficiency virus (HIV)-seropositive and at-risk HIV-seronegative women. J Infect Dis.**188**:128-136.

7. **Strickler HD, Burk RD, Fazzari M, Anastos K, Minkoff H, Massad LS, Hall C, Bacon M, Levine AM, Watts DH, Silverberg MJ, Xue X, Schlecht NF, Melnick S, Palefsky JM.** 2005. Natural history and possible reactivation of human papillomavirus in human immunodeficiency virus-positive women. J Natl Cancer Inst.**97**:577-586.
8. **Qiao YL, Sellors JW, Eder PS, Bao YP, Lim JM, Zhao FH, Weigl B, Zhang WH, Peck RB, Li L, Chen F, Pan QJ, Lorincz AT.** 2008. A new HPV-DNA test for cervical-cancer screening in developing regions: a cross-sectional study of clinical accuracy in rural China. Lancet Oncol.**9**:929-936.
9. **Gage JC, Ajenifuja KO, Wentzensen N, Adepiti AC, Stoler M, Eder PS, Bell L, Shrestha N, Eklund C, Reilly M, Hutchinson M, Wacholder S, Castle PE, Burk RD, Schiffman M.** 2012. Effectiveness of a simple rapid human papillomavirus DNA test in rural Nigeria. Int J Cancer**131**:2903-2909.
10. **Didelot-Rousseau MN, Nagot N, Costes-Martineau V, Vallès X, Ouedraogo A, Konate I, Weiss HA, Van de Perre P, Mayaud P, Segondy M, Yerelon Study Group.** 2006. Human papillomavirus genotype distribution and cervical squamous intraepithelial lesions among high-risk women with and without HIV-1 infection in Burkina Faso. Br J Cancer**95**:355-362.
11. **Castle PE, Solomon D, Wheeler CM, Gravitt PE, Wacholder S, Schiffman M.** 2008. Human papillomavirus genotype specificity of hybrid capture 2. J Clin Microbiol.**46**:2595-2604.

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

	***care*HPV**	
HC2	**Positive**	**Negative**
Positive	50 (33.6%)	2 (1.3%)
Negative	6 (4.0%)	91 (61.1%)

$P < 0.0001$ (McNemar's test)

TABLE 2. Results obtained for 8 samples with discrepant results between *care*HPV and HC2.

Sample No.	Country[a]	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[b]
B331	BF	Negative	Positive	5.07	HPV6
B393	BF	Negative	Positive	10.38	HPV51, HPV69/71[c], HPV70

[a] SA, South Africa; BF, Burkina Faso

[b]Identified by sequencing

[c]No discrimination between HPV69 and HPV71 by the INNO-LiPA HPV genotyping Extra assay.