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Comparison of hybribio-H13 and hybrid capture 2 human papillomavirus tests for detection of CIN2+ and CIN3+

Comparación de las pruebas del virus del papiloma humano hybribio-H13 y captura de híbridos 2 para la detección de NIC2+ y NIC3+

Comparison of two DNA HPV tests for detection of CIN2+ and CIN3+

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Kelly Melisa Castañeda, Mark Stoler, Michael Dean: Acquisition of data and revising it critically for important intellectual.

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Introduction. Low-cost, accurate hrHPV tests are needed for cervical cancer screening in limited-resource settings.

Objective. To carried out to compare the performance of the low-cost H13 test with HC2 to detect CIN2+ and CIN3+.

Materials and methods. Archived baseline samples tested by HC2 from women aged 20–69 years of the ASCUS-COL trial with biopsy-colposcopy directed diagnosis of CIN2+ (n=143) and CIN3+ (n=51) and 632 women with <CIN2, were retrospectively and blindly tested by H13.

Results. The relative sensitivity of H13 versus HC2 for detecting CIN2+ was 0,89 (90% CI:0,80-0,98; pni 0,66) and for CIN3+ was 0,92 (90% CI: 0,85-0,98; pni 0,35). Relative specificity was 1.19 (90% CI: 1.05-1.33; pni <0.00001). In the analysis restricted to ≥30-year-old-women, the relative sensitivity of H13 for CIN3+ was marginally below unity, (ratio: 0.97, 90% CI 0.95-0.99), and the specificity remained higher than HC2.

Conclusion. H13 was as specific but less sensitive than HC2 for detecting CIN2+ or CIN3+. Considering these results and the young age of the population that was recruited for screening because of ASCUS cytology, we suggest our results warrant the evaluation of H13 for screening of cervical cancer, especially in the screening population.

Keywords: Uterine cervical neoplasms; human papillomavirus viruses; human papillomavirus DNA tests.

Introducción. Se necesitan pruebas de alto riesgo VPH precisas y de bajo costo para la tamización del cáncer de cuello uterino en entornos de recursos limitados.

Objetivo. Comparar el desempeño de la prueba H13 de bajo costo con la prueba CH2 para detectar NIC2+ y NIC3+.

Materiales y métodos. Muestras analizadas por CH2 de la línea de base de mujeres entre 20 y 69 años de edad del ensayo ASCUS-COL con diagnóstico dirigido por biopsia-colposcopia de NIC2+ (n=143) y NIC3+ (n=51) y 632 mujeres con <NIC2, fueron retrospectivamente probadas a ciegas por la prueba H13.

Resultados. La sensibilidad relativa de H13 versus CH2 para detectar NIC2+ fue de 0,89 (IC 90 %: 0,80-0,98; pni 0,66) y para NIC3+ fue de 0,92 (IC 90 %: 0,85- 0,98; pni 0,35). La especificidad relativa fue de 1,19 (IC 90%: 1,05-1,33; pni <0,00001). En el análisis restringido a las mujeres ≥30 años, la sensibilidad relativa de H13 para NIC3+ estuvo marginalmente por debajo de la unidad (proporción: 0,97, IC del 90 %: 0,95-0,99) y la especificidad permaneció más alta que CH2.

Conclusión. H13 fue tan específico, pero menos sensible que CH2 para detectar NIC2+ o NIC3+. Teniendo en cuenta estos resultados y la edad joven de la población que se reclutó en la tamización debido a la citología ASCUS, sugerimos que nuestros resultados justifican continuar la evaluación de H13 para la detección del cáncer de cuello uterino, especialmente en la población de tamización.

Palabras clave: neoplasias del cuello uterino; virus del papiloma humano; pruebas de ADN del papillomavirus humano.

In 2020, there were 604.127 new cases and 341.831 deaths from cervical cancer. Around 90% of these cases and deaths occur in Asia, Africa, Latin America, and the Caribbean regions (1). Human Papillomavirus (HPV) is the necessary cause of virtually all cervical cancers, with HPV types 16 and 18 accounting for approximately 70% of cases (2). Prophylactic vaccination against HPV 16 and 18 provides more than 90% protection against infection and HPV 16- and 18-associated high-grade lesions (cervical intraepithelial neoplasia (CIN) grade 2 or 3 (CIN2 and CIN3) or cancer (3). However, because currently implemented HPV vaccines do not eliminate the risk of cervical cancer, early detection remains a public health need.

Cytology-based screening is associated with an important reduction in the incidence and mortality of cervical cancer, especially in high-income countries (HICs), but it has not achieved that impact in Low-Middle Income Countries (LMICs). The main reason is the low sensitivity of cytology, which requires repeated testing that hinders required access to regular screening and follow-up to gynecological management of positive results (4). HPV testing has a sensitivity of around 100% for the detection of cervical high-grade lesions and has a high negative predictive value, which permits the extension of screening to every 5 years. Other important attributes include automation, high reproducibility, and a more rapid turnover of results than cytology (5). Therefore, HPV testing is the superior alternative currently available for cervical screening, especially LMICs where performer-dependable method implementation has been challenging. However, HPV testing has not been widely implemented in routine health care services in most LMICs. Most of the current HPV tests are expensive and require advanced equipment (6).

The H13 HPV test from Hybribio (Hybribio Biotechnology Limited Corp., Hong Kong, China), hereafter referred to as H13, is a low-cost test based on a quantitative

polymerase chain reaction (qPCR) that detects as a pool the HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 genotypes in cervical exfoliates (7). The H13 test does not require complex infrastructure and is robust, with easy interpretation of results obtained in about three hours.

Two studies have compared the performance of the H13 test for the detection of CIN2+ with the reference standard HPV HC2 test (hereafter HC2 test). In the study of 516 women with samples from the Kaiser Permanente Northern California (KPNC) repository, the agreement between H13 and HC2 was found to be good, since H13 correctly identified 91.5% of HPV-positive HC2 samples among CIN2+ cases and correctly identified 92.1% of HPV-negative HC2 samples among ≤CIN2 (7). Within the framework of the VALidation of HPV GENotyping Tests-3 (VALGENT-3) study, an established framework with a repository of 1600 samples for evaluating HPV tests clinical performance relative to validated comparators, it was compared with HC2, a new version of H13 (then called H14), which, in addition to including the HPV 66 genotype, reports genotypes 16 and 18 individually. Relative sensitivity and specificity of H14 versus HC2 for detecting CIN2+ were 0.98 (95% confidence interval [CI], 0,94-1,03; P noninferiority [Pni] 0,01) and 0,97 (95% CI: 0,96-0,99; Pni 0,78), respectively (8).

Although these results suggest that H13 or H14 might be attractive for cervical cancer screening in low-resource settings given its low cost, there are no studies comparing the performance of H13 or H14 to reference standards in samples of women from LMICs. In this secondary analysis of the phase III randomized controlled ASC-US trial (9), we present a head-to-head comparison of the H13 assay with the reference Qiagen HC2 HPV DNA for the detection of CIN2+ and CIN3+ in 842 women that participated in this trial.

Materials and methods

Study design and population

Samples for this study were selected from the ASCUS-COL trial. The ASC-US-COL is a three-arm, non-blinded, parallel group, pragmatic trial. Women aged 20-69 years (n=2,661) with first-time atypical squamous cells of undetermined significance (ASC-US) cytology in the last 2 years were flagged in routine screening services and randomly allocated to receive immediate colposcopy (IC arm; n=882), repeat cytology at 6 and 12 months (RC arm; n=890) or an HPV test within 2 months of recruitment (HPV arm; n=889). Colposcopy and biopsies, according to clinician judgment, were recommended for all women in the IC arm, for women with a repeat ASC-US or worse (ASC-US-positive) cytology in the RC arm, and for hrHPV+ women in the HPV arm. Hybrid Capture 2 HPV DNA test (HC2©, QIAGEN, Germantown, USA) was conducted at the laboratory of Infection and Cancer at the University of Antioquia. All women received invitations, and 80% (n=2,132 women) attended the exit visit after 24 months of follow-up, which included hrHPV and cytology tests (9). All women positive for either test were referred to a certified, well-trained colposcopist using a standardized and controlled protocol of biopsy sampling. After the end of the study, two blinded accredited experts confirmed the histopathological diagnoses of 1,407 women with at least one, and the baseline samples of women in the IC and RC arms were tested for hrHPV by HC2© (QIAGEN) (Supplementary figure 1). ASCUS-COL is registered with ClinicalTrials.gov (NCT02067468).

Selection of participants for sub-study HC2 vs H13 comparison

Women identified after the end of the ASCUS-COL trial with biopsy-colposcopy-directed, adequate diagnosis and with enough remaining archived baseline samples in specimen transport medium (STM; QIAGEN) for further testing (n=1,348) were

considered eligible for this study. We included all women diagnosed with CIN2+ (n=197) and a representative sample of age-matched women (n=645) with a final negative or CIN1 histological diagnosis, as shown in supplementary figure 1. The residual content of the Specimen Transport Medium (STM) tube used for the Qiagen HC2 HPV DNA testing of samples collected at the recruitment visit was used for H13 testing. Data collection and testing of the reference standard (histopathological diagnosis) and comparator test (HC2) were conducted before the index test (H13). The HC2 and H13 assays, as well as the verification of the histological diagnoses, were conducted independently and blindly.

Qiagen HC2 HPV DNA Test

This test is based on a DNA-RNA hybridization that identifies a pool of 13 hrHPV genotypes (*16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68*). Cervical cells were collected from women with a cytobrush (HC Cervical Sampler) and transferred to a tube containing 1 ml of Specimen Transport Medium™ (STM). The Qiagen HC2 HPV DNA Testing (QIAGEN, Gaithersburg, MD, USA) was performed according to the manufacturer instructions at the HPV Lab of the Infection and Cancer Group at the Universidad de Antioquia (UdeA, Medellín, Colombia). Relative light unit values greater than 1 were considered positive.

HybriBio (H13) DNA extraction and testing

The DNA for the HybriBio test was extracted from the denatured residual content of the Specimen Transport Medium (STM) tube used for the Qiagen HC2 HPV DNA Test by a standard protocol (10). In brief, each specimen was digested for 2 h at 55°C in the presence of 200 µg of proteinase K per ml and 1% Laureth-12. The samples were heated to 95°C for 10 minutes to denature the residual protease. After precipitation with ammonium acetate (final concentration 5M) and 70% ethanol, DNA

was washed, dried, and resuspended in 100 µl of TE buffer (10mM TRIS + 0.1mM EDTA) and frozen at -30°C until shipped at room temperature to the Laboratory of Translational Genomics of the National Cancer Institute (NCI/NIH, Bethesda, MD, USA), where testing was performed. The H13 test is a real-time qPCR assay that uses specific primers targeting the HPV E6 and the human beta-globin genes and 2 probes, one labeled with 6-carboxyfluorescein (FAM) fluorescent dye for the detection of a pool of 13 hrHPV genotypes and the other labeled with (6-carboxy-4´,5´-dichloro- 2´,7´-dimethoxyfluorescein, HEX) fluorescent dye, which detects the amplification of the human Beta-globin gene, which works as an internal control for DNA adequacy. The volume of reagents and input DNA were modified from those described in the manufacturer's instructions. The final volume was 11 µL, including 8.75 µL of kit PCR Master Mix, 0.25 µL of DNA Taq Polymerase, and 2 µL of the sample DNA. We have previously shown that this modification results in the minimum assay volume required with equivalent results (11). We used the positive and negative controls included in the H13 kit. A Ct value ≤40 was the threshold for considering a positive result. Negative samples with no positive signal in the cellular internal control were excluded from the analysis. The real-time instrument used was a Roche LightCycler 480 II.

Sequencing of BSGP5+/6+ amplimers

To determine the HPV type of discordant samples (Positive HC2/Negative H13, n=97, and Negative HC2/Positive H13 n=29), DNA purified from exfoliates as described above was amplified with BSGP5+/6+ primers and the amplicon sequenced with Sanger. The conserved BSGP5+/6+ primer pair amplifies a region of 150 bp of the L1 gene that contains unique sequences that distinguish HPV genotypes (12,13). The sequences were used as a query for screening the GenBank

database (www.ncbi.nlm.nih.gov) with BLAST Software 1. HPV types were assigned when a match between the 150 bp interprimer region and an HPV sequence in GenBank was found.

Sample size

We excluded 16 invalid samples -six negative, seven CIN1, and three CIN2-, because of inappropriate signals in the cellular internal control of H13. The final analysis included 826 women. Based on the recommendations of Meijer et al. that at least 60 samples should be analyzed to assess whether a candidate test has a sensitivity for CIN2+ not less than 90% of that of HC2 (14), our study included 194 samples with CIN2+ for a power of 99.6% and 51 samples with CIN3+ for a power of 92.7%. The non-inferiority of H13 to HC2 with respect to the clinical specificity for <CIN2 was assessed in 632 cervical samples of women who did not have histologically confirmed CIN2+ with a power of 90%.

Statistical methods

Sensitivity and specificity with corresponding 95% CIs were estimated for detection of CIN2+ or CIN3+ using <CIN2 (negative and CIN1) as disease-free categories. The McNemar test (McN) was used to compare the differences between matched proportions. A matched non-inferior statistic (ni) with a 90% relative sensitivity threshold and a 98% relative specificity threshold was used when comparing the clinical performance of H13 to HC2. The level of statistical significance for both statistics (pMcN and pni) was set at 0.05. All analyses were conducted using the STATA 13 software (StataCorp LLC, Texas, USA).

Ethical approval

ASCUS-COL complied with Colombian Resolution 8430 of 1993 to conduct studies in humans and was conducted following the CIOMS guidelines (15). The ethics

committees for human experimentation the Sede de Investigación Universitaria (SIU) (Resolution 08-036-171) and the School of Medicine (Resolution 004/2008) from the University of Antioquia approved this study. Participants signed written informed consent, including authorization to use their samples and data for future research.

Results

Figure 1 presents the flowchart for sample selection. Specimens from 842 women that were collected and previously tested with HC2 at the enrolment visit were selected from the 2,661 participants of the ASCUS-COL study with adequate histological diagnosis and retested by H13. Sixteen (1.9%) samples of these residual specimens that tested invalid with H13 were excluded from further analysis, resulting in 826 samples analyzed by both the HC2 and H13 tests. The clinical features of the study population are summarized in Table 1. All women had an ASCUS pap smear for the first time at the screening visit. Most women were under 40 years old (75%), around half started regular sex between 16 and 19 years old, and 54% had between 3 and 4 lifetime sexual partners. Slightly more than half of the women included in this analysis had a definitive histological diagnosis at 6-month follow-up (57%). Supplementary table 1 shows the number of histological diagnoses in the 842 included participants: 506 women without cervical lesions, 139 with CIN1, 146 with CIN2, 47 with CIN3, and four cases of cancer. HC2 and H13 tests were positive in 389 (60%) and 335 (52%) of the 645 women with <CIN2, and 182 (92%) and 160 (81%) of the 197 CIN2+ cases, respectively. For the CIN3+ threshold, HC2 and H13 tests were positive in 48 (94%) and 44 (86%) of 51 women with CIN3+, respectively. Sensitivity and specificity for the detection of CIN2+ or CIN3+ are shown in table 2. The H13 test showed a slightly higher specificity for<CIN2 (47% vs. 39%, difference 7.6, 95% CI: 4.6-10.6), and the HC2 test exhibited higher sensitivity to detect CIN2+

(93% vs. 82%, difference: 10.3, 95% CI: 5.2-15.5) or CIN3+ (94% vs. 86%, difference: 7.9, 95% CI: 0.5-15.2).

Table 3 presents the relative sensitivities for CIN2+ and CIN3+ and the relative specificity for <CIN2 of the H13 test in comparison to the HC2 test. In the analysis with all women (n=826), H13 exhibits inferiority to HC2 with a relative sensitivity of at least 90% for CIN2+ (Pni 0,6584) and CIN3+ (Pni 0,3501), while it was non-inferior to HC2 with a relative specificity for <CIN2+ of at least 98% (Pni ≤0,00001). The relative sensitivity of H13 for CIN2+ and CIN3+ was below unity (ratio: 0,89 90% CI 0,80-0,98 and 0,92 90% CI 0,85-0,98), and the relative specificity for <CIN2 was significantly different from unity (ratio: 1,19, 90% CI 1,05-1,33). Similar results were found when restricting the analysis to women aged 30 and older (n = 454), the relative sensitivity of H13 for CIN2+ and CIN3+ was below unity (ratio: 0,90 90% CI 0,81-0,98 and 0,92 90% CI 0,85-0,98), and the relative specificity for <CIN2 was (ratio: 1,11, 90% CI 0,99-1,24).

We further analyzed the discordance between H13 and HC2 test results by DNA sequencing (Supplementary Table 2). Among the 63 samples with hrHPV genotypes identified by sequencing, 50 samples (79,4%) were HC2+/H13-, and 13 samples (20,6%) were HC2-/H13+. Among the 23 samples negative or with low-risk HPV genotypes identified by sequencing, 16 samples (70%) were HC2+/H13- and 7 samples (30%) were HC2-/H13+.

Discussion

In this study, we compare the clinical accuracy of the Hybribio H13 test in relation to the reference Qiagen HC2 test. Due to the matched design with samples tested with both H13 and HC2, non-inferior statistics could be calculated. Samples were tested immediately after collection by HC2 in Colombia and shipped to the USA, where

testing by H13 was conducted using the minimum assay volume. Under these conditions, the H13 test did not conform to the acceptable standards of clinical performance for sensitivity to detect CIN2+ or CIN3+ but conformed to the acceptable standard of performance for specificity to detect <CIN2, overall and in women ≥30 years old.

Currently, few studies properly comparing the clinical performance of H13 with standard reference tests have been published in peer-reviewed literature. A recent study described the clinical performance between the H13 test and the HC2 test in 373 samples from North America. H13 correctly identified 94% of the HC2 HPV positive CIN2+ cases and 88% of the HC2 HPV negative cases (7). Likewise, in our study, H13 identified 156 of the 180 (87%) of the HC2 positive CIN2+ cases and 224 of the 249 (90%) of the HC2 HPV negative <CIN2 cases. In contrast to that description that reported 143/516 (28%) equivocal results, in our hands, the H13 test was highly robust, as the proportion of samples with equivocal results was very low (16/842, 1,9%). In our study, HC2 samples were processed immediately after collection, and manually extracted DNA was shipped at room temperature to the USA for H13 testing shortly after. We cannot exclude the possibility that the differences between tests could be explained by the modifications to the instructions of manufacturers. Therefore, the results presented here must be interpreted within the scope of this limitation. This is the first study in the international literature that presented a head-to-head comparison of the H13 assay with the reference Qiagen HC2 HPV DNA for the detection of CIN2+ and CIN3+ in a group of samples that allowed the performance of robust statistical tests with adequate power.

In this study, we included women with first-time ASC-US cytology at routine screening visits to health care services, 75% of them between 20 and 39 years of

age. Under these conditions, the H13 test did not conform the acceptable standards of clinical performance for sensitivity to detect CIN2 or CIN3+ but conformed the acceptable standard of the performance for the specificity to detect <CIN2. In the analysis restricted to ≥30 years old women, the relative sensitivity of H13 for CIN3+ was marginally below unity, (ratio: 0,97, 90% CI: 0,95-0,99) and the specificity remained higher than HC2.

In conclusion, this study is the first to compare head-to-head the performance of test H13 with a reference test such as HC2. Test H13 was as specific but less sensitive than HC2 to detect CIN2+ or CIN3+. Considering these results and the young age of the population that was recruited for screening because of ASCUS cytology, we suggest our results warrant the evaluation of H13 for screening of cervical cancer, especially in women over 30 years of age, who are the subject of screening with the HPV test according to Colombian clinical practice guidelines, and in order to have data that contribute to the use of the H13 test as a screening method.

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Conflict of interest

Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer/World Health Organization.

Not conflict of interest by any of the authors is declared. The HC2-hrHPV DNA test was donated by QIAGEN©. The H13 HPV test was donated by Hybribio Biotechnology Limited Corp, Chaozhou, China. The funders had no role in the data collection, analysis, or interpretation of the results.

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FIGURES

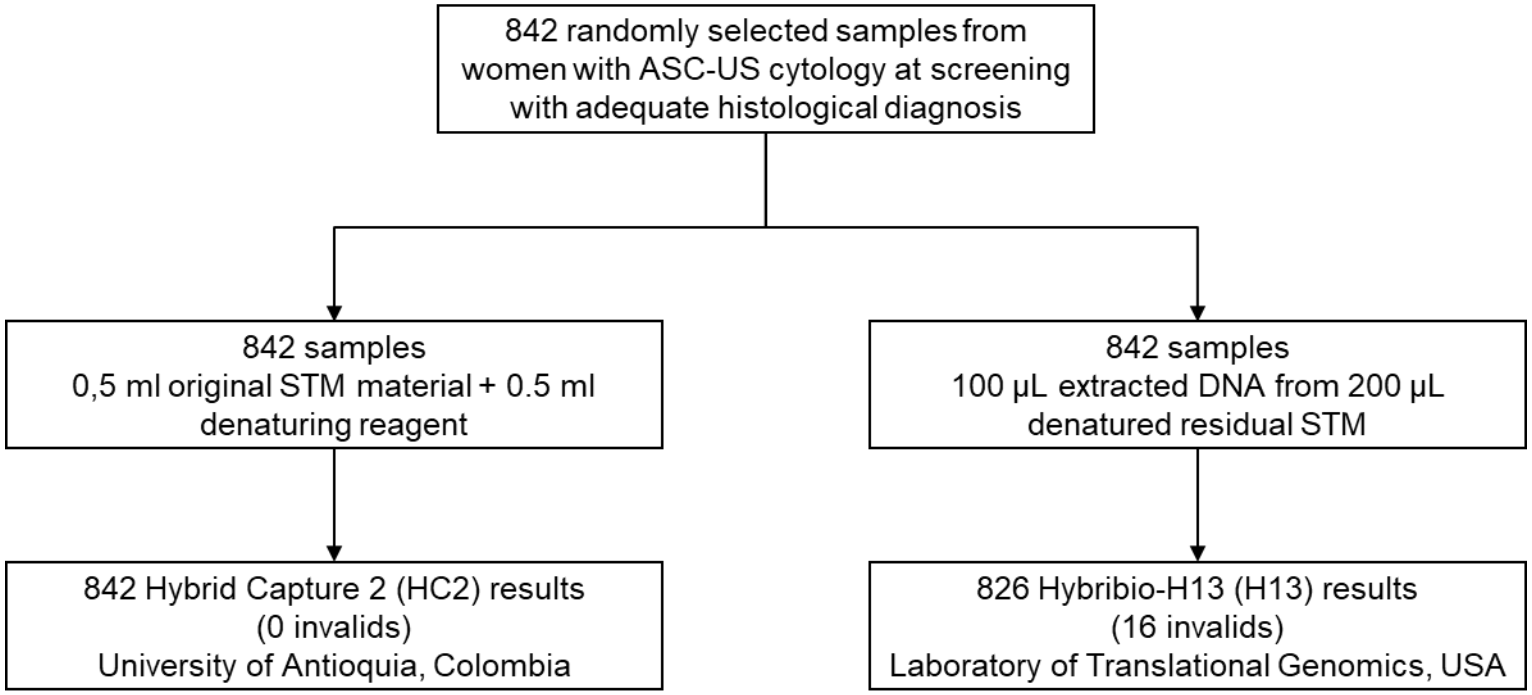


Figure 1. Flow Chart. Presents a flowchart showing the process from the panel collation of samples and the HPV testing to the final endpoint ascertainment for diseased and no disease group.

Table 1. Demographic and clinical characteristics at recruitment visit of 842 women of the ASCUS-COL trial

Characteristic	n (%)
Number	842 (100)
Age (years)	
20-29	379 (45,0)
30-39	252 (29,9)
40-49	151 (17,9)
≥ 50	60 (7,1)
Time to histological diagnosis (months)	
1-6	476 (56,5)
7-12	65 (7,7)
13-18	39 (4,6)
> 18	262 (31,1)
Age of first sexual intercourse (years)	
≤ 15	248 (29,4)
16-19	445 (52,9)
≥ 20	149 (17,7)
Number of lifetime sexual partners	
1-3	458 (54,4)
4-5	203 (24,1)
≥ 6	181 (21,5)
Histological diagnosis	
Negative	506 (60,1)
CIN1	139 (16,5)
CIN2	146 (17,3)
CIN3	47 (5,6)
SCC/ADC	4 (0,5)

CIN1: Cervical intraepithelial neoplasia grade 1. CIN2: Cervical intraepithelial neoplasia grade 2. CIN3: Cervical intraepithelial neoplasia grade 3. SCC: Squamous cell carcinoma. ADC: Adenocarcinoma.

Table 2. Specificity and Sensitivity of HC2 and H13 HPV tests for the detection of CIN2+ and CIN3+

HPV tests	<CIN2 (n = 632)			CIN2+ (n = 194)			CIN3+ (n = 51)		
	TN	FP	Specificity, % (95%CI)	TP	FN	Sensitivity, % (95%CI)	TP	FN	Sensitivity, % (95%CI)
HC2	249	383	39,46 (35,62–43,40)	180	14	92,78 (88,18–96,01)	48	3	94,12 (83,76–98,77)
H13	297	335	47 (43,04–51)	160	34	82,47 (79,40–87,54)	44	7	86,27 (73,74–94,29)

TN= True Negative, FP= False Positive, TP= True Positive, FN= False Negative. (6 biopsy negative, 7 CIN1, 3 CIN2) samples tested invalid by H13 were excluded from the analysis.

Table 3. Relative sensitivity for CIN2+ and CIN3+ and relative specificity for <CIN2 and CIN3+ of the H13 test in comparison to the HC2 test

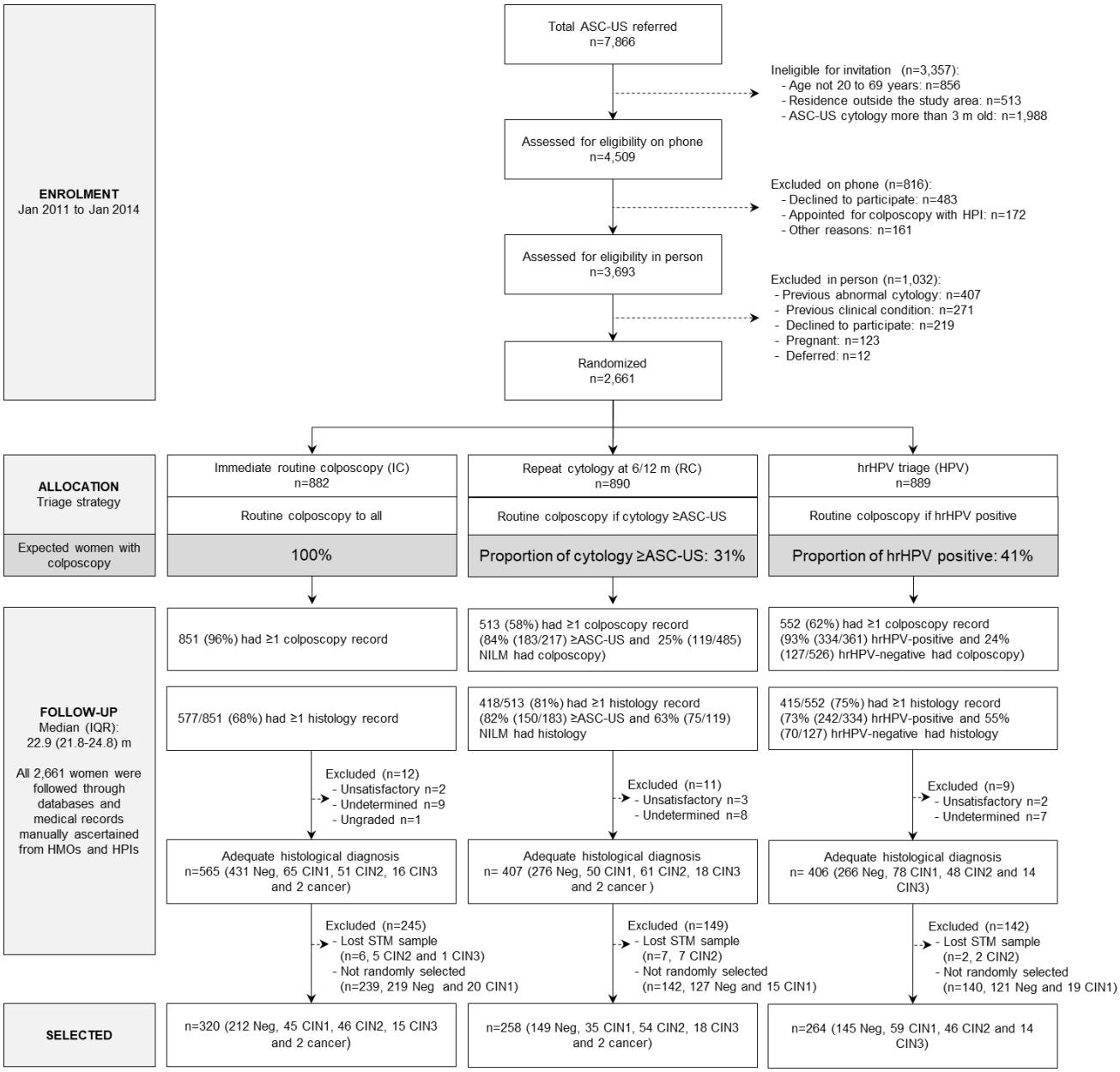
	Relative sensitivity (90% CI)	Relative specificity (90% CI)	P_{McN}[a]	P_{ni}[b]
All n = 826				
CIN2+ (n = 194)	0,89 (0,80-0,98)		0,0002	0,6584
CIN3+ (n = 51)	0,92 (0,85-0,98)		0,125	0,3501
<CIN2 (n = 632)		1,19 (1,05-1,33)	<0,0001	<0,00001
≥30 years n = 454				
CIN2+ (n = 106)	0,90 (0,81 – 0,98)		0,0212	0,5211
CIN3+ (n = 31)	0,92 (0,85 – 0,98)		0,5000	0,2887
<CIN2 (n = 348)		1,11 (0,99 – 1,24)	0,0039	0,0066

[a] p for the McNemar test for a difference between matched proportions.

[b] p for the test for non-inferiority. A matched non-inferior statistic (ni) with a 90% relative sensitivity threshold and 98% relative specificity threshold was used when comparing clinical performance of H13 to HC2.

Sixteen (6 biopsy negative, 7 CIN1, 3 CIN2) samples tested invalid by H13 were excluded from the analysis.

SUPPLEMENTARY MATERIAL



Supplementary figure 1. ASCUS-COL CONSORT Flowchart. The number of women recruited and allocated in each arm. Reports of cytology, colposcopy, and histology were ascertained manually for all women from medical records or databases in the corresponding HMOs and HPIs. Numbers of women with adequate histological diagnosis and retested by H13 are shown.

Supplementary table 1. Distribution of age and HC2 and H13 test results according to histological diagnosis

	Negative	**CIN1**	**CIN2**	**CIN3**	**SCC/ADC**
	n=506	n=139	(n=146)	(n=47)	(n=4)
	n, (%)	n, (%)	n, (%)	n, (%)	n, (%)
Age (years)					
20-29	210 (41.5)	79 (56.8)	70 (47.9)	20 (42.6)	0 (0.0)
30-39	154 (30.4	31 (22.3)	49 (33.6)	17 (36.2)	1 (25.0)
40-49	104 (20.6)	18 (12.9)	19 (3.0)	8 (17.0)	2 (50.0)
≥50	38 (7.5)	11 (7.9)	8 (5.5)	2 (4.3)	1 (25.0)
HPV-HC2					
Positive	277 (54.7)	112 (80.6)	134 (91.8)	44 (93.6)	4 (100.0)
Negative	229 (45.3)	27 (19.4)	12 (8.2)	3 (6.4)	0 (0.0)
HPV-H13					
Positive	240 (47.4)	95 (68.3)	116 (79.5)	40 (85.1)	4 (100)
Negative	260 (51.4)	37 (26.6)	27 (18.5)	7 (14.9)	0 (0.0)
Equivocal	6 (1.2)	7 (5.0)	3 (2.1)	0 (0.0)	0 (0.0)

CIN1: Cervical intraepithelial neoplasia grade 1. CIN2: Cervical intraepithelial neoplasia grade 2. CIN3: Cervical intraepithelial neoplasia grade 3. SCC: Squamous cell carcinoma. ADC: Adenocarcinoma. HPV-HC2: Hybrid Capture 2 Human Papillomavirus test. HPV-13: Hybribio 13-H13 Human Papillomavirus test.

Supplementary table 2. Identification of HPV genotypes by sequencing in discordant HC2/H13 samples

HPV genotype by sequencing	Positive HC2 /Negative H13 n=97	Negative HC2 /Positive H13 n= 29	Total
16, 31, 33,45,	50	13	63
6, 26, 30, 32, 53, 67, 87, 90 or negative	16	7	23
Not sequence obtained	31	9	40

Sequencing analysis included the identification of 13 high-risk HPV types included in HC2 test (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 or 68) as well low-risk HPV types. (6 biopsy negative, 7 CIN1, 3 CIN2) samples tested invalid by H13 were excluded from the analysis. Genotype 66 was not considered since it is not found in either of the two tests.