The PapilloCheck Assay for Detection of High-Grade Cervical Intraepithelial Neoplasia

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Human papillomavirus (HPV) testing is used in primary cervical screening, as an adjunct to cervical cytology for the management of low grade abnormal cytology, and in a test of cure. PapilloCheck (Greiner Bio-One) is a PCR-based DNA microarray system that can individually identify 24 HPV types, including the 13 high-risk (HR) types identified by Hybrid Capture 2 (HC2). Here, we compare PapilloCheck with HC2 for the detection of high-grade cervical intraepithelial neoplasia (CIN2+) in a total of 8,610 cervical cytology samples from the ARTISTIC population-based cervical screening study. We performed a retrospective analysis of 3,518 cytology samples from round 1 ARTISTIC enriched for underlying CIN2+ ($n = 723$) and a prospective analysis of 5,092 samples from round 3 ARTISTIC. Discrepant results were tested using the Roche reverse line blot (RLB) or Linear Array (LA) assay. The relative sensitivity and specificity of HR PapilloCheck compared with that of HC2 for the detection of CIN2+ in women aged over 30 years were 0.94 (95% confidence interval [CI], 0.91, 0.97) and 1.05 (95% CI, 1.04, 1.05), respectively. HC2 missed 44/672 (7%) CIN2+ lesions, while HR PapilloCheck missed 74/672 (11%) CIN2+ lesions. Thirty-six percent of HC2-positive normal cytology samples were HR HPV negative by both PapilloCheck and RLB/LA, indicating that the use of HR PapilloCheck rather than HC2 in population-based primary screening would reduce the number of additional tests required (e.g., reflex cytology) in women where underlying CIN2+ is extremely unlikely. HR PapilloCheck could be a suitable HPV detection assay for use in the cervical screening setting.

Organized cervical screening by cytology has been effective at reducing cervical cancer incidence and mortality in countries where resources permit (1). The established role of persistent high-risk human papillomavirus (HPV) infection in cervical carcinogenesis (2–4) has led to the use of HPV testing alongside cytology to triage minor cytological abnormalities, in posttreatment follow-up, and, more recently, as the primary screen of cervical samples (5). The rationale for this lies in the added sensitivity and high negative predictive value of HPV testing compared to those of cervical cytology (6). Until recently, the only HPV test approved for use in cervical screening programs was the Hybrid Capture 2 (HC2) assay (Qiagen), which employs an RNA cocktail probe to induce a chemiluminescent reaction upon HPV DNA binding with any of 13 high-risk types. HC2 generally has good clinical utility for the detection of high-grade premalignant disease of the cervix (cervical intraepithelial neoplasia 2 [CIN2] or CIN3) (7, 8), but there have been concerns about its low specificity and positive predictive value (PPV). Several reports have indicated that it erroneously detects low-risk HPV types (9–11) and may even cross-react with non-HPV DNA (12). This is seen more in older women, where PCR fails to detect high-risk (HR) HPV in as many as 50% of HC2-positive samples (13). The net result of this is that women may be inaccurately labeled as at risk, leading to anxiety and over-investigation. An HPV test that offered improved specificity would reduce the number of unnecessary tests performed for women at low risk of clinically significant premalignant disease of the cervix.

PapilloCheck (Greiner Bio-One) is a PCR-based DNA microarray system that identifies 24 HPV types individually, including the 13 HR types detected by HC2. The assay involves amplification of the viral E1 gene and hybridization on a chip spotted with DNA probes for the 24 different HPV types. The aim of this study was to compare the clinical performance of HC2 and

PapilloCheck for the detection of underlying CIN2 or worse (CIN2+) using cervical samples from the ARTISTIC study (6), a randomized population-based trial of 24,510 women undergoing routine cervical screening in the United Kingdom National Health Service Cervical Screening program (NHSCSP). We analyzed a total of 8,610 samples comprising 3,518 archival samples enriched for underlying CIN2+ and 5,092 prospectively tested samples using HC2 and PapilloCheck. HPV-positive samples or discrepant results were tested additionally using the Roche prototype reverse line blot assay or the Roche Linear Array assay.

MATERIALS AND METHODS

Study participants and cervical cytology specimens. The ARTISTIC trial methods and design have been reported elsewhere (6). Women in Greater Manchester attending their general practitioner or a sexual health clinic for routine cervical screening in the NHS program between 2001 and 2003 were invited to take part in the ARTISTIC study. After giving written informed consent, cervical cytology samples were collected in PreservCyt liquid-based cytology (LBC) solution, and slides were prepared using the ThinPrep T3000 processor (Hologic) at the Manchester Cytology Centre. Slides were examined by cytoscreeners and graded according to the BSCC classification. Women were randomly allocated in a ratio of 3:1 to have the

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HPV result revealed and acted upon or concealed and further managed based on cytology alone. Management of women with abnormal cytology was identical in both arms, following national guidance for the English Cervical Screening Programme (14). In the second screening round (three years later), colposcopy referral was after two and not three borderline cytology results. Women in the revealed arm with negative cytology who tested HPV positive were invited for repeat HPV testing at 12 months and, if still positive, could choose between immediate colposcopy or a repeat HPV test at 24 months followed by colposcopy if still positive. The study was extended to a third round of screening, where women on both randomized arms were managed solely on the basis of cytology according to national guidelines. Manchester became one of six sentinel sites for HPV triage in England, where HPV triage of low-grade cytological abnormalities was undertaken. Therefore, women with borderline and mild dyskaryosis cytology who tested HPV positive were referred to colposcopy, while those who were negative were returned to routine recall.

Samples selected retrospectively. At the time of testing in ARTISTIC, 4 ml of the residual LBC sample was pelleted, resuspended in phosphate-buffered saline (PBS), and stored at -70°C . Stored aliquots from 3,518 ARTISTIC LBC specimens were selected for testing by the PapilloCheck assay on the basis of their cytology, histology, and HC2 result: HC2-positive normal cytology samples from round 1 ($n = 2,164$, including 32 CIN2+), abnormal cytology collected between July 2001 and December 2001 ($n = 836$, including 155 CIN2+), and 518 cytology samples taken within 30 months preceding CIN2+ diagnosis before August 2008.

Samples selected prospectively. A total of 5,092 liquid-based cytology specimens routinely collected between August 2008 and September 2009 were tested prospectively for HPV DNA using HC2 and PapilloCheck assays. For women with repeat cytology within this period, only the first sample was tested. Women who had previous CIN2+ in the ARTISTIC study period were included ($n = 321$).

HPV testing. (i) HC2 assay. All ARTISTIC LBC specimens were prospectively tested for HR HPV using HC2. Briefly, a 4-ml LBC aliquot was denatured, and the single-stranded HPV DNA was hybridized to a specific probe containing cRNA sequences for 13 HR HPV genotypes (HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, and HPV68) according to the manufacturer's instructions. Positive results were expressed as relative light units (RLU) compared to the result for a high-risk HPV-positive control known to have 1 pg/ml of HPV DNA, which is equivalent to 100,000 HPV copies/ml or 5,000 HPV copies per assay. An RLU/cutoff ratio of ≥ 1 was used as the threshold for an HPV-positive result.

PapilloCheck assay. The PapilloCheck assay was conducted according to the manufacturer's instructions, except that an automated rather than the manual PapilloCheck extraction kit for DNA extraction was used. Extraction was carried out using the NucliSENS easyMAG system (bioMérieux). A 50- μl aliquot of the stored cell pellet was extracted, and purified total nucleic acid was eluted in 100 μl of extraction buffer. Briefly, amplification and detection were carried out using a 5- μl aliquot of extracted DNA added to 20 μl of the PapilloCheck amplification mix combined with 1 U of HotStarTaq DNA polymerase (Qiagen) and 0.005 U of uracil-N-glycosylase (Fermentas). DNA amplification was performed on a GeneAmp 9700 (Applied Biosystems). Following amplification, the PCR products were kept in the dark at -20°C prior to hybridization. Scanning and analysis were carried out using the Greiner Bio-One CheckScanner and CheckReport software, version 2.2.5. For the purposes of this analysis, the same 13 genotypes as those detected by HC2 were considered high risk (as defined by the IARC Monograph working group [15]). Results were expressed as HR PapilloCheck positive or negative. The commercial PapilloCheck high-risk assay now available detects 14 HR types: the same as those for HC2, plus HPV66. Thus, the addition of HPV type 66 also has been assessed separately, and all other types detected by PapilloCheck (types 6, 11, 40, 42, 43, 44, 53, 70, 73, and 82), some of which are considered "probably high risk," have been referred to here as "low-risk" types.

Roche prototype reverse line blot and Roche Linear Array assays.

Either the Roche prototype reverse line blot or the Roche Linear Array assay (collectively referred to as RLB here) was performed on all LBC samples that tested positive for HPV by HC2 or PapilloCheck. These genotyping assays amplify 37 HPV types simultaneously, including the 13 HC2 target types, using PGMY09 to PGMY11 (PGMY09/11) L1 consensus primer PCR. The assays were carried out according to the manufacturer's instructions, except that an automated rather than the manual AmpliLute liquid extraction kit (Roche) for DNA extraction was used. DNA was extracted from a 50- μl aliquot of the stored cell pellet using the automated Roche MagNA Pure LC extraction system in conjunction with the total nucleic acid extraction kit (Roche). The purified total nucleic acid was eluted with a low-salt buffer to a final volume of 100 μl . This automated system was validated and certified by Roche Molecular Diagnostics using HPV test panels before any testing was carried out on clinical material.

Statistical analysis. The clinical performance of PapilloCheck relative to that of HC2 was determined according to its ability to detect underlying CIN2+ by calculating relative sensitivities and specificities with 95% confidence intervals (CI). A power calculation indicated that a study with 2,100 negative results (HPV negative or colposcopy/histology showing less than CIN2) and 400 positive results (underlying CIN2+) would have more than 90% power to demonstrate relative sensitivity of PapilloCheck to HC2 to detect CIN2+ of at least 95% (assuming a proportion of discordant pairs of 0.08) and a sensitivity of HC2 of 95%. Relative sensitivity was calculated as the ratio of positive results (underlying CIN2+) by PapilloCheck to positive results by HC2. The retrospectively selected samples were enriched for underlying CIN2+ to facilitate comparisons of relative sensitivity, and 723 (93%) of the total of 776 CIN2+ samples diagnosed within the study were retrieved. The majority of samples in the prospective study had normal cytology ($n = 4,856$ negative results), which facilitated comparisons of relative specificity for CIN2+ detection. CIN2+ histology following negative cytology was rare unless following a referral based on a positive HC2 test result in the revealed arm of the trial; therefore, calculations of sensitivity were based only on those CIN2+ cases that were diagnosed following referral with abnormal cytology ($n = 672$). Relative specificity was calculated using cases either not referred to colposcopy or negative at colposcopy as the ratio of negative results by PapilloCheck to negative results by HC2. Ninety-five percent confidence intervals for relative sensitivity and relative specificity were calculated (16) to determine differences between the two assays. Meijer et al. (17) proposed that a new test should not have a relative sensitivity of less than 90% of HC2 in women aged over 30 years; thus, results are presented separately for women aged under and over 30 years. Direct comparisons between the assays were made using McNemar's test for paired samples. PapilloCheck also was compared to HC2 with an RLU/cutoff ratio of ≥ 2 , because previous work indicated that this threshold has better clinical utility (13).

RESULTS

Prospective unselected samples (2008 to 2009). Table 1 shows a summary of all test results from the two assays. Of the samples tested prospectively, 4,856/5,092 (95.4%) had normal cytology, reflecting the success of earlier rounds of screening at detecting and treating prevalent premalignant disease of the cervix. The proportion of all cytology samples that tested positive for HR HPV was 12.1% (617/5,092) by HC2 and 7.9% (403/5,092) by PapilloCheck. The proportion of abnormal cytology samples that tested positive for HR HPV was 69.1% (163/236) by HC2 and 54.2% (128/236) by PapilloCheck. There was overall agreement of 94.5% between the tests when all cytology samples were included. By the end of histological follow-up (October 2009), 18 underlying CIN2+ lesions were detected. Seventeen out of 18 were HR HPV positive by HC2, and 16/18 were HR HPV positive by PapilloCheck. The three discordant CIN2+ samples showed one case

TABLE 1 Overall HC2 and PC HPV test results for retrospectively and prospectively collected samples ($n = 8,610$)

Sample	HC2 ⁺						HC2 ⁻						Total no. (%) CIN2+			
	n	HR PC ⁺			HR PC ⁻			n	HR PC ⁺			n		HR PC ⁻		
		n	%	CIN2+ (n)	n	%	CIN2+ (n)		n	%	CIN2+ (n)			n	%	CIN2+ (n)
Prospective unselected (2008–2009)																
Normal cytology ^d	4,856	242	5.0	1	212	4.4	0	33	0.7	0	4369	90.0	0	95.0		
Borderline cytology	118	36	30.5	0	20	16.9	1	1	0.8	1	61	51.7	0	82.2		
Mild cytology	91	70	76.9	6	12	13.2	0	0			9	9.9	0	86.8		
Moderate+ cytology	27	21	77.8	8	4	14.8	1	0			2	7.4	0	85.2		
All cytology	5,092	369	7.3	15	248 ^a	4.9	2	34 ^b	0.7	1	4441	87.2	0	94.5		
Retrospectively selected																
Normal cytology concealed arm ^d (2001–2004)	543	301	55.4	1	242	44.6	0									
Normal cytology revealed arm ^e (2001–2004)	1,621	878	54.2	26	743	45.8	5 ^c							31 (1.9)		
Abnormal cytology (Jul-Dec 2001)																
Borderline	482	109	22.6	19	26	5.4	1	11	2.3	0	336	69.7	12	92.3		
Mild	228	113	49.6	23	31	13.6	0	3	1.3	0	81	35.5	3	85.1		
Moderate+	126	104	82.5	89	8	6.4	3	2	1.6	1	12	9.5	4	92.0		
Additional CIN2+ samples (2002–2007)	518		88.8	460		6.2	32		0.4	2		4.6	24	93.4		
All CIN2+ samples	723		87.6	633		5.9	43		0.6	4		5.9	43	93.5		

^a Twenty-four (10.1%) were high-risk positive by RLB, 214 were HR negative by RLB, and 10 were inhibitory by RLB.^b Twenty-one (63.6%) were high-risk positive by RLB (19 with the same HR types as PapilloCheck), 12 were HR negative by RLB, and 1 was inhibitory (no result by RLB).^c Four out of five of these cytologically normal, HC2⁺, HR-PC⁻ samples were positive for HR types by RLB (1 HPV16, 2 HPV18, and 1 HPV16/18).^d These were not referred for colposcopy based on their HPV status. Any referrals and, hence, CIN2+ diagnoses occurred due to additional follow-up as a result of an abnormal screening history.^e Women who were positive by HC2 on 2 or 3 consecutive occasions were offered colposcopy.

TABLE 2 Sensitivity of HC2 and PapilloCheck HPV tests in 672 abnormal cytology samples preceding a diagnosis of CIN2+ and CIN3+

Age at test (yr) and cytology	Histology	<i>n</i>	HC2 ⁺ (no.)		HC2 [−] (no.)		Sensitivity (%)		Relative sensitivity (95% CI)
			HR PC ⁺	HR PC [−]	HR PC ⁺	HR PC [−]	HC2	HR PC	
20–29									
All abnormal	CIN2+	330	303	12	0	15	95.5	91.8	0.96 (0.94, 0.98)
Borderline		60	49	1	0	10	83.3	81.7	0.98 (0.94, 1.02)
Mild		98	90	6	0	2	98.0	91.8	0.94 (0.89, 0.99)
Moderate+		172	164	5	0	3	98.3	93.3	0.97 (0.95, 1.00)
30–60									
All abnormal	CIN2+	342	291	22	4	25	91.5	86.3	0.94 (0.91, 0.97)
Borderline		65	44	5	1	15	75.4	69.2	0.92 (0.83, 1.02)
Mild		78	71	3	0	4	94.9	91.0	0.96 (0.92, 1.00)
Moderate+		199	176	14	3	6	95.5	89.9	0.94 (0.90, 0.98)
All women									
All abnormal	CIN2+	672	594	34 ^a	4 ^b	40 ^c	93.5	89.0	0.95 (0.93, 0.97)
20–29									
All abnormal	CIN3+	172	162	7	0	3	98.3	94.2	0.96 (0.93, 0.99)
Borderline		27	25	1	0	1	96.3	92.6	0.96 (0.89, 1.04)
Mild		46	43	2	0	1	97.8	93.5	0.96 (0.90, 1.02)
Moderate+		99	94	4	0	1	99.6	94.9	0.96 (0.92, 1.00)
30–60									
All abnormal	CIN3+	186	165	13	4	4	95.7	90.9	0.95 (0.91, 1.00)
Borderline		25	19	2	1	3	84.0	80.0	0.95 (0.81, 1.12)
Mild		24	24	0	0	0	100.0	100.0	1.00
Moderate+		137	122	11	3	1	97.1	91.2	0.94 (0.89, 1.00)
All women									
All abnormal	CIN3+	358	327	20	4	7	96.7	92.5	0.95 (0.93, 0.98)

^a Five out of 14 (36%) CIN2 and 14/20 (70%) CIN3+ samples were HR-RLB positive. Those negative for HR types were mostly positive for LR types (14/15). The five CIN2 samples were positive for types 16, 18 (*n* = 2), 45, and 58, and the 14 CIN3+ samples were positive for types 18 (*n* = 6), 31, 45 (*n* = 5), 51, and 68.

^b Three out of 4 (75%) of these were HR-RLB positive with the same types as the PapilloCheck result (HPV 16, 31, and 45).

^c Fifteen out of 33 of the CIN2 samples were tested with RLB, and all were found HR negative. Two out of 7 CIN3+ samples were positive for HR types by RLB (both for type 16).

missed by HC2 that was positive for HPV31 and one case missed by PapilloCheck that was positive for HPV18; the remaining extra case picked up by HC2 was found positive for HPV70 (Table 1, footnotes *a* and *b*).

Retrospectively selected samples. In contrast to the prospectively tested samples, those collected in July to December 2001 were taken at a time when LBC had just been introduced and cytological abnormalities were being overcalled (the age-adjusted cytological abnormality rate in LBC samples taken at entry into the ARTISTIC trial declined by 40% over the first 2 years of the trial [6]). Hence, the proportion of borderline and mild (LSIL) cytology positive by both assays is lower for these samples (31% in 2001 versus 51% in 2008 to 2009).

A total of 2,164 samples with normal cytology and testing positive by HC2 from round 1 of ARTISTIC were retrospectively selected. Approximately half of these tested negative with PapilloCheck. A total of 1,621 were in the revealed arm, where around 65% of those who had repeatedly positive HC2 tests were referred to colposcopy based only on their HC2 result (and not cytology). CIN2+ was detected in 31 women (6). HR PapilloCheck was positive in 26/31 (84%) of these cases. Of the 5 CIN2+ lesions missed by PapilloCheck, HPV16 or HPV18 was detected by RLB in 4 samples (Table 1, footnote *c*).

Sensitivity. Table 2 shows the sensitivity of each assay for de-

tecting CIN2+ in women with abnormal cytology, stratified by age and cytology result. Sensitivity was consistently higher at younger ages and for more severe cytology, and HC2 was consistently more sensitive than HR PapilloCheck; overall, the sensitivity for detecting CIN2+ was 93.5% for HC2 and 89.0% for HR PapilloCheck, giving PapilloCheck a relative sensitivity of 0.95 (95% CI, 0.93 to 0.97; *P* < 0.0001). HR PapilloCheck missed a total of 34 CIN2+ lesions that were positive by HC2, and HC2 missed 4 CIN2+ lesions that were positive by PapilloCheck. The proportion testing negative by both HC2 and PapilloCheck was 6.0% (40/672) overall. This proportion was much higher for CIN2+ than for CIN3+ and was higher in the first 6 months of the trial (CIN2+, 21.3% [17/80]; CIN3+, 2.7% [2/75]) than later (CIN2+, 6.8% [16/234]; CIN3+, 1.8% [5/283]).

Specificity. Table 3 shows the specificity of the tests based on women without CIN2+ diagnoses by age and cytology in the prospectively collected samples. Overall, PapilloCheck was significantly more specific than HC2 (92.4% versus 88.2%, respectively; *P* < 0.0001), giving a relative specificity of 1.05 (95% CI, 1.04 to 1.06). The specificity decreased with increasing severity of cytological abnormality and remained higher for PapilloCheck.

Further typing. Adding HPV type 66 to the HR PapilloCheck test would have a minimal effect on the results. It increased the overall HR prevalence in the prospective samples from 7.9% to

TABLE 3 Specificity of HC2 and PC among women without CIN2+ histology^b

		HC2 ⁺		HC2 [−]		Specificity (%)		
Age at test (yr) and cytology	<i>n</i>	HR PC ⁺	HR PC [−]	HR PC ⁺	HR PC [−]	HC2	HR PC	Relative specificity (95% CI)
20–29								
All women	397	72	27	2	296	75.1	81.4	1.08 (1.05, 1.12)
Negative	359	49	19	2	289	81.1	85.8	1.06 (1.03, 1.09)
Abnormal	38	23	8	0	7	18.4	39.5	2.14 (1.25, 3.68)
30+								
All women	4,677	282	219	31	4,145	89.3	93.3	1.05 (1.04, 1.05)
Negative	4,496	192	193	31	4,080	91.4	95.0	1.04 (1.03, 1.05)
Abnormal	181	90	26	0	65	35.9	50.3	1.40 (1.23, 1.59)
All women								
All women	5,074	354	246	33	4,441	88.2	92.4	1.05 (1.04, 1.06)
Negative	4,855	241	212	33	4,369	90.7	94.4	1.04 (1.03, 1.05)
Borderline	116	36	19	0	61	52.6	69.0	1.31 (1.16, 1.48)
Mild	85	64	12	0	9	10.6	24.7	2.33 (1.42, 3.82)
Moderate+ ^a	18	13	3	0	2	11.1	27.8	2.50 (0.85, 7.31)

^a CIN2+ was underestimated in these prospectively collected samples due to incomplete histological follow-up. This is most likely to affect the moderate+ cytology group.

^b All samples were collected prospectively.

8.2%. One additional CIN2 case would be picked up by including type 66, but this would hardly alter the sensitivity (89.1% versus 89.0%) or specificity (92.1% versus 92.4%) of the test. Also adding the probable HR types 53, 73, and 82 would pick up an additional 8 CIN2+ cases (HPV53, CIN3; HPV73, 2 CIN2; HPV82, 3 CIN2 and 2 CIN3), increasing the overall sensitivity to detect CIN2+ to 90.3% (with a relative sensitivity compared to that of HC2 of 0.97 [95% CI, 0.95 to 0.98]) and a sensitivity to detect CIN3+ of 93.3% (relative sensitivity of 0.96 [95% CI, 0.94 to 0.99]). An additional 1% of women (51/5,074) would be referred, and the specificity would decrease marginally to 91.4% (relative specificity of 1.04 [95% CI, 1.03 to 1.04]).

Raising the HC2 RLU/cutoff ratio to ≥ 2 slightly decreased the sensitivity to detect CIN2+ from 93.5% to 91.7%, which was still significantly higher than the sensitivity of PapilloCheck (89.0%),

with a sensitivity of 0.97 (95% CI, 0.95 to 0.99; McNemar's $P = 0.006$) relative to that of HC2. Sensitivity to detect CIN3+ also was decreased to 95.5% from 96.7%, again with relative sensitivity of 0.97 (95% CI, 0.94 to 0.99; McNemar's $P = 0.02$). The specificity for the detection of CIN2+ was increased from 88.2% to 90.4% but still was significantly lower than that for PapilloCheck (92.4%), with a relative specificity of 1.02 (95% CI, 1.02 to 1.03; McNemar's $P < 0.0001$). The sensitivities and specificities for the two assays became similar when the HC2 RLU/cutoff ratio was increased to ≥ 5 .

Table 4 shows good agreement between PapilloCheck and Roche line blot (or Linear Array) for HC2-positive samples collected prospectively ($n = 602$) and selected retrospectively ($n = 2,544$). The assays agreed in 82% of women with normal cytology and 91% of those with abnormal cytology (all ages). The false-

TABLE 4 PapilloCheck and Roche reverse line blot HPV test results in HC2-positive samples, prospectively collected ($n = 602$) and retrospectively selected ($n = 2,544$)

Age at test (yr) and cytology		n	HR PC ⁺							HR PC [−]							Total HR (%)		
			HR RLB ⁺			HR RLB [−]				HR RLB ⁺ ^a			HR RLB ^{−b}				Agreement (%)	RLB [−]	PC [−]
			CIN2 ⁺ (n)	n	%	CIN2 ⁺ (n)	n	%	CIN2 ⁺ (n)	n	%	CIN2 ⁺ (n)	n	%	CIN2 ⁺ (n)				
20–29																			
Normal	937	19	586	62.5	17	63	6.7	1	100	10.7	1	188	20.1	0	82.6	26.8	30.7		
Abnormal	264	84	222	84.1	82	9	3.4	1	10	3.8	1	23	8.7	0	92.8	12.1	12.5		
30+																			
Normal	1665	13	619	37.2	9	144	8.7	0	153	9.2	3	749	45.0	1	82.2	45.0	55.6		
Abnormal	280	67	202	72.1	61	18	6.4	1	12	4.3	3	48	17.1	2	89.2	23.6	21.4		
All women ^c																			
Normal	2602	32	1205	46.3	26	207	8.0	1	253	9.7	4	937	36.0	1	82.3	44.0	45.7		
Abnormal	544	151	424	77.9	143	27	5.0	2	22	4.0	4	71	13.0	2	91.0	18.0	17.1		

^a Among 275 samples that were HR PC⁻ and HR RLB⁺, the most common HR types detected by RLB were the following: HPV16 ($n = 65$, 23.5%), HPV52 ($n = 51$, 18.6%), HPV18 ($n = 49$, 17.8%), HPV45 ($n = 27$, 9.8%), and HPV59 ($n = 26$, 9.5%).

^b Among 1,008 samples negative by both tests, 25.0% (235/937) with normal cytology and 62.0% (44/71) with abnormal cytology were positive for LR types by RLB, with the most common types being HPV53 (5.8%), HPV70 (5.3%), and HPV66 (4.9%).

^c Twenty-six out of 3,172 HC2⁺ samples shown in Table 1 were inhibited or insufficient when tested by RLB and are not included here.

positive rate of HC2 in normal cytology samples negative by both RLB and PapilloCheck was estimated to be at least 20% (188/937) in younger women and 45% (749/1,665) in women aged over 30 years. Of the 3,146 samples that tested positive by HC2, 1,283 (40.8%) were negative by PapilloCheck and 1,008 (32.0%) were negative for HR HPV by RLB. RLB detected LR types in 25% of samples with normal cytology and 62% of samples with abnormal cytology. The most common types detected were HPV53 (5.8% samples), HPV70 (5.3% samples), and HPV66 (4.9% samples) (Table 4, footnote b). Of the 275 samples that tested positive for HR HPV by RLB, HPV16 was detected in 65 (53.5%) samples, HPV52 in 51 (18.6%) samples, HPV18 in 49 (17.8%) samples, HPV45 in 27 (9.8%) samples, and HPV59 in 26 (9.5%) samples (Table 4, footnote a).

Of the 5,092 samples tested prospectively, 2.8% ($n = 143$) tested positive by HC2, but no cases of HPV, including low-risk types, were detected with either PapilloCheck or RLB. Of the 617 samples that tested positive by HC2, only 393 (63.7%) had one or more of the 13 HR types detected by PapilloCheck or RLB. Of the remaining 224 where no HR types were detected, 36.2% ($n = 81$) had low-risk types detected: 28.1% (51/177) in women aged over 30 with normal cytology and 63.8% (30/47) in women aged under 30 or with abnormal cytology. The prevalence of low-risk types only by PapilloCheck was very low among the 5,092 samples (3.6%). Forty-four percent (81/183) of these samples with low-risk HPV were positive by HC2, but the proportion was larger for type 53 (20/25), type 66 (11/13), type 70 (15/20), and type 82 (9/20), although the numbers were small.

DISCUSSION

In this study, a total of 8,610 cervical cytology samples from the ARTISTIC trial were tested for HR HPV using the PapilloCheck assay. The higher specificity of PapilloCheck substantially reduced the proportion of women testing HR HPV positive, from 12.1% (617/5,092) by HC2 to 7.9% (403/5,092) in the prospective unselected samples. The majority of these samples had normal cytology (179/214). Thus, if HR PapilloCheck were used instead of HC2 for primary HPV screening, the number of women requiring additional tests (e.g., reflex cytology) would be reduced by about a third in women where the risk of underlying CIN2+ is less than 5%. Prevalent high-grade lesions were detected and treated in the first screening round of the ARTISTIC trial following the introduction of LBC and retraining of cytology readers (6). Therefore, the much lower CIN2+ rate in the prospective study of women screened in 2008 to 2009 is more relevant to future screening practices in the United Kingdom than the prevalence in previous rounds. Overall, the specificity of the PapilloCheck assay was 92.4% compared to 88.2% with HC2, giving a relative specificity of 1.05 (95% CI, 1.04 to 1.06). The specificity of the PapilloCheck assay was significantly higher than that of HC2 for women with borderline or mild cytology, implying that PapilloCheck also could be used as a triage test following cytology.

HC2 is a highly sensitive test for detecting CIN2+, but the relatively low specificity leads to high referral rates, particularly in populations with higher HPV prevalence, such as young women or those with abnormal cytology. HR HPV DNA was not detected by PapilloCheck or RLB in 32.0% (1,008/3,146) of the HC2+ samples (Table 4), which contained either no detectable HPV or only low-risk types which may cause CIN2 or CIN3 but are unlikely to cause invasive cancer and should not be picked up by a

screening test. Similar rates of detection of HR types by Linear Array (69%) were found in a study conducted by Oh et al. (18). They also found low-risk types 53, 66, and 70 in a further 18% of their HC2+ samples and suggest some cross-reaction with HC2 and these types is possible.

Table 4 shows much higher CIN2+ rates among HC2-positive women who also were positive by PapilloCheck (1.9% [27/1,412] in normal cytology and 32.2% [145/451] in abnormal cytology) compared to those who were negative by PapilloCheck (0.4% [5/1,190] in normal cytology and 6.5% [6/93] in abnormal cytology). The relative sensitivity for CIN3+ was 95% (95% CI, 93% to 98%) in all women, where HC2 missed 11/358 (3.1%) CIN3+ cases and PapilloCheck missed 27/358 (7.5%) CIN3+ cases. Eleven of these 27 cases missed by PapilloCheck were positive for HPV18 or HPV45 by RLB, and another 6 were positive for other HR types, leaving 11 that had either low-risk types ($n = 6$, all positive by HC2) or no detectable HPV infection ($n = 5$, all negative by HC2). Meijer et al. (17) stated that an acceptable alternative test to HC2 should have a relative sensitivity for CIN2+ of no less than 90% in women aged over 30 years. PapilloCheck fulfills this criterion with a relative sensitivity of 94% (95% CI, 91% to 97%) in this age group (Table 2). Therefore, PapilloCheck is a suitable alternative to HC2 for primary cervical screening, alongside Abbott M2000, Roche Cobas, Hologic Cervista, and Gen-Probe Aptima HR HPV tests (19).

This is the largest study to date that has compared the clinical performance of PapilloCheck against an established HPV test or genotyping method. Previous studies have included an analysis of baseline samples from women participating in the POBASCAM trial, where 1,665 cervical cytology specimens, including 192 from women with CIN2+, were tested with both PapilloCheck and the GP5+/6+ PCR-enzyme immunoassay. They found that the two HPV testing methods performed similarly in terms of CIN2+ detection (20). A population-based study of 878 cervical cytology specimens, in which there were 32 underlying CIN2+ lesions, also found good correlation between PapilloCheck, HC2, and the GP5+/6+ PCR-enzyme immunoassay (21). Another study tested a colposcopy referral population of 239 women, of whom 93 had CIN2+ using PapilloCheck and the Linear Array assay, and found a high overall concordance rate between the two assays (22).

We conducted parallel retrospective and prospective analyses of ARTISTIC (6) cervical cytology samples in this study. Using ARTISTIC samples had the benefit that the samples were well characterized in terms of clinical follow-up data. The advantage of the retrospective analysis was that we were able to enrich our sample set for underlying CIN2+ lesions, which in turn gave sufficient power to the study to allow a meaningful comparison of HC2 versus PapilloCheck for the detection of CIN2+. Agreement between PapilloCheck and the Roche line blot assays remained good despite the samples having been processed, frozen, and stored for up to 6 years before they were tested with PapilloCheck. In the prospective analysis, we were able to compare the performance of the two tests contemporaneously under the same conditions using fresh cervical cytology material, but there were only 18 underlying CIN2+ lesions. A prospective study sufficiently large to detect small differences in the performance characteristics of these tests would be extremely expensive and is unlikely to be performed.

Greiner Bio-One has developed a microarray-based test kit using the same technology as the approved PapilloCheck which detects the 14 carcinogenic types (REF 505060; Greiner Bio-One).

This is important, because any HPV test used in primary cervical screening must detect high-risk HPV rather than low-risk types or a mixture of the two, and we have shown that PapilloCheck (considering these 14 HR types) is significantly more specific than HC2 with only a small decrease in sensitivity. Compared with the HC2 test, which, using Qiagen QiaSymphony and Rapid Capture, is capable of processing and testing 88 samples in 9 h, the PapilloCheck method employed in this study required 1.5 days to process and test 80 samples. Greiner Bio-One now has developed an automated platform which is expected to launch in early 2016 and will be capable of throughput and ergonomic performance comparable with those of HC2. We and others have shown that PapilloCheck also compares well with other PCR-based systems, such as the Roche Linear Array, and this genotyping capability will have clinical utility for stratifying HPV-positive women, especially as vaccinated cohorts come through to cervical screening. In conclusion, this large study has confirmed that HR PapilloCheck would be a suitable HPV assay in primary screening, providing sufficient sensitivity and improved specificity relative to those of HC2.

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We have no conflicts of interest to report.

E.J.C. and H.C.K. devised the study, analyzed the data, and wrote the manuscript. A.B. and A.S. performed the laboratory investigations and collated the data. H.C.K., C.G., and J.P. provided access to the ARTISTIC study samples and clinical data. C.G. performed the statistical analyses. All authors contributed to data interpretation and reviewed the final manuscript.

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