A new HPV-DNA test for cervical-cancer screening in developing regions: a cross-sectional study of clinical accuracy in rural China

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Summary

Background **A new test (*care*HPV; QIAGEN, Gaithersburg, MD, USA) has been developed to detect 14 high-risk types of carcinogenic human papillomavirus (HPV) in about 2·5 h, to screen women in developing regions for cervical intraepithelial neoplasia (CIN). We did a cross-sectional study to assess the clinical accuracy of *care*HPV as a rapid screening test in two county hospitals in rural China.**

Methods **From May 10 to June 15, 2007, the *care*HPV test was done locally by use of self-obtained vaginal and provider-obtained cervical specimens from a screening population-based set of 2530 women aged 30 to 54 years in Shanxi province, China. All women were assessed by visual inspection with acetic acid (VIA), Digene High-Risk HPV HC2 DNA Test (HC2), liquid-based cytology, and colposcopy with directed biopsy and endocervical curettage as necessary. In 2388 women with complete data, 441 women with negative colposcopy, but unsatisfactory or abnormal cytology or who were positive on HC2 or the new *care*HPV test, were recalled for a second colposcopy, four-quadrant cervical biopsies, and endocervical curettage. An absence of independence between the tests was not adjusted for and the Bonferroni correction was used for multiple comparisons.**

Findings **Complete data were available for 2388 (94·4%) women. 70 women had CIN2+ (moderate or severe CIN or cancer), of whom 23 had CIN3+. By use of CIN2+ as the reference standard and area-under-the-curve analysis with a two-sided alpha error level of 0·0083, the sensitivities and specificities of the *care*HPV test for a cut-off ratio cut-point of 0·5 relative light units, were 90·0% (95% CI 83·0–97·0) and 84·2% (82·7–85·7), respectively, on cervical specimens, and 81·4% (72·3–90·5) and 82·4% (80·8–83·9), respectively, on vaginal specimens (areas under the curve not significantly different, p=0·0596), compared with 41·4% (29·9–53·0) and 94·5% (93·6–95·4) for VIA (areas under the curve significantly different, p=0·0001 and p=0·0031, for cervical and vaginal-specimen comparisons for the *care*HPV test, respectively). The sensitivity and specificity of HC2 for cervical specimens were 97·1% (93·2–100) and 85·6% (84·2–87·1), respectively (areas under the curve not significantly different from the *care*HPV test on cervical specimens, p=0·0163).**

Interpretation **The *care*HPV test is promising as a primary screening method for cervical-cancer prevention in low-resource regions.**

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Introduction

Human papillomavirus (HPV) is the main cause of cervical cancer, affecting nearly 500 000 women around the world every year and killing more than 270 000 women, 80% of whom live in developing countries.[1] Unfortunately, cytology-based programmes rely on a level of infrastructure unattainable in most of the developing world, and few women in these countries have access to effective screening and treatment programmes.[2] This inadequacy has driven the search by the Alliance for Cervical Cancer Prevention to assess alternatives to cytology, such as visual inspection with acetic acid (VIA) and HPV-DNA testing.[3]

On the basis of evidence available up until 2005, the International Agency for Research on Cancer (IARC) made recommendations that supported primary screening based on cytology or HPV-DNA testing.[2] Much of the evidence on the clinical usefulness of primary screening by testing for the presence of carcinogenic types of HPV-DNA in cervical and vaginal samples is based on research with the Digene High-Risk HPV HC2 DNA Test (HC2, QIAGEN Inc, Gaithersburg, MD, USA; formerly Digene Corporation) assay, which tests for 13 carcinogenic HPV types in vaginal or cervical samples.[4–6] A meta-analysis by Cuzick and colleagues[7] has shown the sensitivity of cytology to be 53·0% (95% CI 48·6–57·4) versus a sensitivity of 96·1% (94·2–97·4) for cervical HPV-DNA testing for the detection of moderate or severe cervical intraepithelial neoplasia (CIN).[7] Assuming the initial goal of a cervical-cancer prevention programme in developing countries is to maximise participation and screen women aged 30 to 50 years once or twice in their lifetime, a test that has maximum sensitivity, affordability, and feasibility is desirable.

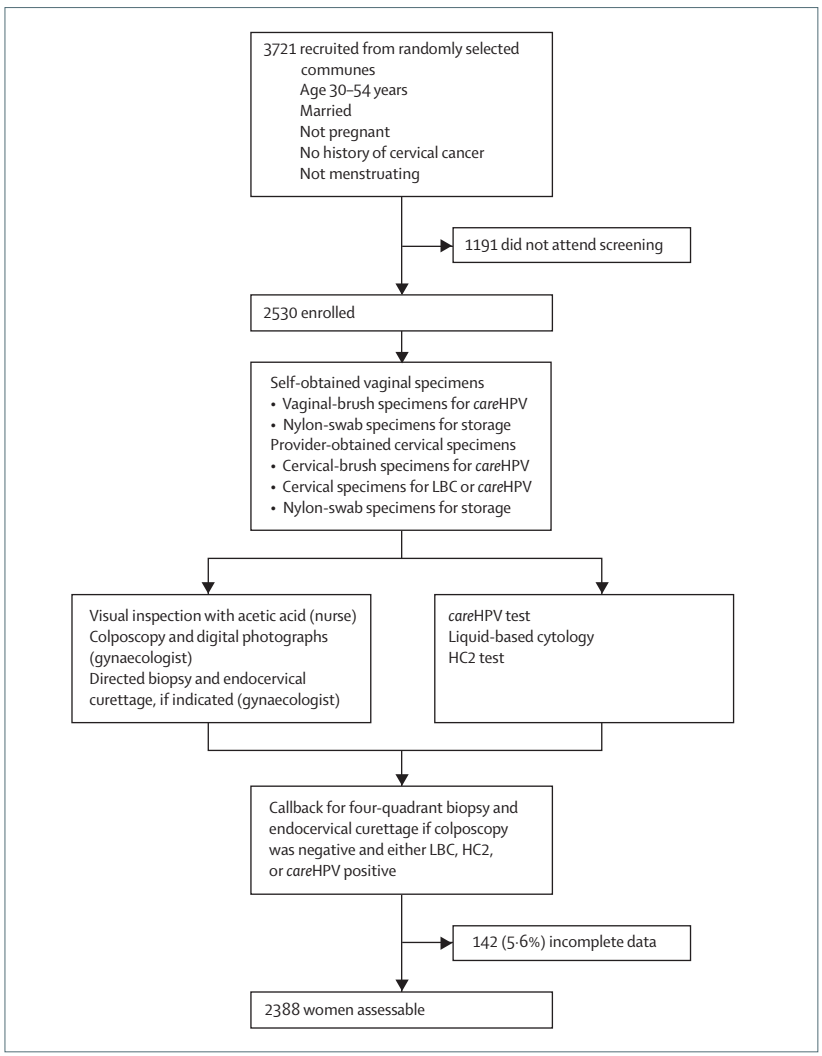

Figure 1: **Flow diagram showing number of women and procedures involved at every step of the study protocol**

Because no HPV-DNA test was deemed appropriate for use in low-resource settings in developing countries, PATH (Seattle, WA, USA) was funded in 2003 by the Bill & Melinda Gates Foundation to lead the research and development of a new HPV-DNA screening test that was rapid, simple, and affordable. On the basis of published evidence and the potential adaptability of the hybrid capture technology, Digene Corporation (now QIAGEN) and PATH entered into a collaborative agreement to design and develop a new HPV-DNA test expressly designed for low-resource settings.[8] The new test, designated *care*HPV, is a signal-amplification assay that detects target HPV-DNA from 14 different carcinogenic HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). The assay needs only a small footprint of bench-top work space (about 25×50 cm), no mains electricity or running water, and can be done by technical support staff in roughly 2·5 h. The short assay time would allow testing and clinical follow-up in the same day. The price for *care*HPV will be negotiated to be feasible for each eligible country or organisation. Here, we report the first clinical validation study of *care*HPV in unscreened 30–54-year old women in a rural area of China, in collaboration with the Cancer Institute, Chinese Academy of Medical Sciences (CICAMS, Beijing, China).

Methods

Patients and procedures

We enrolled women, aged 30 to 54 years, living in rural villages in Shanxi Province, China, and screened them for cervical cancer from May 10 to June 15, 2007. Two communes were selected from each of the Wuxiang and Xiangyuan counties by use of a simple randomised cluster sampling method. All eligible women from the four selected communes were enumerated and invited to participate. Non-pregnant women with no history of CIN, pelvic radiation, or hysterectomy and who were able to provide informed consent were eligible for enrolment (figure 1). The study was approved by the Institutional Review Board of CICAMS and the Human Subjects Protection Committee of PATH.

Screening was done at the Women and Children's Hospital in each of the two counties. The sequence of events from recruitment to study exit is shown in figure 1. On the screening day, women were transported to and from the screening site by bus. Each woman was asked for sociodemographic, reproductive, and behavioural data by trained health workers in confidential settings after informed written consent had been obtained and before clinical examination. A vaginal-brush specimen (Cervical Sampler, QIAGEN) for the *care*HPV test and two nylon-swab specimens (for storage) were self-collected by each woman. The women were instructed to grasp the vaginal brush or swabs at midshaft, to insert them to a depth of about 6 cm or until resistance was met, and then rotate the device two times before withdrawal. Provider-obtained cervical specimens were also collected; a nurse midwife inserted a vaginal speculum using water as a lubricant and collected in order, two nylon-swab specimens from the ectocervix (for storage), a cervical brush (Cervical Sampler) specimen for the *care*HPV test, and a cervical specimen for storage in medium for liquid-based cytology (SurePath, Becton Dickinson, Franklin Lakes, NJ, USA) and HC2 testing. All women then had VIA done by a nurse midwife followed by digital colposcopy (Goldway, Shenzhen, China) by a gynaecologist who was blinded to the findings of the VIA, with directed biopsy and endocervical curettage as necessary. The vaginal and cervical-brush specimens were stored in a collection medium specific for testing by the new test method (new-test storage medium, QIAGEN). The *care*HPV test was done onsite within 3 h by the usual laboratory workers in the Women and Children's Hospital while women waited for their results.

Testing by HC2 was done within 2 weeks as previously described[9] on the residual storage medium after processing for liquid-based cytology in the CICAMS central lab in Beijing, China.

Women who were negative on colposcopy, but had abnormal liquid-based cytology (ie, showing atypical squamous cells—cannot exclude HSIL [ASC-H], low-grade squamous intraepithelial lesions [LSIL], high-grade squamous intraepithelial lesions [HSIL], or cancer) or unsatisfactory cytology according to the Bethesda System, positive HC2 findings, or positive findings on the *care*HPV test were recalled for a second colposcopy and four-quadrant cervical biopsies at the squamo-columnar junction and endocervical curettage. Biopsies were done with a bronchoscopy biopsy instrument that has 2-mm jaws as previously described.[9,10] Patients needing follow-up or treatment for cervical neoplasia were offered care free of charge.

*Care*HPV and HC2

The *care*HPV test is broadly based on the HC2 test with some important differences. The assay time is 2·5 h or less, compared with up to 6 h for HC2. The *care*HPV collection medium, unlike other collection media, contains no toxic chaotropic salts, but rather contains non-toxic surfactants and is specifically formulated for solubilisation of cervical specimens from the collection brush without any requirement for extended mechanical shaking. The capture microplates in HC2 are replaced by magnetic beads coated by a monoclonal antibody with high affinity to RNA-DNA hybrids. Furthermore, the temperatures of some steps in the *care*HPV assay are increased to decrease the overall assay time by more than 2 h. The principle of the assay is as follows: target HPV DNA from lysed cells is denatured and hybridised to full-length complementary RNA, then captured by monoclonal antibodies coated on paramagnetic beads. The captured hybrids on the beads are detected by antihybrid monoclonal antibody conjugated to calf intestine alkaline phosphatase, which reacts with an added chemiluminescent substrate to produce light in proportion to the number of bound alkaline phosphatase molecules along the hundreds of antigenic binding sites per target molecule. Specimen test findings are expressed in relative light units (RLU) and compared with the mean RLU from a minimum positive control set at 1 pg/mL of HPV-16 DNA (expressed numerically as the cutoff) resulting in a ratio, the RLU/cut-off, the proportion of which is indicative of clinical positivity. Because the output signals of both HC2 and the new *care*HPV test are quite linear over a broad range around the cut-off-point ratio (RLU/cutoff) of 1·0, there is the possibility to vary the cut-point by adjusting the calculations to indicate specimen positives at lower or higher values than the value of positive controls, thus a cut-point of 0·5 reflects an assay that can score 0·5 pg/mL of HPV-16 DNA as positive.

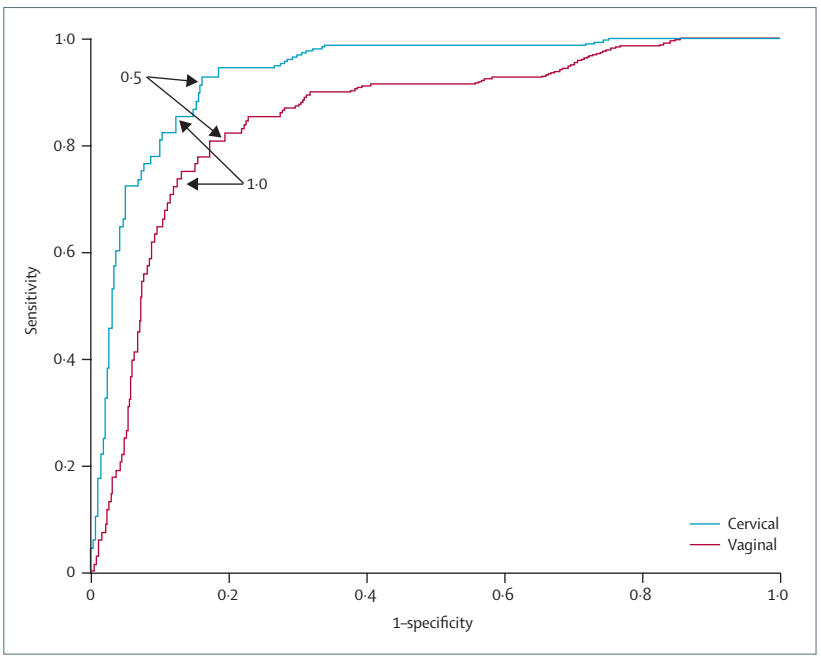

Figure 2: **ROC curves of cervical and vaginal brush specimens using *care*HPV to detect women with CIN2+**
The curve shows the various combinations of sensitivity and 1–specificity over a range of RLU/cut-off ratios. Findings for the 0·5 and 1·0 RLU/cut-off ratios are labelled.

Pathology

The CIN and the Bethesda classification systems were used for histology and cytology, respectively, and pathology processing and reading were done at CICAMS. Liquid-based cytology was deemed positive if ASC-H, LSIL, HSIL or cancer were present (ASC-H+) and histology was assessed as positive if CIN or cancer were present. Two gynaecological pathologists in CICAMS independently read every specimen and a consensus diagnosis was based on the majority assessment, with a third gynaecological pathologist as a tie-breaker. All specimens that were positive by consensus in China and a 10% random sample of negatives were selected to be assessed by an external pathologist in Canada, without any knowledge of the Chinese diagnoses. The final diagnosis was based on the reading of the Canadian pathologist with ample opportunity for discussion of any discordants with the Chinese pathologists. The final diagnosis for each woman was based on the highest reading across all histology findings, including directed and four-quadrant biopsies and endocervical curettage. If a biopsy had not been indicated or the histology finding was negative for a woman, she was assessed as negative for cervical neoplasia.

Statistical analysis

Analyses were mainly focused on the ability of screening tests to detect moderate or severe CIN or cancer (CIN2+). Comparisons that were considered relevant to decisions on the future use of the *care*HPV test in low-resource settings related to HC2 and VIA. Sample size was based

empirically on an estimated prevalence of high-grade disease CIN2+ of 4% in the population in order to yield 100 events of confirmed high-grade disease.[9,10] Receiver operating characteristic (ROC) analysis was used to assess possible cut-points of the RLU/cutoff ratio to define positive findings of the new test for both cervical and vaginal specimens. To choose an optimum RLU/cut-off ratio for *care*HPV using cervical and vaginal specimens, Youden's index [Y=sensitivity–(1–specificity)] was calculated.[11] The accuracies of the four screening tests of primary interest (the new test using cervical and vaginal specimens, HC2 using cervical specimens, and VIA) were compared by use of the z value, where z is defined as:

$$z=\frac{A_1-A_2}{\sqrt{SE^2_1+SE^2_2-2rSE_1SE_2}}$$

(where A_1 refers to the observed area and SE_1 refers to the estimated standard error of the ROC area associated with diagnostic method 1; A_2 and SE_2 refer to corresponding quantities for diagnostic method 2; r represents the estimated correlation between A_1 and A_2; and the quantity z is referred to the normal distribution).[12] We did not adjust for an absence of independence between the tests. Tests were assumed to be independent. Six pairwise comparisons of test accuracies resulted and a two-sided alpha error level of 0·05 was adjusted to 0·0083 on basis of the Bonferroni correction for multiple comparisons. Otherwise, all p values less than 0·05 (two-sided) were considered statistically significant. Analyses were done in SPSS for Windows (version 13.0, SPSS Inc, Chicago, IL, USA).

Role of the funding source

The funding source had no role in the design of the study, collection, analysis, or interpretation of data, or in the writing of the report. Y-lQ, JWS, PSE, Y-pB, JML, F-hZ, and RBP had access to the raw data. The corresponding author (JWS) had full access to all of

	Sensitivity (95% CI), %	Specificity (95% CI), %	PPV (95% CI), %	NPV (95% CI), %	Youden`s index, %	Area under ROC curve (95% CI)
*care*HPV						
Cervical specimens						
0·5 RLU/cut-off ratio cut-point	90·0 (83·0–97·0)	84·2 (82·7–85·7)	14·7 (11·3–18·0)	99·6 (99·4–99·9)	74·2 (72·4–76·0)	0·93 (0·91–0·96)
1·0 RLU/cut-off ratio cut-point	84·3 (75·8–92·8)	87·5 (86·1–88·8)	16·9 (13·0–20·8)	99·5 (99·1–99·8)	71·8 (70·0–73·6)	0·93 (0·91–0·96)
Vaginal specimens						
0·5 RLU/cut-off ratio cut-point	81·4 (72·3–90·5)	82·4 (80·8–83·9)	12·2 (9·3–15·2)	99·3 (99·0–99·7)	63·8 (61·9–65·7)	0·86 (0·81–0·90)
1·0 RLU/cut-off ratio cut-point	72·9 (62·4–83·3)	87·7 (86·3–89·0)	15·1 (11·3–19·0)	99·1 (98·7–99·5)	60·6 (58·6–62·6)	0·86 (0·81–0·90)
HC2 (cervical specimens)	97·1 (93·2–100·0)	85·6 (84·2–87·1)	17·0 (13·3–20·6)	99·9 (99·8–100·0)	82·7 (81·2–84·2)	0·96 (0·94–0·97)
Liquid-based cytology (ASC-H+)	85·3 (76·9–93·7)	97·0 (96·3–97·7)	45·7 (37·0–54·3)	99·5 (99·3–99·8)	82·3 (80·8–83·8)	0·95 (0·92–0·99)
VIA	41·4 (29·9–53·0)	94·5 (93·6–95·4)	18·6 (12·5–24·7)	98·2 (97·6–98·7)	35·9 (34·0–37·8)	0·68 (0·60–0·75)

ROC=receiver operating characteristic. HPV=human papillomavirus. RLU=relative light unit. HC2=Digene High-Risk HPV HC2 DNA Test. ASC-H+=atypical squamous cells—cannot exclude high-grade squamous intraepithelial lesions. VIA=visual inspection with acetic acid.

Table 1: **Sensitivity, specificity, positive-predictive value (PPV), negative-predictive value (NPV), and area under the curve for the various methods in 2388 eligible women who were all subsequently examined by colposcopy, based on the detection of CIN2+ on histology (n=70)**

	Sensitivity (95% CI), %	Specificity (95% CI) %	PPV (95% CI), %	NPV (95% CI), %	Youden`s index, %	Area under ROC curve (95% CI)
*care*HPV						
Cervical specimens						
0·5 RLU/cut-off ratio cut-point	87·0 (73·2–100·0)	82·7 (81·1–84·2)	4·7 (2·7–6·6)	99·8 (99·7–100·0)	69·7 (67·9–71·5)	0·92 (0·85–0·98)
1·0 RLU/cut-off ratio cut-point	87·0 (73·2–100·0)	86·1 (84·7–87·5)	5·7 (3·3–8·2)	99·9 (99·7–100·0)	73·1 (71·3–74·9)	0·92 (0·85–0·98)
Vaginal specimens						
0·5 RLU/cut-off ratio cut-point	82·6 (67·1–98·1)	81·1 (79·5–82·7)	4·1 (2·3–5·9)	99·8 (99·6–100·0)	63·7 (61·8–65·6)	0·84 (0·75–0·93)
1·0 RLU/cut-off ratio cut-point	78·3 (61·4–95·1)	86·5 (85·1–87·9)	5·3 (2·9–7·7)	99·8 (99·5–100·0)	64·8 (62·9–66·7)	0·84 (0·75–0·93)
HC2 (cervical specimens)	95·7 (87·3–100·0)	84·0 (82·5–85·5)	5·5 (3·3–7·7)	99·9 (99·9–100·0)	79·7 (78·1–81·3)	0·94 (0·89–0·99)
Liquid-based cytology (ASC-H+)	87·0 (73·2–100·0)	95·4 (94·5–96·2)	15·7 (9·4–22·1)	99·9 (99·7–100·0)	82·4 (80·9–83·9)	0·94 (0·87–1·00)
VIA	56·5 (36·3–76·8)	94·0 (93·0–94·9)	8·3 (4·0–12·7)	99·6 (99·3–99·8)	50·5 (48·5–52·5)	0·76 (0·63–0·89)

ROC=receiver operating characteristic. HPV=human papillomavirus. RLU=relative light unit. HC2=Digene High-Risk HPV HC2 DNA Test. ASC-H+=atypical squamous cells—cannot exclude high-grade squamous intraepithelial lesions. VIA=visual inspection with acetic acid.

Table 2: **Sensitivity, specificity, positive-predictive value (PPV), negative-predictive value (NPV), and area under the curve for the various methods in 2388 eligible women who were all subsequently examined by colposcopy, based on the detection of CIN3+ on histology (n=23)**

the data and the final responsibility to submit for publication.

Results

Of 3721 eligible women invited to participate, 2530 (68·0%) came for screening. Reasons for non-participation were given by 1055 of 1191 women (88·6%) and included feeling well (287 of 1191 [24·1%]), belief that screening was of no benefit (228 of 1191 [19·1%]), worry about the consequences of a diagnosis of cervical cancer (205 of 1191 [17·2%]), worry about discomfort during the colposcopy examination (197 of 1191 [16·5%]), and belief that cancer was incurable (138 of 1191 [11·6%]). Failure to return for callback examination caused 142 women to be excluded from analysis: one had negative colposcopy and positive HC2 and HSIL cytology, two had negative colposcopy, positive HC2, and negative cytology, and 139 had negative colposcopy, negative HC2, and unsatisfactory cytology. Complete data with adherence to the study protocol were available for 2388 (94·4%) of those screened and our analyses are based on these women (figure 1). Mean age was 43·4 years (SD 6·2; range 30–55) and the mean age of first sexual intercourse was 20·5 years (2·4; 15–33). 0·25% of women (6 of 2388) were currently using hormonal contraceptives, 81·2% (1938 of 2388) had been sterilised, and 16·9% (403 of 2388) were menopausal. The mean number of livebirths was 2·7 (1·1; 0–14). All women were currently married and 98·7% (2356 of 2388) had never smoked. On the basis of colposcopy and histology, 2258 of 2388 (94·6%) were assessed as healthy, 60 of 2388 (2·5%) had CIN1, 47 of 2388 (2·0%) had CIN2, 22 of 2388 (0·9%) had CIN3, and one of 2388 (0·04%) had invasive cervical cancer. Therefore, 70 of 2388 women (2·9%) had CIN2+. Up to now, 68·6% (48 of 70) of these women have been treated and the remainder have refused offers of treatment free of charge, typically saying that they feel well. For categorising histology as negative, CIN1, CIN2, CIN3, or cancer, agreement between the two Chinese pathologists was excellent (kappa coefficient 0·85) and only 10·6% of the specimens needed to be assessed by a third pathologist. Similarly, the agreement between the China consensus diagnosis and the international pathologist was excellent with kappa coefficient of 0·86.

The ROC curves for *care*HPV using cervical and vaginal-brush specimens are shown in figure 2. On the basis of Youden's index and the ability to detect CIN2+, 0·5 was selected as the RLU/cut-off ratio representing a positive cut-point for the *care*HPV test using cervical and vaginal specimens. The sensitivity, specificity, positive-predictive value, negative-predictive value, and area under the ROC curve for *care*HPV, HC2, liquid-based cytology (using the presence of ASC-H, LSIL, HSIL or cancer as the cut-point), and VIA are shown in table 1 for the detection of CIN2+ and in table 2 for the detection of CIN3+. When detection of CIN3+ was the primary goal, a higher RLU/cut-off ratio cut-point of 1·0 seemed to have a slight advantage over 0·5. Table 3 shows the comparison (z score and p value) of area under ROC curve of *care*HPV using cervical and vaginal specimens, HC2 using cervical specimens, and VIA. On the basis of the two-sided alpha error level of 0·05 and the Bonferroni correction for multiple comparisons, a p<0·0083 is considered significant when comparing the six test pairs. There was no significant difference in the area under the curve by use of the *care*HPV test when cervical and vaginal specimens were compared (p=0·060), but *care*HPV using either cervical or vaginal specimens was significantly better than VIA (p<0·0001 and p=0·003, respectively). When findings for cervical specimens were compared, the area under the curve was not statistically different between HC2 and the *care*HPV test (p=0·016).

	*care*HPV (cervical specimens)	*care*HPV (vaginal specimens)	VIA
HC2 (cervical specimens)	z=2·40 (p=0·0163)	z=3·57 (p=0·0004)*	z=5·73 (p<0·0001)*
*care*HPV (cervical specimens)	..	z=1·88 (p=0·0596)	z=4·48 (p=0·0001)*
*care*HPV (vaginal specimens)	..	..	z=2·96 (p=0·0031)*

HPV=human papillomavirus. VIA=visual inspection with acetic acid. *Statistically significant differences (p<0·0083 after Bonferroni correction for multiple comparisons).

Table 3: **Comparison (z value and p value) of area under receiver-operating-characteristic curve of *care*HPV using cervical and vaginal specimens, HC2 using cervical specimens, and VIA**

	Patients, N	HC2 positive, n	*care*HPV (positive vaginal specimen), n	*care*HPV (positive cervical specimen), n	Any positive HPV-DNA test, n	Any negative HPV-DNA test, n
Negative						
<ASC-H	295	192	202	186	294	1
ASC-H+	42	39	25	35	39	3
Unsatisfactory	20	12	15	16	20	0
CIN1						
<ASC-H	25	23	22	21	25	0
ASC-H+	17	16	13	16	17	0
Unsatisfactory	2	2	2	2	2	0
CIN2						
<ASC-H	6	6	5	6	6	0
ASC-H+	24	24	19	24	24	0
CIN3						
<ASC-H	2	1	1	1	2	0
ASC-H+	8	8	6	7	8	0

HC2=Digene High-Risk HPV HC2 DNA Test. HPV=human papillomavirus. ASC-H+=atypical squamous cells—cannot exclude high-grade squamous intraepithelial lesions. CIN=cervical intraepithelial neoplasia.

Table 4: **Findings for all 441 women who were negative on initial colposcopy and were called back for a second colposcopy, including four-quadrant cervical biopsies and endocervical curettage, because of unsatisfactory or ASC-H+ cytology, positive *care*HPV (RLU/cut-off ratio cut-point of 0·5), or positive HC2 findings**

	Patients, N	HC2 positive, n	*care*HPV (positive vaginal specimen), n	*care*HPV (positive cervical specimen), n	Any positive HPV-DNA test, n	Any negative HPV-DNA test, n
Negative						
<ASC-H	2168	226	318	260	466	1702
ASC-H+	46	43	29	39	43	3
Unsatisfactory	44	13	17	20	25	19
CIN1						
<ASC-H	33	27	26	25	29	4
ASC-H+	23	22	17	21	23	0
Unsatisfactory	4	2	2	2	2	2
CIN2						
<ASC-H	7	7	6	7	7	0
ASC-H+	38	37	30	34	37	1
Unsatisfactory	2	2	2	2	2	0
CIN3+						
<ASC-H	3	2	2	2	3	0
ASC-H+	20	20	17	18	20	0

HC2=Digene High-Risk HPV HC2 DNA Test. HPV=human papillomavirus. ASC-H+=atypical squamous cells—cannot exclude high-grade squamous intraepithelial lesions. CIN=cervical intraepithelial neoplasia. The RLU/cut-off ratio of 0·5 is used as the positive cut-point for the *care*HPV test.

Table 5: **Distribution of findings in all 2388 women for whom complete data were available**

The findings for the 441 women who had negative colposcopy, but abnormal liquid-based cytology (ASC-H, LSIL, HSIL or cancer) or an unsatisfactory specimen, positive HC2 findings, or positive findings on *care*HPV are shown in table 4. These women were recalled for a second colposcopy and four-quadrant cervical biopsies and endocervical curettage. In the 441 women, 40 (9·1%) were shown to have CIN2+ by four-quadrant cervical biopsies; eight of these 40 women had a positive HPV-DNA test only and 32 of 40 women had both a positive HPV-DNA test and positive cytology. The findings for all the tests versus histology for the 2388 women are shown in table 5.

Discussion

Our findings clearly show the accuracy of the *care*HPV test for the detection of high-grade cervical neoplasia is substantially better than for VIA. The lower-than-expected number of events of CIN2+ and the consequent decrease in statistical power might account for the absence of any significant difference in the accuracy of the new *care*HPV test and the accuracy of HC2 on cervical specimens—the technology commonly used for HPV-DNA testing in high-resource countries. Future studies using larger sample sizes to increase power and to assess the effect of specimen order on the detection of low-copy HPV infections would be useful to explore the differences between *care*HPV and HC2. The ability of *care*HPV to use vaginal as well as cervical specimens has potentially important implications for the feasibility, flexibility, and acceptability of the test by women and providers for achieving the highest effect possible by optimising coverage of the populations at risk. The lower sensitivity that we noted with vaginal sampling, presumably due to the inability of the sampling device to reach the appropriate area of the cervix in some women, is consistent with findings in other studies.[5,6,9,10]

The *care*HPV test was designed specifically for application in low-resource public-health settings to screen women 30 years of age and older. Simplification of the test procedure compared with the HC2 assay has resulted in a faster running time, a wider scope for the people who can do it, and where they can do it. Accordingly, in this study *care*HPV was done by inexperienced, newly trained, minimally educated technicians on-site under suboptimum conditions of temperature, humidity, lighting, and space; whereas HC2 was done by experienced professionals in experienced laboratories under optimum conditions. *care*HPV has a simplified liquidtransfer process by use of dropper bottles and the reagents are desiccated and stored at suboptimum temperatures. This contrasts with the calibrated pipettors and refrigerated storage of the liquid reagents necessary for HC2. From the perspective of cost-effectiveness, the potential to undertake a *care*HPV test while women wait for results, and to provide further management as necessary, is crucial to successful programmes in low-resource settings where accessibility of services is restricted. Goldie and colleagues[13] have shown, in general, a greater cost-effectiveness of screening and treatment strategies that can be done in one visit, and this should be possible with *care*HPV.[13]

A lower clinical sensitivity and specificity with vaginal sampling compared with cervical sampling for HPV-DNA testing has been shown previously.[9,14] Our findings for VIA are within the range of that reported by others, but compared with a previous screening study in this location, the low sensitivity and high specificity of VIA in our study suggests that providers are using criteria that are too narrow and restrictive for defining a positive VIA assessment.[10]

This is the first report of clinical outcomes for the use of the *care*HPV test as a primary screening test. The study design and conduct were methodologically rigorous and, to the extent possible, adhered to current recommendations on how to assess a new screening test.[15] To increase the external validity of the findings, recruitment was population-based and included women who represent the test's target group. The screening test observers were kept blind to the findings of the other screening tests to ensure independence in the interpretation of each test. To avoid verification bias, each participant had a colposcopy assessment with directed biopsy and endocervical curettage as required. Those who were negative on colposcopy, but positive on *care*HPV testing, liquid-based cytology, or HC2 were recalled for four-quadrant cervical biopsies and

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endocervical curettage to maximise ascertainment of disease. Pathology was assessed by independent observers to keep bias in the categorisation of specimens to a minimum and to decrease misclassification. Although it was important within this study to do the *care*HPV test onsite while women waited, it was not possible to estimate the reproducibility of the new test. To choose an RLU/cut-off ratio as the positive cut-point for the new test in an arbitrary fashion, Youden's index was used. Ideally, the choice of an optimum positive cut-point would be based on evidence from cost-effectiveness data that serve as regional examples for other countries.[13] The use of area-under-the-curve analysis and the z value allowed us to compare the combined effects of sensitivity or specificity for the different types of tests. This strategy, although efficient, has the disadvantage that it weights sensitivity and specificity equally, treating false-positives and false-negatives the same. In the future, it might be necessary to rebalance these two key measures in the context of population-specific HPV and disease prevalence, test positive-predictive value, and affordable costs.

In this study, the accuracy of liquid-based cytology was similar to HC2 testing when both were done in the CICAMS laboratory in Beijing, China, but it would not be feasible to base a rural screening programme in China on the basis of either test. Although cytology-based screening programmes, supported by HPV-DNA testing playing either an adjunctive or triage role, work well in adequately-resourced settings, the infrastructure and trained personnel needed for cytology are not usually feasible for low-resource regions.[16] IARC has done several systematic reviews and is now recommending that HPV-DNA testing can be used for primary screening, as an alternative to cytology.[2] An advantage of HPV-DNA testing is the negative-predictive value that can safely extend the interval for rescreening of negatives to 5 years or more.[17] The findings of a randomised trial of cryotherapy based on VIA versus HPV-DNA testing in South Africa, has prompted the Alliance for Cervical Cancer Prevention to recommend that primary screening in low-resource settings should be based on either VIA or HPV-DNA testing.[18,19] A rapid HPV-DNA test, such as *care*HPV, allows treatment during the same visit as screening, just as VIA does. Although the positive-predictive value of *care*HPV was decreased if a lower (more sensitive) RLU/cut-off ratio cut-point was used to detect women with CIN2+ rather than CIN3+, optimisation of test sensitivity commonly takes precedence over specificity in low-resource settings.[13,19] In such settings, most women have never been screened and the goal of public-health programmes is to attempt to screen older women once in a lifetime with the best test possible. A CIN2+ cut-point is sensible for most policy makers and clinicians in low-resource settings, because this captures women with CIN2 lesions, some of which would progress if untreated, and the usual treatment (cryotherapy) would be safe even if women with minor lesions, such as CIN1, are detected.[13,19]

Overall, *care*HPV seems to have performance characteristics that merit further study and, subject to local cost-effectiveness assessments, might be appropriate for use in resource-constrained screening programmes.

Contributors

Y-lQ and JWS had full access to all the data in the study and had responsibility for the integrity of the data and accuracy of the data analysis. Y-lQ, JML, RBP, and JWS organised the study concept and design. W-hZ, Q-jP, LL, and FC acquired the data. YLQ and JWS analysed and interpreted the data. Y-lQ and JWS drafted the manuscript. RBP, JML, ATL, PSE, BW, and JWS revised the manuscript. Y-pB and F-hZ did statistical analyses. ATL and PSE designed and developed the test.

Conflicts of interest

PSE is an employee of QIAGEN (formerly Digene Corporation) and PSE and ATL own stock in QIAGEN. All other authors declared no conflicts of interest.

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