Chapter 3

Clinical validation of the Cervista HPV HR test in comparison with the Hybrid Capture 2 test according to the guidelines for HPV test requirements for cervical cancer screening


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CHAPTER 3

Abstract

The Cervista HPV HR test is approved in many countries for detection of high-risk human papillomavirus (hrHPV) types in cervical scrapings, and has a similar performance to the hybrid capture 2 (hc2) HPV test. The aim of this study is to demonstrate that the Cervista HPV HR test fulfills the cross-sectional clinical performance and reproducibility criteria of international guidelines for HPV test requirements for primary cervical cancer screening in women 30 years and older. The clinical sensitivity and specificity of Cervista was compared to that of hc2 for detection of high-grade cervical lesions (CIN2+) in women aged ≥30 years in >7,000 screening samples selected from a multisite, population-based, cross-sectional study by non-inferiority analysis. In addition, we also determined the intra- and inter-laboratory reproducibility of the Cervista HPV HR test in 510 scrapings. The Cervista HPV HR test showed a clinical sensitivity for detecting CIN2+ of 89% (95%CI: 81.6-94.2) and a corresponding clinical specificity of 91% (95%CI: 90.5-91.8). Both the relative clinical sensitivity and specificity were non-inferior to that of hc2 (non-inferiority score tests, \( P = 0.043 \) and \( P < 0.0001 \), respectively). Intra- and inter-laboratory agreements were 92% (lower bound 95%CI: 89.7%; kappa = 0.83; \( P < 0.001 \)) and 90.4% (lower bound 95%CI: 88.4%; kappa = 0.80; \( P < 0.001 \)), respectively. In conclusion, the Cervista HPV HR test meets the cross-sectional clinical performance and reproducibility criteria of the international guidelines for HPV test requirements and thus can be considered as clinically validated for primary cervical screening purposes.
Introduction

It is well established that cervical cancer is caused by the persistent infection of cervical epithelial cells by any of the ~14 genotypes of human papillomavirus (HPV) termed high-risk (hrHPV). This knowledge prompted the development of in vitro diagnostic tests for hrHPV testing in clinical specimens. Generally these tests have a high sensitivity and high negative predictive value making them potentially valuable tools for the use in primary screening strategies. Systematic reviews have shown that primary screening using hrHPV testing has a higher sensitivity than cytology for the detection of cervical intraepithelial neoplasia (CIN) grade 2 or higher (CIN2+) \(^1,2\). Although many current international guidelines limit HPV testing to the triage of borderline lesions and to post-CIN follow up, it is believed that in the near future HPV testing will be included as a viable strategy for primary screening in most European official guidelines \(^3\). In line with the international guidelines for HPV DNA testing in primary cervical cancer screening in women 30 years and older described by Meijer et al. \(^4\), recently updated guidelines from the American Society for Colposcopy and Cervical Pathology (ASCCP) emphasize the importance of using a validated HPV test, i.e., an HPV test that has proven acceptable reproducibility, clinical sensitivity, specificity, and positive and negative predictive values for cervical cancer screening of CIN2+ lesions \(^5\).

The Hybrid Capture 2 hrHPV DNA test (hc2; Digene Corp., Gaithersburg, Md) was the first hrHPV test with proven good clinical performance in cervical screening. The hc2 test has a pooled sensitivity of 97.9% (95%CI: 95.9%–99.9%) and a pooled specificity of 91.3% (95% CI: 89.5%–93.1%; range: 85%–95%) in primary screening in North American and European populations \(^6\). The Cervista HPV HR test (Cervista; Hologic Inc., Madison, Wis.) was the second hrHPV assay approved by the FDA in 2009, 10 years after approval of the hc2 test. Cervista has a few advantages over hc2: it requires a sample volume that is half that of hc2, it includes an internal control for sample adequacy, does not cross-react with common non-oncogenic HPV types, and has a shorter processing time. In comparative studies, the Cervista HPV HR test has been shown to have similar sensitivity and specificity results as the hc2 test \(^7-11\).

However, the Cervista HPV HR test has not previously been formally validated for the use in primary cervical screening. International guidelines that have been issued in 2009\(^4\) state that, to be validated for use in primary cervical screening, a candidate hrHPV test should meet the following requirements: (i) a relative clinical sensitivity for CIN2+ of ≥90% compared to the hc2 test as assessed by a non-inferiority score test in women aged ≥30 years; (ii) a relative clinical specificity for CIN2+ of ≥98% compared to the hc2 test as assessed by a non-inferiority score test in women aged ≥30 years; and (iii) an intra-labo-
The aim of the present study was to formally validate the Cervista HPV HR test according to the International Guidelines for HPV DNA testing in primary cervical cancer screening in women 30 years and older by strictly following the validation process recommended by Meijer et al. 4 The relative clinical sensitivity and specificity of the Cervista HPV HR test was compared to that of the hc2 test for detection of CIN2+ in women aged ≥30 years in >7,000 screening samples selected from a multisite, population-based, cross-sectional study by non-inferiority analysis. The intra- and inter-laboratory reproducibility of Cervista was assessed in 510 scraping following this international guideline. We show herein that the Cervista HPV HR test is non-inferior to the hc2 HPV test, and that the intra- and inter-laboratory reproducibility meets the criteria set forth by the international guidelines for HPV test requirements.

**Methods**

**Determination of the Sensitivity and Specificity of Cervista — the SHENCCASTII Study**

**Study Design**

The SHENCCAST II study was a multisite, population-based, cross-sectional study conducted in Guangdong Province in China which enrolled approximately 10,000 women, 25 to 59 years old, who had routine cervical screening.9 Women were included if they had no cervical cancer screening for at least 3 years. Cervical scrapings (in PreservCyt liquid-based cytology medium, Hologic, Marlborough, MA) were collected for each woman; the sample was used first for cytology and then for HPV testing, first by hc2 and then by Cervista. All women with abnormal cytology results (ASCUS or worse) or positive results by either of the HPV tests performed underwent colposcopy and four-quadrant biopsy with endocervical curettage. All specimens were tested at the Royal Ladies Clinic, the POI research center in Shenzhen, China, by trained technicians and according to the assays manufacturer’s instructions. Complete data sets were available for 8,556 subjects. Of the 8556 subject, 7,430 were ≥30 years of age and selected for this study, of which 2.85% were with histological proven CIN2+ lesion. A more detailed methodology of the study has been reported elsewhere.

**Hc2 HPV test**

The hc2 test (Digene Corp., Gaithersburg, Md) is a nucleic acid hybridization test with signal amplification that qualitatively detects the DNA of 13 hrHPV types.12 The targeted HPV DNA is hybridized with HPV-specific RNA probes, and the resulting DNA-RNA hybrids are captured by antibodies immobilized onto a microplate well. Free antibodies conjugated to alkaline phosphatase are added and bind to the hybrids. A chemiluminescent substrate
Clinical validation of the Cervista HPV HR test

is added and is cleaved by the alkaline phosphatase, resulting in light emission, which is measured as relative light unit (RLU) on a luminometer. Results are interpreted as a ratio of RLU to the positive control specimen (RLU/CO). Samples with a RLU/CO ratio >1 are considered hrHPV-positive; samples with RLU <1 are considered hrHPV-negative. In this study, samples with a RLU/CO ratio ≥0.8 and <2.5 (instead of 1.0 to 2.5 as in the package insert) were retested.

Cervista HPV HR test

The Cervista HPV HR test (Hologic Inc., Madison, Wis) is a qualitative test that detects 14 hrHPV types (the same 13 types as Hc2 plus HPV 66) 13, 14. The Cervista test also detects the human histone 2 gene which serves as an internal control for the presence of cellular DNA. Cervista uses the Invader® chemistry, a signal amplification method for detection of specific nucleic acid sequences. As described previously 14, this method utilizes a primary reaction that occurs on the targeted DNA sequence and a secondary reaction that produces a fluorescent signal. Both types of reactions rely on oligonucleotide hybridization, invasive structure formation, and cleavage by the Cleavase® enzyme (Hologic, Inc). Interpretation of HPV results were in accordance with the Cervista HPV HR package insert 13.

Comparison of the Cervista HPV HR test and hc2 assay

The overall sensitivity and specificity (and associated 95% confidence intervals [CI]) of the Cervista HPV HR test and hc2 test for CIN2+ detection in women aged ≥30 years was calculated. To determine whether the performance of Cervista is equivalent to that of the reference test hc2, Cervista was compared with hc2 using a non-inferiority analysis, which involves a multinomial analysis 4. As stipulated in the international guidelines, to determine the clinical specificity, a sample size of at least 800 scrapings was needed to achieve a power of ≥80% in the comparison of the two assays. The relative specificity was calculated from 7218 cervical scrapings of women aged ≥30 years without CIN2+ lesions and having complete data sets available for all tests performed in the SHENCCAST II study. To calculate the relative sensitivity on a representative population-based screening cohort consisting of subjects with a CIN2+ lesion and normal cytomorphology, from the cohort of 212 subjects with histological proven CIN2+ lesions, 78 randomly selected samples with abnormal cytology (≥ASCUS) and 31 samples with normal cytology (NILM) were selected. Cervista and hc2 were compared using the non-inferiority test as described by Meijer et al. 4 using a tabular comparison (Table 1). Under the null hypothesis, the relative sensitivity (when comparing the new test to hc2) is δ₀ and under the alternative hypothesis, the relative sensitivity is greater than δ₀. According to the present guidelines δ₀ should be set to 0.90 for sensitivity and to 0.98 for specificity. The test statistic is defined as:
Where a, b, c, and d are the positive and negative test results (see Table 1), with \( A = n(1 + \delta_0) \), \( B = (a + c)\delta_0^2 - (a + b + 2c) \), and \( C = c(1 - \delta_0)(a + b + c)/n \). The null hypothesis is rejected at nominal significance level \( \delta \) if \( T \) is equal to or greater than the \( 100 \times (1 - \alpha) \) percentile point of the standard normal distribution (\( T \) is interpreted as a z-statistic).

Table 1. Comparison of a new test with hc2

<table>
<thead>
<tr>
<th></th>
<th>hc2 test+</th>
<th>hc2 test-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>New test+</td>
<td>a</td>
<td>b</td>
<td>a + b</td>
</tr>
<tr>
<td>New test-</td>
<td>c</td>
<td>d</td>
<td>c + d</td>
</tr>
<tr>
<td></td>
<td>a + c</td>
<td>b + d</td>
<td>n</td>
</tr>
</tbody>
</table>

Determination of Cervista Reproducibility — the Dutch Study

The international guidelines for HPV DNA testing in primary cervical cancer screening recommend that the intra-laboratory reproducibility in time and inter-laboratory agreement be determined by evaluating at least 500 samples, approximately 30% of which tested hrHPV positive in a reference laboratory using a clinically validated assay. In this study, the intra- and inter-laboratory reproducibility of Cervista was evaluated on 510 scrapings selected from women aged 30 to 60 years participating in the routine national population-based cervical screening program in the Netherlands. These 510 samples comprised 186 hrHPV-hc2 positive and 324 hrHPV-hc2 negative randomly-selected scrapings (36% hrHPV-positivity) following the international guidelines for HPV DNA testing. To determine the intra-laboratory reproducibility, all 510 samples were tested twice with Cervista at a 1- to 3-week interval by the same experienced technician on the same Cervista system at the Department of Pathology and Medical Biology of the University Medical Center Groningen, in the Netherlands (UMCG). For the inter-laboratory agreement, an aliquot (2 mL PreservCyt) of the same samples was sent to an independent reference laboratory (Department of Pathology, AZ St Jan Brugge-Oostende, Brugge, Belgium) which uses Cervista routinely. All samples were randomly renumbered and provided to the reference laboratory without any cytology or hrHPV test results. The percentage agreement between test results was calculated by dividing the number of concordant results by the total number of results; the associated Cohen’s kappa and P-value were determined. A detailed description of the sample selection and analysis of the intra- and inter-laboratory reproducibility is reported elsewhere.
Results and conclusion

Non-inferiority of Cervista versus hc2

The clinical performance of the Cervista HPV HR test was compared to that of the hc2 test in the SHENCCASTII study. To calculate the relative clinical specificity and sensitivity, respectively 7218 samples without CIN2+ lesions and 109 samples with CIN2+ lesions were used for non–inferiority analysis of Cervista versus hc2. The large number of samples analyzed met the sample size requirements for achieving >99% power in the comparison of the two tests (the guideline estimates that >100 samples and >2,500 samples are required to reach a 99% power for comparing the assays’ sensitivity and specificity, respectively).

The overall clinical specificity of hc2 and Cervista for detection of CIN2+ in 7218 women aged ≥30 years were similar, respectively 89% (95%CI: 88.0-89.5) and 91% (95%CI: 90.5-91.8) (Table 2). Recently, we found that the specificity of the Cervista HPV HR test could be further improved by increasing the second cutoff. The overall clinical sensitivity of hc2 and Cervista for detection of CIN2+ in women aged ≥30 years were 94% (95%CI: 87.2-97.4) and 89% (95%CI: 81.6-94.2), respectively. The non-inferiority of the relative sensitivity and relative specificity of the Cervista HPV HR test versus the hc2 test was confirmed as the null hypothesis of inferiority was rejected ($T = 1.76$ and $P = 0.043$ for sensitivity, Table 3; $T = 17.73$, $P<0.0001$ for specificity; Table 4). Therefore, Cervista met the criterion of non-inferiority set forth by the international guidelines, i.e., had a clinical sensitivity not less than 90% of hc2’s sensitivity, and a clinical specificity not less than 98% of hc2’s specificity for detection of CIN2+ in women aged ≥30 years.

Table 2. Sensitivity and specificity of the Cervista HPV HR and the hc2 HPV test for detection of CIN2+ in women aged ≥30 years.

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervista</td>
<td>89% (81.6% - 94.2%)</td>
<td>91% (90.5% - 91.8%)</td>
</tr>
<tr>
<td>hc2</td>
<td>94% (87.2% - 97.4%)</td>
<td>89% (88.0% - 89.5%)</td>
</tr>
</tbody>
</table>

Values are mean (95% CI).

Table 3. Relative sensitivity of the Cervista HPV HR test compared to the hc2 test for CIN2+ cases

<table>
<thead>
<tr>
<th></th>
<th>hc2 test+</th>
<th>hc2 test-</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervista test+</td>
<td>97</td>
<td>0</td>
<td>Test statistics* 1.716</td>
</tr>
<tr>
<td>Cervista test-</td>
<td>5</td>
<td>7</td>
<td>P-value 0.043</td>
</tr>
</tbody>
</table>

* As described in the international guidelines by Meijer et al.
Intra- and Inter-laboratory Reproducibility of Cervista

To determine the reproducibility of the Cervista HPV HR test in clinical practice, we evaluated the intra-laboratory and the inter-laboratory agreement of Cervista results on 510 cytology samples comprising 186 hc2-hrHPV-positive and 324 hc2-hrHPV-negative scrapings. In the intra-laboratory reproducibility evaluation, all 510 samples were tested twice (Test #1 and Test #2) within a 1- to 3-week interval by the same experienced technician on the same Cervista system. The agreement between the two test results was 92.0% (lower bound of 95%CI: 89.7%; kappa = 0.83; \( P<0.001 \)) (Table 5). In the inter-laboratory agreement, aliquots of the same 510 samples tested at UMCG were sent to an independent laboratory (Brugge) that uses Cervista routinely. The agreement between the two laboratories was 90.4% (lower bound of 95%CI: 88.4%; kappa = 0.80; \( P<0.001 \)) (Table 6).

Thus, Cervista’s intra- and inter-laboratory agreement met the guidelines’ requirement of having a lower bound of the 95%CI >87% and a kappa value >0.5.

Table 5. Intra-laboratory reproducibility of the Cervista HPV HR test

<table>
<thead>
<tr>
<th>Test #2 Results</th>
<th>Test #1 Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>174</td>
</tr>
<tr>
<td>Negative</td>
<td>17</td>
</tr>
<tr>
<td>Low gDNA</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>191</td>
</tr>
</tbody>
</table>

Values are number of samples. The agreement between the two test results was 92.0% (lower bound of 95%CI: 89.7%; kappa = 0.83; \( P<0.001 \))

Table 6. Inter-laboratory agreement of the Cervista HPV HR test

<table>
<thead>
<tr>
<th>Brugge Test Results</th>
<th>UMCG Test Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>179</td>
</tr>
<tr>
<td>Negative</td>
<td>35</td>
</tr>
<tr>
<td>Low gDNA</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>214</td>
</tr>
</tbody>
</table>

Values are number of samples. The agreement between the two laboratories was 90.4% (lower bound of 95%CI: 88.4%; kappa = 0.80; \( P<0.001 \))

The aim of the present study was to validate the Cervista HPV HR test for use in primary
screening for the detection of CIN2+ lesions in women aged ≥30 years. Assay validation was performed according to the international guidelines for HPV DNA testing in primary screening and included: (i) determining Cervista’s non-inferiority to the reference hc2 HPV test by comparing Cervista’s clinical sensitivity and specificity to that of hc2 in >7000 cervical samples selected from the SHENCCAST II dataset and (ii) determining Cervista’s intra- and inter-laboratory reproducibility on 510 cervical scraping both selected from women attending routine cervical cancer screening programs in the Netherlands. Cervista met the non-inferiority to hc2 criteria (non-inferiority test) set forth in the international guidelines⁴. Thus, the Cervista HPV HR test fulfills all requirements according to the international guidelines and can be considered formally validated for the use of primary cervical cancer screening in women ≥30 years.
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References


CLINICAL VALIDATION OF THE CERVISTA HPV HR TEST