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Short Communication

Hybrid capture II and PapilloCheck® tests for detection of anal high-risk human papillomavirus

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ABSTRACT

Introduction: This study evaluated the level of concordance between hybrid capture II (HCII) and PapilloCheck® for the detection of high-risk human papillomavirus (HPV) in anal samples. **Methods:** Anal cell samples collected from 42 human immunodeficiency virus (HIV)+ patients were analyzed. **Results:** Considering only the 13 high-risk HPV types that are detectable by both tests, HCII was positive for 52.3% of the samples, and PapilloCheck® was positive for 52.3%. The level of concordance was 80.9% (Kappa = 0.61). **Conclusions:** Good concordance was observed between the tests for the detection of high-risk HPV.

Keywords: Human papillomavirus. PapilloCheck®. Hybrid capture II.

Human papillomavirus (HPV) tests can be very useful for improving the sensitivity of cytology to detect anal intraepithelial neoplasia (AIN) and in post-treatment follow-up because of the tests' excellent negative predictive value[1,2]. Different tests for the detection of high-risk HPV have been used to assess cervical specimens[3]. The hybrid capture II (HCII) test (QIAGEN, Gaithersburg, MD, USA) is approved by the US Food and Drug Administration (FDA) and is used to validate new high-risk HPV tests for cervical screening. Two different probe cocktails are used; one comprises probes for the five low-risk genotypes, 6, 11, 42, 43 and 44, and the other contains probes for the 13 high-risk genotypes, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68[4]. The test recognizes all 13 HPV types, which are classified as class I carcinogenic with respect to cervical cancer by the World Health Organization (WHO)[5]. However, this test has certain limitations, namely that it distinguishes between the high-risk and low-risk groups but does not permit the identification of specific HPV genotypes or multiple HPV infection[6]. In addition, cross-reactivity between the two probe cocktails has been observed[7]. Relative light unit (RLU)/cut-off (CO) values are considered to provide a semiquantitative estimate of viral load[4].

The PapilloCheck® test (Greiner Bio-One, Frickenhausen, Germany) is a polymerase chain reaction (PCR)-based deoxyribonucleic acid (DNA) microarray system that allows the genotyping of 24 different HPV types (six low-risk and 18 high-risk types). The high-risk types include the 13 high-risk types detected by HCII and types 73, 82, 53, 66 and 70. The low-risk types include types 6, 11, 40, 42, 43 and 44/55[8]. The results are expressed qualitatively and semiquantitatively by signal-to-noise ratio (SNR) for 24 low- and high-risk HPV types simultaneously. The PapilloCheck® test distinguishes HPV types and multiple HPV infection and provides the SNR value for each type[8]. The PapilloCheck® test has been considered a reliable screening test for HPV detection and typing[9,10]. Because HCII and PapilloCheck® are commercially available and widely used for routine diagnosis, this study evaluated their level of concordance for the detection of high-risk HPV in anal cytological samples.

This study included 42 human immunodeficiency virus (HIV)-positive patients (30 men and 12 women) older than 18 years with no visible lesions as observed in external visual examinations. An endocervical brush was introduced 4cm into the anal canal, rotated five times, removed and then agitated in the transport solution (PapilloCheck® collection medium and hc2 DNA collection device) provided by the manufacturer. The resulting cell suspension was stored dry at 4-8°C. Two samples were collected from each patient. To avoid false differences between the tests based on the order of collection, this was alternated between patients.

This study was approved by the Ethics in Research Committee of Brasilia University.

The HCII test was performed according to the manufacturer's instructions[8]. The probe cocktail for the detection of the 13 high-risk genotypes was used. A relative light unit/cut-off (RLU/CO) value of 1.0 or greater was considered positive for high-risk HPV detection[7].

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The PapilloCheck® test was performed according to the manufacturer's instructions. This test cannot distinguish between HPV-55 and HPV-44 due to cross-reactivity. Samples with an SNR greater than 20 were considered positive.

Statistical analysis was performed using GraphPad Prism 4 (GraphPad Software, San Diego, CA). The level of concordance between the two tests was determined using Cohen's Kappa coefficient. For multiple infection samples, the SNR values of each identified type were added. Statistical significance was assigned to $p < 0.05$.

The HCII test yielded positive results for 22/42 (52.3%) samples. Considering only the 13 high-risk HPV types that can be detected by both tests, PapilloCheck® was positive for 22/42 (52.3%) samples. All 13 high-risk HPV types that can be detected by both tests were detected, except for HPV 33. Multiple HPV infection was detected in 8/42 (19%) samples. The number of concordant results was 34/42 (80.9%), with a Kappa coefficient value of 0.61, indicating good agreement **(Table 1)**. Of the 8/42 discordant samples, four tested positive by PapilloCheck® but negative by HCII; the HPV types found in these samples by PapilloCheck® were HPV 35, 56 and 68, all of which are high-risk HPV types included in the 13 high-risk HPV-probe set of HCII. The other four discordant samples tested positive by HCII but negative by PapilloCheck®. The PapilloCheck® assay was fully negative for three of these samples (i.e., none of the 24 HPV types that are detectable by PapilloCheck® was observed), but one sample tested positive for HPV 82, a high-risk HPV type detected only by PapilloCheck® and not included in the 13 high-risk HPV-probe set of HCII.

The median (minimum-maximum) RLU/CO ratio was 1.03 (0.26-572.6), and the median (minimum-maximum) SNR value was 21.9 (0-2197). In the discordant samples, the RLU/CO values ranged from 0.3-16.52, and the SNR values ranged from 0-198.1. The RLU/CO and SNR values of all samples are shown in **Tables 2** and **3**.

Overall, 28/42 (66.7%) samples were positive for one or more of the HPV types that can be detected by PapilloCheck®. Of the HPV-positive samples, 27/42 (64.3%) had at least one high-risk HPV type. Multiple infection (more than one HPV subtype) was found in 19/42 (45.2%) samples. All high-risk HPV types were detected, except for HPV 33 and 73. The most frequent high-risk subtype was HPV 16 (7/42, 16.7%), and the most frequent low-risk subtype was HPV 44/55 (8/42, 19%).

TABLE 1 - Frequency of concordant and discordant results between PapilloCheck® and HCII.

	HCII		
PapilloCheck®	positive	negative	Total
Positive	18	4	22
Negative	4	16	20
Total	22	20	42

HCII: hybrid capture II.

TABLE 2 - Relative light unit/cut-off ratio and signal-to-noise ratio values of the samples with concordant results in PapilloCheck® and HCII.

Samples	RLU/CO values	SNRvalues
1	0.61	0
2	1.1	781.8
3	1.05	22.8
4	0.72	0
5	1.17	77.3
8	4.19	791
9	234.73	105
10	0.47	0
11	0.51	0
12	0.41	0
13	572.57	1180.1
14	0.33	0
16	1.02	57.6
17	51.7	321
18	0.51	0
19	2.52	292.1
20	0.35	0
21	0.46	0
22	14.64	574.5
23	5.01	502.3
24	0.35	0
25	0.63	0
26	3.29	550.3
28	0.26	0
29	0.5	0
30	10.39	574.5
31	0.52	0
32	10.85	235
33	45.35	886
34	0.32	0
35	1.86	153.5
37	457.94	365.7
39	457.94	2197
40	0.47	0

HCII: hybrid capture II; RLU/CO: relative light unit/cut-off; SNR: signal-to-noise ratio.

TABLE 3 - Relative light unit/cut-off ratio and signal-to-noise ratio values of the samples with discordant results between PapilloCheck® and HCII.

Samples	RLU/CO values	SNR values
6	1.38	0
7	4.19	0
15	0.51	91.1
27	0.63	21
36	0.3	198.1
38	16.52	0
41	0.48	29
42	3.52	0

HCII: hybrid capture II; RLU/CO: relative light unit/cut-off; SNR: signal-to-noise ratio.

A high level of agreement between the PapilloCheck® and hybrid capture II tests for HPV detection has been shown for cervical samples[9-11]. In a previous study in which 44% of the samples were CIN2+/HSIL, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the PapilloCheck® and HCII tests for CIN2+/HSIL histology detection were 96%, 40%, 61% and 91% and 95%, 42%, 61% and 90%, respectively. None of the comparisons of sensitivities, specificities, PPVs or NPVs showed statistically relevant differences between these tests, and a good sensitivity and NPV (greater than 90%) were observed for both[9]. Consistent with these results, the present study demonstrated an 80.9% concordance between PapilloCheck® and HCII for the detection of the 13 high-risk HPV types that are detectable by both tests in anal cytological samples.

For cervical samples, the concordance between these tests depends on the viral load. Agreement increases with viral load, and a significant number of discordant results were observed for samples with an RLU/CO ratio value between 1.0 and 5.0[10,12]. The proportion of both false negativity and false positivity increased considerably with RLU value proximity to the CO value (i.e., 1.0 RLU/CO), suggesting that these samples should be retested[13]. Similarly, among the values obtained with the PapilloCheck® test, samples with discordant results showed low SNR values (≤ 25)[12]. Regarding the discordant results of the present study, the types detected by PapilloCheck® but not by HCII were HPV 35, 56 and 68, all of which were included in the 13 high-risk HPV-probe set of the HCII test. Didelot et al.[11] also observed one sample in which HPV 16 was detected by the PapilloCheck® test and sequencing, but the sample tested negative for high-risk HPV infection by hybrid capture[11]. In the discordant results of the present study, the SNR values observed by PapilloCheck® were not sufficiently low (21, 29, 91.1 and 198.1) to explain the negative results of the HCII test, and only one sample had an SNR value < 25. For samples that tested positive by HCII and negative by PapilloCheck®, the RLU/CO ratio values were low (1.38, 3.52, 4.19 and 16.52), and only one RLU/CO ratio value was > 5. Certain samples might have tested positive by HCII and negative by PapilloCheck® due to cross-reactivity with HPV types other than those theoretically detected by both tests. Poljak et al.[7] showed that the HCII high-risk probe cocktail detects at least 15 HPV genotypes that are not included in the current HCII high-risk probe cocktail[7].

The HCII test has not been validated as a quantitative test, although RLU/CO values can be considered to provide good estimates of HPV load[14]. In the PapilloCheck® test, as in the HCII test, the viral load is expressed semiquantitatively using SNR values. For cervical samples, HPV load evaluation is important because of its association with lesion severity and persistence, but to the best of our knowledge, there are no studies evaluating the possible role of HPV load as a marker in anal samples[14,15].

In conclusion, the PapilloCheck® and HCII tests have a good concordance level for the detection of the 13 high-risk HPV types that are detectable by both tests in anal cytological samples.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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