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Comparison of the performance of HPV tests in women with abnormal cytology: results of a study within the NHS cervical screening programme

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Objective: The use of testing for human papillomavirus (HPV) is now recognized as an efficient means of triaging women with low-grade cytological abnormalities to either immediate referral to colposcopy or return to routine recall. We aimed to determine the sensitivity and specificity of each of four newer tests for HPV relative to the Qiagen Hybrid Capture 2 (HC2) assay in order to determine whether they could be approved for use in triage in the NHS cervical screening programme.

Methods: We compared the performance of each of four different HPV assays (Abbott M2000, Roche Cobas, Hologic Cervista and Gen-Probe APTIMA) with that of HC2 in order to determine the sensitivity and specificity of each test relative to HC2 for the detection of cervical intraepithelial neoplasia (CIN) grade 2 or worse, using routine cytology samples reported as borderline (atypical squamous cells) or mild dyskaryosis (low-grade squamous intraepithelial lesion) from six laboratories in England. All women who were found to be HPV positive on any test were referred to colposcopy.

Results: Between 2072 and 4217 tests were performed with each assay. All four assays were shown to have a relative sensitivity of no worse than 95% compared with HC2 when a cut-off of 2 relative light units (RLU) was used. All assays had higher relative specificity than HC2 for both borderline and mild cytology referrals (1.06–1.61).

Conclusions: All assays tested met the criteria required. Consequently, all have now been approved for use in HPV triage in the NHS cervical screening programme.

Keywords: human papillomavirus assay, triage, cervical cancer, Papanicolaou test

Introduction

Human papillomavirus (HPV) testing is now becoming an integral part of the English NHS cervical screening programme (NHSCSP). Pilot studies using Hybrid Capture 2 (HC2) have demonstrated that the use of HPV testing to triage women with a cytology result of borderline cytology or mild dyskaryosis (equivalent to atypical squamous cells and low-grade squamous intraepithelial lesion, respectively) would

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allow approximately one-third of such women to be returned immediately to routine recall and for a substantial proportion to be referred for colposcopy without repeat cytology.[1–5] The use of HPV triage is now national policy. HPV as a primary screening method, with the cytology result being used to determine further management in women who are HPV positive, is being piloted.

Now that a number of alternative technologies for HPV testing have become available in recent years, we aimed to assess the performance of four of them (three DNA based and one RNA assay) compared with that of the Qiagen HC2 assay in liquid-based cytology (LBC) samples reported as borderline or mild dyskaryosis. The objectives were to determine the sensitivity and specificity of each of these newer tests relative to HC2, which had already been validated by the pilot, in order to determine whether they could be approved for use in triage in the NHSCSP. The NHSCSP is a highly organized multidisciplinary programme. Potential harms to women include poor sensitivity, but also well-documented distress resulting from non-negative results and unnecessary referral to colposcopy.[6,7] In addition, colposcopy resource is limited and unnecessary referral delays treatment for those with genuine abnormalities. The principal aim of the NHSCSP is to detect high-grade cervical intraepithelial neoplasia (CIN).[8]

The principal aim of our study was to demonstrate the non-inferiority of each alternative assay relative to HC2 in terms of both sensitivity and specificity for the detection of CIN2 or worse (CIN2+). A direct 'head-to-head' comparison of the various assays available was not an objective of this study.

Methods

Cytology samples

Routine cytology samples were obtained from six sites that participated in the HPV triage pilot study,[4] and were continuing to use HPV triage in the management of women with cytology reported as either borderline change or mild dyskaryosis. Borderline change in the British Society for Clinical Cytology (BSCC) 1985 classification is broadly equivalent to atypical squamous cells of undetermined significance (ASC-US) in the Bethesda system. Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H), would also be included as borderline change, but these samples are very few in number in the UK. PreservCyt® [Hologic ThinPrep (TP) system] and SurePath® [BD SurePath (SP)] LBC collection systems are used in England. TP cytology samples were processed using the ThinPrep® 3000 processor, and screened at Norwich, Bristol and Northwick Park. SP samples were prepared using a cell enrichment process and the TriPath Imaging Prep-stain slide processor, and screened at Sheffield, Liverpool and Manchester. Residual TP material from samples which were reported as borderline change or mild dyskaryosis was sent to Bristol Cytology for initial HPV testing by HC2, and aliquots were sent to Bristol Virology for HPV testing with Hologic Cervista HPV, Gen-Probe (now Hologic) HPV APTIMA and Abbott rtHPV, or Manchester Virology for Roche Cobas 4800. HPV tests are subsequently referred to as Cervista, GHPV, rtHPV and Cobas, respectively. For SP samples, the remaining cell pellet obtained after slide preparation was sent to Manchester Virology for HC2 testing, together with 1–2 ml of original SP material for testing by rtHPV, GHPV or Cobas.

As a result of restrictions in the quantity of material available, and also differences in the duration of the study for each test (see below), not all tests were carried out on all samples.

All samples were current and, in the vast majority of cases, a final cytology and HPV report was issued within the normal NHSCSP required timescale of 14 days from the date the sample was taken to receipt of the result by the woman. If any HPV test was positive, the sample was reported as HPV positive.

HPV tests

The HC2 test was carried out using 4 ml of the TP or the entire cell-enriched SP vial by the manual sample preparation and manual assay according to the manufacturer's instructions, but using a cut-off of 2 relative light units (2 RLU) rather than the manufacturer's 1 RLU cut-off.[9] Following initial HC2 testing, further analysis using one of the other HPV testing platforms was carried out. The assays used are summarized in Table 1.

For the Cobas test, the original TP sample was vortexed before directly being placed onto the Cobas x480 extraction instrument and tested according to the Conformité Européenne (CE)-marked manufacturer's instructions. HPV detection was

Table 1. Summary of human papillomavirus (HPV) assays included in the comparison

Test	LBC collection medium	Site	Protocol
HC2	TP	Bristol, Manchester	Use of 2 RLU cut-off at both sites
	SP	Manchester	
rtHPV	TP	Bristol	According to CE-marked M2000SP/RT protocol at both sites
	SP	Manchester	
GHPV	TP	Bristol	According to CE-marked Gen-Probe TIGRIS system
	SP	Manchester	According to CE-marked Gen-Probe TIGRIS system, but with initial proteinase K pre-treatment step
Cervista	TP	Bristol	According to manufacturer's CE-marked protocol, using semi-automated HTA platform (research use only)
Cobas	TP	Manchester	According to CE-marked Cobas 4800 system
	SP	Manchester	

CE, Conformité Européenne; GHPV, Gen-Probe APTIMA HPV; HC2, Hybrid Capture 2; HTA, high throughput automation. LBC, liquid-based cytology; RLU, relative light units; rtHPV, real-time HPV PCR; SP, SurePath; TP, ThinPrep.

then performed on the Cobas z480 using real-time PCR. For SP samples, 1 ml of the original sample was transferred to a secondary tube prior to extraction. All samples for this study were transported and stored at ambient temperature. After this project started, Roche advised that cervical specimens collected in SP preservative fluid and stored at 15–30 °C must be tested within 14 days of the sample being taken.

For the rtHPV test, a 600-µl aliquot of the TP sample and 600 µl of the original SP collection vial following vortexing were transferred to a secondary tube before being placed onto the m2000sp for sample extraction according to the manufacturer's CE-marked instructions, and manually transferred to the m2000rt for amplification and detection.

For the Cervista test, a 2-ml aliquot of TP sample was withdrawn from the original collection vial following vortexing and transferred to a well of a deep well plate. Samples were processed on a Tecan robot and denatured nucleic acids were hybridized for 4 hours at 63 °C with three pools of high-risk HPV-specific probes, Cervista invader probes and Cleavase enzyme. Reactions were then analysed at room temperature in a fluorescence plate reader. Cervista is not validated for SP samples and was therefore not evaluated for these.

For the GHPV test, a 1-ml aliquot of TP was transferred into an APTIMA specimen transfer tube and tested according to the CE-marked manufacturer's instructions. SP samples were in the process of being CE marked by the manufacturer at the time of this study, and it was recommended by Gen-Probe that the sample be pre-treated with a proteinase K solution. Following thorough vortexing of the original sample, a 1-ml aliquot was transferred to an APTIMA specimen transfer tube to which had been added 100 µl of PACE 2 Fast Express solution before being heated at 65 °C for 2 hours. This was then transferred to the Tigris platform for further extraction, followed by amplification and detection.

Sample size

The study aimed to include 2500 samples for each test comparison, which would be expected to yield approximately 2100 negatives (HPV negative or histology less than CIN2) and 400 positives (CIN2+). This would provide 80% power to demonstrate a sensitivity of at least 95% (assuming a proportion of discordant pairs of 0.08 and sensitivity of HC2 of 95%) relative to HC2 in the detection of CIN2+.

Analyses

Data on cytology result, RLU value for HC2 assay, HPV results and management, together with outcome of subsequent referrals to colposcopy, were sent to the coordinating centre for analysis. All samples had been tested by HC2 and by one or more of the alternative assays. Comparisons were made between results of each alternative assay with HC2, based on the paired results of all samples tested by

Table 2. Samples included and numbers positive for individual assays: (a) borderline and mild dyskaryosis samples; (b) borderline samples only

			HC2 positive		HC2 negative				
			Comparison assay		Comparison assay				
LBC type	Comparison assay	*N* (invalid)	Positive *n* (%)	Negative *n* (%)	Positive *n* (%)	Negative *n* (%)	Total positive (%)	Total discordant *n* (%)	Odds ratio (95% CI)
(a)									
SurePath	rtHPV	2114 (6)	1226 (58.0)	243 (11.5)	65 (3.1)	580 (27.4)	72.6	308 (14.6)	0.27 (0.20–0.35)
	GHPV	3486 (13)	2044 (58.6)	454 (13.0)	52 (1.5)	936 (26.8)	73.1	506 (14.5)	0.12 (0.09–0.16)
	Cobas	2373 (33)	1525 (64.3)	185 (7.8)	82 (3.5)	581 (24.5)	75.5	267 (11.3)	0.44 (0.34–0.58)
ThinPrep	rtHPV	2167 (1)	1322 (61.0)	207 (9.6)	42 (1.9)	596 (27.5)	72.5	249 (11.5)	0.20 (0.14–0.28)
	GHPV	2072 (6)	1277 (61.6)	185 (8.9)	27 (1.3)	583 (28.1)	71.9	212 (10.2)	0.15 (0.09–0.22)
	Cervista	4217 (28)	2800 (66.4)	343 (8.1)	128 (3.0)	946 (22.4)	77.6	471 (11.2)	0.38 (0.30–0.46)
	Cobas	2447 (17)	1514 (61.9)	201 (8.2)	63 (2.6)	668 (27.3)	72.7	264 (10.8)	0.31 (0.23–0.42)
(b)									
Surepath	rtHPV	1441 (4)	734 (50.9)	150 (10.4)	54 (3.7)	503 (34.9)	65.1	204 (14.2)	0.36 (0.26–0.49)
	GHPV	2447 (9)	1268 (51.8)	325 (13.3)	40 (1.6)	814 (33.3)	66.7	365 (14.9)	0.12 (0.09–0.17)
	Cobas	1643 (9)	946 (57.6)	121 (7.4)	68 (4.1)	508 (30.9)	69.1	189 (11.5)	0.56 (0.41–0.76)
Thinprep	rtHPV	1503 (0)	816 (54.3)	143 (9.5)	33 (2.2)	511 (34.0)	66.0	176 (11.7)	0.23 (0.15–0.34)
	GHPV	1431 (5)	775 (54.2)	136 9.5)	19 (1.3)	501 (35.0)	65.0	155 (10.8)	0.14 (0.08–0.23)
	Cervista	2966 (15)	1784 (60.1)	249 (8.4)	103 (3.5)	830 (28.0)	72.0	352 (11.9)	0.42 (0.33–0.53)
	Cobas	1752 (12)	963 (55.0)	140 (8.0)	56 (3.2)	593 (33.8)	66.2	196 (11.2)	0.40 (0.29–0.55)

CI, confidence interval; GHPV, Gen-Probe APTIMA HPV; HC2, Hybrid Capture 2; LBC, liquid-based cytology; rtHPV, real-time HPV PCR.

that assay. The agreement on HPV positivity was compared by McNemar's test, and kappa values were calculated. Relative specificity was calculated in cases not referred for colposcopy or found to be negative following colposcopy. The specificity of each alternative test relative to HC2 was calculated as the ratio of the total negative on the alternative test to the total negative on HC2, assuming that there would be no positive disease in those cases that were negatives on both tests and hence not referred. The relative sensitivity of each test in comparison with HC2 was calculated in cases with a positive outcome following colposcopy; the sensitivity of each alternative test relative to HC2 was calculated as the ratio of those positive on the alternative test to those positive on HC2.

Analyses were performed using both CIN2+ and CIN3+ as criteria for a positive outcome; analyses were also performed separately for samples with a result of borderline cytology and mild dyskaryosis, and for the age groups of less than 35 years and 35 years or more. For the relative sensitivity of each

Table 3. Summary of valid colposcopy results

LBC type	Comparison assay	Number with valid colposcopy result: *n* (%) positive	*n* (%) discordant	Total
SurePath	rtHPV	632 (41.2)	124 (40.3)	640
	GHPV	1744 (68.4)	340 (67.2)	1756
	Cobas	1370 (76.5)	189 (70.8)	1373
ThinPrep	rtHPV	1415 (90.1)	223 (89.6)	1487
	GHPV	1338 (89.9)	189 (89.2)	1421
	Cervista	2807 (85.8)	406 (86.2)	2871
	Cobas	1622 (91.2)	241 (91.3)	1674

GHPV, Gen-Probe APTIMA HPV; HC2, Hybrid Capture 2; LBC, liquid-based cytology; rtHPV, real-time HPV PCR.

assay to HC2 for CIN2+, non-inferiority (i.e. relative sensitivity not lower than 95%) was tested using the score test proposed by Meijer *et al.*[10]

Results

A total of 6877 SP and 5571 TP samples was submitted. Of the SP samples, 1161 were tested by two assays in addition to HC2 (all but eight of these by rtHPV and GHPV) and the remainder by a single assay only; 61 samples were excluded from the analysis because of a lack of any valid test result. Of the TP samples, 1115 were tested by all four alternative assays, 1005 by three, 93 by two and 3242 by a single assay only; 116 samples were not tested or had no valid test. A total of 18 881 paired comparisons was therefore available for analysis (7977 on SP and 10 904 on TP samples). The number of samples tested per assay with a valid result ranged from 2072 to 4217.

Table 2a summarizes the data received, the numbers of cytology samples that were successfully tested by each assay, the proportions that were HPV positive, the proportion of discordant pairs and the odds ratios. The proportion of samples positive on HC2 ranged from 69.5% to 74.5% for the different comparisons; the percentage positive on the alternative assays ranged from 60.1% to 69.4%. For all assays, the proportion of positive samples was less than that for HC2. The proportion of discordant pairs ranged from 11.3% to 14.6% for the SP comparisons, and from 10.2% to 11.5% for the TP comparisons. The odds ratios (the ratio of HC2-negative/comparison assay-positive to HC2-positive/comparison assay-negative tests) ranged from 0.12 to 0.44 ($P < 0.001$ for all comparisons). Kappa values for the comparisons ranged from 0.68 to 0.76. Table 2b gives the equivalent data for borderline samples only. The odds ratios are mostly slightly higher than for the overall data, ranging from 0.12 to 0.56.

Table 3 gives the numbers of valid colposcopy results obtained for positive samples and those with discordant results. Overall, the total referred to colposcopy was higher than the number positive on either test, as some samples were included in more than one comparison and were positive on another test, and some women were referred despite no positive HPV result, presumably for clinical reasons. For all except the rtHPV and GHPV comparisons with SP samples, colposcopy outcomes were available on at least 75% of those referred at the time follow-up for the study ended. The percentages of all positive samples and of those with discordant results that had

Table 4. Relative specificity and sensitivity of assays in comparison with HC2

LBC type	Assay	Specificity relative to HC2 (95% CI): <CIN2	<CIN3	Sensitivity relative to HC2 (95% CI): CIN2+	CIN3+
SurePath	rtHPV	1.14 (1.10–1.17)	1.13 (1.09–1.17)	1.05 (0.98–1.12)	1.03 (0.93–1.14)
	GHPV	1.27 (1.23–1.31)	1.27 (1.23–1.31)	0.99 (0.96–1.02)	0.97 (0.94–1.00)
	Cobas	1.12 (1.07–1.16)	1.12 (1.07–1.16)	0.99 (0.96–1.02)	1.00 (0.97–1.04)
ThinPrep	rtHPV	1.22 (1.17–1.27)	1.23 (1.18–1.29)	0.99 (0.95–1.03)	1.02 (0.98–1.06)
	GHPV	1.23 (1.18–1.28)	1.23 (1.19–1.28)	1.00 (0.96–1.04)	1.06 (0.99–1.12)
	Cervista	1.18 (1.14–1.22)	1.18 (1.15–1.23)	0.98 (0.96–1.00)	0.98 (0.95–1.02)
	Cobas	1.17 (1.13–1.22)	1.17 (1.13–1.22)	0.98 (0.95–1.02)	0.98 (0.93–1.04)

CI, confidence interval; CIN, cervical intraepithelial neoplasia; GHPV, Gen-Probe APTIMA HPV; HC2, Hybrid Capture 2; LBC, liquid-based cytology; rtHPV, real-time HPV PCR.

Table 5. Relative specificity and sensitivity of assays for CIN2+ according to cytology result

		Specificity relative to HC2 (95% CI)		Sensitivity relative to HC2 (95% CI)	
LBC type	Comparison test	Borderline	Mild	Borderline	Mild
Surepath	rtHPV	1.08 (1.05–1.11)	1.49 (1.31–1.71)	1.04 (0.96–1.12)	1.06 (0.95–1.19)
	GHPV	1.22 (1.18–1.26)	1.59 (1.42–1.78)	0.98 (0.94–1.01)	1.00 (0.95–1.05)
	Cobas	1.06 (1.02–1.10)	1.51 (1.31–1.75)	0.96 (0.92–1.01)	1.03 (0.98–1.08)
ThinPrep	rtHPV	1.18 (1.13–1.23)	1.47 (1.28–1.68)	0.96 (0.91–1.02)	1.03 (0.99–1.08)
	GHPV	1.21 (1.16–1.26)	1.37 (1.21–1.55)	0.98 (0.93–1.03)	1.03 (0.97–1.10)
	Cervista	1.14 (1.10–1.18)	1.43 (1.26–1.61)	0.99 (0.96–1.01)	0.97 (0.94–1.00)
	Cobas	1.12 (1.08–1.16)	1.61 (1.39–1.86)	0.96 (0.92–1.01)	1.01 (0.96–1.07)

CI, confidence interval; CIN, cervical intraepithelial neoplasia; GHPV, Gen-Probe APTIMA HPV; HC2, Hybrid Capture 2; LBC, liquid-based cytology; rtHPV, real-time HPV PCR.

valid colposcopy outcomes were similar for all assays, apart from Cobas with SP samples, where the proportions were 76.5% and 70.8%, respectively.

Table 4 summarizes the results on specificity and sensitivity for each assay relative to HC2 in both women HPV negative on both tests or with histology result less than CIN2 and in those HPV negative or less than CIN3 on histology. All the alternative assays were significantly more specific than HC2; the relative specificity ranged from 1.12 to 1.27 for both cut-offs. The relative sensitivity of each alternative assay to HC2 in those women with CIN2+ or CIN3+ on histology was demonstrated to be at least 95% ($P = 0.01$–0.05) and ranged from 0.98 to 1.05 for CIN2+ and from 0.97 to 1.06 for CIN3+.

For all assays, the relative specificity (for samples reported negative or with histology less than CIN2) was higher for mild dysksaryosis (range, 1.37–1.61) than for borderline cytology (range, 1.06–1.22) (Table 5). Relative sensitivity ranged from 0.97 to 1.06 for mild dyskaryosis and from 0.96 to 1.04 for borderline cytology. Four tests (GHPV on SP and TP, and rtHPV and Cervista on TP) had significantly higher specificity in samples in the younger age group (Table 6). There were no significant differences between age groups in the relative sensitivities.

Discussion

All of the assays evaluated met the predetermined criteria of at least 95% sensitivity for the detection of CIN2 relative to HC2, confirming the appropriateness of their use in the NHSCSP for the triage of women with borderline or mild cytology to colposcopy or return to routine recall. All assays also demonstrated significantly increased specificity relative to HC2, suggesting the potential to reduce referrals

Table 6. Relative specificity and sensitivity of assays for CIN2+ by age group

		Specificity relative to HC2 (95% CI)		Sensitivity relative to HC2 (95% CI)	
		<CIN2		CIN2+	
LBC type	Assay	<35 years	35+ years	<35 years	35+ years
SurePath	rtHPV	1.16 (1.08–1.24)	1.13 (1.09–1.18)	1.05 (0.97–1.14)	1.04 (0.96–1.13)
	GHPV	1.40 (1.31–1.50)	1.21 (1.17–1.25)	0.98 (0.95–1.02)	0.99 (0.92–1.06)
	Cobas	1.09 (1.01–1.18)	1.13 (1.08–1.18)	1.00 (0.97–1.03)	0.96 (0.88–1.05)
ThinPrep	rtHPV	1.33 (1.23–1.44)	1.17 (1.12–1.23)	0.97 (0.94–1.01)	1.02 (0.94–1.11)
	GHPV	1.34 (1.24–1.46)	1.18 (1.13–1.24)	0.98 (0.95 –1.02)	1.04 (0.94–1.15)
	Cervista	1.30 (1.22–1.38)	1.10 (1.06–1.14)	0.97 (0.95–0.99)	1.01 (0.96–1.06)
	Cobas	1.21 (1.12–1.30)	1.16 (1.11–1.21)	0.99 (0.97–1.02)	0.96 (0.85–1.08)

CI, confidence interval; CIN, cervical intraepithelial neoplasia; GHPV, Gen-Probe APTIMA HPV; HC2, Hybrid Capture 2; LBC, liquid-based cytology; rtHPV, real-time HPV PCR.

to colposcopy. It should be noted that the cut-off used for the HC2 assay was 2 RLU; had the manufacturer's recommended cut-off of 1 RLU been used, the sensitivity of HC2 would have been increased, but the specificity decreased. The increase in relative sensitivity cannot be estimated as samples positive on HC2 using a cut-off of 1 RLU, but negative on the alternative assay, will not have been referred. However, in the ARTISTIC randomized trial, the proportions of CIN2+ lesions detected in women with low-grade cytology were 17.3% at an RLU/cut-off (Co) ratio of greater than 1 and 18.0% at an RLU/Co ratio of greater than 2, with relative sensitivities of 87.7% and 84.2%, respectively.[9] The authors concluded that increasing the threshold for positivity from an RLU/Co ratio of greater than 1 to an RLU/Co ratio of greater than 2 resulted in a beneficial balance between relative sensitivity and the proportion of CIN2+ lesions detected, for both routine screening and triage for low-grade cytology.

The proportion of samples with an outcome of CIN2+ was lower than that used as the basis for the calculation of the sample size. Therefore, this required longer testing with some assays until a sufficient number of positive samples had been accrued. The total number tested and the number of confirmed CIN2+ remained lower than originally estimated for some assays, but the proportion of discordant pairs was lower than predicted (6% on average) and the numbers obtained were sufficient for the assessment of all assays despite the more stringent criteria for relative sensitivity than those recommended by Meijer *et al.*[10]

These data relate to HPV triage of cytology samples reported as borderline or mild dyskaryosis. It is possible, given different clinical circumstances, that the performance of the alternative assays with regard to sensitivity and specificity will differ when these assays are used in either primary screening or test of cure settings. Recently, Cuzick *et al.*[11] reported the performance of six assays in a screening population, and found that HC2 and three of the alternative assays included in the present study all showed high sensitivity for high-grade lesions positive by cytology. Mesher *et al.*[12] reported the sensitivity of a range of assays (including HC2) in women referred to colposcopy with low-grade abnormalities, and found that five tests had very high sensitivity in such a setting. In the Predictors 2 study, Szarewski *et al.*[13] studied the performance of seven tests in 1099 samples of women referred for colposcopy on the basis of abnormal cytology, and found that five showed high sensitivity (93% or above) for CIN2+. Other studies have compared the performance of several assays, including rtHPV, GHPV and Cobas, with that of HC2 in different settings and have also shown satisfactory performance.[14–17] Recent reviews of commercially available assays have highlighted the potential for the use of assays that include HPV-16 and HPV-18 genotyping to improve test accuracy.[18,19]

Although lower for borderline cytology than for mild dyskaryosis, the specificity relative to HC2 for borderline cytology was still significantly increased for all assays. This is of relevance to settings in which only ASC-US cytology is triaged with HPV testing. As expected, overall HPV-positive rates were higher in women under the age of 35 years (84% versus 62%) and, for some but not all assays, this was reflected in a higher relative specificity in this age group.

Our study has the advantage that it was performed in a routine setting of HPV triage in population screening using the current policy of the NHSCSP. Limitations include the fact that colposcopy outcomes were not available for a proportion of samples with a positive HPV result. However, we have no reason to believe that this will have led to any bias in the findings. Paired testing was performed in order to ensure that new commercially available HPV tests would not result in significant and ineffective changes in colposcopy referrals. The study has demonstrated that, across the full screening age range, and across several sites in routine service, the four tests chosen had adequate sensitivity and enhanced specificity when compared with HC2 at a cut off of 2 RLU. Consequently, they have been accepted for use as a triage in the English NHSCSP.

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Conflicts of interest

H.K. is chair of the Advisory Committee for Cervical Cancer, but any view expressed in this paper is that of the authors and in no way reflects the view of Public Health England. K.D. has received sponsorship from Hologic and MTM (subsequently

acquired by Roche) to attend scientific meetings within the last 5 years. H.C. has received an unrestricted educational grant from GSK and occasional honoraria/consultation fees from Abbott, Hologic/Gen-Probe, Qiagen and Roche. P.M. has received sponsorship from Roche to attend national and international scientific meetings in the past 5 years, has received payment and expenses from Abbott UK to present at a UK Users Group meeting, and is in receipt of funding from Gen-Probe (now part of Hologic) for the evaluation of unrelated Gen-Probe products.

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