Qiao, YL; Jeronimo, J; Zhao, FH; Schweizer, J; Chen, W; Valdez, M; Lu, P; Zhang, X; Kang, LN; Bansil, P; +9 more... Paul, P; Mahoney, C; Berard-Bergery, M; Bai, P; Peck, R; Li, J; Chen, F; Stoler, MH; Castle, PE; (2013) Lower cost strategies for triage of human papillomavirus DNA-positive women. International journal of cancer Journal international du cancer, 134 (12). pp. 2891-901. ISSN 0020-7136 DOI: https://doi.org/10.1002/ijc.28616

Downloaded from: http://researchonline.lshtm.ac.uk/4649193/

DOI: https://doi.org/10.1002/ijc.28616

Usage Guidelines:

Please refer to usage guidelines at https://researchonline.lshtm.ac.uk/policies.html or alternatively contact researchonline@lshtm.ac.uk.

Available under license: http://creativecommons.org/licenses/by-nc-nd/2.5/
Lower cost strategies for triage of human papillomavirus DNA-positive women

You-Lin Qiao1, Jose Jeronimo2, Fang-Hui Zhao1, Johannes Schweizer3, Wen Chen1, Melissa Valdez2, Peter Lu3, Xun Zhang1, Le-Ni Kang1, Pooja Bansil2, Proma Paul2, Charles Mahoney3, Marthe Berard-Bergery3, Ping Bai1, Roger Peck3, Jing Li1, Feng Chen1, Mark H. Stoler4 and Philip E. Castle5

1 Cancer Institute and Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, People’s Republic of China
2 Arbor Vita Corporation, 6611 Dumbarton Circle, Fremont, CA
3 Department of Pathology, University of Virginia, Charlottesville, VA
4 Global Cancer Initiative, 100 Radcliff Drive, Chestertown, MD
5 Arbor Vita Corporation, 6611 Dumbarton Circle, Fremont, CA

Using human papillomavirus (HPV) testing for cervical cancer screening in lower-resource settings (LRS) will result in a significant number of screen-positive women. This analysis compares different triage strategies for detecting cervical precancer and cancer among HPV-positive women in LRS. This was a population-based study of women aged 25–65 years living in China (n = 7,541). Each woman provided a self-collected and two clinician-collected specimens. The self-collected and one clinician-collected specimen were tested by two HPV DNA tests—careHPV™ and Hybrid Capture 2; the other clinician-collected specimen was tested for HPV16/18/45 E6 protein. CareHPV™-positive specimens were tested for HPV16/18/45 DNA. HPV DNA-positive women underwent visual inspection with acetic acid (VIA) and then colposcopic evaluation with biopsies. The performance for detection of cervical intraepithelial neoplasia grade 3 or cancer (CIN3+) among HPV DNA-positive women was assessed for different triage strategies: HPV16/18/45 E6 or DNA detection, VIA, colposcopic impression, or higher signal strength (≥10 relative light units/positive control [rlu/pc]). The percent triage positive ranges were 14.8–17.4% for VIA, 17.8–20.9% for an abnormal colposcopic impression; 7.9–10.5% for HPV16/18/45 E6; 23.4–28.4% for HPV16/18/45 DNA; and 48.0–62.6% for higher signal strength (≥10 rlu/pc), depending on the HPV test/specimen combination. The positivity for all triage tests increased with severity of diagnosis. HPV16/18/45 DNA detection was approximately 70% sensitive and had positive predictive values (PPV) of approximately 25% for CIN3+. HPV16/18/45 E6 detection was approximately 50% sensitive with a PPV of nearly 50% for CIN3+. Different triage strategies for HPV DNA-positive women provide important tradeoffs in colposcopy or treatment referral percentages and sensitivity for prevalent CIN3+.

Cervical cancer incidence and mortality have declined significantly in those places that have effectively implemented Papanicolaou (Pap) test-based screening.1 Yet cervical cancer remains the second most common female cancer and third most common cause of female cancer-related mortality globally, with an annual incidence of approximately 530,000 and mortality of 275,000, respectively.2 This seeming contradiction is explained by the fact that cervical cancer incidence and mortality are approximately 10-fold greater in low- and middle-income countries (LMIC), where Pap programs have failed to be established because of the technical and financial barriers to implementation.1,3

Because of these limitations, alternative screening strategies have been developed and evaluated, including molecular testing for the necessary cause of cervical cancer, carcinogenic human papillomavirus (HPV). DNA testing for HPV has been shown to be more sensitive4–9 and more reliable10–12 than Pap testing. A key attribute of HPV testing related to its high sensitivity is its excellent negative predictive value, providing near complete reassurance following a negative test that the woman does not have cancer or precancer.13–15 Thus, a negative HPV DNA test does an excellent job of
screening by ruling out disease in the primarily healthy population, permitting fewer screens of the general population in lifetime. Affordable tests like careHPV™ (careHPV™; QIAGEN, Gaithersburg, MD) and “tiered pricing” of higher cost tests will make HPV testing increasingly more available to LMIC. However, the challenge of using HPV testing, or any screening test, is the management of screen-positive women, as most women with a positive screening test (≈80% to 90%) will not have concurrent disease (i.e., cervical precancer or cancer). This is an especially perplexing problem in LMIC, where there are limited numbers of clinics, colposcopists, pathologists and clinicians qualified to provide diagnosis and treatment, and services must be prioritized for women at highest risk for harboring precancer or cancer. In China, findings showed a higher prevalence of HR-HPV infection and CIN2+, which suggests that the burden of cervical cancer in China especially in some rural areas is more substantial than was previously reported with a much higher need for comprehensive screening and will result in many more HPV positive women to manage. Moreover, in the context of a screen-and-treat program, where treatment is provided without colposcopy or biopsy, it may be desirable to immediately treat only those at a higher risk of cervical precancer and cancer among the screen-positives to minimize overtreatment.

In 2010, we launched a clinical study in 7,500 women living in rural China as part of the Screening Technologies to Advance Rapid Testing for Cervical Cancer Prevention—Utility and Program Planning (START-UP) Project. The stated goals of this study were to evaluate new strategies for screening and management of screen positives that might be employed in LMIC. Here, we report on our evaluation of different management or “triage” strategies for HPV DNA-positive women. We explored both visual and molecular methods to distinguish between HPV DNA-positive women at high and low risk of cervical precancer and cancer. Further management of the latter might be deferred until there is evidence of increased risk (e.g., HPV persistence), thereby increasing the “predictive value” of the intervention and decreasing the use of more invasive procedures and resources in lower-risk women.

Material and Methods

Population

The population for this study was recruited as follows: First, we selected two high-risk communes from each county (Yangcheng, Xinmi and Tonggu) according to the proposed sample size. Second, the number of women aged 25–65 years in each commune was collected from the local residence registry of the police office. Third, we determined the candidate villages for the study considering the size of the village and the transportation conditions. Fourth, all the women aged 25–65 years and living in the chosen villages who had not undergone screening in the last five years were invited to participate in the study if they met the study criteria. The recruitment was stopped when the target sample size was reached. We noted a challenge in the recruitment of the oldest and youngest women as older women were less willing to undergo screening and many of the younger women were transient and could not be located. Thus, our study population was biased toward women who were 35–50 years old.

Women aged 25–65 years were considered eligible if they (i) had a cervix, (ii) had not been previously diagnosed with cervical cancer, (iii) were not pregnant, (iv) were physically able to undergo routine cervical cancer screening and (v) were able to provide informed consent. We did not exclude women if they had previous cervical cancer screening because we assumed that even if a few women had been screened for cervical cancer, the quality of cytology screening was likely to be very poor. Women were excluded if they were not married and reported never having had sexual intercourse. Local doctors conducted the initial recruitment and eligibility screening. Eligibility was confirmed at the study clinic. Eligible women were then educated about the study and asked to complete the written informed consent in order to participate in the study.

Enrollment visit

Participants were given an education session about cervical cancer prior to the start of the study procedures. First, women were asked to complete a short risk-factor survey administered by study personnel. Then, women were given instructions on how to self-collect a vaginal sample and were provided a private room to self-collect their vaginal specimen. Next, women underwent a routine pelvic exam at which time two cervical specimens were collected, the first into a dry tube for OncoE6™ Cervical Test (Arbor Vita Corporation) testing and the second into dcm buffer (QIAGEN) for HPV DNA testing. Then visual inspection with acetic acid (VIA) was done and results were recorded.
Clinical management

Women who tested positive for any of the six screening tests performed (VIA, HPV E6 and HC2 and careHPVTM on clinician-collected and self-collected specimens) were referred to colposcopy and approximately 10% random sample of women who tested negative for all screening tests (screen-negative women) underwent a second VIA and a rigorous colposcopic evaluation that included using a microbiopsy protocol as previously described. As dictated by the IRBs, women who had no visible lesions had their screening result revealed and if there were still no visible lesions, no biopsies were taken.

Laboratory tests

CareHPVTM was done as previously described at the clinical sites by a laboratory technician who had a general level of training comparable to the local hospital staff and who was trained to run careHPVTM by a senior CICAMS technician. A research-use only pooled probe set targeting HPV16, 18 and 45 was developed for the study and ran on the same careHPVTM platform with the same protocol on all careHPVTM-positive specimens. The HPV DNA 16/18/45 test was run periodically when there were sufficient numbers to nearly or completely fill a batch of 90. A signal strength of 1.0 relative light units per positive control (rlu/pc) or greater was considered positive for both tests.

HC2 was performed per the manufacturer’s instruction, except that 50 μL of the dcm specimen was combined with 25 μL kit denaturation reagent rather than combining 1,000 μL of the STM specimen with 500 μL kit denaturation reagent. Because HC2 cannot be set up at local clinical sites, training comparable to the local hospital staff and who had a general level of training was supervised by CICAMS staff. Briefly, a cervical specimen collected using a polyester swab was stored in a tube without buffer until tested. The swab specimen was treated in a two-step process, first with 933 μL of lysis solution and next with 87 μL of conditioning solution, both with 15-minute incubation under gentle agitation. Next, the specimen solution was clarified from insoluble components by centrifugation in a table-top microcentrifuge for 10 minutes at >10,000 rpm. A 200-μL aliquot of the sample solution was then transferred into a vial with lyophilized detector mAb; the test unit was next inserted into the detector mAb vials, and the specimen solutions ran up the test strips by capillary action. After 55 minutes, the test was transferred into vials with wash solution, and after a 12-minute washing the test unit was immersed into another set of vials containing developing solution. After 15–25 minutes (depending on the ambient temperature), the test unit was removed from the developing solution vials and placed onto a reading guide, allowing for visual inspection. Appearance of one or more test lines indicated E6 oncoprotein of the corresponding HPV type present in the initial cervical swab specimen.

Pathology

A CICAMS pathologist (Professor Xun Zhang) provided the primary diagnosis of biopsies and surgical specimens, and the worst of the two was used for the final diagnosis in these analyses. All initial biopsy diagnoses of CIN2+ and a random sample of <CIN2 were independently reviewed by an expert US pathologist (MHS) to confirm the results. There was no qualitative difference in the results of this analysis using either set of diagnoses (data not shown).

Analyses

We evaluated five different strategies to triage an HPV-positive test by any of the four combinations of HPV tests (careHPVTM or HC2) and specimen collection (clinician or self): (i) colposcopic impression of low-grade disease or worse, (ii) the second VIA conducted among the screen-positive population referred to colposcopy, (iii) using a higher cutoff of 10.0 rlu/pc for the HPV DNA screening test, (iv) the E6 test for HPV16/18/45 and (v) the HC2 DNA test for HPV16/18/45 (among careHPVTM positives only). We selected a cutoff for colposcopy based on receiver-operator curve analysis for the detection of CIN2+ shown in Supporting Information Figure 1; using metaplasia or worse added little sensitivity and decreased specificity significantly while using a cutoff of high-grade or worse was too insensitive. We selected a DNA triage cutoff of 10.0 rlu/pc based on reports that the signal strength near the 1.0 rlu/pc positive cutoff were less predictive of CIN2+ and CIN3+, and we wanted to evaluate HPV DNA test performance by reducing the referral by approximately 50%.

We calculated the percent positive of each triage test among any HPV-positive test result overall, by age groups (<30, 30–39, 40–49 and 50 years and older) and by severity
of histologic diagnosis. We calculated sensitivity, specificity, positive and negative predictive values and odds ratio (OR) for CIN2+ and CIN3+ for all triage tests among those women who tested HPV DNA positive. A McNemar chi-square test was used to assess differences in sensitivity and specificity for CIN2+ and CIN3+ for the other triage tests between the different specimens.

The clinical performance of the triage tests among HPV DNA-positive women for CIN2+ is shown in Table 3 and for CIN3+ is shown in Table 4. The 10 rlu/pc cutpoint was generally the most sensitive and least specific of triage tests for both endpoints (p < 0.0001) except for the 10 rlu/pc cutpoint for self-collected specimens tested by careHPV™. DNA detection of HPV16/18/45 among careHPV™ positives was generally the next most sensitive and the second least specific. E6 detection of HPV16/18/45 was by far the most specific for CIN2+ (p < 0.0001 for all) and CIN3+ (p < 0.0001 for all), and its positive predictive value was greater than 50% for CIN2+ and almost 50% for CIN3+. For visual methods, colposcopy tended to be slightly more sensitive and slightly less specific than VIA, although the differences were not statistically significant. For CIN2+, the sensitivity of colposcopy ranged from 55.3% to 58.8% and the specificity ranged from 84.2% to 86.9%. On the other hand, the sensitivity for VIA ranged between 46.0% and 46.8% and its specificity was between 86.1% and 88.6%. Similar results were observed for the CIN3+ endpoint: the sensitivity for colposcopy was between 63.3% and 63.9% and its specificity was between 83.3% and 86.1%; the sensitivity for VIA ranged from 52.6% to 54.2% and its specificity ranged from 85.3% to 87.9%.

We also calculated the OR and 95% confidence interval (CI) as a summary measure of the association of positive triage tests with the endpoints among HPV DNA-positive women. E6 was the most strongly associated with CIN2+ and CIN3+. For example, E6 detection was strongly associated with CIN3+ among women who tested careHPV™ positive on their clinician-collected specimen (OR: 17.9, 95% CI: 11.1–28.9) and on their self-collected specimen (OR: 28.8, 95% CI: 17.0–48.8). The OR of 10 rlu/pc cutpoint (for clinician-collected specimens only), VIA, colposcopy and DNA for HPV16/18/45 were between 5 and 15 for CIN2+ and between 5 and 15 for CIN3+. At the other extreme, the 10 rlu/pc cutpoint for HC2 on self-collected specimens was only weakly associated with CIN3+ (OR: 2.3, 95% CI: 1.4–3.6) and the 10 rlu/pc cutpoint for careHPV™ on self-collected specimens was not associated with CIN3+ (OR: 1.2, 95% CI: 0.8–1.9).

**Discussion**

We evaluated multiple strategies for managing HPV DNA-positive women (triage) to expand the menu of options for secondary cervical cancer prevention through screening, management of screen positives, diagnosis and early treatment of precancer and early cancer. More sensitive methods of triaging HPV DNA-positive women to detect CIN3+, such as using a higher cutpoint or HPV16/18/45 DNA detection, were generally less specific, i.e., more false positives. Conversely, highly specific methods for CIN3+, such as HPV16/18/45 E6, were less sensitive. In general, the relative
Table 1. The percent positive of the triage tests/methods among HPV DNA positives by specimen collection method (clinician or self) and test (careHPV™ or Hybrid Capture 2 [HC2]), overall and by age group.

| Age group (years) | Clinician collection | | | | Self collection | | | |
|-------------------|----------------------|------------------|-----------------|-----------------|------------------|-----------------|-----------------|-----------------|
|                   | careHPV+              | careHPV+         | HC2              | HC2+             | careHPV+         | careHPV+         | HC2              | HC2+             |
| N                 | careHPV (≥10)         | DNA (rlu/pc)     | E6 (rlu/pc)     | impression (%)   | N                | careHPV (≥10)    | DNA (rlu/pc)     | E6 (rlu/pc)     | impression (%)   |
| All               | 1065                 | 58.3             | 28.4            | 10.5             | 16.4             | 1076             | 62.6             | 10.5             | 20.9             | 17.4             |
| <30               | 46                   | 58.7             | 30.4            | 2.2              | 37.0             | 47               | 53.2             | 2.1              | 34.0             | 27.3             |
| 30–39             | 241                  | 59.8             | 28.2            | 7.5              | 29.9             | 240              | 65.8             | 7.5              | 32.5             | 28.7             |
| 40–49             | 401                  | 58.4             | 27.2            | 9.2              | 20.2             | 410              | 62.4             | 9.0              | 21.0             | 17.2             |
| 50+               | 377                  | 57.3             | 29.4            | 14.9             | 11.1             | 379              | 61.7             | 15.0             | 11.9             | 9.5              |

| All               | 1074                 | 48.0             | 23.4            | 8.7              | 17.8             | 1329             | 52.1             | 7.9              | 18.0             | 15.1             |
| <30               | 51                   | 47.1             | 25.5            | 2.0              | 27.5             | 54               | 64.8             | 1.9              | 29.6             | 25.0             |
| 30–39             | 232                  | 45.3             | 23.7            | 6.0              | 28.9             | 315              | 47.9             | 5.4              | 28.6             | 24.6             |
| 40–49             | 406                  | 47.8             | 20.9            | 8.1              | 17.5             | 525              | 51.2             | 7.2              | 17.0             | 14.1             |
| 50+               | 385                  | 50.1             | 25.5            | 11.7             | 10.1             | 435              | 54.7             | 11.3             | 10.1             | 8.5              |

1 careHPV™ test.

Abbreviations: VIA, visual inspection after acetic acid; rlu/pc, relative light units per positive control (signal strength).
Table 2. The percent positive of the triage tests/methods for increasing severity of diagnosis among HPV DNA positives by sampling method (clinician or self) and test (careHPV™ or HC2)

| careHPV+ (clinician) | HC2+ (clinician) |
|----------------------|------------------|
| n                    | careHPV (≥10)   | E6 | Colpo Impression | VIA (2nd) | n | HC2 (≥10)   | E6 | Colpo Impression | VIA (2nd) |
| Negative¹            | 770             | 47.4 | 21.3 | 4.9 | 11.2 | 10.0 | 771             | 52.9 | 4.9 | 12.2 | 11.0 |
| CIN1                 | 157             | 85.4 | 27.4 | 9.6 | 31.2 | 26.0 | 167             | 83.8 | 9.6 | 32.3 | 27.5 |
| CIN2                 | 42              | 88.1 | 50.0 | 16.7 | 38.1 | 33.3 | 42              | 90.5 | 16.7 | 38.1 | 33.3 |
| CIN3                 | 83              | 91.6 | 74.7 | 49.4 | 60.2 | 50.7 | 83              | 91.6 | 49.4 | 60.2 | 50.7 |
| Cancer               | 13              | 69.2 | 92.3 | 84.6 | 84.6 | 71.4 | 13              | 84.6 | 84.6 | 71.4 | 50.7 |
| CIN2+                | 138             | 88.4 | 68.8 | 42.8 | 55.8 | 46.1 | 138             | 90.6 | 42.8 | 55.8 | 46.1 |
| CIN3+                | 96              | 88.5 | 77.1 | 54.2 | 63.5 | 52.6 | 96              | 90.6 | 54.2 | 63.5 | 52.6 |

| careHPV+ (self)      | HC2+ (self)     |
|----------------------|-----------------|
| n                    | careHPV (≥10)   | E6 | Colpo Impression | VIA (2<sup>nd</sup>) | n | HC2 (≥10)   | E6 | Colpo Impression | VIA (2<sup>nd</sup>) |
| Negative¹            | 811             | 42.7 | 16.9 | 3.1 | 9.5   | 8.7 | 1033            | 46.5 | 3.1 | 10.4 | 9.3  |
| CIN1                 | 144             | 72.9 | 22.9 | 9.0 | 33.3 | 27.7 | 164             | 73.8 | 9.2 | 36.0 | 30.8 |
| CIN2                 | 36              | 58.3 | 47.2 | 19.4 | 36.1 | 30.3 | 42              | 69.1 | 19.1 | 38.1 | 33.3 |
| CIN3                 | 72              | 50.0 | 73.6 | 52.8 | 59.7 | 50.8 | 79              | 69.6 | 50.6 | 59.5 | 51.5 |
| Cancer               | 11              | 72.7 | 100.0 | 90.9 | 90.9 | 83.3 | 11              | 72.7 | 90.9 | 90.9 | 83.3 |
| CIN2+                | 119             | 54.6 | 68.1 | 46.2 | 55.5 | 46.0 | 132             | 69.7 | 43.9 | 55.3 | 46.9 |
| CIN3+                | 83              | 53.0 | 77.1 | 57.8 | 63.9 | 53.7 | 90              | 70.0 | 55.6 | 63.3 | 54.2 |

¹Includes women who did not have biopsies and biopsies that were diagnosed as negative.
²careHPV™ test.

Abbreviations: CIN, cervical intraepithelial neoplasia; CIN1, CIN grade 1; CIN2, CIN grade 2; CIN3, CIN grade 3, CIN2+, CIN2 or more severe; CIN3+, CIN3 or more severe; VIA, visual inspection with acetic acid; rlu/pc, relative light units per positive control (signal strength).
Table 3. The clinical performance (sensitivity, specificity, PPV and NPV) with 95% confidence intervals of the triage tests/methods for detection of CIN2 or CIN2+ among HPV DNA positives by sampling method (clinician or self) and test (careHPV<sup>TM</sup> or HC2)

| careHPV+ (clinician) |          |          |          | HC2+ (clinician) |          |          |
|----------------------|----------|----------|----------|-----------------|----------|----------|
|                      | careHPV (>10) | HPV16/18/45 | Colpo Impression | VIA (2<sup>nd</sup>) |          |          |
| Sensitivity          | 88.4 (81.9, 93.2) | 68.8 (60.4, 76.4) | 42.8 (34.4, 51.5) | 55.8 (47.1, 64.2) | 46.1 (36.8, 55.6) |
| Specificity          | 46.2 (42.9, 49.4) | 77.7 (74.8, 80.3) | 94.3 (92.6, 95.7) | 85.4 (83.0, 87.6) | 87.4 (85.1, 89.5) |
| PPV                  | 19.6 (16.6, 23.0) | 31.5 (26.3, 37.0) | 52.7 (43.0, 62.2) | 36.3 (29.8, 43.2) | 31.5 (24.6, 39.2) |
| NPV                  | 96.40 (94.21, 97.93) | 94.36 (92.48, 95.89) | 91.71 (89.78, 93.38) | 92.85 (90.91, 94.49) | 92.78 (90.84, 94.42) |
| OR                   | 6.5 (3.8, 11) | 7.7 (5.2, 11) | 12 (8.0, 19) | 7.4 (5.1, 11) | 5.9 (3.9, 9.0) |

| careHPV+ (self) |          |          |          | HC2+ (self) |          |          |
|-----------------|----------|----------|----------|-------------|----------|----------|
|                  | careHPV (>10) | HPV16/18/45 | Colpo Impression | VIA (2<sup>nd</sup>) |          |          |
| Sensitivity      | 54.6 (45.2, 63.8) | 68.1 (58.9, 76.3) | 46.2 (37.0, 55.6) | 55.5 (46.1, 64.6) | 46.0 (36.0, 56.3) |
| Specificity      | 52.8 (49.6, 56.0) | 82.2 (79.6, 84.6) | 96.0 (94.6, 97.2) | 86.9 (84.6, 89.0) | 88.6 (86.4, 90.5) |
| PPV              | 12.6 (9.86, 15.8) | 32.3 (26.5, 38.4) | 59.1 (48.5, 69.2) | 34.6 (27.8, 41.8) | 29.9 (22.8, 37.8) |
| NPV              | 90.32 (87.56, 92.65) | 95.38 (93.72, 96.71) | 92.48 (91.74, 94.94) | 94.00 (92.22, 95.47) | 92.93 (92.16, 95.41) |
| OR               | 1.4 (0.92, 2.0) | 9.8 (6.5, 15) | 21 (13, 34) | 8.3 (5.5, 12) | 6.6 (4.3, 10) |

|                  | HPV16/18/45 | E6 | Colpo Impression | VIA (2<sup>nd</sup>) |          |          |
| Sensitivity      | 90.6 (84.4, 94.9) | 42.8 (34.4, 51.5) | 55.8 (47.1, 64.2) | 46.1 (36.8, 55.6) |
| Specificity      | 46.2 (38.4, 44.8) | 94.2 (92.6, 95.6) | 84.2 (81.7, 86.5) | 86.1 (83.7, 88.3) |
| PPV              | 18.6 (15.7, 21.7) | 52.2 (42.6, 61.7) | 34.2 (28.0, 40.8) | 29.3 (22.8, 63.5) |
| NPV              | 96.77 (94.55, 98.27) | 91.80 (89.88, 93.45) | 92.83 (90.89, 94.47) | 92.77 (90.82, 94.41) |
| OR               | 6.8 (3.8, 12) | 12.2 (7.9, 19) | 6.7 (4.6, 9.8) | 5.3 (3.5, 8.0) |

Odds ratios with 95% confidence intervals as a measure of association are also shown. Abbreviations: CIN, cervical intraepithelial neoplasia; CIN2, grade 2; CIN2+, grade 2 or more severe; NPV, negative predictive value; OR, odds ratio; PPV, positive predictive value; rlu/pc, relative light units per positive control (signal strength); VIA, visual inspection with acetic acid.
Table 4. The clinical performance (sensitivity, specificity, PPV and NPV) with 95% confidence intervals of the triage tests/methods for detection of CIN3 or more severe (CIN3+) among HPV DNA positives by sampling method (clinician or self) and test (careHPV™ or HC2).

|                      | careHPV+ (clinician) |                      |                      |                      | HC2+ (clinician) |                      |                      |
|----------------------|----------------------|----------------------|----------------------|----------------------|------------------|----------------------|----------------------|
|                      |                      |                      |                      |                      |                  |                      |                      |
|                      | careHPV (>10)        |                      |                      |                      |                  |                      |                      |
|                      |                      |                      |                      |                      |                  |                      |                      |
| Sensitivity          | 88.5 (80.4, 94.1)    | 77.1 (67.4, 85.0)    | 54.2 (43.7, 64.4)    | 63.5 (53.1, 73.1)   | 52.6 (40.8, 64.2) | 90.6 (82.9, 95.6)   | 54.2 (43.7, 64.4)    |
| Specificity          | 44.7 (41.5, 47.9)    | 76.5 (73.7, 79.1)    | 93.8 (92.1, 95.2)    | 84.4 (82.0, 86.6)   | 86.5 (84.2, 88.6) | 40.2 (37.1, 43.4)   | 93.8 (92.1, 95.2)    |
| PPV                  | 13.7 (11.1, 16.6)    | 24.5 (19.8, 29.8)    | 46.4 (37.0, 56.1)    | 28.8 (22.8, 35.4)   | 23.8 (17.6, 31.0) | 12.9 (10.5, 15.7)   | 46.0 (36.6, 55.6)    |
| NPV                  | 97.52 (95.61, 98.76) | 97.12 (95.67, 98.18) | 95.38 (93.85, 96.63) | 95.90 (94.34, 97.13)| 95.81 (94.24, 97.05)| 97.77 (95.80, 98.97)| 95.43 (93.91, 96.66) |
| OR                   | 6.2 (3.3, 12)        | 11 (6.7, 18)         | 18 (11, 29)          | 9.4 (6.0, 15)       | 7.1 (4.4, 12)    | 6.5 (3.3, 13)      | 18 (11, 29)          |
|                      |                      |                      |                      |                      |                  |                      |                      |
|                      |                      |                      |                      |                      |                  |                      |                      |
|                      | careHPV+ (self)      |                      |                      |                      |                  |                      |                      |
|                      |                      |                      |                      |                      |                  |                      |                      |
| Sensitivity          | 53.0 (41.7, 64.1)    | 77.1 (66.6, 85.6)    | 57.8 (46.5, 68.6)    | 63.9 (52.6, 74.1)   | 53.7 (41.1, 66.0) | 70.0 (59.4, 79.2)   | 55.6 (44.7, 66.0)    |
| Specificity          | 52.4 (49.2, 55.5)    | 81.1 (78.6, 83.5)    | 95.5 (94.0, 96.7)    | 86.1 (83.8, 88.2)   | 87.9 (85.7, 89.9) | 49.2 (46.3, 52.0)   | 95.6 (94.3, 96.6)    |
| PPV                  | 8.53 (6.26, 11.3)    | 25.5 (20.2, 31.4)    | 51.6 (41.0, 62.1)    | 27.7 (21.5, 34.7)   | 23.4 (16.9, 30.9) | 9.09 (7.06, 11.5)   | 47.6 (37.8, 57.6)    |
| NPV                  | 93.01 (90.57, 94.98) | 97.69 (96.42, 98.60) | 96.43 (95.07, 97.50) | 96.60 (95.18, 97.70)| 96.52 (95.09, 97.62)| 95.75 (93.88, 97.18)| 96.73 (95.58, 97.66) |
| OR                   | 1.2 (0.79, 1.9)      | 15 (8.5, 25)         | 29 (17, 49)          | 11 (6.8, 18)        | 8.5 (5.1, 14)    | 2.3 (1.4, 3.6)      | 27 (16, 44)          |

Odds ratios (OR) with 95% confidence intervals as a measure of association are also shown. Abbreviations: CIN, cervical intraepithelial neoplasia; CIN2, grade 2; CIN3, grade 3; CIN2+, grade 2 or more severe; CIN3+, grade 3 or more severe; NPV, negative predictive value; OR, odds ratio; PPV, positive predictive value; rlu/pc, relative light units per positive control (signal strength); VIA, visual inspection with acetic acid.
performance of the different management strategies did not depend on the method of screening to identify HPV-positive women.

Appropriate screening strategies for LMICs differ from the criteria for strategies appropriate for a high-income country. Screening programs, including the management strategies, will need to be tailored to meet local needs in terms of financial and human resources, infrastructure and capacities, societal norms and patient acceptability and level of cancer risk reduction desired. For instance, the Chinese government launched a cervical cancer prevention program targeting 10 million rural women using a Pap smear or VIA during the year of 2009–2011. However, China lacks a sufficient number of cytopathologists or trained health care workers to screen an estimated 500 million women in rural areas by Pap smear or VIA. Objective, efficient and high reproducible screening tests are more desirable in China. The affordable HPV DNA test (careHPV™) has been developed for developing countries. Testing with careHPV™ at five-year intervals was reported to be the optimal cervical cancer screening strategy for China and has the best cost-effectiveness performance and the highest benefit-cost ratio with moderate life outcomes in a modeling study. The decision to triage and by what method will depend greatly on the weighting of the benefits (e.g., cancer prevention), the harms (e.g., false positive results leading to unnecessary colposcopy, biopsy and possibly treatment) and programmatic issues (e.g., losses to follow-up and availability of health services) in the local context.

HPV DNA testing is uniquely suited as a screening test because of its ability to “rule out” disease (i.e., women who test negative for the necessary causal factor, HPV, are on average many years away from developing invasive cervical cancer). One of the challenges to widespread implementation of HPV DNA testing is its lower specificity. Some countries may have insufficient capacity to act (e.g., colposcopy or immediate treatment) on a HPV DNA-positive result. In such places, strategies to prioritize women into higher-risk groups in need of immediate intervention and lower-risk groups who might be deferred for a period of time (e.g., 6–24 months) and then re-evaluated would be valuable.

Here we presented a variety of methods/tests that might be employed to make the distinction between higher- and lower-risk HPV DNA-positive women. Each method has distinct advantage(s) in terms of its performance and/or applicability. Simply using a higher cutoffpoint of 10 rlu/pc as the triage, with ≥10 rlu/pc for immediate intervention and 1.0–9.9 rlu/pc deferred to follow-up, could be seen perhaps as the easiest to implement because it requires no additional tests, if the rlu/pc value was made available. Previous findings of primary screening strategies showed that the performance of HPV DNA testing with an increased cutoff-point of 10 rlu/pc may be ideal for a population such as China that could utilize a single test primary screening strategy to allow for infrequent screening and minimal infrastructure requirements. In this study using a cutpoint of 10 rlu/pc as the triage was the most sensitive and least specific triage for CIN2+ and CIN3+ when using a clinician-collected specimen but was much less effective when using a self-collected specimen, such that it was no better than chance when tested with careHPV™.

Detection of HPV16/18/45 using a research-use only assay on the careHPV™ platform was very sensitive, identifying nearly 70% of all CIN2+ and nearly 80% of all CIN3+, and had positive predictive value (PPV) around 25%, as predicted by the etiologic fraction of cervical cancer and precancer caused by these three HPV genotypes. Our findings are consistent with data reported for a recently US Food and Drug Administration-approved HPV DNA test that includes HPV16 and HPV18 detection and previous reports from epidemiologic studies of the clinical utility of HPV16 or HPV16 and 18 detection. Newly developed US screening guidelines have reaffirmed the use of HPV16 or HPV16 and HPV18 detection for management of HPV-positive, Pap-negative women. Since HPV16/18/45 DNA testing was done reflexively from a careHPV™-positive specimen, there was no additional specimen or visit required, making it relatively simple to implement. One caveat is that in order to maximize its cost-effectiveness, HPV16/18/45 DNA testing on this platform would need to be done in batches, which could take several days to accumulate enough specimens and may preclude same-day “screen-and-intervene” programs in clinics with smaller numbers of women or a low HPV prevalence.

Visual methods of triage, such as colposcopy and VIA, had lower sensitivity and better specificity for CIN2+ and CIN3+ than DNA methods of triage. Colposcopy was more sensitive but less specific than VIA. These methods could be done on the same day or with a subsequent visit and followed by the appropriate intervention (diagnosis and/or treatment). Not surprisingly, visual methods have been proposed as the triage method for HPV DNA testing in low-resource settings, especially since VIA is already being used in many countries. A review of the criteria used for VIA or colposcopy could be required when working with women known to be infected with carcinogenic HPV types.

We noted that the VIA performance was less sensitive but more specific in this setting than reported in recent meta-analyses/systematic reviews. We also observed that colposcopy missed approximately 35% of disease (i.e., 35% of disease was found by random biopsies after negative colposcopic assessment). Given the possible bias of using colposcopy to assess VIA performance, we suggest that in order to accurately assess the true performance of VIA, random biopsies are required to identify lesions not visually apparent.

The detection of HPV16/18/45 E6 had sensitivity comparable to VIA but had the best specificity of any triage test or method and predicted that one out of every two HPV16/18/45 E6 positives had clinically important disease (CIN3+). Thus, in the programmatic context in which referral to colposcopy or overtreatment in the context of a screen-and-treat program needed to be minimized, detection of HPV16/18/45
E6 by OncoE6™ Cervical Test might be one option to consider. One caveat is that OncoE6™ Cervical Test only targets HPV16, 18 or 45; targeting additional types is expected to increase sensitivity without significant impact on specificity, since E6 overexpression is expected to be a characteristic of precancer of any type rather than infection. Another limitation is that a second dedicated sample was needed for testing, so either co-collection of a clinician specimen for OncoE6™ Cervical Testing or a second visit of the HPV DNA-positive women would be required. Adapting the OncoE6™ Cervical Test for use with specimens stored in buffers/transport medium employed for HPV DNA testing would increase its usability as a triage method.

As noted above, the sensitivity of the 10 rlu/pc cutoff for triage dropped significantly with the use of the self-collected specimen compared to the clinician-collected specimen. The consequence of this is that the 10 rlu/pc cutoff is a significantly more sensitive triage method using the clinician-collected specimen ($p < 0.0001$ for CIN3+) but less sensitive using the self-collected specimen ($p = 0.0003$ for CIN3+) compared to HPV16/18/45 DNA detection. A comparison of signal strength (rlu/pc) and HPV16/18/45 detection (Supporting Information Table 1) shows that low signal strength ($1 \leq 10$ rlu/pc), HPV16/18/45 DNA-positive results from the clinician-collected specimens identified fewer cases of CIN2+ and CIN3+ than high signal strength ($\geq 10$ rlu/pc), HPV16/18/45 DNA-negative results but the converse was true for self-collected specimens.

We acknowledge the following limitations in our study. First, we did not conduct cytology, which is one of the standard methods being considered for triage of HPV positives in higher-resource countries. Collection of a third cervical specimen (fourth specimen overall) might have otherwise regressed in the triage-negative group was detected and treated immediately and a small number of CIN3 could have developed into invasive cancer during the time interval.

Our findings show that increasing the evidence and knowledge about these programmatic choices will enable countries, specifically LMICs, to make an informed decision about what strategies might work best for screening their women. The next step forward is large, realistic demonstrations to show how assembling these different components into a cogent program can perform in the real world.

**Acknowledgements**

We thank Wen-Hua Zhang for training local gynecologists; Zhi-Xia Li, Qiao-Yun Dai, Yu Wang, Yu-Qian Sun, Hui-Jing Luo and Chun-Jing Fu for doing the laboratory testing; Qing Li and Xiao-Yang Liu for reviewing the pathological slides; and all members of the START-UP project. We also thank all the participants in this study.

**References**

1. International Agency for Research on Cancer. IARC handbooks of cancer prevention: cervix cancer screening. Lyon, France: IARC Press, 2005.
2. Ferlay J, Shin HR, Bray F, et al. Globocan 2008 V1.2, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10 [Internet]. International Agency for Research on Cancer 2010 [cited April 2, 2013]; Available from: URL. http://globocan.iarc.fr/.
3. Kitchener HC, Castle PE, Cox JT. Chapter 7: achievements and limitations of cervical cytology screening. Vaccine 2006;24:53–63–70.
4. Cuzick J, Clavel C, Petry KU, et al. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. Int J Cancer 2006;119:1095–101.
5. Mayrand MH, Duarte-Franco E, Rodrigues I, et al. Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer. N Engl J Med 2007;357:1579–88.
6. Naude P, Ryd W, Tornberg S, et al. Human papillomavirus and Papanicolaou tests to screen for cervical cancer. N Engl J Med 2007;357:1589–97.
7. Rijkaart DC, Berkhoef J, Rozenwald L, et al. Human papillomavirus testing for the detection of high-grade cervical intraepithelial neoplasia and cancer: final results of the PORASCAM randomised controlled trial. Lancet Oncol 2012;13:78–88.
8. Ronco G, Giorgi-Rossi P, Carozzi F, et al. Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomised controlled trial. Lancet Oncol 2010;11:249–57.
9. Castle PE, Stoler MH, Wright TC, Jr., et al. Performance of carcinogenic human papillomavirus (HPV) testing and HPV16 or HPV18 genotyping for cervical cancer screening of women aged 25 years and older: a subanalysis of the ATHENA study. Lancet Oncol 2011;12:880–90.
10. Stoler MH, Schiffman M. Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL Triage Study. JAMA 2001;285:1500–5.
11. Castle PE, Wheeler CM, Solomon D, et al. Inter-laboratory reliability of Hybrid Capture 2. Am J Clin Pathol 2004;122:238–45.
12. Carozzi FM, Del MA, Confortini M, et al. Reproducibility of HPV DNA testing by Hybrid Capture 2 in a screening setting. Am J Clin Pathol 2005;124:76–80.
13. Dillner J, Rebolj M, Biembaum P, et al. Long term predictive values of cytology and human papillomavirus testing in cervical cancer screening: joint European cohort study. BMJ 2008;337: a1754.
14. Castle PE, Glass AG, Rush BB, et al. Clinical human papillomavirus detection forecasts cervical cancer risk in women over 18 years of follow-up. J Clin Oncol 2012;30:3044–50.
15. Kjaer SK, Frederiksen K, Munk C, et al. Longterm absolute risk of cervical intraepithelial neoplasia grade 3 or worse following human papillomavirus infection: role of persistence. J Natl Cancer Inst 2010;102:1478–88.
16. Qiao YL, Sellers JW, Eder PS, et al. A new HPV- DNA test for cervical-cancer screening in developing regions: a cross-sectional study of clinical accuracy in rural China. Lancet Oncol 2008;9:929–36.
17. Adesina A, Chumba D, Nelson AM, et al. Improvement of pathology in sub-Saharan Africa. Lancet Oncol 2013;14:e152–157.
18. Zhao FH, Lekwokwitz AK, Hu SY, et al. Prevalence of human papillomavirus and cervical intraepithelial neoplasia in China: a pooled analysis of 17 population-based studies. Int J Cancer 2012;131:2929–38.
19. Shi JF, Canfell K, Lew JH, et al. The burden of cervical cancer in China: synthesis of the evidence. Int J Cancer 2012;130:641–52.
20. Koshy J, Lindsay L, Pimenta JM, et al. Persistent human papillomavirus infection and cervical neoplasia: a systematic review and meta-analysis. Am J Epidemiol 2008;168:123–37.
21. Castle PE, Rodriguez AC, Burk RD, et al. Short term persistence of human papillomavirus and risk of cervical precancer and cancer: population based cohort study. BMJ 2009;339:b2569.
22. Pretorius RG, Zhang WH, Belinson JL, et al. Colposcopically directed biopsy, random cervical
biopsy, and endocervical curettage in the diagnosis of cervical intraepithelial neoplasia II or worse. Am J Obstet Gynecol 2004;191:430–4.

23. Gage JC, Ajenifuja KO, Wentzensen N, et al. Effectiveness of a simple rapid human papillomavirus DNA test in rural Nigeria. Int J Cancer 2012;131:2903–9.

24. Schweizer J, Lu PS, Mahoney CW, et al. Feasibility study of a human papillomavirus E6 oncoprotein test for diagnosis of cervical pre-cancer and cancer. J Clin Microbiol 2010;48:4646–8.

25. Moy LM, Zhao FH, Li LY, et al. Human papillomavirus testing and cervical cytology in primary screening for cervical cancer among women in rural China: comparison of sensitivity, specificity, and frequency of referral. Int J Cancer 2010;127:646–56.

26. Women’s health in rural China. Lancet 2009;374:358.

27. Zhao FH, Chen JF, Gao XH, et al. Effectiveness and health economic analysis of strategies on cervical screening and early diagnosis and treatment. Zhonghua Zhong Liu Za Zhi 2012;34:632–6.

28. Schiffman M, Castle PE, Jeronimo J, et al. Human papillomavirus and cervical cancer. Lancet 2007;370:890–907.

29. Zhao FH, Lin MJ, Chen F, et al. Performance of high-risk human papillomavirus DNA testing as a primary screen for cervical cancer: a pooled analysis of individual patient data from 17 population-based studies from China. Lancet Oncol 2010;11:1160–71.

30. de Sanjose S, Quint WGV, Alemany L, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol 2010;11:1048–56.

31. Smith JS, Lindsay L, Hootts B, et al. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: A meta-analysis update. Int J Cancer 2007;121:621–32.

32. Castle PE, Solomon D, Schiffman M, et al. Human papillomavirus type 16 infections and 2-year absolute risk of cervical precancer in women with equivocal or mild cytologic abnormalities. J Natl Cancer Inst 2005;97:1066–71.

33. Saslow D, Solomon D, Lawson HW, et al. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. CA Cancer J Clin 2012;62:147–72.

34. Sauvaget C, Fayette JM, Mwinege R, et al. Accuracy of visual inspection with acetic acid for cervical cancer screening. Int J Gynecol Obstet 2011;113:14–24.

35. Sritipsukho P, Thaweekul Y. Accuracy of visual inspection with acetic acid (VIA) for cervical cancer screening: a systematic review. J Med Assoc Thai 2010;93:5254–5261.

36. Pretorius RG, Kim RJ, Belinson JL, et al. Inflation of sensitivity of cervical cancer screening tests secondary to correlated error in colposcopy. J Low Genit Tract Dis 2006;10:5–9.

37. Trimble CL, Piantadosi S, Gravitt P, et al. Spontaneous regression of high-grade cervical dysplasia: effects of human papillomavirus type and HLA phenotype. Clin Cancer Res 2005;11:4717–23.

38. Castle PE, Schiffman M, Wheeler CM, et al. Evidence for frequent regression of cervical intraepithelial neoplasia-grade 2. Obstet Gynecol 2009;113:18–25.