Head-to-head comparison of the RNA-based Aptima® HPV assay and the DNA-based HC2 HPV test in a routine screening population of women aged 30 to 60 years in Germany

Running title: Comparison of Aptima® HPV and HC2

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ABSTRACT

Testing for E6/E7 mRNA of high-risk (HR) HPV infected cells might improve the specificity of HPV testing for identification of cervical pre-cancer lesions. We here compared the RNA-based Aptima® HPV assay (AHPV; Hologic) and the DNA-based Hybrid Capture 2 (HC2; Qiagen) HPV test to liquid-based cytology (LBC) in women attending routine cervical screening. N=10,040 women aged 30-60 were invited to participate in the study; 9,451 of which were included in the analysis. Specimens were tested centrally by LBC, AHPV and HC2 and women positive on any test were referred to colposcopy. Genotyping was performed on all HR HPV positive samples. Test characteristics were calculated based on review histology. As a result, we identified 90 women with CIN2+ including 43 women with CIN3+. Sensitivity differences in detecting CIN2+ (p=0.180) or CIN3+ lesions (p=0.0625) between AHPV and HC2 were statistically non-significant. Out of three CIN3 cases missed by AHPV, two cases presented a lesion-free cone and one had a non-HR HPV67-infection. Specificity (<CIN2) and PPV (CIN2+) of AHPV were significantly higher (both p<0.001) than those of HC2. The overall agreement between both tests was substantial at κ=0.77. Finally, we present results of several possible triage strategies based on the primary screening test being either AHPV or HC2. In summary, the AHPV assay is both specific and sensitive for the detection of high-grade pre-cancerous lesions and may be used in primary cervical cancer screening for women 30 years and older.

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INTRODUCTION

The cervical cancer mortality rate has dramatically decreased in Germany since the introduction of gynaecological screening for cervical cancer in 1971 (1). Annual opportunistic screening is performed by conventional cytology (Pap-smear) and is covered by health insurances for women aged 20 years and older. Despite this extensive effort, 4,600 new cases and approximately 1,500 deaths of cervical cancer (2) and 150,000 cases of cervical cancer precursors (CIN3) are diagnosed each year (3). Persistent infection with high-risk human papillomaviruses (HR HPV) has been shown to be causal for the development of cervical precancerous lesions and cancer. This has led to the development and investigation of various HPV detection methods and HR HPV testing in addition to cytology is nowadays widely applied in cervical cancer screening programmes (4-7). Three DNA-based and one RNA-based assay for HR HPV group detection, and two HPV16/18 genotyping assays have been approved by the US Food and Drug Administration (FDA) for cervical cancer screening (http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm330711.htm). These include the Digene Hybrid Capture 2 High-Risk HPV DNA test (HC2; QIAGEN, Hilden, Germany), the Cervista HPV HR test (Hologic, San Diego, CA), the cobas® HPV Test (Roche, Pleasanton, USA) and the Aptima® HPV Assay (AHPV; Hologic, San Diego, CA) as well as the HPV16/18 genotyping tests Aptima HPV 16 18/45 Genotype Assay (Hologic, San Diego, CA) and Cervista HPV 16/18 (Hologic, San Diego, CA). The cobas® HPV Test with concurrent HPV16/18 genotyping has recently been approved by the FDA for primary screening (www.fda.gov).

The HC2 test for the collective detection of at least 13 carcinogenic HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) (8) is a nucleic acid hybridisation assay with signal

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amplification using microplate chemiluminescence for semi-quantitative detection of HPV-DNA in cervical specimens.

The AHPV assay detects the HPV E6 and E7 mRNA of the 13 HR HPV types also targeted by HC2 and in addition the class 2B type HPV66 (9). The AHPV test has previously been compared to HC2 (10-18) however, only three studies were conducted in a routine screening population (12, 14, 18) of which only two studies performed a split sample comparison with liquid based cytology (LBC) and HC2 (12, 14).

The objective of this study was therefore to evaluate the AHPV assay in comparison to LBC and HC2 regarding clinical sensitivity and specificity for the detection of high grade CIN in women of a German routine screening population aged 30-60 years using split cervical samples collected in ThinPrep collection medium. Cervical samples from 9,451 women were analyzed by LBC, AHPV and HC2 and subsequently evaluated based on respective reviewed histology findings.

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METHODS

Participants. Women aged 30-60 years attending routine cervical screening in three German centres in Tübingen, Saarbrücken and Freiburg were invited to participate in the study (n=10,040). Exclusion criteria for this study were hysterectomy or destructive therapy of the cervix, pregnancy, an abnormal cytology result within the past 6 months, HIV infection and organ transplantation. Written informed consent of each participant was obtained and the study protocol was approved by all relevant ethics committees (Ethik-Kommission Universitätsklinikum Tübingen, reference number: 475/2008MPG1; Ethik-Kommission Alfred-Ludwigs-Universität Freiburg, reference number: EK Freiburg 63/09; Ethik-Kommission Landesärztekammer Baden-Württemberg, reference number: B-2009-030 f; Ethik-Kommission Ärztekammer des Saarlandes, reference number: 02/10).

Study Design. Eligible consenting women (n=10,040) had a single liquid based cytology sample taken using a Rovers CervexBrush during the annual routine speculum examination. Samples were placed into ThinPrep transport medium according to the manufacturer's guidelines. Liquid-based cytology (LBC), Digene Hybrid Capture 2 High-Risk HPV DNA test (HC2; QIAGEN GmbH, Hilden, Germany) and Aptima® HPV Assay (AHPV; Hologic, San Diego, CA) were performed on all samples. All HR HPV positive samples were also genotyped by the INNO-LiPA HPV Genotyping Extra test. All women with a positive result in any of the three screening tests (n=699) were invited for colposcopy within 8 weeks of receiving their test results along with a random 5% sample of women with triple negative results (n=438). Colposcopy was carried out in specialized colposcopy clinics and histology results were reported according to the CIN nomenclature. Biopsies were taken from areas with a

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colposcopic impression of CIN. The colposcopist was not blinded to the screening test results.

Liquid based cytology. All samples were first analyzed by LBC. LBC results were evaluated according to the Munich nomenclature II and were translated into The Bethesda System (TBS) as previously described (19, 20). LBC results were considered negative when the result was Pap I/II (equivalent to NILM) or Pap IIW (equivalent to inadequate and ASCUS); all other results were considered positive and resulted in referral to colposcopy.

HPV testing and genotyping. Residual LBC samples were sent to Tübingen for HPV testing. In Tübingen samples were aliquoted and processed as follows. One 4ml aliquot was subjected to HC2. One 1ml aliquot was subjected to AHPV and another 1ml aliquot was used for HPV genotyping. Sample processing was performed according to the manufacturer's specifications. Remaining samples were stored for quality assurance purposes.

Digene Hybrid Capture 2 High-Risk HPV DNA (QIAGEN GmbH, Hilden, Germany) testing was performed as previously described (20) using the Rapid Capture System 1 (RCS-1) according to the instructions. The cut-off value of RLU/CO=1, equivalent to 1pg HPV DNA per 1ml of sampling buffer for positive test results, was used in this study. PreservCyt® specimens were retested when RLU/CO ratios between ≥1.0 and <2.5 were obtained. If the initial retest result was positive (RLU/CO of ≥1.0), the specimen was reported as "positive". If the retest was negative (RLU/CO of <1.0), a second repeat test (third result) was performed to generate a final result.

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The Aptima® HPV Assay (Hologic, San Diego, CA) was performed following the manufacturer's instructions. The recommended cut-off value of 1.0 signal-to-cut off (S/CO) ratio at the time of study initiation was used in this study.

HPV genotyping on ThinPrep samples as well as paraffin-embedded conisation biopsies was carried out using the INNO-LiPA HPV Genotyping Extra test as previously described (21, 22). One conisation biopsy which returned an "HPV X" LiPA result, was genotyped by nested PCR and subsequent sequence analysis. For the nested PCR MY09/MY11 primers were used as the outer pair and GP5+/GP6+ primers were used as the inner pair amplifying the L1 conserved region, as previously described (23).

Cytology and Histology Reviews. All LBC-positive samples and samples with abnormal histology were collected by the respective clinical departments and a blinded review was performed by independent external experts for quality control. In case of a discrepant review reading, a second review was performed. The result was considered final when two out of three diagnoses were identical.

Statistical Analyses. Women with adequate results from all three screening tests were included in the analysis (n=9,451). Analyses are based on the original screening results and on the reviewed histology results.

As 93 women with abnormal screening results did not attend colposcopy and 27 had an inadequate colposcopy (17% of all women referred), the verification bias stemming from an incomplete colposcopy needed to be adjusted by estimating what colposcopy results would have been observed had all women participated. Conditional on the LBC results (cytology negative (NILM or ASCUS: Pap I/II or Pap IIw), low-grade positive (LSIL: Pap III or Pap IIID) or

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high-grade positive (HSIL: Pap IVa or Pap IVb)) and the HPV detection (negative by AHPV and HC2, positive by AHPV or HC2, positive by AHPV and HC2, positive to HPV16 or HPV16/18), we used inverse probability of colposcopy weights in order to estimate what would have been observed, had all women with at least one positive test an adequate colposcopy as well as an adequate histology in case of an abnormal colposcopy.

Because only 3.6% of the women who tested triple negative had a colposcopy performed, we did not use inverse probability of colposcopy weights for this group as this would have resulted in unstable estimates of sensitivity with wide confidence intervals. Instead we assumed that LBC and HPV testing are conditionally independent in women with disease (CIN2+ or CIN3+ depending on analysis) in order to estimate the number of pathologic cases of CIN2 and CIN3+ among triple negative women without an adequate colposcopy examination (24). Hence, the assumption was that the sensitivity of LBC to detect disease identified by HPV testing is the same as the sensitivity of LBC to detect disease missed by HPV testing. With missing histology results imputed, we subsequently were able to estimate sensitivity, specificity, negative predictive value and positive predictive value of all three tests. When the sensitivity and/or the specificity of a test were 100%, the confidence intervals were based on the exact binomial for the observed number otherwise the confidence intervals were calculated using a normal approximation to the binomial distribution. Using the discordant pairs and assuming a binomial distribution, we tested whether there was a significant difference in the sensitivity and specificity of HC2 compared to AHPV and the confidence interval for the difference in specificity was calculated using the Wald test with Bonett-Price Laplace adjustment (25). Kappa statistics were used to assess agreement between HPV tests, these statistics were not adjusted for verification bias.

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RESULTS

Screening results. A screening sample was obtained from 10,040 women, of whom 9,451 were included in the analysis. The 589 excluded subjects comprised 181 who were ineligible and 408 for whom at least one of the three screening test results was not available mostly due to insufficient residual material (see Figure 1). Of the 9,451 eligible women, 130 (1.4%) tested positive on at least one HPV test and had abnormal cytology, 64 (0.7%) had abnormal cytology but were negative on both HPV tests, 505 were positive on at least one of the two HPV tests but were cytology normal, and 8,752 (92.6%) were negative on all three screening tests. A total of 699 samples tested positive in at least one test and the numbers testing positive on each screening test were 194 (2.15%) for LBC, 464 (4.9%) for AHPV, and 580 (6.1%) for HC2 (Table 1). All 635 HPV-positive samples were genotyped using the INNO-LiPA HPV Genotyping Extra test, determining that 400 women (63%) presented with single HPV infections and 167 (26.3%) with multiple infections while 63 samples (9.9%) were LiPA negative and five samples failed. Further details about the genotype distribution are summarized in Table 2.

Of the 699 women with an abnormal screening result, 606 (86%) attended colposcopy and 579 (83%) either had adequate negative colposcopy or an adequate biopsy (Figure 1). Among the 438 women with negative screening invited for colposcopy, 312 (71%) attended and 267 (61%) either had adequate negative colposcopy or an adequate biopsy.

A total of 90 women were identified with CIN2 or worse (1% of the cohort). N=47 of which were confirmed cases of CIN2, 43 had CIN3 or AIS and no cases of cancer were detected (Table 3A). HC2 detected four more high-grade pre-cancerous lesions than AHPV including three CIN3 cases of which two presented with a lesion-free cone and one had an infection with HPV67 (which is not targeted by either HC2 or AHPV) as determined by PCR and

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subsequent sequencing analysis using the original ThinPrep sample as well as material from the paraffin-embedded conisation biopsy.

Of the 9,257 women with negative cytology, 286 (3%) had Pap IIw (inadequate/ASCUS). The proportion of women with Pap IIw and a positive HPV result found to have CIN2+ was 2.1% (6/286) compared to 0.5% (46/8971) of women with Pap I or II (NILM). By comparison 10.6% (17/161) of women with Pap III (ASC-H) or IIID (LSIL to HSIL) and 63.6% (21/33) of women with Pap IVa or IVb (HSIL) had CIN2+ identified by histology (Table 3A). The corresponding unadjusted positive predictive values for AHPV and HC2 were 17.7% (82/464) and 14.8% (86/580), respectively. Among those testing negative for AHPV and HC2 the percentages found to have CIN2+ were 0.09% (8/8,987) and 0.05% (4/8,871), respectively. The extremely low-rates of CIN2+ detected in women negative for AHPV and HC2 may partly be due to the lack of colposcopy in these women. The results adjusted for verification bias are presented in Table 3B. We estimate that in addition to the 90 confirmed cases of CIN2+ there were 21.7 undetected cases of CIN2+ among the study cohort (12.7 extra CIN2 and 9.0 CIN3+).

Cytology and histology reviews were performed by independent experts for quality assurance. In summary, 2% of the cytology negative samples were upgraded while 6% of cytology positive samples were negative at rescreening. Histology review of negative samples resulted in an upgrade of 1.6% of initially histology negative results and 15% of histology positive samples were normal during review.

Comparison of test characteristics. After adjusting for verification bias, we estimated the sensitivity, specificity, positive predictive value and negative predictive value of each screening test (Table 4). The sensitivities of the tests are slightly lower after adjustment. For the detection of CIN2+ they are: 39.5% for LBC, 87.8% for AHPV, and 93.2% for HC2. For the

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detection of CIN3+ sensitivities are: 49.8% for LBC, 90.9% for AHPV, and 100.0% for HC2. The corresponding specificities are as follows: 98.4% for LBC, 96.1% for AHPV, and 94.9% for HC2. Consequently, the PPV of AHPV (21.1%) is somewhat better than that of HC2 (17.9%). Indeed, it is estimated that only 10.0 of 171 women who tested positive for HC2 but negative for AHPV had CIN2+ yielding a PPV for this combination of 5.8%. Women testing positive in at least one HPV test had their samples typed for 24 HPV genotypes including HPV16/18. HPV16 typing had a sensitivity of 44.8% for CIN2+ and 59.0% for CIN3+, but a high PPV of 38.8% (Table 4). The addition of HPV18 positive samples added 5.5 additional CIN2+ cases out of 29 who tested positive for HPV18 but negative for HPV16 giving a PPV of 19%.

The difference in the sensitivity of HC2 and AHPV for CIN2+ and CIN3+ was not statistically significant (p value=0.180 and 0.0625 respectively), however the difference in specificity of 96.1% for AHPV as compared to 94.9% for HC2 (difference 1.2%, 95% CI 0.87%-1.48%) was significantly higher ($p<0.001$).

Of special interest is the comparison of the AHPV results to those of HC2 on individual samples. Overall, there was 97.6% agreement on the 9,451 samples with a Cohen's kappa value of 0.77. Although the agreement was 88.9% among the 90 known cases of CIN2+, the kappa value was only 0.11. It is also of interest to note the lack of agreement (only 78.6%) among samples shown to be positive for either HPV16 or HPV18 DNA: 21 samples were positive for HC2 but negative by AHPV and 13 were AHPV positive but HC2 negative.

Triage strategies. In Table 5 we present the results of a number of possible triage strategies based on the primary screening test being either AHPV or HC2. Triage using LBC or HPV16/18 would yield a higher PPV for immediate colposcopy, but a smaller proportion of

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cases would be diagnosed immediately. For instance using HPV16/18 in AHPV positive women would yield a PPV for immediate colposcopy of 36.9% with an immediate sensitivity (for CIN2+) of 45.8%. Triaging AHPV positive women using LBC and HPV16/18 typing and referring women positive on either triage test would increase the sensitivity of immediate colposcopy to 60.0% with a PPV of 32.6%. The sensitivity of such a strategy for CIN3+ is increased to 72.0%.

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DISCUSSION

This study evaluated and compared the performances of the Digene Hybrid Capture 2 High-Risk HPV DNA test (HC2; QIAGEN GmbH, Hilden, Germany) and the Aptima® HPV Assay (AHPV; Hologic, San Diego, CA) to liquid based cytology (LBC) and histology in a routine screening cohort of 10,040 women in Germany. We found that both HPV tests were highly sensitive in detecting high grade cervical lesions (CIN2+ and CIN3+).

HC2 detected four more high-grade pre-cancerous lesions than AHPV including three CIN3 cases. Of the three CIN3+ cases missed by AHPV, two presented with a lesion-free cone and one had an infection with the non-HR type HPV67. The detection of the two lesion-free cases by HC2 may be explained by the fact that the HC2 test targets HPV DNA, which is more stable than mRNA. Some rare HPV67-related invasive cervical carcinoma (ICC) cases have previously been reported (26, 27) and HPV67 prevalence is higher in ICC than in women with normal cytology (28), which is why the IARC has classified HPV67 as possibly carcinogenic (class 2B carcinogens) (9). Despite HPV67 not being a target of the HC2 test, previous reports demonstrated cross-reactivity with HPV67 (29), which explains why this case was detected by HC2.

The non-significantly lower sensitivity of AHPV in detecting CIN2+ ($p=0.180$) and CIN3+ ($p=0.0625$) is in line with earlier studies (11, 12, 17). However, more recent reports demonstrate an equal (10, 14-16) or higher (13, 18) sensitivity of AHPV as compared to that of HC2. All these studies also reported a higher specificity for AHPV, which is also supported by our data showing a significantly ($p<0.001$) increased specificity of 96.1% for AHPV as compared to 94.9% for HC2 (difference 1.2%, 95% CI 0.87%-1.48%). This might possibly be due to the fact that by measuring mRNA levels, the AHPV assay detects actively infected cells whereas DNA-based assays such as HC2 do not distinguish between intra- or

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extracellular viral DNA, which may represent contamination with e.g. virus particles as previously shown in transmission studies in young couples (30).

The Cohen's kappa value confirmed a substantial agreement between the overall test performances. The small value (κ=0.11) in high-grade lesions is due to the different CIN2+ detection rates of both assays.

Despite our finding that AHPV has a lower positivity rate than HC2, the generally high test positivity rates of both assays as standalone tests suggest that one might not wish to refer all HPV-positive women to immediate colposcopy. It is noteworthy that we have used the manufacturer recommended cut-off for the AHPV assay, which was valid at the beginning of this study (S/CO 1.0). When we applied the new FDA approved cut-off value of S/CO=0.5 to our data set, 46 additional HR HPV-positive cases were detected of which only four histology results were available (all ≤CIN1). The cut-off of S/CO=0.5 did not have any effect on the sensitivity and specificity results of the AHPV assay.

Current practice in Germany involves an annual cytology screening. However, poor sensitivity of cytology demands the development of more accurate screening schemes. Strategies for the improvement of early diagnosis of CIN2+ cases have been assessed for AHPV based on the primary screening test being cytology (10-18). Adjunctive testing to cytology leads, however, to a reduced combined sensitivity. AHPV has also been suggested as the primary cervical cancer screening test in few studies (10, 13, 14). HPV DNA screening – though under certain requirements – has already been identified as an alternative option for primary cervical cancer screening (31-33). A triage scenario in which AHPV positive women are tested for HPV16 or 18 and/or cytology showed a similar immediate sensitivity for CIN2+ as triage of HC2 positive women by the same methods, but led to 9.3% fewer referrals to colposcopy (Table 5). Our results also imply that only slightly more women will

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be referred (206 for AHPV vs 194 for cytology only) while a larger number (>50%) of additional CIN2+ cases will be identified compared to cytology as primary standalone test (71.2 for AHPV vs 44 for cytology only). Current practice (cytology screening) would have referred 194 cytology-positive women to colposcopy, immediately detecting 44 (39.4%) CIN2+ cases within our study cohort. Using cytology as triage for either HPV test (AHPV and HC2), respectively 111 and 129 women would be referred with 39.2 and 43 cases detected immediately. This means the number of referrals may be drastically reduced by more than 30% while the number of detected cases remains unchanged. A similar result was obtained for HPV16/18 screening as the triage test, but the referral-to-cases ratios of 2.6 for HC2 and 2.7 for AHPV are more in favor of HPV16/18 genotyping. This result highlights the importance of HPV16/18 genotyping, as the estimated relative risk for prevalent high-grade disease is significantly higher for those who test HPV16/18-positive as compared to HR-HPV-positive (34-36).

It should generally be noted that without increasing the number of referrals (from 194 for cytology only screening), only a maximum of 48.7% of the total CIN2+ patients would have been identified in any tested triage scenario. Therefore it seems plausible to refer double-positive patients to colposcopy, while the follow-up strategy for HPV-positive women who were negative on triage would be based on shorter repeat screening intervals, which means cases that have been missed in the first screening round would likely be identified during follow-up.

In summary, AHPV was found to be both more specific and to have a higher PPV than HC2. Although AHPV appeared to be less sensitive, we estimate that only 5.8% of those testing positive by HC2 but negative by AHPV had CIN2 or worse. In fact, triaging AHPV positive women using LBC and/or HPV16/18 typing and referring women positive on either triage

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test would increase the sensitivity of immediate colposcopy to 60.0% with a PPV of 32.6%, very similar results as one would obtain by triaging HC2 tests in the same way. As a conclusion, AHPV is both sensitive and specific for the detection of high-grade intraepithelial neoplasia of the cervix and with an appropriate triage strategy can be considered as a primary cervical cancer screening option for women 30 years and older.

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472 **FIGURE LEGEND**

473 **Figure 1.** Study flow chart.

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475 **TABLES**

476

Table 1: Cytology results by HPV test result for those women included in the analysis

	LBC positive		LBC negative		
	AHPV+	AHPV-	AHPV+	AHPV-	**Total**
HR HC2+	110	19	299	152	**580**
HR HC2-	1	64	54	8752	**8871**
Total	**111**	**83**	**353**	**8904**	**9451**

477 +: positive; -: negative

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480 **Table 2:** Summary of the genotype distribution as determined by INNO-LiPA Genotyping

481 Extra.

HPV Genotype	Single infection N	Multiple infection N	Total N
16	72	55	127
31	64	32	96
52	33	29	62
53	30	29	59
51	21	25	46
66	21	25	46
18	20	15	35
56	16	17	33
68	15	16	31
70	13	17	30
39	14	15	29
33	20	7	27
74	3	23	26
45	9	12	21
58	7	13	20
54	3	12	15
35	8	5	13
6	1	11	12
69	3	9	12
71	3	9	12
44	1	10	11
59	4	2	6
82	2	4	6
73	1	2	3
X	16		16
Total	**400**	**399**	**799**
Sample failed			5
HPV negative			63
Total			**867**

482 X: HPV DNA was detected by LiPA, but could not be correlated to a specific type

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Table 3: Detailed data of screening results comparing LBC results to the histology result by high risk HPV results.

	Histology				
LBC	Not available N(APHV+, HC2+)	<CIN2 N(APHV+, HC2+)	CIN2 N(APHV+, HC2+)	CIN3 or worse N(APHV+, HC2+)	Total N(%APHV+,%HC2+)
A: Observed results					
Normal (Pap I,II)	8582(80, 119)	343(192, 245)	26(23, 23)	20(18, 20)	8971(3.5, 4.5)
ASC-US, AGC (Pap IIw)	236(10, 10)	44(24, 28)	5(5, 5)	1(1, 1)	286 (13.9, 15.4)
ASC-H & AGUS (Pap III)	6(0, 0)	23(7, 7)	2(2, 2)	2(1, 2)	33(30.3, 33.3)
LSIL, HSIL (Pap IIID)	21(15, 17)	94(45, 59)	8(6, 7)	5(5, 5)	128(55.5, 68.8)
HSIL, CIS, Micro (Pap IVa, IVb)	3(3, 3)	9(6, 6)	6(6, 6)	15(15, 15)	33(90.9, 90.9)
Total	8848(108, 149)	513(274, 345)	47(42, 43)	43(40, 43)	9,451(4.9, 6.1)
B: Verification bias adjusted results					
Normal (Pap I,II and Pap IIw)		9189.4(294.8, 389.9)	41.5(34.8, 35.0)	26.1(23.4, 26.1)	9257(3.8, 4.9)
Low grade (Pap III and Pap IIID)		140.2(64.4, 79.4)	11.6(9.4, 10.5)	9.1(7.1, 9.1)	161(5.0, 6.1)
High grade, CIS, Micro (Pap IVa,IVb)		9.7(6.7, 6.7)	6.6(6.6, 6.6)	16.7(16.7, 16.7)	33(90.9, 90.9)
Total		9339.3(365.9, 476.0)	59.7(50.8, 52.1)	52.0(47.2, 52.0)	9,451.0(4.9, 6.1)

+: positive

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Table 4: Sensitivity, specificity, positive and negative predictive values.

	Sensitivity (CIN3+)		Sensitivity (CIN2+)		Specificity (<CIN2)		PPV (CIN2+)		NPV(<CIN2)	
	%	95% CI	%	95% CI	%	95% CI	%	95% CI	%	95% CI
LBC	49.8	(34.7-64.9)	39.5	(29.4-49.5)	98.4	(98.1-98.7)	22.7	(16.3-29.1)	99.3	(99.1-99.5)
HR HC2	100	(91.8-100)	93.2	(87.1-99.2)	94.9	(94.1-95.7)	17.9	(14.5-21.4)	99.9	(99.8-100)
AHPV	90.9	(81.1-100)	87.8	(80.2-95.5)	96.1	(95.5-96.7)	21.1	(17.0-25.2)	99.8	(99.7-100)
HPV 16	59.0	(44.2-73.7)	44.8	(34.5-55.1)	99.2	(98.9-99.4)	38.8	(29.4-48.1)	99.3	(99.1-99.5)
HPV 16/18	63.1	(48.6-77.6)	49.7	(39.3-60.1)	98.9	(98.6-99.2)	35.2	(27.0-43.4)	99.4	(99.2-99.6)

+: and worse

Table 5: Strategies for triage to colposcopy adjusted for verification bias for women who test HPV positive, Sensitivity and PPV for CIN2 or worse histology.

	Number referred to colposcopy	Cases found immediately	Ratio referrals/ cases	Immediate sensitivity (CIN2+)	PPV of referral (CIN2+)
	N	N		%(95% CI)	%(95% CI)
Cytology only	194	44	4.4	39.5(29.4-49.5)	22.7(16.3-29.1)
Triage of HR HC2 positive					
All to colposcopy	580	104.0	5.6	93.2(87.1-99.2)	17.9(14.5-21.4)
Refer if positive to AHPV	409	94.1	4.3	84.2(75.9-92.6)	23.0(18.5-27.5)
Refer if HPV16/18 positive	146	54.4	2.6	48.7(38.3-59.1)	37.3(28.6-46.0)
Refer if cytology positive	129	43.0	3.0	38.5(28.4-48.5)	33.3(24.5-42.1)
Refer if HPV16/18 positive and/or cytology positive	227	71.2	3.2	63.8(53.6-74.0)	31.4(24.8-38.1)
Triage of AHPV positive					
All to colposcopy	464	98.1	4.7	87.8(80.2-95.5)	21.1(17.0-25.2)
Refer if positive to HR HC2	409	94.1	4.3	84.2(75.9-92.6)	23.0(18.5-27.5)
Refer if HPV16/18 positive	139	51.2	2.7	45.8(35.5-56.2)	36.9(28.0-45.7)
Refer if cytology positive	111	39.9	2.8	35.7(25.9-45.5)	35.9(26.3-45.6)
Refer if HPV16/18 positive and/or cytology positive	206	67.0	3.1	60.0(49.6-70.3)	32.6(25.6-39.6)

Verification bias adjusted results; Total CIN2+ = 111.7; +: and worse

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