JCM
Journals.ASM.org

Clinical Evaluation of the Cartridge-Based GeneXpert Human Papillomavirus Assay in Women Referred for Colposcopy

Mark H. Einstein,[a] Katherine M. Smith,[b] Thomas E. Davis,[c] Kathleen M. Schmeler,[d] Daron G. Ferris,[e] Ashlyn H. Savage,[f] Jermaine E. Gray,[g] Mark H. Stoler,[h] Thomas C. Wright, Jr.,[i] Alex Ferenczy,[j] Philip E. Castle[k]

Montefiore Medical Center and Albert Einstein College of Medicine, Bronx, New York, USA[a]; Oklahoma University Health Sciences Center, Oklahoma City, Oklahoma, USA[b]; Indiana University, Indianapolis, Indiana, USA[c]; The University of Texas MD Anderson Cancer Center, Houston, Texas, USA[d]; Georgia Regents University, Augusta, Georgia, USA[e]; Medical University of South Carolina, Charleston, South Carolina, USA[f]; University of Mississippi Medical Center, Jackson, Mississippi, USA[g]; University of Virginia, Charlottesville, Virginia, USA[h]; Columbia University, New York, New York, USA[i]; McGill University and Jewish General Hospital, Montreal, Quebec, Canada[j]; Global Cancer Initiative, Chestertown, Maryland, USA[k]

High-risk human papillomavirus (hrHPV) testing is now being introduced as a potential primary screening test for improved detection of cervical precancer and cancer. Current U.S. Food and Drug Administration-approved tests are batch tests that take several hours to complete. A rapid, non-batch test might permit point-of-care (POC) testing, which can facilitate same-day screen and management strategies. For a non-batch, random-access platform (GeneXpert; Cepheid, Sunnyvale, CA), a prototype hrHPV assay (Xpert) has been developed where testing for 14 hrHPV types can be completed in 1 h. In the first clinical evaluation, Xpert was compared to two validated hrHPV tests, the cobas HPV test (cobas, Roche Molecular Systems) and Hybrid Capture 2 (hc2, Qiagen), and to histologic outcomes using specimens from colposcopy referral populations at 7 clinical sites in the United States ($n = 697$). The sensitivity of Xpert for cervical intraepithelial neoplasia grade 2 or more severe diagnoses (CIN2+) ($n = 141$) was equal to that of cobas (90.8% versus 90.8%, $P = 1$) and greater than that of hc2 (90.8% versus 81.6%, $P = 0.004$). Xpert was more specific than cobas (42.6% versus 39.6%, $P = 0.02$) and less specific than hc2 (42.6% versus 47.7%, $P < 0.001$). Similar results were observed for cervical intraepithelial neoplasia grade 3 or higher (CIN3+) ($n = 91$). HPV16 detection by Xpert identified 41.8% of the CIN2+ specimens with a positive predictive value (PPV) of 54.6%. By comparison, HPV16 detection by cobas identified 42.6% of the CIN2+ specimens with a PPV of 55.0%. hrHPV detection by the Xpert demonstrated excellent clinical performance for identifying women with CIN2+ and CIN3+ that was comparable to that of currently available clinically validated tests.

There is now significant evidence that molecular testing for the ~15 high-risk human papillomavirus (hrHPV) types that cause virtually all cervical cancer is more sensitive and less specific for the detection of cancer, cervical intraepithelial neoplasia grade 2 (CIN2), more-severe CIN2 (CIN2+), or CIN3+ than cervical cytology (1–7). hrHPV testing and the associated treatment for high-grade disease can reduce the risk of incident cervical cancer within 4 to 5 years (5) and the risk of cervical cancer-related death within 8 years (7). Because hrHPV testing is more sensitive than cervical cytology for cervical precancer and cancer, a negative hrHPV result provides more-robust information regarding the absence of incident cervical precancer and cancer (8, 9).

hrHPV testing is now recommended for cervical cancer screening in several evidence-based guidelines. hrHPV and cervical cytology "cotesting" every 5 years in women 30 and older is recommended in the United States (10). The World Health Organization recently recommended hrHPV testing for cervical cancer screening in places where cervical cytology has not been established (http://apps.who.int/iris/bitstream/10665/94830/1/9789241548694_eng.pdf). Several countries are now in the process of considering or performing evaluations for modifying a program relying on cervical cytology to incorporate hrHPV testing (11).

There are 4 U.S. Food and Drug Administration (FDA)-approved hrHPV tests: Hybrid Capture 2 (hc2; Qiagen, Germantown, MD) (2003), Cervista (Hologic, Bedford, MA) (2009), the cobas HPV test (cobas; Roche Molecular Systems, Pleasanton, CA) (2011), and Aptima (Gen-Probe/Hologic, San Diego, CA) (2011). All are batch tests that take several hours to complete.

The Cepheid Xpert HPV assay (referred to as "Xpert" here) is a new, qualitative, real-time PCR assay for the detection of hrHPV DNA. The assay is formatted in a single-use GeneXpert test cartridge and is run on the Cepheid GeneXpert system, a multianalyte, random access, molecular-diagnostic platform ranging in capacity from 1 to 80 test processing modules. Importantly, a single hrHPV DNA test can be completed in 1 h, permitting same-day screening, diagnosis, and treatment which reduce the potential loss to follow-up in lower-resource settings and permit decentralized, clinic-based (versus lab-based) testing in higher-resource settings.

To identify the preliminary clinical cutoffs for Xpert and compare performance to that of two benchmark assays, cobas and hc2, a study of hrHPV detection was conducted on cervical specimens collected from women undergoing colposcopy for an abnormal

Received 22 January 2014 **Returned for modification** 27 February 2014
Accepted 30 March 2014
Published ahead of print 9 April 2014
Editor: A. M. Caliendo
Address correspondence to Mark H. Einstein, meinstei@montefiore.org.
Supplemental material for this article may be found at http://dx.doi.org/10.1128/JCM.00176-14.

doi:10.1128/JCM.00176-14
The authors have paid a fee to allow immediate free access to this article.

cervical cytology result. The results of the three tests were compared to each other for the detection of hrHPV and to the severity of disease as determined by biopsy-confirmed diagnoses. The clinical parameters for each test for detection of women with cervical precancer and cancer were calculated.

MATERIALS AND METHODS

Study population and design. This study was a two-stage, multicenter (7 U.S. sites), prospective study that enrolled women of all ages referred for colposcopy evaluation based on one or more prior abnormal Pap test results or an abnormal Pap test result in combination with a positive hrHPV test result or other clinical suspicion of cervical cancer. Two Pap specimens (specimen A and specimen B) were collected and placed into ThinPrep (Hologic) collection vials from each subject immediately before colposcopy. Specimen A was processed for cytology review followed by analysis with Xpert. Specimen B was reserved for comparator hrHPV analysis with hc2, cobas, and, finally, Xpert. Both specimens were collected using an endocervical brush/spatula combination per the ThinPrep package insert instructions. A minimum of two cervical biopsy specimens were collected from each subject as well as an endocervical curettage (ECC) in cases of unsatisfactory colposcopy results. The study was approved by the Institutional Review Board at each participating site.

Laboratory testing. Xpert includes reagents for the simultaneous detection of 13 hrHPV types (HPV16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, and -68) and 1 possible hrHPV type (HPV66), a human reference gene (HMBS [hydroxymethylbilane synthase]), and an internal Probe Check Control (PCC). The 14 targeted HPV types are detected in five fluorescent channels: fluorescent channel 1 (HPV16), fluorescent channel 2 (HPV18 and -45) ("HPV18/45"), fluorescent channel 3 (HPV31, -33, -35, -52, and -58), fluorescent channel 4 (HPV51 and HPV59), and fluorescent channel 5 (HPV39, -56, -66, and -68). The human reference gene (fluorescent channel 6) verifies specimen adequacy. The PCC verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability. In total, the assay utilizes six fluorescent channels for the detection of individual types of HPV, groups of HPV, and the human reference gene. Each fluorescent channel has its own cutoff parameters for target detection/validity. If sufficient signal is detected by the human reference gene (i.e., if the sample has sufficient cellularity), the assay results are reported as an overall "positive" if any type of targeted HPV is detected, but, additionally, HPV16 and HPV18/45 and, collectively, the other high-risk HPV types detected by the assay are reported specifically as "positive" or "negative." The Xpert HPV test result from specimen B was used for analyses described below.

Stage I recruited 144 subjects with 31 cases of CIN2+. Data from stage I were used to estimate a set of clinical cutoffs for the assay relative to CIN2+ (and separate analysis of CIN3+) disease endpoints using a receiver operating characteristic (ROC) approach. Stage II recruited 564 subjects with 111 cases of CIN2+. Data from stage II were used to refine the clinical cutoffs relative to CIN2+ (and CIN3+) disease endpoints also using an ROC approach (see Table S1 in the supplemental material).

Retrospectively, a homogeneity analysis was conducted to confirm that the results from stage I and stage II could be pooled. This analysis examined subject population characteristics (racial/ethnic group and subject age) and specimen properties (cytology status and the threshold cycle [C_T] distributions of the human reference gene and the HPV targets). Stage I had a statistically greater proportion of African-American subjects than stage II (54.2% versus 41.7%; Fisher's exact P value < 0.05). Stage II had a statistically larger proportion of Hispanic or Latina subjects than stage I (25.9% versus 11.1%; Fisher's exact P value < 0.05). However, these two racial/ethnic groups are well represented in both stages, comprising ~60% of each study population, and thus, the statistical difference was deemed to represent a minimal risk with respect to combining across stages. Other than these two racial/ethnic groups, stage I and stage II had statistically similar populations (Fisher's exact P value $= 0.1$). Stage II had a statistically greater proportion of subjects over the age of 60 than stage I (14.5% versus 4.2%; Fisher's exact P value $= 0.01$). For subjects under the age of 60, stage I and stage II had statistically equivalent distributions of the various age groups (Fisher's exact P value $= 0.6$). Stage I and stage II were statistically equivalent in the proportions of subjects represented in each cytology category (Fisher's exact P value $= 0.16$). The mean, minimum, and maximum C_T values observed in each reporting channel of Xpert were relatively consistent between stage I and stage II of the study. In a test for similarity of the mean C_T values for the channels, only the mean C_T value for HMBS was statistically different ($P < 0.0001$) between stage I (mean C_T, 30.0) and stage II (mean C_T, 31.0). The difference in mean C_T values between stage I and stage II has little impact on study findings, as both mean C_T values were substantially removed from the maximum C_T value for the channel that defines specimen adequacy. The subject population characteristics and the specimen properties from stage I and stage II indicate that the data from these stages could be pooled for the analyses presented below.

Xpert results were compared to the results from hc2 testing and cobas testing, which were conducted per their respective U.S. *in vitro* diagnostic (US-IVD) package inserts. hc2 testing was performed at the Wishard Hospital (Indianapolis, IN) (T.E.D.) and cobas testing was performed at Purdue University (Indianapolis, IN) (B. van der Pol). cobas targets the same 14 HPV types targeted by Xpert. hc2 is a signal amplification DNA test for the same 13 hrHPV types as Xpert and cobas and is known to detect HPV66 due to cross-reactivity (12). For this analysis, we assumed that all 3 tests detect 14 hrHPV types. All testing was done blind to the histological diagnosis or the results of the other hrHPV tests.

Test results for Xpert were categorized hierarchically according to *a priori* cancer risk: (i) HPV16 positive or (ii) HPV16 negative and HPV18/45 positive or (iii) HPV16 and HPV18/45 negative and positive for other hrHPV types or (iv) hrHPV negative. Likewise, results for cobas were categorized as follows: (i) HPV16 positive or (ii) HPV16 negative and HPV18 positive or (iii) HPV16 and HPV18 negative and positive for other hrHPV types or (iv) hrHPV negative.

Consensus pathology diagnoses of CIN2+ that tested negative by any hrHPV test in stage I and stage II of the study were subjected to further molecular analysis to describe the nature or cause of the apparent false-negative result. These biopsy specimens underwent $p16^{INK4a}$ immunohistochemistry analysis (CINtec; Roche and Ventana Medical Systems, Inc., Tucson, AZ). ThinPrep specimens underwent a two-stage HPV genotyping at an independent laboratory (K. Cuschieri) with the Linear Array HPV Genotyping Test (LA; Roche Molecular Systems, Pleasanton, CA) for 37 HPV genotypes, including the 14 HPV types detected by the clinical assays being evaluated (13–15). Specimens negative by LA were retested by a multiplex, laboratory-developed bead array assay with higher analytical sensitivity.

Pathology review. A pathology review of the biopsy and ECC specimens was first made locally for standard of care/patient management and then retrospectively, in a blind fashion, by a panel of three expert review pathologists (M.H.S, T.C.W., and A.F.) to determine a consensus final cervical disease status, which was used in this analysis.

Statistical analysis. The study targeted the recruitment of 150 CIN2+ cases, which with a clinical sensitivity of 92% for CIN2+ would result in a 95% confidence interval of approximately ±6%, i.e., 86% to 98%. The number of subjects required to achieve the sample size of cases was dependent upon disease prevalence in the study population. In women with atypical squamous cell of undetermined significance (ASC-US) or low-grade squamous intraepithelial lesion (LSIL) cytology referred to immediate colposcopy in the ASCUS-LSIL Triage Study (ALTS) ($n = 1{,}163$), 15.3% had a consensus pathology review diagnosis of CIN2+ at enrollment (16). In women with only one mildly dyskaryotic smears referred for colposcopy in the United Kingdom ($n = 510$), the overall prevalence of CIN2+ was 28.7% (17). In a preclinical validation study of an mRNA-based hrHPV assay in a colposcopy referral population (18), the prevalence of CIN2+ was 18.7%. Given the variability in CIN2+ prevalence in different populations, the *a priori* target recruitment was 600 to 1,000

TABLE 1 Sociodemographics of the 697 women referred to colposcopy and included in this analysis

Parameter	No. of women	%
Clinical site		
1	117	16.8
2	69	9.9
3	151	21.7
4	109	15.6
5	81	11.6
6	121	17.4
7	49	7.0
Total	697	100
Race or ethnicity		
American Indian/Alaskan native	13	1.9
Asian	5	0.7
Black or African American	308	44.2
Hispanic or Latina	158	22.7
White, not Hispanic or Latina	203	29.1
Other	10	1.4
Total	697	100
Age group (yrs)		
18–29	244	35.0
30–39	137	19.7
40–49	139	19.9
50–59	89	12.8
60–75	88	12.6
Total	697	100

women referred to colposcopy, corresponding to 25% to 15% CIN2+ prevalence, respectively.

For the three pairwise comparisons of hrHPV detection by the three hrHPV tests, kappa values with 95% confidence intervals (95% CI), percent total agreement, and percent positive agreement were calculated. Binomial 95% CI values were calculated for the percent test positive. Differences in percent test positive were tested for statistical significance ($P < 0.05$) using an exact version of the McNemar chi-square test. The percent positive for each histologic diagnosis was calculated. Differences in percent positive for a histologic diagnosis were tested for statistical significance using a Pearson chi-square test.

The clinical performance parameters of sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV), odds ratios (OR), and positive likelihood ratios (PLR) and negative likelihood ratios (NLR) for cervical intraepithelial neoplasia grade 2 (CIN2) or more-severe CIN2 (CIN2+) and grade 3 (CIN3) or more-severe CIN3 (CIN3+) were calculated for all three tests. Pairwise differences in sensitivity and specificity between tests were tested for statistical significance using an exact version of the McNemar chi-square test. Clinical performance parameters were stratified by age groups (18 to 29 years, 30 to 39 years, and 40 to 75 years and older). The risk of CIN2+ and CIN3+ was calculated for hierarchical HPV risk groups for Xpert and cobas.

A P value of <0.05 was considered statistically significant. Analyses were done using Excel software and STAT 12.1 (College Station, Texas).

TABLE 3 Distribution of histologic diagnoses and the percentages of positive test results for high-risk human papillomavirus DNA detection by Xpert (Cepheid), cobas (Roche), and hc2 (Qiagen)[a]

Diagnosis	No. of tests	% of tests	Xpert %Pos	cobas %Pos	hc2 %Pos
Negative	418	60.0	50.5	54.8	45.7
CIN1	138	19.8	78.3	77.5	72.5
CIN2	50	7.2	88.0	88.0	84.0
CIN3/AIS	89	12.8	92.1	92.1	79.8
Cancer	2	0.3	100.0	100.0	100.0
<CIN2	556	79.8	57.4	60.4	52.3
CIN2+	141	20.2	90.8	90.8	81.6

[a] Abbreviations: CIN, cervical intraepithelial neoplasia; AIS, adenocarcinoma *in situ*; %Pos, percentage of positive test results.

RESULTS

Of the 708 women recruited into the study, 697 (98.4%) had all three test results and were included in the analysis. Of the 11 women excluded, 9 had indeterminate Xpert results and 2 had indeterminate hc2 results. Table 1 shows the sociodemographics of the 697 women. The number enrolled from each site ranged from 49 (7.0%) from site 7 to 151 (21.7%) from site 3. Most women were African-American (44.2%), followed by white (29.1%) and Hispanic or Latina (22.7%). The mean age, median age, and range of ages were 35.2, 33, and 18 to 75 years, respectively; 35.0% of the women were 18 to 29 years of age, and 12.6% were 60 to 75 years of age.

Table 2 shows the three pairwise results for the three hrHPV tests. The percent hrHPV positive for Xpert was 64.1% (95% CI = 60.4% to 67.7%), for cobas was 66.6% (95% CI = 62.9% to 70.0%), and for hc2 was 58.2% (95% CI = 54.5% to 61.9%). cobas was more likely to test positive than Xpert ($P = 0.02$) and hc2 ($P < 0.0001$), and Xpert was more likely to test positive than hc2 ($P < 0.0001$). The kappa value for Xpert and cobas was 0.84 (95% CI = 0.80 to 0.88), for Xpert and hc2 was 0.73 (95% CI = 0.67 to 0.78), and for cobas and hc2 was 0.67 (95% CI = 0.62 to 0.73).

There were 60.0% of women with negative histology, 19.8% with CIN1, 7.2% with CIN2, 12.8% with CIN3/AIS, and 0.3% with cervical cancer (Table 3); 20.2% had CIN2 or a more severe diagnosis (CIN2+) ($n = 141$). The percent positive increased with increasing severity of diagnosis for all three tests ($p_{trend} < 0.0001$ for all tests); the percent positive for hc2 was consistently lower

TABLE 2 Pairwise comparisons and agreement statistics for high-risk human papillomavirus DNA detection by Xpert (Cepheid), cobas (Roche), and hc2 (Qiagen)[a]

Comparison	No. (%) of results						Total no. of results	Kappa	% total agreement	% positive agreement	P
	Test 1 Pos	Test 2 Pos	Test 1 Pos, test 2 Pos	Test 1 Pos, test 2 Neg	Test 1 Neg, test 2 Pos	Test 1 Neg, test 2 Neg					
Xpert vs cobas	447 (64.1)	464 (66.6)	430 (61.7)	17 (2.4)	34 (4.9)	216 (31.0)	697	0.84	92.7	96.2	0.02
Xpert vs HC2	447 (64.1)	406 (58.2)	381 (54.7)	66 (9.5)	25 (3.6)	225 (32.3)	697	0.73	86.9	85.2	<0.0001
cobas vs HC2	464 (66.6)	406 (58.2)	381 (54.7)	83 (11.9)	25 (3.6)	208 (29.8)	697	0.67	84.5	82.1	<0.0001

[a] An exact version of the McNemar chi-square test was used to test for statistically significant differences ($P < 0.05$) between tests in percentages of positive tests. Abbreviations: Pos, positive; Neg, negative.

TABLE 4 Clinical performance of Xpert (Cepheid), cobas (Roche), and hc2 (Qiagen) for the detection of CIN2 or CIN2+ and CIN3 or CIN3+[a]

Endpoint and parameter	Xpert		cobas		hc2	
	Value	95% CI	Value	95% CI	Value	95% CI
CIN2+						
Sensitivity	90.8%	84.7–95.0%	90.8%	84.7–95.0%	81.6%	74.2–87.6%
Specificity	42.6%	38.5–46.9%	39.6%	35.5–43.8%	47.7%	43.4–51.9%
Positive predictive value	28.6%	24.5–33.1%	27.6%	23.6–31.9%	28.3%	24.0–33.0%
Negative predictive value	94.8%	91.3–97.2%	94.4%	90.6–97.0%	91.1%	87.2–94.1%
Odds ratio	7.32	4.07–13.2	6.45	3.58–11.6	4.03	2.56–6.34
Positive-likelihood ratio	1.58	1.45–1.73	1.50	1.38–1.64	1.56	1.39–1.74
Negative-likelihood ratio	0.216	0.128–0.216	0.233	0.137–0.395	0.387	0.270–0.553
CIN3+						
Sensitivity	92.3%	84.8–96.9%	92.3%	84.8–96.9%	80.2%	70.6–87.8%
Specificity	40.0%	36.1–44.0%	37.2%	33.3–41.2%	45.0%	40.9–49.0%
Positive predictive value	18.8%	15.3–22.7%	18.1%	14.7–21.9%	18.0%	14.4–22.1%
Negative predictive value	97.2%	94.3–98.9%	97.0%	93.9–98.8%	93.8%	90.4–96.3%
Odds ratio	8.00	3.70–17.3	7.11	3.29–15.3	3.31	1.94–5.65
Positive-likelihood ratio	1.54	1.41–1.68	1.47	1.35–1.60	1.46	1.29–1.65
Negative-likelihood ratio	0.192	0.0938–0.394	0.207	0.101–0.425	0.440	0.288–0.672

[a] Abbreviations: CIN2, cervical intraepithelial neoplasia grade 2; CIN2+, more-severe cervical intraepithelial neoplasia grade 2; CIN3, cervical intraepithelial neoplasia grade 3; CIN3+, more-severe cervical intraepithelial neoplasia grade 3; 95% CI, 95% confidence interval.

than for the other two tests for noncancer diagnoses. The difference in testing hrHPV positive was significant in women with a negative diagnosis ($P = 0.03$).

The clinical performance of each test for CIN2+ and CIN3+ is shown in Table 4. Xpert and cobas were equally sensitive for CIN2+ (90.8%) and CIN3+ (92.3%) and were more sensitive than hc2 for CIN2+ (81.6%) ($P = 0.004$ versus Xpert; $P = 0.002$ versus cobas) and for CIN3+ (80.2%) ($P = 0.003$ versus Xpert; $P = 0.003$ versus cobas). In contrast, hc2 was more specific than Xpert or cobas for either endpoint ($P < 0.0001$), and Xpert was more specific than cobas with the CIN2+ endpoint ($P = 0.02$) and the CIN3+ endpoint ($P = 0.02$). All three tests had very similar PLR (~28% for the CIN2+ endpoint and ~18% for the CIN3+ endpoint). The OR for CIN2+ and CIN3+ were 7.32 and 8.00 for Xpert, 6.45 and 7.11 for cobas, and 4.03 and 3.11 for hc2.

Overall, in stage I and stage II combined, there were 13 (9.2%) Xpert-, 13 (9.2%) cobas-, and 26 (18.4%) hc2-negative results for women diagnosed with CIN2+. The laboratory and pathology results for any CIN2+ from stage II of the study ($n = 19$) with at least one false-negative hrHPV test result are shown in Table S2 in the supplemental material. Of the 19 CIN2+ women, 2 tested Xpert negative alone, none tested cobas negative alone, 8 tested hc2 negative alone, 2 tested Xpert and cobas negative, 1 tested Xpert and hc2 negative, 1 tested cobas and hc2 negative, and 5 tested negative by all three tests.

There were 11 very clear examples of false-negative results—cases of CIN3 or $p16^{INK4a}$-positive CIN2 and/or independent confirmation by LA of the presence of a targeted hrHPV genotype—that tested negative by one of the three tests being compared. A specimen from another case of $p16^{INK4a}$-negative CIN2 (CXH162050) which was negative by hc2 contained HPV52 but may not have represented a true case of precancer. Five cases were negative by all three tests; of those, 3 were questionable cases, 1 was a $p16^{INK4a}$-positive CIN3 case (CXH035066) that was caused by an untargeted HPV genotype (HPV69), and 1 was a $p16^{INK4a}$-positive CIN2 case (CXH162068) that may have been caused by a HPV type not detected by any of the HPV assays, including LA. There were two cases that were positive only by hc2, most likely due to cross-reactivity with untargeted, borderline carcinogenic HPV types (12): a $p16^{INK4a}$-positive CIN2 case (CXH162063) was caused by HPV70 and a rare $p16^{INK4a}$-negative CIN3 case (CXH014105) that was positive for both HPV51 and HPV82 but for which both Xpert and cobas were negative, suggesting that HPV82 might have been the causal HPV type.

Figure 1A shows the sensitivity for CIN3+, panel B the percent test positive, and panel C the positive predictive value for CIN3+ for each test stratified by age groups. For all three tests, sensitivity was highest in women 18 to 29 years of age whereas there was a notable decrease in sensitivity and increase in false-negative results in women of age 30 to 39. Percent test positive decreased with increasing age for all tests. The positive predictive values for all three tests were similar and remained relatively constant in the three age groups.

For HPV16 detection by Xpert and cobas, the percent total agreement was 99.0%, the percent positive agreement was 93.8%, and the kappa value was 0.96 (95% CI = 0.89 to 1.00). Table 5 shows the absolute risk (PPV) of CIN2+ and CIN3+ by HPV risk groups defined by Xpert or cobas. The risk of CIN2+ and CIN3+ was highest for HPV16 detected by either of the tests, 54.6% for CIN2+ and 43.5% for CIN3+ by Xpert, and 55.0% for CIN2+ and 44.0% for CIN3+ by cobas. The risks for CIN2+ and CIN3+ were similar for HPV18/45 detected by Xpert and HPV18 detected by cobas; while the percent positive for HPV18/45 by Xpert was greater than for HPV18 by cobas (8.8% versus 4.9%), HPV18/45 detection by Xpert identified more CIN2+ (9.2% versus 5.0%) and more CIN3+ (7.7% versus 4.4%) than HPV18 detection by cobas. Like many cross-sectional studies and analyses (1, 3, 16, 19, 20), the risk of CIN2+ and CIN3+ for HPV18/45- or HP18-positive women was similar to that for women testing positive for carcinogenic HPV types other than HPV16 and HPV18/45 or HPV18.

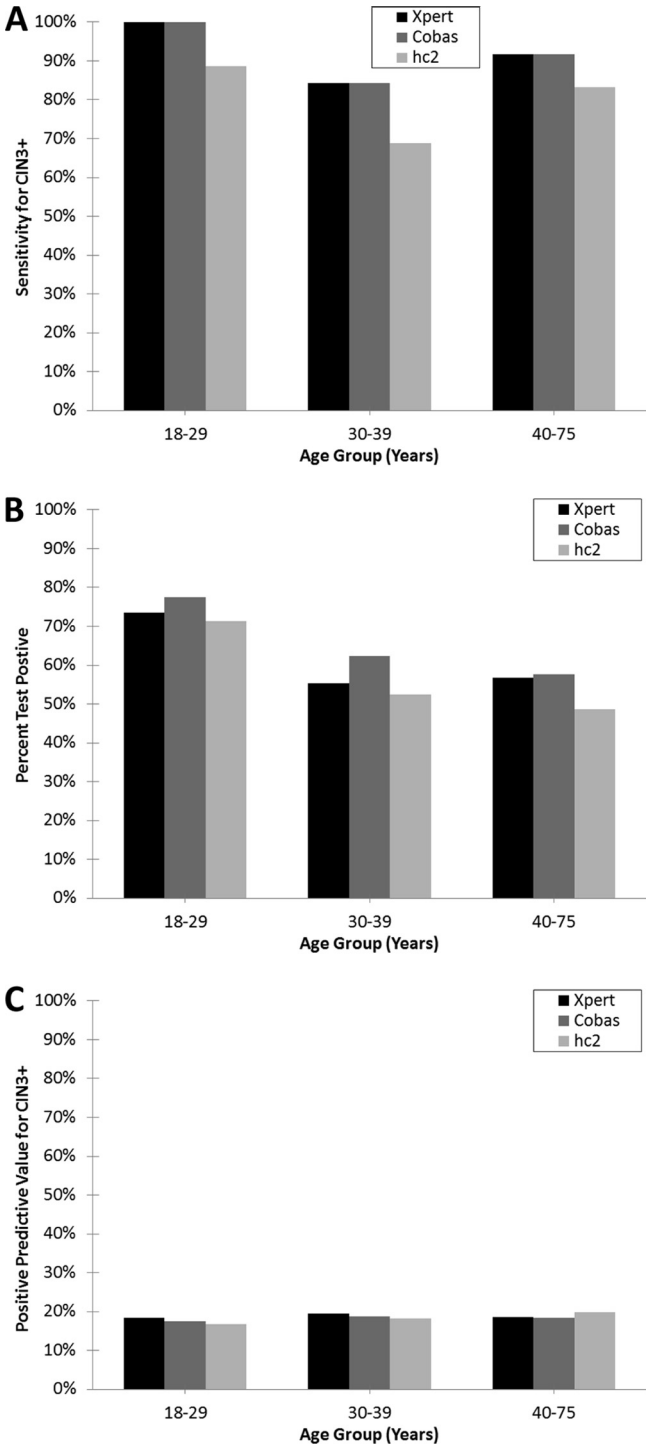

FIG 1 Sensitivity of Xpert (Cepheid), cobas (Roche), and hc2 (Qiagen) for cervical intraepithelial neoplasia grade 3 (CIN2) or more-severe CIN (CIN3+) (A), percent positive specimens (B), and positive predictive value for CIN3+ by age group (C).

DISCUSSION

This is the first report on the performance of a novel hrHPV DNA assay, the Xpert HPV assay, on the GeneXpert clinical laboratory platform. Xpert is a rapid (1-h), non-batch HPV test that might permit point-of-care (POC) testing, which can facilitate same-day screen and management strategies. Xpert results were comparable to the results from two U.S. FDA-approved tests, cobas and hc2. There was good agreement between all assays for hrHPV detection, with the best pairwise agreement between Xpert and cobas. Surprisingly, Xpert was more sensitive albeit less specific than hc2 and more specific than cobas for CIN2+.

Also, HPV16 detection by Xpert was comparable to detection by cobas and was strongly associated with the presence of CIN2+ and CIN3+. HPV18/45 detection by Xpert and HPV18 detection by cobas had similar predicted CIN2+ and CIN3+ results, with HPV18/45 detection being more sensitive and HPV18 detection being more specific for those endpoints.

A detailed analysis of 19 cases with at least one negative hrHPV test showed that there were a variety of causes of apparent false-negative results. Some were truly false negatives, i.e., cases of CIN2+ that should have been detected by all three assays. Others were questionable cases of CIN2+, despite a rigorous pathology review, and/or caused by untargeted HPV genotypes, some of which have the ability to occasionally cause cervical cancer (19, 21, 22). Similar cases of hrHPV-negative CIN3 were observed in ALTS (23).

The target sample size of the study was 150 CIN2+ in order to achieve a 95% confidence interval of ±6% for a test that was 92% sensitive for CIN2+. The actual sensitivity for Xpert was 90.8%, and 141 cases of CIN2+ were diagnosed. Thus, the final 95% confidence interval was 84.7% to 95.0%, consistent with the design goal of the study established *a priori*.

We noted two important limitations of the study. First, the study was conducted in colposcopy referral populations to enrich for CIN2+ endpoints and not in the intended-use populations, which are general screening populations. Women are referred to colposcopy for an abnormal cytology, which is associated with higher hrHPV viral loads than in women with normal cytology (24–26). Therefore, the results are not generalizable to the intended-use screening population. However, we noted that the results of Xpert were comparable to those of two other assays that have been validated and approved by the U.S. FDA for general screening. Second, Pap test results leading to the referral for colposcopy were not available for this analysis. We therefore could not compare the performance of Xpert with that of cobas and hc2 for another regularly used clinical indication for reflex hrHPV testing of the ASC-US population.

GeneXpert platforms are now widely available where there is endemic tuberculosis since the World Health Organization endorsement of Cepheid's Xpert MTB/RIF technology for molecular detection of TB and drug-resistant TB in late 2010. For example, South Africa is rolling out a national program for TB detection and treatment based on the Xpert test (27). These same regions often have a high incidence of cervical cancer due to limited preventive services and high prevalence of HIV, which increases the risk of cervical cancer substantially (28, 29). Once fully clinically validated, the Xpert HPV assay could be easily deployed to these regions to provide rapid point-of-care screening, including same-day screen-and-treat programs to minimize losses to follow-up that substantially reduce the effectiveness of screening programs. The GeneXpert can be configured to meet a wide range of testing volumes and so can be customized to meet the specific local demands for hrHPV testing. In conclusion, the Xpert HPV assay is a

TABLE 5 Risk of CIN2 or CIN2+ and CIN3 or CIN3+ determined by Xpert (Cepheid) and cobas (Roche) results categorized hierarchically according to cancer risk[a]

Test and risk category	All		CIN2+			CIN3+		
	No. of results	% of results	No. of results	% of results	% risk	No. of results	% of results	% risk
Xpert								
HPV16+	108	15.5	59	41.8	54.6	47	51.6	43.5
HPV18/45+	61	8.8	13	9.2	21.3	7	7.7	11.5
Other hrHPV+	278	39.9	56	39.7	20.1	30	33.0	10.8
hrHPV−	250	35.9	13	9.2	5.2	7	7.7	2.8
cobas								
HPV16+	109	15.6	60	42.6	55.0	48	52.7	44.0
HPV18+	34	4.9	7	5.0	20.6	4	4.4	11.8
Other hrHPV+	321	46.1	61	43.3	19.0	32	35.2	10.0
hrHPV−	233	33.4	13	9.2	5.6	7	7.7	3.0

[a] Abbreviations: CIN2, cervical intraepithelial neoplasia grade 2; CIN2+, more-severe cervical intraepithelial neoplasia grade 2; CIN3, cervical intraepithelial neoplasia grade 3; CIN3+, more-severe cervical intraepithelial neoplasia grade 3; HPV16+, HPV16 positive; HPV18/45+, HPV16 negative and HPV18/45+ positive (Xpert); HPV18+, positive for HPV18+ (cobas); HPVhr+, negative for HPV16 and HPV18/HPV45 or HPV18 and positive for other high-risk human papillomavirus types; hrHPV−, negative for high-risk human papillomavirus.

promising new assay for hrHPV DNA detection that warrants further evaluation.

ACKNOWLEDGMENTS

We thank Cepheid for specimen analysis, for logistical support in the execution of this study, and for assistance in data collection and analysis. We also thank Barbara van der Pol, Indiana University-Purdue University, Indianapolis, IN, and Kate Cuschieri, Royal Infirmary of Edinburgh, Edinburgh, United Kingdom, for providing reference laboratory services.

M.H.E. has advised or participated in educational speaking activities but does not receive an honorarium from any companies. In specific cases, Montefiore Medical Center has received payment from Roche and Hologic for time spent by M.H.E. on these activities. If travel is required for meetings with any industry, the company pays travel expenses for M.H.E. Also, Montefiore has received grant funding from Cepheid, Roche, Becton Dickinson, and Hologic for research-related costs of clinical trials for which M.H.E. has been the overall principal investigator (PI) or Montefiore PI.

D.G.F. has served as a consultant and member of the HPV advisory board for Merck. Georgia Regents University has received grant funding for his clinical trials from Cepheid, Merck, Roche, Gen-Probe (Hologic), and GSK. M.H.S. is a consultant in clinical trial designs and expert pathologist for Merck, BD, Roche, Gen-Probe (Hologic), Inovio Pharma, and Cepheid. T.C.W. is a consultant in clinical trial designs and expert pathologist for Merck, BD, Roche, Gen-Probe (Hologic), Inovio Pharma, and Cepheid. A.F. is an expert pathologist for Merck, BD, Roche, GenProbe (Hologic), Inovio Pharma, and Cepheid. P.E.C. is a consultant for Cepheid as well as for BD, Gen-Probe (Hologic), GE Healthcare, and Roche. He has received HPV tests and testing reagents for reduced cost or no cost from Qiagen, Roche, mtm, and Norchip. He is compensated as a member of a Merck data and safety monitoring board for HPV vaccines.

Cepheid was the funding source for this work.

REFERENCES

1. **Clifford GM, Smith JS, Aguado T, Franceschi S.** 2003. Comparison of HPV type distribution in high-grade cervical lesions and cervical cancer: a meta-analysis. Br. J. Cancer **89:**101–105. http://dx.doi.org/10.1038/sj.bjc.6601024.
2. **Grulich AE, van Leeuwen MT, Falster MO, Vajdic CM.** 2007. Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. Lancet **370:**59–67. http://dx.doi.org/10.1016/S0140-6736(07)61050-2.
3. **Lorincz AT, Reid R, Jenson AB, Greenberg MD, Lancaster W, Kurman RJ.** 1992. Human papillomavirus infection of the cervix: relative risk associations of 15 common anogenital types. Obstet. Gynecol. **79:**328–337. http://dx.doi.org/10.1097/00006250-199203000-00002.
4. **Meyer-Rath G, Schnippel K, Long L, MacLeod W, Sanne I, Stevens W, Pillay S, Pillay Y, Rosen S.** 2012. The impact and cost of scaling up GeneXpert MTB/RIF in South Africa. PLoS One **7:**e36966. http://dx.doi.org/10.1371/journal.pone.0036966.
5. **Naucler P, Ryd W, Tornberg S, Strand A, Wadell G, Elfgren K, Radberg T, Strander B, Forslund O, Hansson BG, Rylander E, Dillner J.** 2007. Human papillomavirus and Papanicolaou tests to screen for cervical cancer. N. Engl. J. Med. **357:**1589–1597. http://dx.doi.org/10.1056/NEJMoa073204.
6. **Peyton CL, Gravitt PE, Hunt WC, Hundley RS, Zhao M, Apple RJ, Wheeler CM.** 2001. Determinants of genital human papillomavirus detection in a US population. J. Infect. Dis. **183:**1554–1564. http://dx.doi.org/10.1086/320696.
7. **Rijkaart DC, Berkhof J, Rozendaal L, van Kemenade FJ, Bulkmans NW, Heideman DA, Kenter GG, Cuzick J, Snijders PJ, Meijer CJ.** 2012. Human papillomavirus testing for the detection of high-grade cervical intraepithelial neoplasia and cancer: final results of the POBASCAM randomised controlled trial. Lancet Oncol. **13:**78–88. http://dx.doi.org/10.1016/S1470-2045(11)70296-0.
8. **Andrae B, Kemetli L, Sparen P, Silfverdal L, Strander B, Ryd W, Dillner J, Tornberg S.** 2008. Screening-preventable cervical cancer risks: evidence from a nationwide audit in Sweden. J. Natl. Cancer Inst. **100:**622–629. http://dx.doi.org/10.1093/jnci/djn099.
9. **Cuzick J, Clavel C, Petry KU, Meijer CJ, Hoyer H, Ratnam S, Szarewski A, Birembaut P, Kulasingam S, Sasieni P, Iftner T.** 2006. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. Int. J. Cancer **119:**1095–1101. http://dx.doi.org/10.1002/ijc.21955.
10. **Ronco G, Dillner J, Elfstrom KM, Tunesi S, Snijders PJ, Arbyn M, Kitchener H, Segnan N, Gilham C, Giorgi-Rossi P, Berkhof J, Peto J, Meijer CJ.** 3 November 2013. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. Lancet http://dx.doi.org/10.1016/S0140-6736(13)62218-7.
11. **Castle PE, de Sanjosé S, Qiao YL, Belinson JL, Lazcano-Ponce E, Kinney W.** 2012. Introduction of human papillomavirus DNA screening in the world: 15 years of experience. Vaccine **30**(Suppl 5):F117–F122. http://dx.doi.org/10.1016/j.vaccine.2012.05.071.
12. **Castle PE, Solomon D, Wheeler CM, Gravitt PE, Wacholder S, Schiffman M.** 2008. Human papillomavirus genotype specificity of hybrid capture 2. J. Clin. Microbiol. **46:**2595–2604. http://dx.doi.org/10.1128/JCM.00824-08.
13. **Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM.** 2010. Globocan 2008: cancer incidence and mortality worldwide. IARC Canc-

erBase no. 10. International Agency for Research on Cancer, Lyon, France. http://globocan.iarc.fr/Default.aspx.
14. **Giannopoulos T, Butler-Manuel S, Tailor A, Demetriou E, Daborn L.** 2005. Prevalence of high-grade CIN following mild dyskaryotic smears in different age groups. Cytopathology **16:**277–280. http://dx.doi.org/10.1111/j.1365-2303.2005.00301.x.
15. **Mayrand MH, Duarte-Franco E, Rodrigues I, Walter SD, Hanley J, Ferenczy A, Ratnam S, Coutlee F, Franco EL.** 2007. Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer. N. Engl. J. Med. **357:**1579–1588. http://dx.doi.org/10.1056/NEJMoa071430.
16. **Safaeian M, Schiffman M, Gage J, Solomon D, Wheeler CM, Castle PE.** 2009. Detection of precancerous cervical lesions is differential by human papillomavirus type. Cancer Res. **69:**3262–3266. http://dx.doi.org/10.1158/0008-5472.CAN-08-4192.
17. **Dockter J, Schroder A, Hill C, Guzenski L, Monsonego J, Giachetti C.** 2009. Clinical performance of the APTIMA HPV Assay for the detection of high-risk HPV and high-grade cervical lesions. J. Clin. Virol. **45**(Suppl 1):S55–S61. http://dx.doi.org/10.1016/S1386-6532(09)70009-5.
18. **de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, Tous S, Felix A, Bravo LE, Shin HR, Vallejos CS, de Ruiz PA, Lima MA, Guimera N, Clavero O, Alejo M, Llombart-Bosch A, Cheng-Yang C, Tatti SA, Kasamatsu E, Iljazovic E, Odida M, Prado R, Seoud M, Grce M, Usubutun A, Jain A, Suarez GA, Lombardi LE, Banjo A, Menéndez C, Domingo EJ, Velasco J, Nessa A, Chichareon SC, Qiao YL, Lerma E, Garland SM, Sasagawa T, Ferrera A, Hammouda D, Mariani L, Pelayo A, Steiner I, Oliva E, Meijer CJ, Al-Jassar WF, Cruz E, Wright TC, et al.** 2010. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol. **11:**1048–1056. http://dx.doi.org/10.1016/S1470-2045(10)70230-8.
19. **Clifford GM, Smith JS, Plummer M, Munoz N, Franceschi S.** 2003. Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. Br. J. Cancer **88:**63–73. http://dx.doi.org/10.1038/sj.bjc.6600688.
20. **Smith JS, Lindsay L, Hoots B, Keys J, Franceschi S, Winer R, Clifford GM.** 2007. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. Int. J. Cancer **121:**621–632. http://dx.doi.org/10.1002/ijc.22527.
21. **Gravitt PE, Schiffman M, Solomon D, Wheeler CM, Castle PE.** 2008. A comparison of linear array and hybrid capture 2 for detection of carcinogenic human papillomavirus and cervical precancer in ASCUS-LSIL triage study. Cancer Epidemiol. Biomarkers Prev. **17:**1248–1254. http://dx.doi.org/10.1158/1055-9965.EPI-07-2904.
22. **Ronco G, Giorgi-Rossi P, Carozzi F, Confortini M, Dalla Palma P, Del Mistro A, Ghiringhello B, Girlando S, Gillio-Tos A, De Marco L, Naldoni C, Pierotti P, Rizzolo R, Schincaglia P, Zorzi M, Zappa M, Segnan N, Cuzick J, New Technologies for Cervical Cancer screening (NTCC) Working Group.** 18 January 2010. Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomised controlled trial. Lancet Oncol. http://dx.doi.org/10.1016/S1470-2045(09)70360-2.
23. **Castle PE, Cox JT, Jeronimo J, Solomon D, Wheeler CM, Gravitt PE, Schiffman M.** 2008. An analysis of high-risk human papillomavirus DNA-negative cervical precancers in the ASCUS-LSIL Triage Study (ALTS). Obstet. Gynecol. **111:**847–856. http://dx.doi.org/10.1097/AOG.0b013e318168460b.
24. **Schlecht NF, Trevisan A, Duarte-Franco E, Rohan TE, Ferenczy A, Villa LL, Franco EL.** 2003. Viral load as a predictor of the risk of cervical intraepithelial neoplasia. Int. J. Cancer **103:**519–524. http://dx.doi.org/10.1002/ijc.10846.
25. **van Duin M, Snijders PJ, Schrijnemakers HF, Voorhorst FJ, Rozendaal L, Nobbenhuis MA, van den Brule AJ, Verheijen RH, Helmerhorst TJ, Meijer CJ.** 2002. Human papillomavirus 16 load in normal and abnormal cervical scrapes: an indicator of CIN II/III and viral clearance. Int. J. Cancer **98:**590–595. http://dx.doi.org/10.1002/ijc.10232.
26. **Xi LF, Kiviat NB, Galloway DA, Zhou XH, Ho J, Koutsky LA.** 2008. Effect of cervical cytologic status on the association between human papillomavirus type 16 DNA load and the risk of cervical intraepithelial neoplasia grade 3. J. Infect. Dis. **198:**324–331. http://dx.doi.org/10.1086/589715.
27. **Guimerà N, Lloveras B, Lindeman J, Alemany L, van de Sandt M, Alejo M, Hernandez-Suarez G, Bravo IG, Molijn A, Jenkins D, Cubilla A, Muñoz N, de Sanjose S, Bosch FX, Quint W, RIS HPV TTHPV VVAPO study groups.** 2013. The occasional role of low-risk human papillomaviruses 6, 11, 42, 44, and 70 in anogenital carcinoma defined by laser capture microdissection/PCR methodology: results from a global study. Am. J. Surg. Pathol. **37:**1299–1310. http://dx.doi.org/10.1097/PAS.0b013e31828b6be4.
28. **Dillner J, Rebolj M, Birembaut P, Petry KU, Szarewski A, Munk C, de Sanjose S, Naucler P, Lloveras B, Kjaer S, Cuzick J, van Ballegooijen M, Clavel C, Iftner T, Joint European Cohort Study.** 2008. Long term predictive values of cytology and human papillomavirus testing in cervical cancer screening: joint European cohort study. BMJ **337:**a1754. http://dx.doi.org/10.1136/bmj.a1754.
29. **Gravitt PE, Peyton CL, Alessi TQ, Wheeler CM, Coutlee F, Hildesheim A, Schiffman MH, Scott DR, Apple RJ.** 2000. Improved amplification of genital human papillomaviruses. J. Clin. Microbiol. **38:**357–361.