JCP Online First, published on September 26, 2016 as 10.1136/jclinpath-2016-204044

HPV self-sampling in CIN2+ detection: sensitivity and specificity of different RLU cut-off of HC2 in specimens from 786 women

F Bottari,[1] S Igidbashian,[2] S Boveri,[2] A Tricca,[1] C Gulmini,[1] M Sesia,[1] N Spolti,[2] M Sideri,[†] F Landoni,[2] M T Sandri[1]

▶ Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/jclinpath-2016-204044).

[1]Division of Laboratory Medicine, European Institute of Oncology, Milan, Italy
[2]Preventive Gynecology Unit, European Institute of Oncology, Milan, Italy

Correspondence to
Dr F Bottari, European Institute of Oncology, via Ripamonti 435, Milan 20141, Italy; fabio.bottari@ieo.it

[†]Deceased

Received 22 July 2016
Accepted 26 August 2016

ABSTRACT

Aims Mortality for cervical cancer varies between the different regions of the world, with high rates in low-income countries where screening programmes are not present and organised. However, increasing screening coverage is still a priority in all countries: one way to do that is to base screening on self-sampled screening. The success of a self-sampling screening strategy depends on capacity to recruit unscreened women, on the performance and acceptability of the device and on the clinical performance of the high-risk human papillomavirus (HPV) test.

Methods This study based on 786 enrolled women investigates the best cut-off value of Hybrid Capture 2 HPV test (HC2) for self-sampled specimens in terms of sensitivity and specificity.

Results In this population, we found that the sensitivity and the specificity for cervical intraepithelial neoplasia grade 2 or more detection of HC2 performed on self-sampled specimens were 82.5% and 82.8%, respectively considering the relative light units (RLU) cut-off value of 1. Increasing the cut-off value the sensitivity decreases and the specificity raises and the best area under the curve for the RLU cut-off value is 1.

Conclusions Our results confirm that the cut-off value of 1 suggested by Qiagen for PreservCyt specimen is the best cut-off value also for self-sampled specimens.

INTRODUCTION

Cervical cancer is the fourth most common cause of cancer-related death in women worldwide: mortality considerably varies between the different regions of the world, with high rates in low-income countries where screening programmes are not present and organised.[1]

The identification of oncogenic human papillomavirus (HPV) as the necessary cause of cervical cancer and its precursor lesions[2] opened a new scenario for cervical cancer screening, introducing HPV DNA detection as screening test: strong evidences showed that high-risk HPV (HR HPV) testing is more sensitive than cytology in detecting high-grade cervical intraepithelial neoplasia (CIN). Moreover, HPV testing permits extension of screening intervals resulting in less screening rounds and lower surveillance costs.[3]

Increasing screening coverage is still a priority in all countries:[4] common barriers to screening are accessibility to and acceptability of the pelvic examination needed for the cervical Pap smear or HPV tests,[5] and the lack of participation in screening or follow-up could be considered one of the major risk factors for cervical cancer in industrialised countries:[6] encouraging these women to participate translates in saving lives and reducing the costs of treatment for invasive cancer.

While attempts to introduce self-sampling in cytology-based screening failed,[7] HPV testing can be performed on samples collected by women themselves and previous studies indicate that self-collected and clinician-collected HR HPV testing have comparable results.[8]

Offering self-sampling of cervical and/or vaginal material for HR HPV testing has shown to be effective in increase screening compliance: about one-third of the non-attendees submit self-sampled material for HPV testing when HPV self-sampling is offered.[9 10]

Nevertheless, previous studies demonstrated that PCR-based HPV DNA tests in self-collected samples have higher sensitivity than Pap test and comparable sensitivity to samples obtained by physicians.[8 11 12]

This study evaluates the results of HR HPV self-sample assayed by Qiagen Hybrid Capture 2 (HC2) HPV DNA test, and investigates if the cut-off value of HC2 for clinician-collected LBC as described in package insert could be the best cut-off value for self-sampling specimen too in terms of sensitivity and specificity. In fact, according to the manufacturer's instructions, the HC2 HPV DNA test cut-off is 1 pg/mL equivalent to 100 000 HPV copies/mL, but when testing PreservCyt specimens (as in case of self-sampled specimens), if the relative light unit (RLU) ratio of sample is ≥1 and <2.5, Qiagen recommends to retest the specimen. If the first result of retest is positive (≥1 RLU), the specimen can be reported as positive; in the other case, if the initial retest result is negative (<1 RLU), a second retest (third result) needs to be done to generate a final result.

Considering this, the aim of this study was to evaluate if an increase in the cut-off for self-sampled samples can be useful in terms of sensitivity and specificity of test.

MATERIALS AND METHODS

Patients

Patient recruitment was carried out by the Preventive Gynecology Unit of the European Institute of Oncology (IEO) from May 2006 until August 2007 and the median follow-up period was 33.8 months. All women scheduled for cervical cytology for any reason or for a cervical conservative treatment for CIN at our Institute were asked

To cite: Bottari F, Igidbashian S, Boveri S, *et al*. *J Clin Pathol* Published Online First: [*please include* Day Month Year] doi:10.1136/jclinpath-2016-204044

to take part in the study and received written information on the trial, a self-sampling kit with written instructions and pictures on how to perform the sampling and an informed consent form. Exclusion criteria were: age <18 years, pregnancy and refusal to participate. All women who agreed to participate signed the informed consent form. The study was approved by the IEO Ethics Committee.

Self-sampling collection

Patients self-collected a cervicovaginal sample for HPV testing with the cervical sampler in the restroom adjacent to the examination room or on the gynaecological bed before the excisional procedure.

The self-sampler used in the sample collection was the Hybrid Capture (HC) Cervical Sampler (Qiagen, Hilden, Germany) that has a conical brush with a smooth plastic tip. Women were instructed to insert the device 5–6 cm into the vagina and rotate it three times in the same direction. After taking the sample, women placed the brush at the bottom of the specimen transport tube snapping off the shaft at the score line and leaving the end of the brush inside the closed tube.

Hybrid Capture 2

All samples were sent to the IEO Laboratory Medicine Division and there the Qiagen HC2 HPV DNA test was performed according to the manufacturer's instructions.[13] HC2 is a sandwich capture molecular hybridisation assay: it is a signal amplification detection method based on chemiluminescence that detects 13 HR HPV types HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68). The DNA:RNA hybrids are captured on a microplate, and the emitted light is measured in a luminometer as RLU. Samples were considered as positive if the ratio RLU/cut-off was >1.0 (equivalent to 1.0 pg HPV DNA/mL or 100 000 HPV copies/mL) as described in package insert.

Statistical analysis

The characteristics of population were summarised and described using either counts and percentage or mean, SD and median. Patients with histological diagnosis of CIN2, CIN3 and cervical carcinoma were classified as CIN2+. Differences among levels of RLU were compared using the Kruskal-Wallis test. The two-sample two-sided Wilcoxon test was used for group comparisons in cases of non-normally distributed continuous data.

Overall sensitivity and specificity were calculated for self-sampling test in relation to CIN2+ pathological category. The accuracy, sensitivity and specificity of RLU of self-sampling test to discriminate CIN2+ level from lower cervical disease were estimated in a receiver operating characteristics (ROC) analysis. ROC area point estimates of sensitivities and specificities with their 95% CIs were tabulated for 1, 2, 3, 5 and 10 RLU value.

All tests were considered statistically significant at the 0.05 significance level. All statistical analyses were performed using SAS V.9.3 (SAS Institute, Cary, North Carolina, USA).

RESULTS

Median age of 786 enrolled women was 43 years (range 19–72 years). Subsequent histology was available for 143 women (18%) and among them we found 82 ≥CIN2 (10.4%): in particular, 25 of 30 CIN2, 36 of 44 CIN3 and 6 of 8 cervical carcinoma tested positive with HC2 (figure 1). Women with no histological evaluation were considered negative if they resulted negative to cytology, if they had a cytology result ≤ atypical squamous cells of undetermined significance (ASCUS) with a negative HPV test or if colposcopy after a positive cytology resulted negative.

The distribution of the subjects in terms of RLU cut-off (positive RLU≥1 and negative <1) and disease category is shown in table 1: ≥CIN2 were 82.9% HC2 positive and 17.1% negative. Sensitivity and specificity of HC2 test performed on self-sampled specimens were 81.7 and 82.8, respectively: positive predictive value was 35.6 and negative predictive value was 97.5.

In our population, HC2 categorises well the population as described in table 2: RLU median value was 0.2, 3.13 and 44.0 for negative, CIN1 and ≥CIN2 category, respectively, and Kruskal-Wallis test was calculated, showing a $p<0.001$ for the three categories.

The diagnostic performance of HC2 in discriminating ≥CIN2 lesions is verified using ROC analysis (≥CIN2 in screening or follow-up rounds). Sensitivity and specificity varying the cut-off value (1, 2, 3, 5) are shown in table 3 and the resultant accuracy (ROC area) values for HC2 and their corresponding ROC curves are shown in figure 2.

DISCUSSION

The success of a self-sampling strategy depends on the capacity to recruit unscreened women, on the performance and acceptability of the device and on the clinical performance of the HR HPV testing of the self-sample. A previous meta-analysis demonstrated that the sensitivity and specificity of HPV tests may be different when signal amplification-based HPV assays are applied.[14] This meta-analysis showed that HPV testing with signal-based assays on self-samples was less sensitive and specific

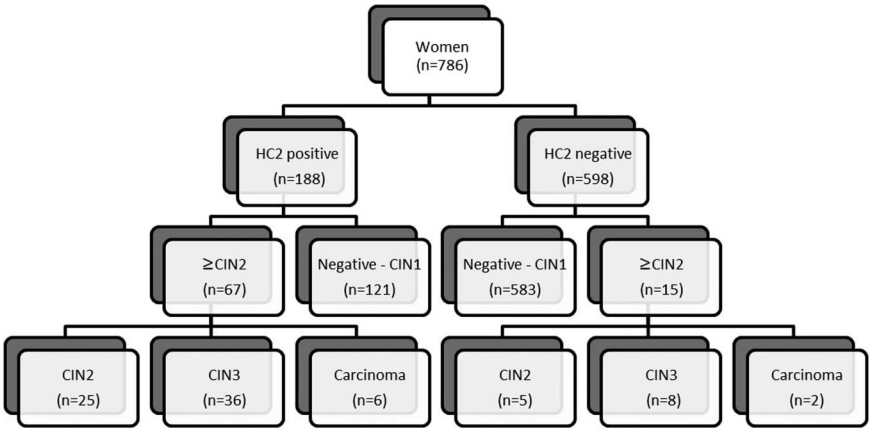

Figure 1 Flow diagram for Hybrid Capture 2 (HC2) and histology results.

Table 1 Sensitivity, specificity, PPV and NPV of HC2 in self-sampled specimens

	Disease			
			Sensitivity	81.7 (71.6–89.4)
HPV self	<CIN2	≥CIN2	Specificity	82.8 (79.8–85.5)
Negative	583	15	PPV	35.6 (28.8–42.9)
Positive	121	67	NPV	97.5 (95.9–98.6)

Negative is HC2 RLU<1 and positive is HC2 RLU≥1.
CIN, cervical intraepithelial neoplasia; HC, Hybrid Capture; HPV, human papillomavirus; NPV, negative predictive value; PPV, positive predictive value; RLU, relative light units.

Table 2 Mean, median, minimum and maximum HC2 value (in terms of RLU) for disease category

	N	Mean	Median	Minimum	Maximum	SD
Negative	653	16.2	0.25	0.10	1947.3	117.9
CIN1	51	97.2	3.46	0.13	1651.9	271.8
≥CIN2	82	315.6	44.0	0.13	2485.5	592.8

Kruskal-Wallis test p<0.001; negative versus CIN1 p<0.001, negative versus ≥CIN2 p<0.001, CIN1 versus ≥CIN2 p=0.003.
CIN, cervical intraepithelial neoplasia; HC, Hybrid Capture; RLU, relative light units.

Table 3 Sensitivity and specificity increasing the RLU cut-off value*

RLU	Sensitivity (%) 95% CI	Specificity (%) 95% CI	AUC (95% CI)
1.00	82.5 (75.5 to 88.6)	82.8 (80.0 to 85.7)	0.88 (0.84 to 0.92)
2.08	76.4 (69.1 to 83.6)	86.5 (83.9 to 89.1)	
3.10	71.8 (64.1 to 79.5)	88.0 (85.5 to 90.5)	
5.19	68.0 (60.0 to 76.0)	89.3 (86.9 to 91.7)	

*CIN2 and CIN3 at screening or follow-up rounds.
AUC, area under the curve; CIN, cervical intraepithelial neoplasia; RLU, relative light units.

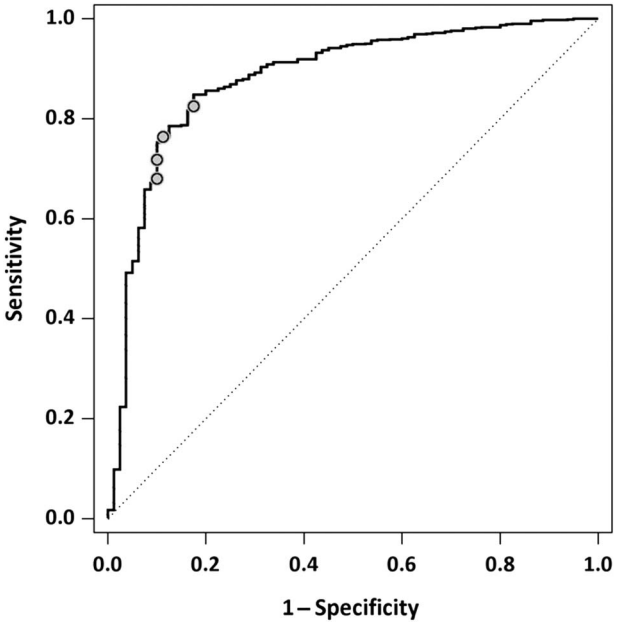

Figure 2 Receiver operating characteristics (ROC) curve for Hybrid Capture 2 (HC2) high-risk human papillomavirus (HR HPV) test (cut-off value 1). The relative cut-off values (1, 2, 3, 5) are marked with o.

than testing on clinician-based samples; on the other hand, some PCR-based HPV tests showed similar sensitivity on both self-samples and clinician-based samples. The authors concluded that HPV testing on a self-sample can be suggested as an additional strategy to reach women not participating in the regular screening programmes.

No data are available in other clinical setting, for example, follow-up of previously treated women or after a positive cytology. In our study, we analysed a group of 786 women who were scheduled for a Pap smear in our Institute for different clinical reasons: for screening, for diagnosis after a positive cytology and before a conservative treatment or during the follow-up visit after ≥CIN2 treatment. Overall, we had a quite high prevalence of women with high-grade lesions (≥CIN2 10.4%). In this population, we found that the sensitivity and the specificity for ≥CIN2 detection of HC2 performed on self-sampled specimens were 82.5% and 82.8%, respectively considering the RLU cut-off of 1. In the meta-analysis recently published by Arbyn *et al*,[14] 18 papers could be included in which HC2 was used. They reported a cumulative sensitivity of 76% (95% CI 69% to 82%) in CIN2+ detection, slightly lower than what we found. As far as specificity is concerned, they found a higher value of 86% (95% CI 83% to 89%) compared with our study. A possible explanation for this difference may be that we did not examine a screening population, but women in whom a higher positivity of HPV testing is expected, for example, in case of follow-up after treatment where the positivity might be due to slow clearance of the virus.

In order to increase the clinical performance of HC2 HPV test on self-sampled specimens, we evaluated the changes of sensitivity and specificity while increasing the cut-off value of RLU. We found that increasing the RLU cut-off from 1 to 5 determined a substantial decrease of sensitivity (from 82.5% to 68%) with a minor increase of specificity (from 80.7% to 87.7%), and the combined evaluation of sensitivity and specificity through ROC curves evidenced that also in case of self-sampling the best RLU cut-off value is 1. Moreover, looking at the absolute values of RLU in the different cytological categories (negative, CIN1 and ≥CIN2), we found a significant increase of RLU in these three groups. This is in agreement with the previous study by Origoni *et al*,[15] who showed that the value of RLU, although it cannot be considered a real and reliable measure of viral load, is associated with the severity of the lesion, with higher values of RLU in case of more severe disease.

As already suggested, the use of self-sampling may be of great help in increasing the coverage in the screening settings. However, another key point in cervical cancer control is the follow-up of patients treated for a ≥CIN2 lesion. It is widely known that a percentage from 5% to 15% of these women will develop a recurrence of the disease within the first 2 years from treatment, and all the guidelines suggest strict monitoring.[16 17] Unfortunately, some of these women are lost at follow-up: in these cases, a possible strategy to catch them up could be the invitation to perform a self-sampling, the results of which can identify women needing a gynaecological control.

Our study confirms that the sensitivity of HC2, although not ideal, is however higher than the sensitivity of the Pap smear.[18] In future, new PCR-based HPV test will be available and they will have to be evaluated also in this setting.

In conclusion, the results of our study performed in a setting with a high prevalence of high-grade lesions showed that the

cut-off currently used for HC2 testing is adequate also in case of self-sampled specimens, maximising sensitivity and specificity. These data indicate that self-sampling may be a valuable option to increase coverage in screening programmes and also to decrease the women lost at follow-up after treatment for cervical lesions.

Take home messages

- The study investigates the performance of Hybrid Capture 2 (HC2) test on 786 self-sampled specimens.
- In our population, the sensitivity and specificity were 82.5% and 82.8%, respectively.
- The results confirm that 1 is the best cut-off of HC2 test also for self-sampled specimens.

Handling editor Runjan Chetty

Competing interests None declared.

Patient consent Obtained.

Ethics approval IEO Ethics Committee.

Provenance and peer review Not commissioned; externally peer reviewed.

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