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Optimal Cut-off for a Positive Hybrid Capture 2 Test for the detection of Human Papillomavirus; data from the ARTISTIC trial

Running title: Optimal cut-off for a positive Hybrid Capture 2 test

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Abstract

We present data on Hybrid Capture 2 (HC2) for the detection of high risk human papillomavirus (HR HPV) using different cut-off values, within a primary screening setting and as a triage for low-grade cytology. In the ARTISTIC population-based trial 18,386 women were screened by cytology and for HPV. Cervical Intraepithelial Neoplasia (CIN2+) lesions were identified amongst 453 women within 30 months of an abnormal baseline sample. At a HC2 cut-off of ≥1RLU/Co, 15.6% were positive and the proportion of CIN2+ in this group was 14.7%. The relative sensitivity for CIN2+ was 93.4%. At ≥2RLU/Co there was a 2.5% reduction in positivity with an increase in the proportion of CIN2+ detected. The relative sensitivity slightly decreased to 90.3%. Amongst women with low-grade cytology HPV prevalence was 43.7% and 40.3% at ≥1 and ≥2RLU/Co respectively. The proportion of CIN2+ detected was 17.3% and 18.0% with a relative sensitivity of 87.7% at ≥1RLU/Co and 84.2% at ≥2RLU/Co.

At ≥1RLU/Co, 68.3% of HC2 positives were confirmed by the Roche line blot compared to 77.2% at ≥2RLU/Co. Fewer HC2 positives were confirmed amongst the 35-64 year olds (50.3% at >1RLU/Co and 63.2% at >2RLU/Co) compared to 20-34 year olds (78.7% at >1RLU/Co and 83.7% at >2RLU/Co).

If used for routine screening as an initial test or as a triage for low-grade cytology we would suggest increasing to ≥2RLU/Co from the ≥1RLU/Co recommended by the manufacturer as this study has shown that a beneficial balance between relative sensitivity and the proportion of CIN2+ detected is achieved.

Introduction

Persistent infection with any of the 15 cancer associated high risk human papillomavirus (HR HPV) genotypes is now well recognised as essential for the subsequent development of cervical cancer and its high grade precursor lesions (15, 16, 2). Due to the very high prevalence of HPV infection especially in younger women which typically resolve within 1 to 2 years (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, Qiagen formally Digene). Hybrid Capture 2 technology is a nucleic acid hybridisation assay with signal amplification that utilizes microplate chemiluminescence for the qualitative detection of HPV. There is increasing interest in the use of HC2 within the cervical screening programme either as a stand alone screening test or in conjunction with cytology. Other important clinical applications is the use of HC2 as a triage test for women with low grade cytological changes (borderline or mild dyskaryosis) or as a test for monitoring and predicting therapeutic outcome of women treated for high grade Cervical Intraepithelial Neoplasia (CIN3). Whilst 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) there nevertheless remain, some concerns over its specificity and positive predictive value (PPV). There have been a number of reports suggesting that it detects not only non-high risk HPV types which are clinically insignificant but may also cross react with non HPV nucleic acid (10, 14, 3, 9). This, 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 over investigated. One method of improving the clinical utility of the HC2 assay in terms of specificity of

HPV testing is to raise the cut-off value to achieve a clinically relevant balance between sensitivity and specificity.

ARTISTIC (A Randomized Trial In Screening To Improve Cytology) was a randomised population based screening trial of liquid based cervical cytology plus HPV testing, with accrual of 24,510 women undergoing routine primary screening in the UK National Health Service Cervical Screening programme (NHSCSP) in Greater Manchester. Women were randomised in a ratio of 3:1 to have the HPV test result revealed and acted upon if persistently positive in cytology-negative cases (revealed arm) or concealed (concealed arm). The primary analysis has recently been published (7), which showed that only 3% of women with negative cytology and persistently HPV DNA positive were found to have CIN2+, and that over two screening rounds there was no significant difference in CIN2+ or CIN3+ between the two arms of the trial.

We wished to determine the optimal cut-off, with respect to clinical utility, for a positive HC2 test using the large dataset 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 examine the impact of HPV 16 and 18 typing.

Materials and Methods

As part of the ARTISTIC trial study design, consenting women attending for routine cervical screening in Greater Manchester 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) 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 first screening modality and all abnormal slides were checked by a biomedical scientist or cytopathologist before reports were authorised. A rapid screening quality control of all negative and inadequate slides was performed before such reports were authorised. 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 4ml aliquot of the LBC sample, single stranded HPV DNA present in the sample was hybridised with a specific probe containing complementary RNA sequences to the 13 HR HPV genotypes. The resultant RNA/DNA hybrid was then captured onto the surface of an antibody coated micro-titre plate. Immobilised hybrids were then reacted with alkaline phosphatase conjugated antibodies specific for RNA/DNA hybrids and detected with a chemiluminescent substrate which is cleaved by the action of alkaline phosphatase to produce light. Positive results were expressed as relative light units compared to a positive high risk control containing 1 pg/ml of HPV DNA equivalent to 100,000 HPV copies/ml or 5000 HPV copies per assay.

A total of 24,510 adequate cytology and HPV results were obtained from women aged 20-64. Women were followed up according to the ARTISTIC study design in which participants were randomised in a ratio of 3:1 either to have the HPV test result revealed and acted upon (revealed arm), or concealed from the women and her doctor (concealed

arm). There were a total of 18,386 and 6,124 women in the revealed and concealed arm 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 (moderate or severe dyskaryosis) cytological abnormalities; two consecutive mild dyskaryosis or three consecutive borderline results. Women in the revealed arm who were cytology normal but persistently tested HPV positive over a period of at least one year were also referred to colposcopy. Punch biopsies 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 or cytology normal but persistently HPV positive triggering referral to colposcopy.

Cells from an additional 4ml aliquot of the LBC samples were pelleted, re-suspended in Phosphate buffered saline (PBS) and stored at -70^{O}C. Stored aliquots from all HC2 positive samples were retrospectively tested using the prototype Line Blot Assay (LBA; kindly donated by Roche). The LBA is a genotyping assay that amplifies 37 HPV types simultaneously including the 13 HC2 target types using PGMY09/11 L1 consensus primer PCR. The assay was carried out essentially according to the manufacturer's instructions as previously described (12) with the exceptions of using the Roche MagNa Pure LC automated extraction system instead of the manual Qiagen vacuum extraction and amplifying 50µl of DNA instead of 5µl as suggested by the Roche protocol. Whilst 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 was an attractive alternative for DNA extraction because of its ease of use. The LBA was validated for the manual extraction method using 250µl of the original LBC sample. We had available a five times concentrated cell pellet. We therefore extracted DNA from a 50µl aliquot of cell pellet using the automated MagNaPure. HPV test panels provided by Roche were initially validated using the MagNA Pure and results were certified by Roche Molecular Diagnostics before any testing on clinical material was carried out. Following denaturation of the amplified product, HPV and beta-globin sequences if present were hybridised to oligonucleotide-coated genotyping strips before colour 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 was to use the recommended threshold for a positive HC2 test result of RLU/Co ≥1. The semi-quantitative nature of the HC2 assay allows for a range of cut-off values and the large size of the study, has therefore allowed retrospective analysis of different cut-off 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 to minimize verification bias in terms of differences in rates of referral to colposcopy between the two study arms. The sensitivity is referred to as relative sensitivity as 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 (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 cut-off values described. A positive HC2 result 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 cut-offs when the HC2 positive result was categorised as either HPV16/18 positive or non 16/18 positive.

Results

Proportion of CIN2+ lesions identified and the relative sensitivity for women who tested HPV positive at a HC2 RLU/Co value of ≥1

The prevalence of HPV according to the manufacturer's criteria with a positive relative light units/cut-off (RLU/Co) of ≥1.0 was 15.6% (2860/18386) for women in the revealed arm at baseline in the ARTISTIC study (Table 1). In total, 26% (744/2860) of the positive samples had an RLU/Co value between 1 and < 4 whilst 74% (2116/2860) had an RLU/Co value of ≥4. With increasing severity of cytology grade there was a strong linear trend of increasing HPV prevalence from 10.4% (1675/16042) for cytological normal, 43.7% (867/1986) for borderline/mild dyskaryosis, and 88.8% (318/358) for moderate or worse dyskaryosis (P_{Trend} <0.0001). Histological data were available from 453 women in whom CIN2+ lesions were identified within 30 months of entry into the trial. The proportion of CIN2+ detected amongst women who had a HC2 RLU/Co value of >1 at baseline was 14.7% (423/2860). A high proportion of these women (94.8%) had an RLU/Co value of >4. The relative sensitivity of the HC2 assay for the detection of CIN2+

171 was 93.4% (423/453) increasing to 97.0% (226/233) for women who had underlying
172 histologically confirmed CIN3+. In total, 10.5 % (23/220) of women with CIN2 lesions
173 were found to be HC2 negative, whilst only 3% (7/233) of CIN3+ lesions were HC2
174 negative.

175 **Proportion of CIN2+ lesions identified and the relative sensitivity for women who**
176 **tested HPV positive after adjustment of the HC2 RLU/Co value**
177 Table 1 shows that adjustment of the HC2 cut-off to ≥2.0 RLU/Co resulted in a 2.5%
178 reduction in the overall test positivity (CI 2.26 to 2.72). The reduction in test positivity at
179 ≥2.0 RLU/Co by cytology grade versus the lower ≥1.0 RLU/Co was statistically
180 significant for women with normal cytology (1675/16042, 10.4% versus 1286/16042,
181 8.0%; CI 2.20 to 2.67) and borderline/mild cytology (867/1986, 43.7% versus 802/1986,
182 40.4%; CI 2.53 to 4.15). There was not a statistically significant reduction amongst
183 women with moderate/worse cytology (318/358, 88.8% versus 315/358, 88.0%; CI 0.17
184 to 2.43). Adjustment of the HC2 cut-off to ≥2.0 RLU/Co would have resulted in a loss of
185 14 out of 423 (3.3%) CIN2+ lesions (including four out of 226 (1.8%) CIN3+) that were
186 HC2 positive at ≥1 RLU/Co. This would result in a slight decrease in the relative
187 sensitivities to 90.3% (409/453) and 95.3% (222/233) for CIN2+ and CIN3+ lesions
188 respectively. An increase in the proportion of CIN2+ detected amongst women who were
189 positive at an RLU/Co of >2 was observed compared to a 1 RLU/Co increasing from
190 14.7% (423/2860) to 17.0% (409/2403).

If different grades of cytology are compared, amongst women with normal cytology an increase from 1 RLU/Co to 2 RLU/Co would have resulted in five out of 32 (15.6%) CIN2+ lesions (including one out of 10 (10%) CIN3+) that would have been missed with a reduction of 389 out of 1675 (23.2%) HC2 positive test results (Table 1). Amongst women with borderline/mild cytology, another six out of 150 (4%) CIN2+ lesions (including 2 out of 66 (3%) CIN3+) would have been missed, with the benefit of 65 out of 867 (7.5%) fewer colposcopies if HPV testing were being used to triage.

Increasing the HC2 cut-off to ≥4.0 (Table 1), results in a further decrease in the overall HPV prevalence to 11.5% (2116/18386) equating to 744 fewer HC2 positive test results compared with a cut-off of ≥1.0, whilst reducing the overall sensitivity for the detection of CIN2+ lesions to 88.5% (401/453). At varying cut-off values women at a higher RLU/Co ≥4 were at a six times greater risk of CIN2+ compared with women who had an RLU/Co value between 1 and 3.999. The proportion of CIN2+ detected were 19% (401/2116) and 3.0% (22/744) respectively.

Confirmatory testing of HC2 positive samples by the Roche prototype Line Blot Assay

Further testing of the 2860 HC2 positive samples by the Roche prototype LBA found that 35 (1.2%) had invalid LBA results testing either beta-globin negative or were of insufficient volume for additional testing. The analytical specificity of HC2 positive samples investigated using the LBA was therefore based on 2825 samples. Results revealed that at an RLU/Co value of ≥1, 68.3% (1935/2825) of the HC2 positive samples

were confirmed by the LBA and found to contain one or more of the 13 HC2 target types (Table 2). There were significantly more confirmed HC2 at a cut-off between 2 and <4 RLU/Co compared to a cut-off between 1 and <2 RLU/Co (45.4% versus 22.6%; P_{Trend}= <0.0001). At a cut-off of ≥2 RLU/Co, 77.2% (1833/2374) of HC2 positive samples were confirmed increasing to 81.5% (1704/2090) at a cut-off of ≥4.

When we examined the proportion of confirmed HC2 positive samples by age group we found that at an RLU/Co of ≥1, HC2 samples were confirmed in 78.7% (1423/1807) of 20-34 year olds whilst significantly fewer (50.3%, 512/1018) were confirmed amongst the 35-64 year olds (P_{Trend} =<0.0001). Raising the cut-off to an RLU/Co of ≥2 increased the proportion of HC2 positive samples being confirmed to 83.7% (1355/1618) and 63.2% (478/756) amongst the 20-34 and 35-64 year olds respectively.

The proportion of confirmed HC2 is shown to increase with cytology grade (Table 2) from 57.9% (962/1661) amongst cytological normal to 96.8% (302/312) amongst moderate/severe dyskaryosis (P_{Trend}= <0.0001) at an RLU/Co value of ≥1. Significantly more confirmed HC2 at ≥2.0 RLU/Co was observed compared with a ≥1.0 RLU/Co for women who had normal cytology (69.1% versus 57.9%; P_{Trend}= <0.0001) and borderline/mild cytology (82.7% versus 78.8%; P_{Trend}= 0.0443). This trend was not apparent amongst women with moderate/severe dyskaryosis cytology (96.8% versus 96.8%). The risk of CIN2+ lesions during round 1 was significantly higher amongst the HC2 confirmed positive samples at an RLU/Co value of ≥1 compared to the unconfirmed samples (95.9% versus 4.1% respectively).

Table 2 also categorised HC2 positive women who were either HPV 16 and/or 18 DNA positive and non HPV 16 or 18 as detected by the LBA. As expected CIN2+ was significantly (P = <0.0001) more prevalent amongst HPV16/18 confirmed women compared with non 16/18 confirmed women irrespective of varying RLU/Co cut-off values and across all grades of cytology. The impact of altering the HC2 cut-off value on relative sensitivity of CIN2+ lesions was affected less in women with confirmed HPV 16 and / or 18.

Discussion

Before any implementation of HPV testing into a national cervical screening programme, the optimal balance between clinical sensitivity and specificity of any HPV assay either as a primary screening or triage test is necessary to maximize the benefit of HPV testing and to minimize unnecessary diagnostic procedures. These data have been obtained from a trial setting embedded in the national cervical screening programme, 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 of up to 30 months following repeat cytology (if low grade and repeat HPV testing if cytology was normal).

If HPV DNA testing were used in primary screening, increasing the threshold from 1 RLU/Co to ≥2 RLU/Co, no clinically important loss in sensitivity would have been observed with 96% of CIN3+ still testing positive. If HC2 were used as the only indication for colposcopy referral, at a 1 RLU/Co cut-off value, 2860 (15.6%) of the

253 population would require further investigation which could have a major impact on
254 colposcopy rates. A substantial proportion of these referrals would be unnecessary with
255 only 423 CIN2+ lesions found in this group. By increasing to ≥2 RLU/Co, a significant
256 reduction in test positivity would be observed resulting in 16% fewer referrals without
257 significantly reducing test positives in CIN3+ lesions. A loss of only four (1.8%) CIN3+
258 was observed. On increasing to ≥4 RLU/Co we begin to see a significant loss in relative
259 sensitivity for CIN3+.

260 Using the HC2 assay in the triage setting to aid referral to colposcopy amongst women
261 who test borderline/mild by cytology raising the cut-off to ≥2RLU/Co would have
262 resulted in 65 (7.5%) fewer procedures with an almost identical proportion of CIN2+
263 lesions detected amongst HC2 positive women (17.3% (150/867) at ≥1 RLU/Co
264 compared with 18.0% (144/802) at ≥2 RLU/Co). Increasing the cut-off to ≥4 RLU/Co
265 would mean a further 34 fewer colposcopies would be performed with the subsequent
266 non detection of a further three CIN2 lesions however no additional CIN3+ lesions would
267 have gone undetected. Increasing the cut-off to ≥4 did not result in a significant increase
268 in the proportion of CIN2+ lesions detected when compared to a cut-off of ≥2 RLU/CO
269 (18.4% versus 18.0%).

270 Confirmation of HC2 positive samples by the LBA showed that 32.3% could not be
271 confirmed as containing a HC2 target type. This was surprising given the lower analytical
272 sensitivity of the HC2 assay compared with the LBA which involves PGMY09/11 L1
273 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 called the Linear Array (LA) (Roche Molecular Systems) (4). For 3,335 paired test results an increase in analytical sensitivity was observed by the LA compared with the LBA resulting in greater detection for most of the 37 HPV genotypes targeted by both assays. Our method in ARTISTIC used 50µl of the pelleted sample instead of 5µl suggested for the LBA which is comparable to the recommended volume for the LA. We therefore would not expect to see an increase in analytical sensitivity if HC2 positive samples had been 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 – 7000 copies /ml depending on genotype. PCR failures due to inhibitors, is an unlikely explanation for this large number of unconfirmed samples, as we only found 35 out of 2860 (1.2%) samples had unsatisfactory beta-globin housekeeping genes. One possible reason may be the presence of totally integrated HPV DNA 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 in high-grade lesions. Our data show that the majority of unconfirmed HC2 positives were found mainly amongst women with normal or low grade cytology and is likely to reflect a certain level of background noise with the HC2 assay at an RLU/Co of 1.0. This theory is further supported by the low number of CIN2+ lesions detected amongst women who tested positive with low level RLU/Co values. This data again supports an increase in the RLU/Co to ≥2. Another benefit for raising the cut-off was observed amongst older women (35-64 year olds) were a far greater proportion of HC2 positive samples were

confirmed at an RLU/Co of ≥2 compared to an RLU/Co of ≥1. This would result in fewer older women being referred to colposcopy.

Limitations of this study include an underestimation in the number of CIN2+ detected as 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 cytology normal and HPV negative were not colposcoped as part of the ARTISTIC protocol as they were at such low risk. The sensitivity of HC2 can therefore only be an estimate in terms of the true prevalence of CIN2+ but the relative sensitivity for different cut-offs is a valid measure. Previous studies have reported virtually no CIN2+ occurring among women who tested cytology normal and HPV negative (13, 1).

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

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Table 1: Cytology and histology by varying HC2 cut off values (revealed arm only *N* = 18386)

Cut-off point	Total Number	<1 RLU N (%)	1 - <2 RLU N (%)	2 - <4 RLU N (%)	≥4 RLU N (%)
Cytology					
Normal	16042	14367 (89.6)	389 (2.4)	249 (1.6)	1037 (6.5)
Borderline/Mild	1986	1119 (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	18386	15526 (84.4)	457 (2.5)	287 (1.6)	2116 (11.5)
Histology by cytology in round 1					
Normal *N* =16042					
CIN2	22	-	4 (18.2)	0 (0)	18 (81.8)
CIN3+	10	-	1 (10)	2 (20)	7 (70)
Borderline/ Mild *N* = 1986					
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* = 3158					
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* = 18386					
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)

Table 2 Confirmatory testing of HC2 positive samples by the Roche prototype Line Blot Assay

HC2 cut-off	1-<2 RLU/Co				2-<4 RLU/Co				≥4 RLU/Co			
Cytology grade at baseline	HC2 pos with valid LBA result [a]	HC2 pos and target type confirmed by LBA [b]	HC2 pos 16 and / or 18 detected by LBA	HC2 pos LBA non 16 or 18	HC2 pos with valid LBA result [a]	HC2 pos and target type confirmed by LBA [b]	HC2 pos 16 and / or 18 detected by LBA	HC2 pos LBA non 16 or 18	HC2 pos with valid LBA result [a]	HC2 pos and target type confirmed by LBA [b]	HC2 pos 16 and / or 18 detected by LBA	HC2 pos LBA non 16 or 18
	N	**N (% of HC2 pos)**	**N (% of HC2 pos)**	**N (% of HC2 pos)**	**N**	**N (% of HC2 pos)**	**N (% of HC2 pos)**	**N (% of HC2 pos)**	**N**	**N (% of HC2 pos)**	**N (% of HC2 pos)**	**N (% of HC2 pos)**
Normal (N = 16028)	387	82 (21.2)	37 (9.6)	350 (90.4)	247	108 (43.7)	40 (16.2)	207 (83.8)	1027	772 (75.2)	274 (26.7)	753 (73.3)
Bord / Mild (N = 1971)	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 = 18351)	451	102 (22.6)	50 (11.1)	401 (88.9)	284	129 (45.4)	45 (15.8)	239 (84.0)	2090	1704 (81.5)	698 (33.4)	1392 (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 absence of inhibitors
b HC2 positive samples were confirmed by the LBA if they were found to contain one or more of the 13 HC2 target types