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Optimal Threshold for a Positive Hybrid Capture 2 Test for Detection of Human Papillomavirus: Data from the ARTISTIC Trial[▽]

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We present data on the use of the Hybrid Capture 2 (HC2) test for the detection of high-risk human papillomavirus (HR HPV) with different thresholds for positivity within a primary screening setting and as a method of triage for low-grade cytology. In the ARTISTIC population-based trial, 18,386 women were screened by cytology and for HPV. Cervical intraepithelial neoplasia lesions of grade two and higher (CIN2+ lesions) were identified for 453 women within 30 months of an abnormal baseline sample. When a relative light unit/cutoff (RLU/Co) ratio of ≥1 was used as the threshold for considering an HC2 result positive, 15.6% of results were positive, and the proportion of CIN2+ lesions in this group was 14.7%. The relative sensitivity for CIN2+ lesion detection was 93.4%. When an RLU/Co ratio of ≥2 was used as the threshold, there was a 2.5% reduction in positivity, with an increase in the proportion of CIN2+ lesions detected. The relative sensitivity decreased slightly, to 90.3%. Among women with low-grade cytology, HPV prevalences were 43.7% and 40.3% at RLU/Co ratios of ≥1 and ≥2, respectively. The proportions of CIN2+ lesions detected were 17.3% and 18.0%, with relative sensitivities of 87.7% at an RLU/Co ratio of ≥1 and 84.2% at an RLU/Co ratio of ≥2. At an RLU/Co ratio of ≥1, 68.3% of HC2-positive results were confirmed by the Roche line blot assay, compared to 77.2% of those at an RLU/Co ratio of ≥2. Fewer HC2-positive results were confirmed for 35- to 64-year-olds (50.3% at an RLU/Co ratio of ≥1 and 63.2% at an RLU/Co ratio of >2) than for 20- to 34-year-olds (78.7% at an RLU/Co ratio of ≥1 and 83.7% at an RLU/Co ratio of >2). If the HC2 test is used for routine screening as an initial test or as a method of triage for low-grade cytology, we would suggest increasing the threshold for positivity from the RLU/Co ratio of ≥1, recommended by the manufacturer, to an RLU/Co ratio of ≥2, since this study has shown that a beneficial balance between relative sensitivity and the proportion of CIN2+ lesions detected is achieved at this threshold.

Persistent infection with any of the 15 cancer-associated high-risk human papillomavirus (HR HPV) genotypes is now well recognized as essential for the subsequent development of cervical cancer and its high-grade precursor lesions (2, 15, 16). Due to the very high prevalence of HPV infections that typically resolve within 1 to 2 years, especially in younger women (6, 11), the role of HPV testing in the early detection of cervical lesions remains controversial. The most widely used test for the detection of a group of 13 HR HPV genotypes is the commercially available, FDA-approved Hybrid Capture 2 high-risk HPV DNA test (HC2 test; Qiagen [formally known as Digene]). Hybrid Capture 2 technology consists of a nucleic acid hybridization assay with signal amplification that utilizes microplate chemiluminescence for the qualitative detection of HPV. There is increasing interest in the use of HC2 technology within the cervical screening program either as a stand-alone screening test or in conjunction with cytology. Other important clinical applications include the use of the HC2 assay as a triage test for women with low-grade cytological changes (borderline or mild dyskaryosis) or as a test for monitoring and predicting therapeutic outcomes for women treated for high-grade (grade 3) cervical intraepithelial neoplasia (CIN3). While this assay is reliable and has been well validated in terms of its clinical sensitivity for the detection of CIN3 and cervical cancer (5, 8), some concerns over its specificity and positive predictive value (PPV) nevertheless remain. A number of reports have suggested that it not only detects non-high-risk HPV types, which are clinically insignificant, but may also cross-react with non-HPV nucleic acid (3, 9, 10, 14). These features, combined with the high prevalence of cervical HPV infections, would lead to a large number of women being identified as "at risk" and therefore needlessly overinvestigated. One method of improving the clinical utility of the HC2 assay in terms of the specificity of HPV testing is to raise the threshold at which results are considered positive in order to achieve a clinically relevant balance between sensitivity and specificity.

ARTISTIC (A Randomized Trial In Screening To Improve Cytology) was a randomized population-based screening trial of liquid-based cervical cytology plus HPV testing, with accrual of 24,510 women undergoing routine primary screening in the United Kingdom National Health Service Cervical Screening program (NHSCSP) in Greater Manchester. Women were randomized at a ratio of 3:1 to have the HPV test result revealed and acted upon if it was persistently positive in cytology-neg-

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ative cases (revealed arm) or to have the result concealed (concealed arm). The primary analysis has been published recently (7); it showed that only 3% of women with negative cytology and persistently HPV DNA positive results were found to have CIN of grade 2 or higher (CIN2+) and that over two screening rounds there was no significant difference in CIN2+ or CIN3+ between the two arms of the trial.

We wanted to determine the optimal threshold, with respect to clinical utility, for a positive HC2 test by using the large data set collected from the first round of the ARTISTIC trial. Our secondary objective was to further investigate all positive samples by the Roche prototype line blot assay (LBA) as a means of confirming the HC2 result and examining the impact of typing for HPV type 16 (HPV 16) and HPV 18.

MATERIALS AND METHODS

As part of the ARTISTIC trial study design, consenting women attending offices of general practitioners and family planning clinics for routine cervical screening in Greater Manchester, United Kingdom, between 2001 and 2003 (round 1) were tested for HPV using the HR HC2 test. Cervical samples were collected into PreservCyt (Cytyc) liquid-based cytology (LBC) solution, and slides were prepared on the ThinPrep T3000 processor (Hologic) at the Manchester Cytology Centre. Cytology results were reported using the BSCC classification. All smears were read by the cytoscreeners as the first screening modality, and all abnormal slides were checked by a biomedical scientist or cytopathologist before reports were authorized. A rapid screening quality control of all negative and inadequate slides was performed before such reports were authorized. After processing for cytology, residual material was tested for HR HPV at the Manchester Virology laboratory using the HC2 assay, which targets a group of 13 HR HPV genotypes. Briefly, after denaturation of a 4-ml aliquot of the LBC sample, the single-stranded HPV DNA present in the sample was hybridized with a specific probe containing cRNA sequences for the 13 HR HPV genotypes. The resultant RNA-DNA hybrid was then captured on the surface of an antibody-coated microtiter plate. Immobilized hybrids were then reacted with alkaline phosphatase-conjugated antibodies specific for RNA-DNA hybrids and were detected with a chemiluminescent substrate that is cleaved by the action of alkaline phosphatase to produce light. Positive results were expressed as relative light units (RLU) compared to the result for a high-risk positive control containing 1 pg/ml of HPV DNA, equivalent to 100,000 HPV copies/ml or 5,000 HPV copies per assay.

A total of 24,510 adequate cytology and HPV results were obtained for women 20 to 64 years old. Women were followed up according to the ARTISTIC study design, in which participants were randomized at a ratio of 3:1 either to have the HPV test result revealed and acted upon (revealed arm) or to have it concealed from the woman and her doctor (concealed arm). There were a total of 18,386 and 6,124 women in the revealed and concealed arms, respectively. The mean age of women in the revealed arm was 40.16 years, and the mean age of women in the concealed arm was 40.21 years ($P = >0.05$). Women were referred to colposcopy if they had either high-grade cytological abnormalities (moderate or severe dyskaryosis), two consecutive mild dyskaryosis results, or three consecutive borderline results. Women in the revealed arm who were normal by cytology but persistently tested HPV positive over a period of at least 1 year were also referred to colposcopy. Punch biopsy specimens and excisional specimens were taken in the presence of an abnormality and provided the histological data for this analysis. Women were classified histologically at round 1 on the basis of the highest grade of histology within 30 months of an abnormal round 1 cytology result or a normal cytology result but persistently HPV positive test results, triggering referral to colposcopy.

Cells from an additional 4-ml aliquot of the LBC samples were pelleted, resuspended in phosphate buffered saline (PBS), and stored at −70°C. Stored aliquots from all HC2-positive samples were retrospectively tested using the prototype LBA (kindly donated by Roche). The LBA is a genotyping assay that amplifies 37 HPV types simultaneously, including the 13 HC2 target types, using PGMY09–PGMY11 (PGMY09/11) L1 consensus primer PCR. The assay was carried out essentially according to the manufacturer's instructions as described previously (12), except that the Roche MagNA Pure LC automated extraction system was used instead of the manual Qiagen vacuum extraction method and 50 μl of DNA was amplified instead of 5 μl as suggested by the Roche protocol. While the manual extraction method was the only method validated for extraction of LBC samples prior to detection with LBA, this extraction procedure is laborious and time-consuming and therefore was unrealistic for the extraction of all HC2-positive samples generated in the ARTISTIC trial. The MagNA Pure system was an attractive alternative for DNA extraction because of its ease of use. The LBA was validated for the manual extraction method using 250 μl of the original LBC sample. We had available a five-times-concentrated cell pellet. We therefore extracted DNA from a 50-μl aliquot of the cell pellet using the automated MagNA Pure system. HPV test panels provided by Roche were initially validated using the MagNA Pure system, and results were certified by Roche Molecular Diagnostics before any testing was carried out on clinical material. Following denaturation of the amplified product, HPV and beta-globin sequences, if present, were hybridized to oligonucleotide-coated genotyping strips before color development and interpretation using the template provided.

Data analysis. Samples were classified as HC2 HR HPV positive for the purpose of the ARTISTIC trial, as well as for this study, according to the manufacturer's instructions, which were to use an RLU/cutoff (RLU/Co) ratio of ≥1 as the threshold for a positive HC2 test result. The semiquantitative nature of the HC2 assay allows for a range of threshold values, and the large size of the study has therefore allowed retrospective analysis of different threshold values using data from round 1. The higher RLU/Co values that we considered were ≥2 and ≥4. This evaluation was confined to women from the revealed arm in order to minimize verification bias in terms of differences in rates of referral to colposcopy between the two study arms. The sensitivity of the assay is referred to as relative sensitivity, because only women who were HPV positive over a 12-month period were referred to colposcopy. The prototype LBA, which detects 37 HPV types, including the 13 target HC2 types (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68), was used to confirm the detection of HC2-positive samples for the varying threshold values described. A positive HC2 result for which the DNA was found to contain one or more of the 13 target HC2 types was described as a "confirmed HC2" sample. We also examined the impact of the different thresholds when the HC2-positive result was categorized as either HPV 16 and/or HPV 18 (HPV 16/18) positive or non-HPV 16, non-HPV 18 positive.

RESULTS

Proportion of CIN2+ lesions identified and relative sensitivity for women who tested HPV positive at an HC2 RLU/Co ratio of ≥1. The prevalence of HPV according to the manufacturer's criteria, with a positive RLU/Co ratio of ≥1, was 15.6% (2,860/18,386) for women in the revealed arm at baseline in the ARTISTIC study (Table 1). In total, 26% (744/2,860) of the positive samples had an RLU/Co value between 1 and <4, while 74% (2,116/2,860) had an RLU/Co value of ≥4. With increasing severity of the cytology grade, there was a strong linear trend of increasing HPV prevalence, from 10.4% (1,675/16,042) for cytological normality to 43.7% (867/1,986) for borderline/mild dyskaryosis and 88.8% (318/358) for moderate or worse dyskaryosis (P_{Trend}, <0.0001). Histological data were available for 453 women in whom CIN2+ lesions were identified within 30 months of entry into the trial. The proportion of women with HC2 RLU/Co values of ≥1 at baseline for whom CIN2+ was detected was 14.7% (423/2,860). A high proportion of these women (94.8%) had RLU/Co values of >4. The relative sensitivity of the HC2 assay for the detection of CIN2+ was 93.4% (423/453) and increased to 97.0% (226/233) for women who had underlying histologically confirmed CIN3+. In total, 10.5% (23/220) of women with CIN2 lesions were found to be HC2 negative, while only 3% (7/233) of women with CIN3+ lesions were HC2 negative.

Proportion of CIN2+ lesions identified and relative sensitivity for women who tested HPV positive after adjustment of the HC2 RLU/Co value. Table 1 shows that adjustment of the HC2 positivity threshold to an RLU/Co ratio of ≥2 resulted in a 2.5% (confidence interval [CI], 2.26 to 2.72%) reduction in overall test positivity. The reduction in test positivity at an RLU/Co ratio of ≥2 by cytology grade from that at the lower RLU/Co

TABLE 1. Cytology and histology by varying HC2 positivity thresholds[a]

Parameter and group	Total no.	No. (%) of HC2 results			
		<1 RLU	1 to <2 RLU	2 to <4 RLU	≥4 RLU
Cytology					
Normal	16,042	14,367 (89.6)	389 (2.4)	249 (1.6)	1,037 (6.5)
Borderline/mild	1,986	1,119 (56.3)	65 (3.3)	34 (1.7)	768 (38.7)
Moderate/worse	358	40 (11.2)	3 (0.8)	4 (1.1)	311 (86.9)
All women	18,386	15,526 (84.4)	457 (2.5)	287 (1.6)	2,116 (11.5)
Histology by cytology in round 1					
Normal (n = 16,042)					
CIN2	22		4 (18.2)	0 (0)	18 (81.8)
CIN3+	10		1 (10)	2 (20)	7 (70)
Borderline/mild (n = 1,986)					
CIN2	100	18 (18)	4 (4)	3 (3)	75 (75)
CIN3+	71	3 (4.2)	2 (2.8)	0 (0)	66 (93)
Moderate/worse (n = 358)					
CIN2	98	5 (5.1)	2 (2.0)	1 (1.0)	90 (91.8)
CIN3+	152	4 (2.6)	1 (0.7)	2 (1.3)	145 (95.4)
All cytology grades (n = 18,386)					
CIN2 in round 1	220	23 (10.5)	10 (4.5)	4 (1.8)	183 (83.2)
CIN3+ in round 1	233	7 (3.0)	4 (1.7)	4 (1.7)	218 (93.6)

[a] Revealed arm only (n = 18,386).

ratio of ≥1 was statistically significant for women with normal cytology (1,675/16,042 [10.4%] versus 1,286/16,042 [8.0%] positive; reduction, 2.4% [CI, 2.20 to 2.67%]) or borderline/mild cytology (867/1,986 [43.7%] versus 802/1,986 [40.4%] positive; reduction, 3.3% [CI, 2.53 to 4.15%]). There was not a statistically significant reduction for women with moderate/worse cytology (318/358 [88.8%] versus 315/358 [88.0%] positive; reduction, 0.8% [CI, 0.17 to 2.43%]). Adjustment of the HC2 positivity threshold to an RLU/Co ratio of ≥2 would have resulted in a loss of 14 out of 423 (3.3%) CIN2+ lesions (including 4 out of 226 [1.8%] CIN3+ lesions) that were HC2 positive at an RLU/Co ratio of ≥1. This would result in slight decreases in the relative sensitivities for CIN2+ and CIN3+ lesions, to 90.3% (409/453) and 95.3% (222/233), respectively. An increase in the proportion of CIN2+ lesions detected among women who were positive at an RLU/Co ratio of ≥2 over that among women positive at an RLU/Co ratio of ≥1, from 14.7% (423/2,860) to 17.0% (409/2,403), was observed.

If different grades of cytology are compared, among women with normal cytology, an increase in the positivity threshold from an RLU/Co ratio of ≥1 to an RLU/Co ratio of ≥2 would have resulted in 5 out of 32 (15.6%) CIN2+ lesions (including 1 out of 10 [10%] CIN3+ lesions) being missed, with a reduction of 389 out of 1,675 (23.2%) HC2-positive test results (Table 1). Among women with borderline/mild cytology, another 6 out of 150 (4%) CIN2+ lesions (including 2 out of 66 [3%] CIN3+ lesions) would have been missed, with the benefit of 65 out of 867 (7.5%) fewer colposcopies if HPV testing was being used for triage.

Increasing the HC2 positivity threshold to an RLU/Co ratio of ≥4 (Table 1) results in a further decrease in the overall HPV prevalence to 11.5% (2,116/18,386), equating to 744 fewer HC2-positive test results than would be obtained with a threshold of ≥1, and a reduction in the overall sensitivity for the detection of CIN2+ lesions to 88.5% (401/453). Women with test results at a higher RLU/Co value, ≥4, were at a six-times-greater risk of CIN2+ than women who had an RLU/Co value between 1 and 3.999. The proportions of CIN2+ lesions detected were 19% (401/2,116) and 3.0% (22/744), respectively.

Confirmatory testing of HC2-positive samples by the Roche prototype line blot assay. Further testing of the 2,860 HC2-positive samples by the Roche prototype LBA found that 35 (1.2%) samples had invalid LBA results, either testing beta-globin negative or having insufficient volume for additional testing. The analytical specificity of HC2-positive samples investigated using the LBA was therefore based on 2,825 samples. The results revealed that at an RLU/Co value of ≥1, 68.3% (1,935/2,825) of the HC2-positive samples were confirmed by the LBA and were found to contain one or more of the 13 HC2 target types (Table 2). Significantly more HC2-positive samples were confirmed by the LBA at an HC2 positivity threshold consisting of an RLU/Co ratio between 2 and <4 than at an RLU/Co threshold between 1 and <2 (45.4% versus 22.6%; P_{Trend}, <0.0001). At an RLU/Co threshold of ≥2, 77.2% (1,833/2,374) of HC2-positive samples were confirmed, and at a threshold of ≥4, this proportion increased to 81.5% (1,704/2,090).

When we examined the proportion of confirmed HC2-positive samples by age group, we found that at an RLU/Co ratio of ≥1, HC2-positive samples were confirmed for 78.7% (1,423/1,807) of 20- to 34-year-olds, while significantly fewer HC2-positive samples (50.3% [512/1,018]) were confirmed for 35- to 64-year-olds (P_{Trend}, <0.0001). Raising the threshold to an RLU/Co ratio of ≥2 increased the proportions of HC2-positive samples confirmed by the LBA to 83.7% (1,355/1,618) for 20- to 34-year-olds and 63.2% (478/756) for 35- to 64-year-olds.

The proportion of confirmed HC2-positive samples is shown to increase with the cytology grade (Table 2), from 57.9% (962/1,661) for cytologically normal samples to 96.8% (302/312) for samples showing moderate/severe dyskaryosis (P_{Trend}, <0.0001) at an RLU/Co value of ≥1. Significantly higher proportions of confirmed HC2-positive samples were observed at an RLU/Co ratio of ≥2 than at an RLU/Co ratio of ≥1 for

women who had normal cytology (69.1% versus 57.9%; P_{Trend}, <0.0001) or borderline/mild cytology (82.7% versus 78.8%; P_{Trend}, 0.0443). This trend was not apparent among women with moderate/severe dyskaryosis (96.8% versus 96.8%). The risk of CIN2+ lesions during round 1 was significantly higher among women with confirmed HC2-positive samples at an RLU/Co value of ≥1 than among women with unconfirmed samples (95.9% versus 4.1%, respectively).

Table 2 also categorized HC2-positive women who were either positive or negative for HPV 16 and/or 18 DNA by the LBA. As expected, CIN2+ was significantly ($P < 0.0001$) more prevalent among HPV 16/18-confirmed women than among women with confirmed non-type 16, non-type 18 HPV, irrespective of varying positivity thresholds and across all grades of cytology. The impact of altering the HC2 positivity threshold on the relative sensitivity of detection of CIN2+ lesions was lower for women with confirmed HPV 16 and/or HPV 18.

TABLE 2. Confirmatory testing of HC2-positive samples by the Roche prototype line blot assay

Parameter and group	Result with the following HC2 positivity threshold:											
	RLU/Co, 1 to <2				RLU/Co, 2 to <4				RLU/Co, ≥4			
	No. of HC2-pos results with valid LBA result[a]	No. (%) of HC2-pos[b] results with:			No. of HC2-pos results with valid LBA result	No. (%) of HC2-pos results with:			No. of HC2-pos results with valid LBA result	No. (%) of HC2-pos results		
		Target type confirmed by LBA[c]	HPV 16 and/or HPV 18 detected by LBA	Non-HPV 16, non-HPV 18 result by LBA		Target type confirmed by LBA	HPV 16 and/or HPV 18 detected by LBA	Non-HPV 16, non-HPV 18 result by LBA		Target type confirmed by LBA	HPV 16 and/or HPV 18 detected by LBA	Non-HPV 16, non-HPV 18 result by LBA
Cytology grade at baseline												
Normal (n = 16,028)	387	82 (21.2)	37 (9.6)	350 (90.4)	247	108 (43.7)	40 (16.2)	207 (83.8)	1,027	772 (75.2)	274 (26.7)	753 (73.3)
Borderline/mild (n = 1,971)	61	17 (27.8)	10 (16.4)	51 (83.6)	33	18 (54.5)	5 (15.2)	28 (84.8)	758	636 (83.9)	244 (32.2)	514 (67.8)
Moderate/worse (n = 352)	3	3 (100)	3 (100)	0 (0)	4	3 (75.0)	0 (0)	4 (100)	305	296 (97.0)	180 (59.0)	125 (41.0)
All cytology grades (n = 18,351)	451	102 (22.6)	50 (11.1)	401 (88.9)	284	129 (45.4)	45 (15.8)	239 (84.0)	2,090	1,704 (81.5)	698 (33.4)	1,392 (66.6)
Histology in round 1												
CIN2 (n = 193)	9	7	5	4	4	3	1	3	180	170	80	100
CIN3+(n = 225)	4	3	2	2	4	4	2	2	217	214	145	72

[a] A valid LBA result was defined by adequate specimen collection, processing, and the absence of inhibitors.
[b] pos, positive.
[c] HC2-positive samples were confirmed by the LBA if they were found to contain one or more of the 13 HC2 target types.

DISCUSSION

Before any implementation of HPV testing in a national cervical screening program, it is necessary to achieve the optimal balance between the clinical sensitivity and specificity of any HPV assay to be used either as a primary screen or as a triage test in order to maximize the benefit of HPV testing and minimize unnecessary diagnostic procedures. These data have been obtained from a trial setting embedded in the national cervical screening program, which provides a "real-life" dimension. The HPV test was not always a single test in round 1, because histological outcomes were obtained over a period as long as 30 months following repeat cytology (if low-grade lesions were found) and repeat HPV testing (if cytology was normal).

If HPV DNA testing were used in primary screening and the threshold were increased from an RLU/Co ratio of ≥1 to ≥2, no clinically important loss in sensitivity would have been observed, with 96% of CIN3+ lesions still testing positive. If the HC2 assay were used as the only indication for colposcopy referral, at an RLU/Co threshold of ≥1, 2,860 (15.6%) women in the population would require further investigation, which could have a major impact on colposcopy rates. A substantial proportion of these referrals would be unnecessary, with only 423 CIN2+ lesions found in this group. By increasing the threshold to an RLU/Co of ≥2, a significant reduction in test positivity would be observed, resulting in 16% fewer referrals, without significantly reducing the number of CIN3+ lesions testing positive. A loss of detection of only four (1.8%) CIN3+ lesions was observed. On increasing the threshold to an RLU/Co ratio of ≥4, we begin to see a significant loss in relative sensitivity for CIN3+.

If the HC2 assay were used in the triage setting to aid referral to colposcopy among women who test borderline/mild by cytology, raising the threshold to an RLU/Co ratio of ≥2 would have resulted in 65 (7.5%) fewer procedures, with an almost identical proportion of CIN2+ lesions detected among HC2-positive women (17.3% [150/867] at an RLU/Co ratio of ≥1 compared with 18.0% [144/802] at an RLU/Co ratio of ≥2). Increasing the threshold to an RLU/Co ratio of ≥4 would mean that a further 34 fewer colposcopies would be performed, with the subsequent failure to detect a further 3 CIN2 lesions; however, no additional CIN3+ lesions would have

gone undetected. Increasing the threshold to an RLU/Co ratio of ≥4 did not result in a significant increase in the proportion of CIN2+ lesions detected over that at an RLU/Co threshold of ≥2 (18.4% versus 18.0%).

Confirmation of HC2-positive samples by the LBA showed that 32.3% could not be confirmed as containing an HC2 target type. This was surprising given the lower analytical sensitivity of the HC2 assay than of the LBA, which uses PGMY09/11 L1 consensus primers to amplify the target HPV DNA. A recent publication described an improvement in analytical sensitivity with the commercialized version of the LBA, which is now available and is called the Linear Array (LA) (Roche Molecular Systems) (4). For 3,335 paired test results, an increase in analytical sensitivity by the LA over that by the LBA was observed, resulting in greater detection for most of the 37 HPV genotypes targeted by both assays. Our method in ARTISTIC used 50 μl of the pelleted sample instead of the 5 μl suggested for the LBA, the former of which is comparable to the volume recommended for the LA. We therefore would not expect to see an increase in analytical sensitivity if HC2-positive samples were investigated using the LA. According to the manufacturer's specifications for the LA, the 13 HC2 target types are detected in the range of 50 to 7,000 copies/ml depending on the genotype. PCR failures due to inhibitors are an unlikely explanation for this large number of unconfirmed samples, since we found only 35 out of 2,860 (1.2%) samples with unsatisfactory beta-globin housekeeping genes. One possible reason may be the presence of HPV DNA that is totally integrated into the host genome, which can result in the subsequent disruption or deletion of the L1 region, the region targeted by the PGMY primers. This is a rare event, however, and most commonly occurs with high-grade lesions. Our data show that the majority of unconfirmed HC2-positive results were found among women with normal or low-grade cytology results and are likely to reflect a certain level of background noise with the HC2 assay at an RLU/Co ratio of 1. This theory is further supported by the low number of CIN2+ lesions detected among women who tested positive with low-level RLU/Co values. These data, again, support an increase in the RLU/Co threshold to ≥2. Another benefit for raising the threshold was observed among older women (35- to 64-year-olds), for whom a far greater proportion of HC2-positive samples were confirmed at an RLU/Co ratio of ≥2 than at an RLU/Co ratio of ≥1. Raising the threshold would thus result in fewer older women being referred to colposcopy.

The limitations of this study include an underestimation of the number of CIN2+ lesions detected, since a number of women invited for a repeat HPV test or to attend colposcopy did not adhere to the protocol. In addition, women who were cytologically normal and HPV negative were not subjected to colposcopy as part of the ARTISTIC protocol, because they were at very low risk. The sensitivity of the HC2 assay can therefore only be estimated in terms of the true prevalence of CIN2+, but the relative sensitivity for different positivity thresholds is a valid measure. Previous studies have reported virtually no CIN2+ occurring among women who tested cytologically normal and HPV negative (1, 13).

In conclusion, our data show that the HC2 assay at an RLU/Co threshold of ≥2 could be used safely and practically both in primary cervical screening and for triage of low-grade cytological abnormalities without significantly reducing the frequency of positive test results in true precursor CIN3 lesions and could thus reduce the number of colposcopic referrals. Confirmatory data from other studies in other settings could result in a recommendation to change to an RLU/Co threshold of ≥2 for the HC2 assay.

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