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Comparison of Clinical Performance of Abbott RealTi*m*e High Risk HPV Test with That of Hybrid Capture 2 Assay in a Screening Setting▽

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Randomized trials have produced sound evidence about the efficacy of screening with human papillomavirus (HPV) DNA tests in reducing cervical cancer incidence and mortality. We evaluated the clinical performance and reproducibility of the Abbott RealTi*m*e High Risk (HR) HPV test compared with that of the HR hybrid capture 2 (HC2) assay as assessed by a noninferiority score test. A random sample of 998 cervical specimens (914 specimens of cervical intraepithelial neoplasia less severe than grade 2 [<CIN2] and 84 specimens of cervical intraepithelial neoplasia grade 2 or more severe lesions [≥CIN2]), collected in the Florence and Catania cervical Cancer Screening Programs from women aged 25 to 64 and already tested by HR HC2, were retested with the Abbott RealTi*m*e HR HPV test. Absolute specificity was 92.3% (95% confidence interval [CI], 90.4 to 94.0) and 92.6% (95% CI, 90.7 to 94.2) for the Abbott RealTi*m*e HR HPV test and the HR HC2, respectively. Absolute sensitivity was 96.4% (95% CI, 89.9 to 99.3) and 97.6% (95% CI, 91.7 to 99.7) for the Abbott RealTi*m*e HR HPV test and the HR HC2, respectively. The noninferiority score test revealed that the clinical sensitivity and specificity of the Abbott RealTi*m*e HR HPV test were not inferior ($P = 0.004$ and 0.009, respectively) to those of HR HC2. Overall agreement between the two assays was 96.5%, with a k value of 0.86 (CI 95%, 0.82 to 0.91). We evaluated the intralaboratory reproducibility by retesting 521 samples at least 4 weeks after the first test; the crude agreement between the first and second test was 98.5%, with an overall k value of 0.97 (CI 95%, 0.95 to 0.99). This test fully satisfies the requirements of a primary cervical cancer screening test. This assay differentiates between HPV16, HPV18, and non-HPV16/18 types in every specimen, but how to use this information in a screening setting still is unclear.

The etiologic link between persistent high-risk human papillomavirus (HR HPV) infections and cervical cancer and its immediate precancerous lesions has been widely demonstrated. A recent IARC classification reports solid evidence for a causal link to cervical cancer for only 12 HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59), which are defined as high-risk HPV (2).

Large randomized trials produced sound evidence about the efficacy of screening with an HPV DNA test in reducing cervical cancer incidence (19) and mortality (20). According to trial results, an HPV test used as a cervical cancer screening test has three advantages: a higher long-term negative predictive value (NPV) that permits extending the screening interval without increasing the interval risk of cancer, a clinical sensitivity of 90 to 95% for cervical intraepithelial neoplasia grade 2 or 3 (CIN2/3) (1, 3, 6, 7, 13, 17, 18), and a marked reduction of CIN2/3 and cancer among test-negative women in the subsequent screening round (19).

Several studies (12, 21) suggest that infections supported by HPV16 and HPV18 are associated with a higher risk for the progression of cervical cancer. Consequently, the genotyping of HPV16 and HPV18 has been proposed to guide the management of HPV-positive women throughout the follow-up procedures (12). Usually, viral tests are used to understand the etiology of symptomatic diseases. However, the HR HPV test in screening is aimed at preventing cervical cancer in an asymptomatic population, therefore it is useful only when it is able to detect clinically relevant infections. In other words, HPV testing for screening purposes needs optimal balance between clinical sensitivity and specificity. At present, the HPV assays considered clinically validated for screening purposes are the hybrid capture 2 HPV test (HC2) and the GP5+/6+-PCR enzyme immunoassay (EIA) (24). New candidate assays should prove their value in large prospective screening studies or should prove to be noninferior to a validated reference assay in clinical equivalence studies on specimens from a cervical screening cohort. An international consortium recently published guidelines (14) defining the appropriate study design and sample size to measure the sensitivity, specificity, and reproducibility of a new HPV DNA test to validate it for screening.

In accordance with those guidelines, the aim of this study was to assess the clinical specificity and sensitivity compared to the hybrid capture 2 (HC2) HR HPV assay and the intralaboratory reproducibility of the Abbott RealTi*m*e High Risk HPV, a new method that is able to detect 12 HR HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) and 2 uncertain-risk HPV types (66 and 68).

Moreover, because the Abbott RealTi*m*e HR HPV test allows partial typing (HPV16 and HPV18 versus other risk types), we evaluated the accuracy of typing test results in samples with infections of known types.

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MATERIALS AND METHODS

Study population. The study was based on a sample of 998 women collected in the Florence and Catania Cervical Cancer Screening Programs, which regularly invite all resident women aged 25 to 64 years.

To assess the noninferiority of the specificity of the Abbott RealTi*m*e HR HPV test (i.e., relative specificity was not lower than 98%), 914 samples (median subject age, 44.6 years) without CIN2 or with more severe lesions were tested. The samples were randomly selected, according to Meijer and colleagues (14), from a population-based screening population recruited in two short periods, June to September 2008 in Catania and November 2009 to January 2010 in Florence, during which women were tested with the HR HC2 test. According to the screening protocol, women were always directly referred to colposcopy if the HR HC2 was positive (≥1 relative light unit [RLU]) or cytology was atypical squamous cells of undetermined significance (ASCUS) or more severe.

To assess the noninferiority of the sensitivity (i.e., the relative sensitivity was not lower than 90%), 84 cervical scrapes (median subject age, 35 years) with histologically confirmed cervical intraepithelial neoplasia grade 2 or more severe lesions (≥CIN2) were tested. All of the samples came from a representative set of women of the same screening population, specifically from women referred to colposcopy for an abnormal Pap test (ASCUS or more) between January 2007 and January 2010 in Florence and in Catania. In both cases, suspicious areas were biopsied, and histology was read locally and was not blind to cytology or HPV results.

The intralaboratory reproducibility was determined by retesting 521 samples, 234 (44.9%) of which were HR HPV positive, 4 to 5 weeks after the first test. These samples came from the same screening programs, recruited between January 2007 and January 2010, to have a sufficient number of HR HC2-positive samples, as suggested by Meijer et al. (14).

During the entire study period (2007 to 2010 in Florence and 2008 in Catania), informed consent was obtained from all study participants, and this study followed local ethical guidelines.

All of the samples were selected to evaluate the sensitivity, specificity, and reproducibility of the new test by closely following the recommendations reported by Meijer et al. (14).

Laboratory procedures. At enrollment, cervical specimens were collected using a broom-like device (cervical sampler; Digene Corporation, Gaithersburg, MD) and put in specimen transport medium (STM; Digene Corporation). An aliquot of 800 μl of STM was used for HR HC2, while 200 μl of each STM specimen was stored at −80°C in the ISPO biological bank for future studies (8).

HC2 test. At enrollment, all samples were tested by HR HC2 assay (Qiagen, Gaithersburg, MD), according to the manufacturer's protocol, in the same laboratory in Florence. We used only probe mix B, which is specific for 12 high-risk HPV types, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59, and for 1 uncertain-risk (IR) HPV type, HPV68, which was classified (2) as probably carcinogenic. HC2 is a sandwich capture molecular hybridization assay that utilizes chemiluminescent detection to provide a semiquantitative result. Briefly, after denaturation, the single-stranded HPV DNA present in the sample was hybridized with a mixture of single-stranded full-genomic-length RNA probes specific for 13 HR HPV genotypes. The RNA-DNA hybrids then were captured on the surface of an antibody-coated microtiter plate. Immobilized hybrids are detected by adding an alkaline phosphatase-conjugated antibody to RNA-DNA hybrids, followed by the addition of a chemiluminescent substrate that is cleaved by the action of alkaline phosphatase to produce light. The emission of light is measured semiquantitatively as RLU in a luminometer. The assay is calibrated to detect approximately 4,700 genome equivalents (or 1 pg/ml) of HPV target, represented by an RLU measurement of greater than or equal to the cutoff value calculated in each run by a series of standards. Measurements below the cutoff were scored as negative. Positive and negative controls (provided by the manufacturer) were included in each run.

Abbott RealTi*m*e HR HPV test. The Abbott RealTi*m*e HR HPV test (Abbott, Wiesbaden, Germany) was performed in a Florence (ISPO) laboratory. The Abbott RealTi*m*e HR HPV test is a qualitative *in vitro* test for the detection of DNA from 12 high-risk human papillomavirus genotypes, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59, and from 2 HPVs recently classified as uncertain-risk types: HPV68 has been classified as probably carcinogenic to humans and HPV66 as possibly carcinogenic by the IARC Monograph Working Group (2).

For each sample, 25 μl was added to 475 μl *m*Lysis$_{DNA}$ (Abbott *m*Sample Preparation System$_{DNA}$; Abbott, Wiesbaden, Germany), of which 400 μl was used for DNA extraction.

DNA was isolated using the Abbott *m*2000sp. automated sample preparation system using magnetic particles to capture the nucleic acid (Abbott *m*Sample Preparation System$_{DNA}$ for RealTi*m*e HR HPV; Wiesbaden, Germany). The Abbott *m*2000sp. automatically prepares the master mix and transfers DNA isolated from samples and a master mix volume to a 96-well optical reaction plate. The master mix (Abbott RealTi*m*e HR HPV amplification reagent kit; Wiesbaden, Germany) contains a modified GP5+/6+ primer mix consisting of three forward primers and two reverse primers targeting the conserved L1 region of HPV and an internal control primer pair targets a human beta-globin sequence. The Abbott RealTi*m*e HR HPV test provides four results: the qualitative detection of 14 HPV types (12 HR HPV and 2 uncertain risk [IR] HPV), HPV16 and HPV18 genotyping, and the evaluation of an internal control (human beta-globin). HPV16- and HPV18-specific probes and a probe for human beta-globin are labeled with different dyes, while the other HR HPV probes (for HPV types 31, 33, 35, 39, 45, 51, 52, 56, 56, and 59) and uncertain-risk probes (HPV66 and HPV68) are labeled with the same dye.

One positive control (linearized DNA plasmid with HPV16, HPV18, HPV58, and human beta-globin sequences) and one negative control (plasmid DNA with human beta-globin sequences) are included in each run and are processed using the same sample preparation procedures as those for the specimens. The system will validate the run only if the negative control shows a signal in the Cy5 signal channel and the positive control shows four different signals for HPVs and the internal control.

After the plate is sealed, it is placed manually on an Abbott *m*2000*rt* for PCR amplification.

The HPV target cutoff (32.00 cycle threshold [Ct]) as well as the internal control target cutoff (35.00 Ct) is already established by the manufacturer, and samples with insufficient content of cervical samples are automatically invalidated.

HPV typing. All HPV-positive samples (positive by HR HC2 or by Abbott RealTi*m*e HR HPV test) were typed according to the manufacturer's instructions using GP5+/6+ primers (Ampliquality HPV HS BIO, variant *Single Step*; Ab Analitica, Padua, Italy) that amplify a broad spectrum of HPV genotypes by targeting a 150-bp fragment within the L1 open reading frame (ORF) of the HPV genome. All PCR products were genotyped, regardless of gel result, by reverse-line blot hybridization for the detection of 12 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59), 7 uncertain-risk HPV types (26, 53, 66, 68, 70, 73, and 82), and 10 low-risk HPV types (6, 11, 40, 42, 43, 44, 54, 61, 72, and 81) according to the manufacturer's instructions (Ampliquality HPV type; Ab Analitica, Padua, Italy).

All PCR- and reverse line blot (RLB)-negative samples were amplified with a nested PCR with the MY09/11 primer pair and GP5+/6+ inner primer pair, followed by reverse-line blot hybridization as reported above (16). All untyped samples (PCR-positive but RLB-negative samples) underwent direct Sanger sequencing of the GP5+/6+ PCR product using BigDye Terminator 1.1 chemistry (Applied Biosystems) on an ABI 310 genetic analyzer (Applied Biosystems) to identify a specific HPV type probably not included in our set of probes. A sequence was considered a match if it had >90% nucleotide similarity to an HPV sequence in GenBank. The HPV16 E7 PCR assay (25) was used for samples with discordant HPV16 results between the Abbott RealTi*m*e HR HPV test and HPV genotyping. The final results of these typing processes were considered the gold standard to measure the analytical accuracy of the Abbott RealTi*m*e HR HPV and HR HC2 tests.

Analytical sensitivity of Abbott RealTi*m*e HR HPV test. The analytical sensitivity to HPV16 of the Abbott RealTi*m*e HR HPV test at a cutoff value established by the manufacturer was further evaluated in our laboratory using the HR HC2-positive calibrator (high-risk HPV calibrator [1pg/ml]; cloned HPV16 DNA and carrier DNA in STM with 0.05% [wt/vol of sodium azide, equivalent to 100,000 copies of HPV16/ml) at three different dilutions (500 HPV16 copies/test, 400 HPV16 copies/test, and 320 HPV16 copies/test) using five replicates in five different experiments. The HR HC2 calibrator permits the analysis of the whole procedure of the Abbott RealTi*m*e HR HPV assay, from extraction to DNA amplification.

Statistical analysis. To compare the clinical sensitivity and specificity for ≥CIN2 of the Abbott RealTi*m*e HR HPV test to that of HR HC2, a noninferiority score test ($P < 0.05$) was performed (22). The thresholds used for noninferiority were 90 and 98% for relative sensitivity and specificity, respectively. The thresholds were recommended by previously published guidelines (14) and guarantee much higher negative predictive value than cytology and a limited number of false-positive test results. The level of agreement was determined using kappa statistics (Cohen's *k*) (5). All estimates are presented with their 95% confidence intervals.

Receiver operating characteristic (ROC) curves were elaborated to evaluate the effect of the cycle number cutoff setting on test performance for the detection of ≥CIN2 samples.

TABLE 1. Comparison of the Abbott RealTi*m*e HR HPV and the HR Hybrid Capture 2 findings stratified for controls and cases

Sample	Assay result (no. of samples)			Total
	Abbott RealTi*m*e HR HPV	HR HPV HC2		
		+	−	
Controls	+	52[c]	18	70
	−	16	828[c]	844[a]
Total		68	846[b]	914
Cases (≥CIN2)	+	81[f]	0	81[d]
	−	1	2[f]	3
Total		82[e]	2	84

[a] Absolute specificity for the Abbott RealTi*m*e HR HPV test was 92.3% (95% CI, 90.4 to 94.0).
[b] Absolute specificity for HR HC2 was 92.6% (95% CI, 90.7 to 94.2).
[c] Overall agreement for controls was 96.3% with a *k* value of 0.73 (95% CI, 0.65 to 0.82).
[d] Absolute sensitivity for the Abbott RealTi*m*e HR HPV test was 96.4% (95% CI, 89.9 to 99.3).
[e] Absolute sensitivity for HR HC2 was 97.6% (95% CI, 91.7 to 99.7).
[f] Overall agreement for cases was 96.4% with a *k* value of 0.79 (95% CI, 0.402 to 1.00).

RESULTS

Clinical sensitivity and specificity. Absolute specificity was 92.3% (844/914; 95% CI, 90.4 to 94.0) and 92.6% (846/914; 95% CI, 90.7 to 94.2) for the Abbott RealTi*m*e HR HPV test and HR HC2, respectively. The relative specificity was 99.8%, and the probability of being less than 98% is 0.009.

Absolute sensitivity was 96.4% (81/84; 95% CI, 89.9 to 99.3) and 97.6% (82/84; 95% CI, 91.7 to 99.7) for Abbott RealTi*m*e HR HPV test and HR HC2, respectively. The relative sensitivity was 98.8%, and the probability of being less than 90% is 0.004.

Overall agreement between the two assays was 96.5% (963/998), with a *k* value of 0.86 (95% CI, 0.82 to 0.91). Agreement on the 914 control samples without high-grade lesions was 96.3% (880/914), with a *k* value of 0.73 (95% CI, 0.65 to 0.82), while the agreement on the 84 cervical samples with histologically confirmed cervical intraepithelial neoplasia grade 2 or more severe lesions was 96.4% (81/84), with a *k* value of 0.79 (95% CI, 0.40 to 1.00) (Table 1).

Among HR HC2-negative samples (<1 relative light unit/cutoff [RLU/CO]), the Abbott RealTi*m*e HR HPV test showed a gradual increase of positivity rate in samples, with higher RLU/CO for the HR HC2 test (Table 2).

TABLE 2. Agreement and disagreement among 998 specimens between the Abbott RealTi*m*e HR HPV and the HR HC2 results by HC2 signal intensity ratio

HR HPV HC2 score (RLU/CO)	No. of agreements between Abbott RealTi*m*e HR HPV and HR HC2 (%)	No. of disagreements between Abbott RealTi*m*e HR HPV and HR HC2	
		HR HC2 negative/Abbott RealTi*m*e HR HPV positive (%)	HR HC2 positive/Abbott RealTi*m*e HR HPV negative (%)
<0.20	467/467 (100)	None	
0.21–0.30	245/246 (99.6)	1/246 (0.4)	
0.31–0.50	92/100 (92.0)	8/100 (8.0)	
0.51–0.99	26/35 (74.3)	9/35 (25.7)	
1–5	23/31 (74.2)		8/31 (25.8)
>5	110/119 (92.4)		9/119 (7.6)

TABLE 3. Results of reproducibility between Abbott RealTi*m*e HR HPV first and second tests among 521 samples[b]

Second test	First test (no. of samples)		Total (no. of samples)
	Positive	Negative	
Positive	234 (44.9)[a]	3 (0.6)	237 (45.5)
Negative	5 (1.0)	279 (53.5)[a]	284 (54.5)
Total	239 (45.9)	282 (54.1)	521 (100)

[a] The agreement between the first and second test was 98.46%, with an overall *k* value of 0.97 (CI 95%, 0.95 to 0.99).
[b] Values in parentheses are cell percentages.

Intralaboratory reproducibility. We evaluated the Abbott RealTi*m*e HR HPV test reproducibility with 521 specimens, 44.9% of which tested positive by the HR HC2 test. These samples were repeated at least 4 to 5 weeks after the first test. The crude agreement between the first and second test was 98.5% (513/521), with an overall *k* value of 0.97 (CI 95% 0.95 to 0.99). The crude agreement between positive and negative samples was similar, 97.9% (234/239) and 98.9% (279/282), respectively (Table 3).

Evaluation of specimen adequacy. All specimens tested by the Abbott RealTi*m*e HR HPV test had valid beta-globin results (under 35 Ct), suggesting that specimen collection, preparation, and processing (sample extraction and amplification efficiency) were satisfactory. As a result, we did not exclude any result obtained or reprocess any sample.

Results of HPV genotyping with GP5+/6+ RLB hybridization in samples positive by HR HC2 and Abbott RealTi*m*e HR HPV tests. Table 4 shows genotyping results of 168 HR HPV-positive samples (HR HC2 positive and/or Abbott RealTi*m*e HR HPV positive). Among the 150 HR HC2-positive cervical samples, the genotyping results by RLB confirmed HR HC2 HPV target types in 138 (92.0%) samples. Among the 151 RealTi*m*e HR HPV-positive cervical samples, genotyping con-

TABLE 4. Typing with GP5+/GP6+ RLB hybridization in positive HR HPV samples stratified by type of test

Classification	No. (%) HR HC2 positive (out of 150)	No. (%) Abbott RealTi*m*e HR HPV positive (out of 151)
HR positive[a,b]	139 (92.0)	147 (97.3)
False positive	11 (7.3)	4 (2.6)
Uncertain-risk HPV[c]	7	3
LR HPV[d]	2	1
Negative	2	0

[a] HR HPV types included in HC2 probe B were 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.
[b] HR HPV types included in the Abbott RealTi*m*e HR HPV test were 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.
[c] Uncertain-risk HPV types were 26, 53, 67, 70, 73, 82, and 85 (IARC classification).
[d] Low-risk (LR) HPV types were 6, 11, 40, 42, 43, 44, 54, 55, 61, 72, 81, and 84 (IARC classification).

TABLE 5. Comparison between the Abbott RealTi*m*e HR HPV-positive samples (cases and controls together) and typing by GP5+/GP6+ RLB hybridization[d]

Abbott RealTi*m*e HR HPV finding	GP5+/GP6+ RLB hybridization finding[e] (no. of samples)								
	HPV16	HPV16 + other HR HPV	HPV18	HPV18 + other HR HPV	HPV16 + HPV18	Other HR HPV	Uncertain-risk HPV[b]	LR HPV[c]	Negative
HPV16	**36**	0	0	0	0	0	0	0	0
HPV16 + other HR HPV	**4**	**8**	0	0	0	1	0	0	0
HPV18	0	0	**8**	0	0	0	0	0	0
HPV18 + other HR HPV	0	0	**2**	0	0	0	0	0	0
HPV16 + HPV18	0	0	0	0	**1**	0	0	0	0
Other HR HPV	1[a]	5[a]	0	0	0	**81**	3	1	0

[a] All were confirmed to be HPV16 by PCR with HPV16 E6/E7 primers.
[b] Uncertain-risk HPV types were 26, 53, 67, 70, 73, 82, and 85 (IARC classification).
[c] Low-risk (LR) HPV types were 6, 11, 40, 42, 43, 44, 54, 55, 61, 72, 81, and 84 (IARC classification).
[d] Other HR HPV types included in the Abbott test were 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.
[e] Numbers in boldface are concordant samples; underlined numbers are discordant samples.

firmed Abbott RealTi*m*e HR HPV target types in 148 (98.0%) samples.

Among all 998 samples analyzed, 35 (3.5%) showed discordant results by the two HPV methods (HR HC2 and Abbott RealTi*m*e HR HPV tests). Among 18 (1.8%) HR HC2-negative/Abbott RealTi*m*e HR HPV-positive samples, the proportion of Abbott RealTi*m*e HR HPV target types was 83.3% (15/18). Among 17 (1.7%) HR HC2-positive/Abbott RealTi*m*e HR HPV-negative samples, HR HC2 HPV target types were identified in 41.2% of samples (7/17). It is interesting that two ≥CIN2 specimens that were HR HPV negative with the Abbott RealTi*m*e HR HPV test and with HR HC2 were HPV positive only in nested PCR; typing by RLB showed HPV52 and HPV58, respectively. The ≥CIN2 specimen that was HPV negative by the Abbott RealTi*m*e HR HPV test and positive by HR HC2 was HPV negative by all PCR systems used for typing.

Table 5 compares the results of partial genotyping by Abbott RealTi*m*e HR HPV test with GP5+/GP6+ RLB hybridization typing. Among 49 HPV16 infections (both single infections and those with other HR HPV) identified by Abbott RealTi*m*e HR HPV partial genotyping, 48 (98.0%) were confirmed by RLB. Similarly, all HPV18 samples positive by the Abbott RealTi*m*e HR HPV test were confirmed by RLB. Among 91 samples classified by the Abbott RealTi*m*e HR HPV test as other HR HPV types, 81 (89.0%) were confirmed as other HR HPV types by RLB, 5 samples were identified by RLB as coinfections with HPV16 and other HR HPV types, and one sample was classified by RLB as HPV16; HPV16 in these six samples also was confirmed by typing with HPV16 E7-specific primers.

The analytical sensitivity of HPV16 and HPV18 Abbott RealTi*m*e HR HPV typing was 88.1% (59/67) (95% CI, 77.8 to 94.7), while the analytical specificity of the partial genotyping of Abbott RealTi*m*e HR HPV with HPV-positive samples was 98.8% (85/86) (95% CI, 93.7 to 100).

Abbott RealTi*m*e HR HPV test ROC curves. Considering CIN2 or more severe lesions as the final outcome, the ROC curve analysis indicates that the percentage of samples classified correctly was higher with a 32 Ct cutoff (accuracy, 94.4%) than with a 30 Ct cutoff (accuracy, 93.7%). Indeed, in changing the cutoff cycle number from ≤32 to ≤30 there is a slight gain in specificity, 93.4% versus 92.3%, that leads to a reduction in false-positive samples from 18 to 11 and a reduction in sensitivity from 96.4 to 94%. These data suggest that there is no increase in test performance by decreasing the cutoff to 30 cycle numbers to increase test performance for the detection of ≥CIN2 lesions.

Abbott RealTi*m*e HR HPV analytical sensitivity. Five replicates for every HR HC2 calibrator dilution (500 HPV16 copies/test, 400 HPV16 copies/test, and 320 HPV16 copies/test) have been tested in five different experiments. The samples containing 500 HPV16 copies/test showed 100% detection of HPV16 in all five experiments, while samples with 400 HPV16 copies/test and 320 HPV16 copies/test showed a reproducibility of less than 95%.

DISCUSSION

The aim of the present study was to compare the clinical accuracy of the Abbott RealTi*m*e HR HPV test with a clinically validated reference HPV test, hybrid capture 2, on samples from women enrolled in an organized screening program. The results show that the clinical sensitivity of the Abbott RealTi*m*e HR HPV test and specificity for ≥CIN2 are almost identical to those of HR HC2. In fact, the Abbott RealTi*m*e HR HPV test showed 98.8% relative sensitivity for ≥CIN2 and a 99.8% specificity relative to that of HR HC2. For the relative sensitivity and specificity for ≥CIN2, the Abbott RealTi*m*e HR HPV test has probabilities of 0.996 and 0.991, values that differ by less than 10 and 2%, respectively, from the thresholds fixed by the guidelines for HPV DNA test requirements for primary cervical cancer screening (14), confirming previous studies (7, 11, 23). It must be noted that relative specificity may be slightly underestimated. Since the management of the women in the screening program was driven by the HR HC2 test and cytology and not by the Abbott RealTi*m*e HR HPV, some of the HR HC2-negative/Abbott RealTi*m*e HR HPV-positive women in the specificity sample may have an undetected ≥CIN2. Nevertheless, the rationale of the guidelines of Meijer et al. is to be conservative in the estimates. This does not apply to the relative sensitivity estimate, since the ≥CIN2 all were cytology positive independently from the HR HC2 result.

Another requirement of a screening test, especially if it is applied as the primary test in large programs, is reproducibility. The Meijer et al. guidelines also recommend evaluating

intra- and interlaboratory reproducibility on at least 500 specimens, 30% of which tested positive for a clinically validated test. The Abbott RealTi*m*e HR HPV test showed good reproducibility of results, with a *k* value of 0.97, much above the *k* value of 0.5 indicated by the guidelines.

Moreover, if the Abbott RealTi*m*e HR HPV assay is used for cervical cancer screening, the contemporary amplification of an internal control target sequence will be very important for the HPV-negative women who will be advised to repeat the test after 3 to 5 years. This added value could be essential if screening by self-sampling is introduced in the cervical cancer screening for nonscreened women.

Overall, high agreement (96.5%) for HR HPV detection was observed between the two assays, as observed in previous studies (9, 15). The agreement on misclassified cases indicates that HPV DNA-negative ≥CIN2 are not due simply to a technical malfunctioning of the test that randomly reduces analytical sensitivity but probably share characteristics reducing the probability of detecting DNA by any means. On the other hand, the agreement on misclassified controls is expected according to what we know about the natural history of the disease, and it reflects all of the infections that do not cause any high-grade lesions.

Even though several studies evaluating the clinical performance of the Abbott RealTi*m*e HR HPV test have been published recently (7, 9, 10, 11), our study is, to our knowledge, the first application of Meijer's guidelines to this new HPV detection system. Cuzick et al. (7), for example, compared the Abbott RealTi*m*e HR HPV test to other tests, including the HR HC2, in women with abnormal cervical cytology smears, but the subjects were not from a screening population. Their results suggested that the Abbott RealTi*m*e HR HPV test is a promising candidate for use in primary screening, but further study is needed to evaluate its performance directly in a screening contest.

Compared with the results of our typing gold standard, Abbott RealTi*m*e HR HPV showed higher clinical specificity than HC2 for HR HPV infections; the high clinical specificity has been observed already by Poljak et al. (15). High analytical specificity is one of the only necessary conditions for high clinical specificity and by itself does not guarantee good performance in screening.

Abbott RealTi*m*e HR HPV assay also provides HPV16 and HPV18 typing. Comparing Abbott RealTi*m*e HR HPV partial typing results to our gold standard typing, the specificity for HPV16 or HPV18 was very high (98.8%), but the sensitivity was lower (88.1%). In particular, we observed less sensitivity of the Abbott RealTi*m*e HR HPV test for HPV16 when HPV16 was a coinfection with other HR HPVs. In fact, out of 13 women infected with HPV16 and other HR HPV types according to gold standard typing, only 8 were correctly identified by the Abbott RealTi*m*e HR HPV test; the other 5 samples were classified as other HR HPV types by the Abbott RealTi*m*e HR HPV test. These samples probably had a lower HPV16 viral load, below the Abbott RealTi*m*e HR HPV cutoff, compared to those of the other HPV types that were identified. It is interesting that none of these five samples was ≥CIN2, possibly confirming that the established cutoff correlates with clinical results. Our results about analytical sensitivity for HPV16 are extremely consistent with those obtained by Huang et al. (10) with similar methods. This is not surprising, since the analytical accuracy of many HPV DNA tests have been demonstrated to be highly consistent among laboratories (4).

Recently, Kjær et al. (12) showed that the main predictor of the subsequent risk of CIN3 or worse was HPV16 persistence, indicating the potential value of partial genotyping in cervical cancer screening, although several issues remain to be resolved before HPV16 persistence can be used in a primary screening program.

In conclusion, the Abbott RealTi*m*e HR HPV test showed relative sensitivity and specificity for ≥CIN2 that were very close to those of the HR HC2 test, and it had good reproducibility. This test fully satisfied the requirements for a primary cervical cancer screening test. Further investigations are needed to understand the clinical utility and accuracy of identifying HPV16 and HPV18 types in a screening setting.

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