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Clinical Validation of the Abbott RealTi*m*e High Risk HPV Assay According to the Guidelines for Human Papillomavirus DNA Test Requirements for Cervical Screening

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This study showed that the Abbott RealTi*m*e High Risk HPV assay fulfilled cross-sectional clinical equivalence and reproducibility criteria of international consensus guidelines, which indicates that this assay can be considered clinically validated for cervical cancer screening purposes.

Since infection with high-risk human papillomavirus (hrHPV) is the main causative factor for cervical cancer development, hrHPV testing has been recognized as primary cervical screening tool and is likely to be adopted soon in many countries. However, it is imperative that hrHPV DNA assays to be used for primary cervical screening be clinically validated to ensure, over and above a high clinical sensitivity, a high clinical specificity for cervical intraepithelial neoplasia grade 2/3 and cervical cancer (CIN2+). The latter is important to minimize follow-up procedures done for hrHPV test-positive women without clinically meaningful disease (<CIN2), whose infection will generally disappear spontaneously. Clinical validation of a candidate hrHPV DNA assay for screening can be performed by cross-sectional clinical equivalence analysis of the assay relative to one of the established clinically validated reference assays, i.e., high-risk HPV hybrid capture 2 (HC2) or GP5+/6+ PCR, as outlined by an international consortium (1, 2). According to these guidelines, candidate assays should exhibit clinical noninferiority to HC2 or GP5+/6+ PCR (i.e., relative sensitivity for CIN2+ of ≥0.90 and specificity for CIN2+ of ≥0.98), and show a sufficiently high intralaboratory reproducibility and interlaboratory agreement (i.e., both showing a percentage of agreement with a lower confidence bound not less than 87% [kappa value of at least 0.5]) to be clinically validated. Several hrHPV assays recently have been partially or completely clinically validated using these criteria (3–5).

The Abbott RealTi*m*e High Risk (HR) HPV assay is an automated multiplex real-time PCR test that targets the (GP5+/6+) L1 region of 14 hrHPV types (6). It includes an internal sample control (IC; human β-globin PCR), and four distinct fluorescent labels allow the separate detection of DNA from HPV16, HPV18, a pool of 12 other high-risk genotypes (i.e., HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66, and HPV68), and IC. The present study set out to clinically validate the Abbott RealTi*m*e HR HPV assay according to the international guidelines by comparing its clinical sensitivity and specificity for CIN2+ to that of GP5+/6+ PCR and assessing intralaboratory reproducibility and interlaboratory agreement.

For clinical sensitivity analysis, a representative set of 68 cervical samples was used. These cervical scrapes were collected within a population-based screening setting in the Utrecht and North-Holland region of the Netherlands and were derived from women diagnosed with histologically confirmed CIN2+ (i.e., 30 CIN2, 34 CIN3, and 4 SCC diagnosed within 12 months from baseline sampling). These clinical cases were detected by cervical screening on the basis of an abnormal Pap smear and/or a positive hrHPV GP5+/6+ PCR result. The hrHPV GP5+/6+ PCR, with enzyme immunoassay read-out for the pooled detection of the same 14 hrHPV types as the Abbott RealTi*m*e HR HPV assay, was performed as described previously (7). The median age of case women was 40 (range, 30 to 60) years. The samples from 49 (72%) of these women revealed abnormal cytology (7 borderline or mild dyskaryosis [BMD] and 42 >BMD), and 19 (28%) had normal cytology. The GP5+/6+ PCR positivity rate is shown in Table 1.

For clinical specificity analysis, we used 859 consecutive scrapes from the screening population of the Utrecht region (median age of 41 [range, 31 to 60] years) with normal cytology and without evidence of CIN2+ diagnosis within a 12-month period. This study was approved by the Institutional Review Board via protocol 2011-03 of the Department of Pathology, VU University Medical Center. This study followed the local ethical guidelines of the VU University medical center. All samples were collected in PreservCyt medium.

For Abbott RealTi*m*e HR HPV assay, DNA extraction was performed with the fully automated extraction instrument *m*2000sp and subsequent testing with the *m*2000rt real-time PCR instrument according to the manufacturer's instructions (Abbott GmbH & Co. KG, Wiesbaden, Germany). Testing was performed blinded from GP5+/6+ PCR (7) results and cyto-/histopathology outcome. Over 99.8% (926/927) of the specimens gave valid results with the Abbott RealTi*m*e HR HPV assay (Table 1). The Abbott RealTi*m*e HR HPV assay was positive for 65 women with CIN2+, resulting in a clinical sensitivity for CIN2+ of 95.6% (65/68; 95% confidence interval [CI], 87.2 to 98.6). The clinical specificity for CIN2+ was 92.0% (789/858; 95% CI, 90.0 to 93.5) (Table 1). By comparison, these figures were 98.5% (67/68; 95% CI, 90.3 to 99.8) and 91.8% (788/858; 95% CI, 89.9 to 93.4), re-

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TABLE 1 Abbott RealTi*me* HR HPV assay results for 926 cervical scrapings from population-based screening stratified by case-control status

Sample type and test	Result	No. of samples with GP5+6+ PCR result		
		Negative	Positive	Total
Control (<CIN2)				
Abbott RealTi*me*	Negative	782	7	789
HR HPV assay	Positive	6	63	69
Total		788	70	858
Case (CIN2+)				
Abbott RealTi*me*	Negative	1	2	3
HR HPV assay	Positive	0	65	65
Total		1	67	68

spectively, for GP5+/6+ PCR (Table 1). Agreement between Abbott RealTi*me* HR HPV assay and GP5+6+ PCR was strong, i.e., 97.0% (95% CI, 89.0 to 99.3) and 98.5% (95% CI, 97.4 to 99.1) for cases and controls, respectively. Clinical sensitivity and specificity values of the Abbott RealTi*me* HR HPV assay were compared to those of GP5+6+ PCR using a noninferiority score test (R package version 2.8.1) with a relative sensitivity threshold for CIN2+ of 90% and a relative specificity threshold for CIN2+ of 98% using hrHPV GP5+6+ PCR as a reference (1). Both, clinical sensitivity and specificity for CIN2+ of the Abbott RealTi*me* HR HPV assay were noninferior to that of GP5+6+ PCR ($P = 0.028$ and $P = 0.0003$, respectively).

To assess intralaboratory reproducibility of the Abbott RealTi*me* HR HPV assay, two equal portions of a total of 504 cervical PreservCyt samples were used, of which one-third was positive by high-risk HPV HC2. These portions were tested with the Abbott RealTi*me* HR HPV assay at different time points with an interval of 2 to 8 weeks. The resulting intralaboratory reproducibility was 98.4% (496/504; 95% CI, 97.2 to 99.2 [Table 2]) with a kappa value of 0.96.

The interlaboratory agreement analysis of the Abbott RealTi*me* HR HPV assay was determined based on 500 samples that initially had been tested at the Institute of Microbiology and Immunology, University of Ljubljana, Ljubljana, Slovenia, and subsequently in a blinded manner at the VU University Medical Center lab. The interlaboratory agreement was 99.8% (499/500; 95% CI, 99.1 to 99.9 [Table 3]) with a kappa value of 0.99. For both the intra- and interlaboratory agreement, the lower confidence bounds were >87%, with kappa values of >0.5, and the values thus complied with the guidelines (1).

Our findings are in line with previous studies showing a good agreement between Abbott RealTi*me* HR HPV assay and HC2 (8, 9). The current study is more comprehensive in that it addresses all aspects put forward in the guidelines (i.e., clinical sensitivity, clinical specificity, intralaboratory reproducibility, and interlaboratory agreement) and additionally proves clinical noninferiority to the other clinically validated assay, i.e., GP5+/6+ PCR (1).

In conclusion, this study shows that the Abbott RealTi*me* HR HPV assay is clinically comparable to hrHPV GP5+/6+ PCR. Since the assay meets the criteria for cross-sectional clinical equivalence and reproducibility of the international guidelines, the Abbott RealTi*me* HR HPV assay can be considered clinically validated for cervical screening purposes.

TABLE 2 Intralaboratory reproducibility over time of the Abbott RealTi*me* HR HPV assay[a]

First result	No. of samples with second result		
	Negative	Positive	Total
Negative	328	5	333
Positive	3	168	171
Total	331	173	504

[a] Agreement: 98.4% (95% CI, 97.2 to 99.2) and kappa value of 0.96.

TABLE 3 Interlaboratory agreement of the Abbott RealTi*me* HR HPV assay[a]

Result at Ljubljana lab	No. of samples with result at Amsterdam lab		
	Negative	Positive	Total
Negative	332	0	332
Positive	1	167	168
Total	333	167	500

[a] Agreement: 99.8% (95% CI, 99.1 to 99.9) and kappa value of 0.99.

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