Title: Evaluation of the clinical performance of the cobas® 4800 HPV test in a colposcopy referred population

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Abstract

The potential value of HPV testing in cervical screening and management has led to the development of a range of HPV detection technologies. The clinical performance of the cobas® 4800 HPV test for detection of high-grade disease in a colposcopy referred population was compared with the gold standard HPV test, Hybrid Capture 2. ThinPrep cervical smears were collected from 558 women referred to colposcopy with repeat abnormal cytology. Histological confirmed diagnosis was available for 491 patients. Biopsy confirmed CIN 1, CIN 2 and CIN 3 were identified in 29.7%, 22.8% and 20.2% respectively, 23.8% were normal on histology, 3.5% had an inadequate biopsy. The overall agreement between the cobas® 4800 HPV test and Hybrid Capture 2 was 92.3% (95% CI 91.7%-92.9%). In women with CIN 2+, HPV DNA was detected in 90.0% (95% CI 88.8%-91.3%) and 90.5% (95% CI 89.4%-91.7%) by the cobas® 4800 HPV test and Hybrid Capture 2 respectively. A subset of discordant results (n=23) were tested with Linear Array HPV Genotyping Test (Roche Diagnostics). This identified a small number of Hybrid Capture 2 positive/cobas® 4800 HPV negative which were positive for low-risk HPV types only with HPV 53 most commonly detected. The overall clinical sensitivity and specificity for detection CIN 2+ was 90.0% (95% CI 88.8-91.3) and 55.5% (95% CI 52.5-58.5) for the cobas® 4800 HPV test and 90.5% (95% CI 89.4-91.7)

and 50.2% (95% CI 47.2-53.2) for Hybrid Capture 2. Clinical performance comparable irrespective of age or referral smear with the exception of those referred to colposcopy with LSIL. The cobas® 4800 HPV test demonstrated enhanced specificity than hc2 for detection of CIN 2+ in women presenting with LSIL (55% vs 35%). In conclusion, the cobas® 4800 HPV test showed overall comparable performance to hc2 for detection of CIN 2+.

Introduction

Based on the known causal relationship between HR HPV and cervical cancer (37), HPV has now become an important tool in developing strategies for cancer prevention. As papillomaviruses cannot be cultured reliably *in vitro,* detection relies on molecular technologies that detect HPV nucleic acids in cervical smears.

Hybrid Capture 2 (hc2) was the first HPV DNA detection test to receive FDA approval in March 2003 for use in conjunction with routine Pap screening in women over 30 years and women with ASCUS cytology. There is a strong body of evidence which demonstrates its good clinical sensitivity and high Negative Predictive Value (NPV) for detection of high grade abnormalities (1, 2). Its use is believed to improve patient management by providing a more accurate assessment of risk of cervical cancer and its precursors (3, 4, 5). Based on the success of hc2, it was recognized that many new HPV DNA detection tests would populate the

market and it was recommended that the performance of all new HPV detection tests should be assessed relative to hc2 (as the "the gold standard"), (6).

While HPV DNA testing has been demonstrated to be accurate for detection of high grade disease i.e. cervical intraepithelial neoplasia grade 2+ (CIN 2+) there remain limitations. For example, a proportion of HPV DNA positive women will not develop CIN 2+ or cervical cancer. Despite the fact that a number of HPV types have been characterised as high risk for the development of cancer, not all high risk types have the same carcinogenic potential (10,11). Kjaer et al found absolute risk of CIN 3 or worse after infection with HR HPV types other than 16, 18, 31, and 33 to be 6%. HPV 16 and HPV 18 account for approximately 70% of all invasive cervical cancer cases (12). This suggests that genotyping for HPV 16/18 might provide useful risk stratification for high grade disease (9). Today, many commercially available HPV tests now include a genotyping capability.

Testing for carcinogenic HPV DNA has been proposed for triage of low grade abnormalities. In the case of ASCUS there is general agreement that HPV triage has improved accuracy for detection of CIN 2+ compared to cytology. A meta-analysis by Arbyn et al reported a pooled estimated sensitivity and specificity of 94.8% (95% CI: 92.7%-96.9%) and 67.3% (95% CI: 58.2%–76.4%) respectively (14). However, in the case of LSIL,

the evidence on the value of HPV testing is conflicting. The ALTS (ASCUS-LSIL Triage Study) study reported findings indicating that HPV DNA has improved accuracy in the detection of CIN 2+ in women with ASCUS over the age of 30 years (15, 16). However, due to the high HPV positivity rate in LSIL the test is likely to be of limited value (15, 16). Findings from the NHSCP in England program differ slightly. The Sentinel Sites program (17) reported that HPV DNA triage of LSIL and ASCUS was well accepted, cost effective and resulted in a more rapid return to routine recall compared to repeat cytology.

Relatively new to the market, the cobas® 4800 HPV test has been analytically and clinically validated (7, 8, 9) and in April 2011 it received FDA approval for use in cervical screening in women over the age of 30 years and in those with ASCUS cytology. The cobas® 4800 HPV test detects a pool of 12 HPV types with separate detection of HPV 16 and HPV 18. In this study the clinical performance of the cobas® 4800 HPV test and hc2 are compared in a colposcopy referred population. The assays have been validated and compared in previous studies in women with ASCUS cytology and ≥30 years (7, 8, 9, 18). This study seeks to add further knowledge to the performance of both tests in detecting CIN 2+, taking into consideration age and cytological classification, in particular LSIL. In order to resolve discordant results a subset of cervical cytology specimens were genotyped using Linear Array.

Materials and Methods

Study population

Based on guidelines from the national cervical screening program in Ireland (CervicalCheck), women are referred to colposcopy based on the following criteria: cervical cytology graded HSIL, three consecutive smears graded ASCUS, two consecutive smears graded LSIL, two consecutive smears graded a combination of ASC-US and LSIL or any 3 smear test results that are not normal in the previous 10 years without referral to colposcopy.

Eligible women who provided informed consent were enrolled into CERVIVA studies, through the colposcopy clinics at the Coombe Women's and Infants University Hospital, Dublin and the National Maternity Hospital, Dublin between July 2010 and July 2011. Eligibility was on the basis of having an abnormal smear of any grade and over the age of 18 years. Women who were pregnant at the time of clinic attendance or who had had a previous treatment for cervical neoplasia were excluded. A cervical smear was obtained and collected in PreservCyt medium prior to colposcopic examination. At colposcopy women were managed according to standard protocol of the clinic outlined by the Irish cervical screening program CervicalCheck quality assurance guidelines. Cervical smears from a total of 558 women between the ages of 18-65 years were included in

the study. Colposcopy-guided biopsy specimens (punch biopsy or LLETZ) were taken if an area of abnormality was identified in 491 out of 558 patients. Histological diagnosis was made based on standard protocol outlined in the CervicalCheck quality assurance guidelines.

Hybrid Capture 2

HR HPV DNA testing was performed on 4mls of specimen using the Hybrid Capture 2 (hc2) (Qiagen, UK) as described by the manufacturer. hc2 is a semi-quantitative nucleic acid hybridisation assay with signal amplification that utilizes chemiluminescent detection for the quantitative detection of 13 HR HPV types. HR HPV DNA is detected by a full length RNA probe cocktail for the detection of oncogenic HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. The RLU negative cut off value is 1.0pg, representing 5000 copies of HPV genome. Specimens below this detection limit are considered negative. A value between 1-2.5 RLU was considered to be borderline and retested where possible.

The cobas® 4800 HPV test

The cobas® 4800 HPV test (Roche Diagnostics) is a fully automated system involving sample preparation combined with real-time PCR technology from 400µl of cervical smear sample. The test was carried out as described by the manufacturer. The cobas® 4800 HPV test individually detects HPV 16 and HPV 18 and 12 pooled HR HPV genotypes (31, 33, 35,

39, 45, 51, 52, 56, 58, 59, 66 and 68). Complementary primer pairs are used to amplify a sequence of approximately 200 base pairs within the L1 region of the HPV genome and fluorescent oligonucleotide probes specific for HPV16, HPV18, and the 12 other HR HPV types. The assay also detects the human β-globin gene as an internal control and to provide a measure of sample adequacy.

HPV genotyping using the Linear Array HPV Genotyping Test

HPV genotyping was performed on a subset of 23 discordant patient samples using the Linear Array HPV Genotyping test (Roche Diagnostics) for the detection of 37 HPV genotypes (6, 11, 16, 18, 26, 31,33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55,56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73 (MM9), 81, 82 (MM4), 83 (MM7), 84 (MM8), IS39, and CP6108), as described by the manufacturer. DNA was amplified using biotinylated primers targeting a sequence of nucleotides within the L1 region of the HPV genome. Hybridisation of amplicons to probes which are bound to test strips was performed to detect the various genotypes. The strips were then read visually by comparing the pattern of blue lines to the linear array HPV genotyping test reference guide.

Statistical Analysis

Agreement between the cobas® 4800 HPV test and hc2 was calculated for HR HPV detection, based on concordant positive or negative results, irrespective of HPV type, by both assays. Cohen's kappa coefficient was used to ascertain the overall agreement between the cobas® 4800 HPV test and hc2 in addition to agreement for each histologic diagnosis Normal, CIN 1 and CIN 2+. McNemar's test was used to compare rates of HPV detection between tests. The clinical performance of each test was assessed by calculating the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and the relative 95% confidence intervals (CI) for detecting (a) CIN 2+ and (b) CIN 2/3. This analysis was done for all women with histologically confirmed diagnosis (n=491) and repeated for those with low-grade referral cytology (ASCUS/LSIL; n=404). Logistic regression was used to calculate crude odds ratios to determine the risk of CIN 2+ associated with infection with HPV 16, HPV 18 or HR HPV. HPV 16 positive results were classified as previously shown by Stoler et al whereby HPV 16 positive alone or HPV 16 in the presence of HPV 18 and/or 12 other HR HPV. HPV 18 positive results included those positive for HPV 18 alone and positive for HPV 18 in the presence of 12 other HR HPV. HR-HPV was classified as those positive for 12 other HR HPV alone (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and

68) (n=165) (9). A p value of <0.05 was considered statistically significant.

Results

Concordance between the cobas® 4800 HPV test and hc2

Results were obtained from 558 women attending their first colposcopy visit with a median age of 32 years (interquartile range 28-39). The study population comprised of 465 women presenting with minor cytological abnormalities, LSIL or ASCUS, and 96 women presenting with HSIL. When all women were included (n=558), the overall prevalence of HPV DNA detected by the cobas 4800® HPV test was lower, 62.7% (350/558; 95% CI 60.8%-64.7%), compared to 65.8% (367/558; 95% CI 63.9%-67.6%) for hc2 (McNemar p=0.015). A comparison of the cobas® 4800 HPV test and hc2 on the 558 cases demonstrated an overall agreement of 92.3% (95% CI 91.7%-92.9%) (Cohen's Kappa Coefficient 0.834; 95% CI 0.784-0.881). Both tests were positive in 60.4% (337/558) and both tests were negative in 31.9% (178/558) of cases. There were 43 discordant results; among them 69.8% (30/43) were cobas® 4800 HPV negative, hc2 positive. The remaining 30.2% (13/43) were hc2 negative, cobas® 4800 HPV positive.

HPV detection rates across various grades CIN

Histological confirmed diagnosis was available for 491 of the 558 women (88%). Biopsy confirmed CIN 1, CIN 2 and CIN 3 were identified in 29.7% (146/491), 22.8% (112/491) and 20.2% (99/491) respectively, 23.8% (117/491) were normal on histology. There was 3 uncertain grade CIN and 14 inadequate biopsy samples which were excluded from analysis resulting 474 for analysis. The positivity rate for both tests and test agreement increased with increasing grades of CIN (figure 1). Women with a histological diagnosis of normal had a HPV positivity rate of 45.3% and 39.3% for hc2 and cobas® 4800 HPV test, respectively. HPV DNA was detected in 52.7% and 48.6% for CIN 1 and 90.5% and 90.0% in CIN 2+ by hc2 and the cobas® 4800 HPV test, respectively.

Assessment of discordant HPV test results using a third HPV detection test, the Linear Array HPV Genotyping Test (Roche Diagnostics)

In 43 cases the results did not match between the cobas and hc2. Samples from 30 women were reported as HR HPV positive by hc2 and HPV negative by the cobas® 4800 HPV test. Conversely, samples from 13 women were reported as HPV positive by the cobas® 4800 HPV test and HPV negative by hc2. An additional procedure, Linear Array (LA), was employed on all samples with adequate material remaining, n=23 (17 hc2

positive/cobas® 4800 HPV negative; 6 hc2 negative/cobas® 4800 HPV positive). In the case of HPV test results hc2 positive/cobas® 4800 HPV negative, 70.6% (12/17) were found to contain low-risk HPV types only. The most common genotype in this subset was HPV 53, which was detected in 41% (7/17) of cases. 23% (4/17) were negative for HPV of which 3 cases were histologically normal on biopsy. There were 6 cases which were hc2 negative/cobas® 4800 HPV positive, 83% (5/6) of this sub-group were found to contain multiple HPV infections, at least one of which was high-risk.

Clinical performance of the cobas® 4800 HPV test

Table 1 shows the sensitivity, specificity, NPV and PPV for detection of CIN2+ and CIN 3 for the cobas® 4800 HPV test and hc2. Sensitivity of the cobas® 4800 HPV test for detection of CIN 2+ was comparable to hc2 (90.0% vs 90.5%). The specificity (55.5%) and PPV (61.9%) of the cobas® 4800 HPV to detect CIN 2+ was marginally higher than hc2 but this did not reach significance. Both tests demonstrated comparable NPVs. When histologically confirmed CIN 3 was considered, sensitivity increased to 98.0% for hc2 and reached 100% for the cobas® 4800 HPV test. Specificity fell by around 10%, to 40.0% and 44.5% for hc2 and the cobas® 4800 HPV test respectively.

When the analysis was restricted to women of the age of 30 years and older, the sensitivity for detection of CIN 2+ decreased for both tests slightly, but not significantly, to 89.1% for both cobas and hc2. For both tests, specificity increased significantly by approximately 10% to 64.8% and 60.2% for cobas® 4800 HPV and hc2 respectively.

The performance of the cobas® 4800 HPV test was evaluated in a subpopulation of n=465 presenting at colposcopy with LSIL/ASCUS. The HPV positivity rate for LSIL was 64.5% (158/245) and 71.8% (176/245) for cobas® 4800 HPV test and hc2 respectively (McNemar p=0.803). The overall agreement between the cobas and hc2 in women presenting with LSIL/ASCUS was 91.0% (95% CI 90.2-91.7). In contrast, the positivity for ASCUS was 47.7% (105/220) for the cobas® 4800 HPV test and for hc2 (this does not represent 100% agreement, it is merely coincidence that the number of positive cases for each test is n=105. Within the 105 positive cases for each test there were 16 discordant results). The sensitivity, specificity, PPV and NPV for detection of CIN 2+ The clinical performance of the cobas® 4800 HPV test for detection of CIN2+ in women presenting with LSIL and ASCUS was evaluated in those with confirmed histological diagnosis resulting in 404 available for analysis (table 2). The cobas® 4800 HPV test had comparable sensitivity to hc2 for detection of CIN 2+ in both LSIL (86.8% vs 89.7%) and ASCUS (81.0% for both tests) referral groups. Specificity of the cobas® 4800 HPV test was comparable to hc2 in ASCUS referral. However, in women

297 referred with LSIL cytology, the cobas® 4800 HPV test demonstrated a
298 higher specificity at 55.2% compared to 35.1% for hc2.

299 **Discussion**

300 The aim of this study was to assess the performance of the cobas® 4800
301 HPV test for detection of HR HPV in women attending colposcopy with
302 cytological abnormalities. Overall, the cobas® 4800 HPV test had a lower
303 positivity rate of 62.7% compared to hc2 at 65.7% (McNemar p=0.015),
304 however agreement between the two tests remained high at 92.3%
305 producing a kappa value of 0.832 (95% CI 0.784-0.881). The strength of
306 agreement appeared to increase with severity of the lesion. A higher level
307 of agreement was identified in CIN 2+ cases, 93.8% (95% CI 93.1%-
308 94.6%) agreement compared to 87.2% (95% CI 85.2%-89.2%) in
309 women who were histologically normal. Similar findings have been found
310 in previous studies; these have reported both a high concordance (>87%)
311 and that this tended to increase with lesion severity (8, 9, 19).
312
313 Recommended guidelines by Stoler et al 2007, state that a HPV test
314 should include at least 13 HR HPV types (16, 18, 31, 33, 35, 39, 45, 51,
315 52, 56, 58, 59, and 68). They also endorse HPV 66 an emerging possible
316 carcinogenic HPV type which should be considered for inclusion in HPV
317 tests going forward (20). Interestingly, HPV 66 has been identified as one
318 of the top 10 genotypes detected in the Irish cervical screening population
319 (Cerviva data in press). The cobas® 4800 HPV test detects HPV 66 in

addition to the 13 HR HPV types detected by hc2. Although there have been suggestions that hc2 can detect HPV 66 due to cross-reactivity with other HPV types including some low risk (LR) HPV types (21), HPV 66 alone does not explain all the discordant samples observed here. Only one hc2 positive case was confirmed as HPV 66 positive in the subset (n=23) of samples tested using the linear array HPV genotyping test (Roche Diagnostics).

HPV genotyping by linear array was performed on 17 samples within this population found to be hc2 positive/cobas® 4800 HPV negative. LR HPV types were identified in 12 of these samples, in particular HPV 53 representing 58% (7/12) of those cases with LR HPV infections. This is consistent with reports that the hc2 test detects up to 15 different HPV genotypes, not included in the high-risk probe cocktail, with low-risk HPV 53 observed to be the most common (21, 22). In fact, the manufacture states that a small amount of cross hybridisation can occur in cases of high levels (≥4ng/ml) of low risk types (Hybrid Capture 2 package insert). In our study, the remaining 23% (4/17) were found to be HPV negative using the Linear Array. This may be a result of differences in the amplification targets between the hc2, which targets the full length probe, while the cobas® 4800 HPV test and linear array HPV genotyping tests, only target the L1 region of the genome. Loss or partial loss of L1 has been reported following viral DNA integration into the host genome (23,

24, 25). However, of these four cases 3 were normal and 1 had CIN 1 on histology suggesting viral integration was unlikely.

In total five of the six samples which produced negative hc2 results but positive cobas® 4800 HPV test results were found to contain multiple infections, at least one of which was a HR HPV. The negative result produced by hc2 may be a result of analytical sensitivity of the test which is slightly less than PCR-based target amplified techniques. The limit of detection of hc2 is reported to be between 0.2-1pg HPV-DNA/ml (approximately 5000 copies for HPV 16) (hybrid capture 2 product insert) compared to a limit of detection for the cobas® 4800 HPV test reported as 600 copies/ml for HPV 16 and 18 (cobas® 4800 HPV test product insert). For the Linear Array HPV Genotyping assay the limit of detection varies according to the genotype studied, ranging from 76 copies/ml for HPV59 to 200 copies /ml for HPV16 to 20,000 copies/ml for HPV82 (Linear Array HPV Genotyping Test product insert).

Evaluation of the clinical performance of the cobas® 4800 HPV test was achieved by determining its ability to detect CIN 2+ and CIN 3. This was accomplished by calculating sensitivity, specificity, PPV and NPV for cobas® 4800 HPV test and comparing it the performance of hc2 in this study population. The sensitivity and specificity of the cobas® 4800 HPV test for detection of CIN 2+ were 90.0% (95% CI 88.8-91.3) and 55.5% (95% CI 52.5-58.5), respectively. This was comparable with hc2 which

demonstrated sensitivity and specificity of 90.5% (95% CI 89.4-91.7) and 50.2% (95% CI 47.2-53.2). When disease endpoint was confined to detection of CIN 3, sensitivity for both the cobas® 4800 HPV test and hc2 increased to 100% and 98.0% (95% CI 97.6-98.4) respectively. However this resulted in a significant loss of specificity by approximately 10% for both tests, and PPV fell by half; in contrast, the NPV increased and was very high for both tests.

Most previous studies have evaluated the cobas® 4800 HPV test for triage of ASCUS smears (8, 9, 35). Focusing on the ASCUS population in our study the cobas® 4800 HPV test had a sensitivity and specificity of 81.0% (77.1-85.0) and 63.3% (59.6-67.1) respectively. Lapierre et al showed equivalent results, reporting sensitivity of 89.7% (95% CI 72.8-97.2) in a population of women aged over 24 with at least one ASCUS (8). Stoler et al 2011 evaluated the clinical performance of the cobas® 4800 HPV test in a population of women aged over 21 with ASCUS, reporting higher sensitivity and specificity for detection of CIN 2+ as 90.0% (95% CI: 81.5-94.8) and (70.5% 95% CI: 68.1-72.7) respectively and lower PPV (14.0% 95% CI: 12.8-15.3) (9) than the current study. This may be, in part, due to the higher prevalence of high grade disease in our ASCUS population (26.0% vs 5.1%) (9). This difference in the prevalence of CIN2+ may be attributed to the a number of factors most notably the different management strategies of ASCUS adapted in different settings. In Ireland, ASCUS is managed by repeat cytology;

391 women are referred to colposcopy after three consecutive smears graded
392 ASCUS. In comparison, in the United States women with ASCUS on
393 cytology are triaged by HPV detection, a positive result leading to
394 colposcopy referral (30). Furthermore it is important to note that this
395 study population represents a population of women attending colposcopy
396 on the bases of repeat minor cytology and not a women undergoing
397 routine screening. Consequently the study is enriched for cervical disease
398 producing a higher PPV than what would be seen in a screening
399 population.
400
401 It is recognised that any improvement in clinical sensitivity almost always
402 results in a reduction in clinical specificity and vice versa. It is important
403 to recognise that a balance between clinical sensitivity and specificity for
404 detection of CIN 2+ is important in order to identify women at risk of high
405 grade disease while minimising unnecessary follow up procedures in those
406 who are not. The use of HPV detection is not without its limitations and,
407 due to the repeatedly reported low specificity compared to cytology (5),
408 there is evidence that HPV triage of ASCUS/LSIL can result in unnecessary
409 referrals to colposcopy (15, 36). Thus appropriate management strategies
410 are needed for HPV positive cases. In England, the NHS have incorporated
411 HPV DNA triage for LSIL and ASCUS, with a higher RLU/Co cut off value of
412 2.0 for hc2 was adapted in 2007, in their Sentinel Sites Studies (26)
413 rather than that recommended by the manufacturer of a RLU/Co ratio of
414 1.0. This is based on data from the ARTISTIC trial, which found that

increasing the threshold RLU/Co ratio to ≥2 generated a positive balance between sensitivity and detection of CIN 3 lesions, reducing unnecessary colposcopy procedures without reducing detection rates of CIN 3 (27). Other recommendations have suggested that identification of the most carcinogenic HPV genotypes (eg. HPV16 and HPV18) could be useful (9, 34).

When our analysis was restricted to those referred with minor cytology overall the cobas® 4800 HPV test demonstrated comparable clinical performance to hc2. Both tests had a similar sensitivity in both ASCUS and LSIL referred population. For LSIL referrals, a higher sensitivity and significantly lower specificity for detection of CIN 2+ was demonstrated compared to those referred with ASCUS, which is consistent with findings previously reported using hc2 (4, 5). Interestingly, the cobas® 4800 HPV test was more specific than hc2 for detection of CIN 2+, and had high PPV, in women presenting to colposcopy with LSIL cytology 55.2% (95% CI 51.3-59.1) and displayed a specificity comparable with that shown by hc2 in ASCUS cytology (62.7%, 95% CI 58.9-66.4). However, it should be noted that the present study included only 245 women presenting with LSIL; these observations therefore require confirmation in larger populations.

The strengths of this study are that enrolment was systematic through the Irish national screening program, CervicalCheck. Women were

managed under a standard protocol outlined by CervicalCheck guidelines. These attributes allowed the test performance to be evaluated in a routine population-based setting. In addition, the clinical performance of the cobas was assessed in LSIL, which has not been previously reported. The weakness of the study is that test performance of the HPV tests is not evaluated in a primary screening setting. However, given the high prevalence minor cytological abnormalities in Ireland, which account for over 13% of cervical smears (7), this study has particular relevance in an Irish setting.

In summary, the cobas® 4800 HPV test demonstrated a high level of agreement with hc2 and comparable clinical performance in the overall population, women over the age of 30 and in those referred with ASCUS cytology. The cobas® 4800 HPV test demonstrated a higher specificity in women referred to colposcopy with LSIL, however study numbers are low and this would to be confirmed in larger populations. The cobas® 4800 HPV test may offer some potential advantages to the hc2, in that it permits individual identification of HPV 16 and 18, which together are responsible for up to 70% of cervical cancer (12). HPV persistence is considered one of the most important predictors for high-risk of cervical disease and requires the exact HPV genotype to be identified. Therefore, tests which allow the specific HPV genotype to be classified appear to have great potential for improving future screening programs. Furthermore the cobas® 4800 HPV test requires a smaller specimen

volume of 400µl compared to 4mls for hc2 and contains an internal control for each sample, which the hc2 lacks. Overall in terms of clinical performance neither test offered benefit over the other for detection of CIN 2+.

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Disclosures:

Dr Cara Martin and Professor John O'Leary have had a cobas® 4800 system placed into their laboratory by Roche Diagnostics. All HPV testing kits and associated reagents for both the cobas® 4800 HPV test and hc2 were purchased for this study. Roche Diagnostics and Qiagen are commercial partners in CERVIVA.

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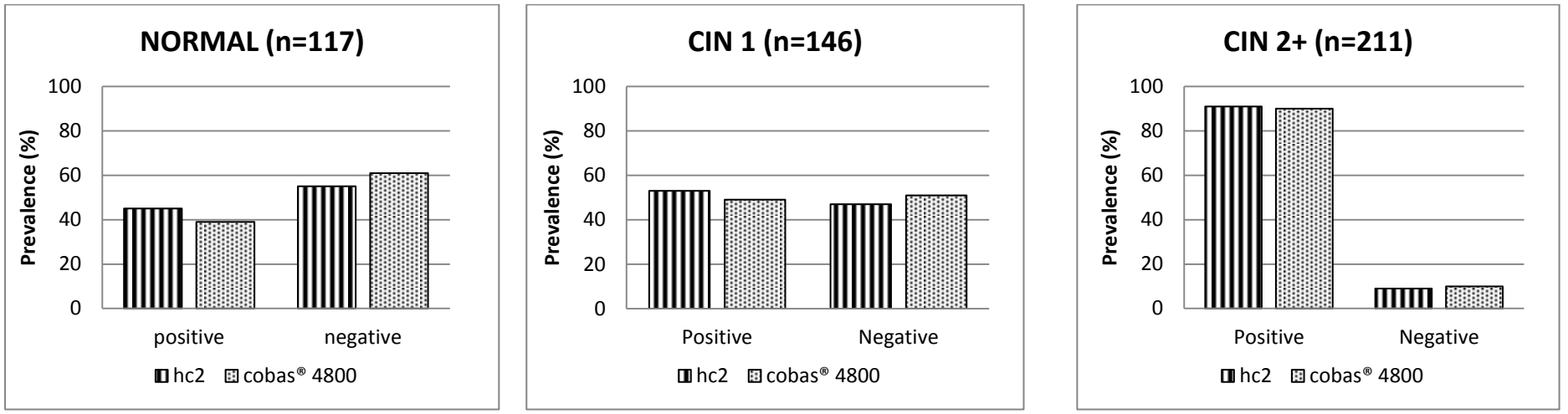

Figure 1: HR HPV detection by cobas® 4800 HPV test and hc2 across the different histological confirmed grades of CIN in women referred to colposcopy with an abnormal smear. A) Normal: Agreement 87.2% (95% CI 85.2%-89.2%) Kappa 0.594 B) CIN 1 Agreement: 92.5% (95% CI 91.35-93.6%) Kappa: 0.850 C) Agreement: 93.8% (95% CI 93.1%-94.6%) Kappa: 0.649

Table 1: Clinical performance of the cobas® 4800 HPV test and hc2 test for detection of CIN 2+ and CIN 3 in all women with histologically confirmed diagnosis (n=474); percentages and 95% confidence intervals

	CIN 2+ (n=211)		CIN 3 (n=99)	
	cobas® 4800	hc2	cobas® 4800	hc 2
Sensitivity	90.0% (88.8-91.3)	90.5% (89.4-91.7)	100% (-)	98.0% (97.6-98.4)
Specificity	55.5% (52.5-58.5)	50.2% (47.2-53.2)	44.5% (42.0-47.0)	40.0% (37.6-42.4)
PPV	61.9% (59.3-64.5)	59.3% (56.7-62.0)	31.6% (29.2-34.0)	30.1% (27.8-32.4)
NPV	87.4% (85.8-89.1)	86.8% (85.0-88.7)	100% (-)	98.7% (98.5-98.9)

711 Table 2: Clinical performance of the cobas® 4800 HPV and hc2 test for
712 detection of CIN 2+ in women with minor referral cytology and
713 histologically confirmed diagnosis (n=404); percentages and 95%
714 confidence intervals
715

	LSIL (n=214)		ASCUS (n=190)	
	cobas® 4800	Hc2	cobas® 4800	Hc2
Sensitivity	86.8% (84.0-89.5)	89.7% (87.5-91.9)	81.0% (77.1-85.0)	81.0% (77.1-85.0)
Specificity	55.2% (51.3-59.1)	35.1% (31.5-41.6)	63.3% (59.6-67.1)	62.7% (58.9-66.4)
PPV	46.1% (41.8-50.4)	37.9% (34.3-41.6)	46.1% (41.3-50.9)	45.6% (40.8-50.4)
NPV	90.4% (88.7-92.2)	88.5% (86.0-91.1)	89.6% (87.9-91.4)	89.5% (87.7-91.3)

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