Author’s response to reviews

Title: Feasibility and Accuracy Evaluation of Three Human Papillomavirus Assays for FTA Card-based Sampling: a Pilot Study in Cervical Cancer Screening

Authors:

Shao-Ming Wang (wangshaoming@cicams.ac.cn)
Shang-Ying Hu (shangyinghu@cicams.ac.cn)
Wen Chen (chenwen@cicams.ac.cn)
Feng Chen (chenfeng915@tom.com)
Fang-Hui Zhao (zhaofangh@cicams.ac.cn)
Wei He (545875763@qq.com)
Xin-Ming Ma (maxinming2009@163.com)
Yu-Qing Zhang (yqzhang2010@hotmail.com)
Jian Wang (591430941@qq.com)
Priya Sivasubramaniam (priyagsiva@gmail.com)
You-Lin Qiao (qiaoy@cicams.ac.cn)

Version: 3
Date: 4 June 2015

Author’s response to reviews: see over
**Point-by-point response to the editor’s comments**

Comment 1#: It is unclear how screened women were recruited. The authors should describe the procedures, how many women were approached, how many refused, and what were their characteristics compared to women who participated. How many women were referred for diagnostics but did not show up?

*Re: Thanks for the editor’s comment. As we stated in the method, our study was based on a large project called “LCMCCSS”. Per editor’s suggestion, we have added the screening procedures in the method section:” From May to June of 2012, we conducted the study in women who were called back for colposcopy examination of LCMCCSS. The recruitment was stopped when the target sample size (n=396) was reached.” and added the participant rates in the result section:” A total of 3191 women were invited to attend the screening tests of LCMCCSS study in 2012, among which 370 (11.7%) refused to participate. The participants had a higher marriage rate (96.7% vs. 93.8%, P=0.005) and a lower smoking rate (0.3% vs. 1.6%, P=0.003) than those who didn’t. According to the testing results, 1392 women were referred to colposcopy diagnosis, among which 106 (7.6%) refused to come back. The called back women (n=1286) had a lower smoking rate (0.1% vs. 1.9%, P=0.016) and less numbers of live birth (no. of less than 3 live births: 91.2% vs. 84.9%, P=0.031) than those who refused to come back.”*

Comment 2#: What was the source of information for socio-demographic information?

*Re: Thanks for the editor’s comment. The source of information was a questionnaire. We have added this information in the method section with the following revision:” After a socio-demographic questionnaire survey, each woman provided a self-collected and two clinician-collected specimens.”*

Comment 3#: If a comparison is made between several tests/procedures, then their outcomes should be compared on samples with results on all compared tests. Please revise the analysis to reflect this.

*Re: Thanks for the editor’s comment. A total of 368 samples had results on all compared tests. Per the editor’s suggestion, we have revised the analysis throughout the manuscript including result section, discussion section, figure and tables.*

Comment 4#: Please structure the Discussion, e.g. into general findings, strengths and weaknesses, comparison with other literature, and clinical implications.

*Re: Thanks for the editor’s comment. We have restructured the discussion section with the following revision:” general findings, comparison with other literature, strengths and weaknesses, and clinical implications”.*
Point-by-point response to the reviewer’s comments

Reviewer 1: Jesper Bonde

General comments:
Comment 1.1#:
The manuscript is well written, and I fully understand the logistic clinical challenge of expanding cervical cancer screening coverage in less developed regions confronted by infrastructural issues. Yet, I need the authors to more clearly describe the advantages of FTA cards, whereas now the introduction mainly states the disadvantages of liquid based sampling. What are the determining advantages of FTA cards?

Re: Thanks for reviewer’s suggestion. We have added the advantage of FTA card in the introduction section with the following revision: “A solid card which called Whatman Indicating FTA Elute® card (FTA card, GE Healthcare, Buckinghamshire, UK) is a potential sampling medium for DNA sample collection. The indicating FTA Elute Card is small cards which provide a cost effective room temperature method for collecting, shipping, archiving and processing nucleic acids from a wide variety of biological samples. It contains an inert dye that changes from purple to white indicating the location of a clear, colorless sample. Moreover, indicating FTA Elute cards facilitate rapid purification of nucleic acids in less than 30 min per sample and provide DNA in solution for multiple amplification reactions with high stability. The current data found that genomic DNA stored on FTA cards at room temperature for more than 17.5 years still can be successfully amplified by PCR.”

Comment 1.2#:
What are the criteria’s for the number of included women? To show any differences in testing proficiency I miss a power calculation; what is the needed sample size to show equal performance in HPV detection between the sub groups?

Re: Thanks for reviewer’s comment. We have added the sample size and power calculation in the statistical analysis section: “According to previous studies, we assumed the sensitivity ranged from 0.85 to 0.96, the specificity ranged from 0.60 to 0.80, and the CIN2+ prevalence was about 4%. At this circumstance, the maximal sample size was 375 with 0.05 of alpha and 0.80 of power [19]. Considering the 5% of testing failure rate, a maximum of 395 women was required.”

Comment 1.3#:
Is there any stability data on the FTA cards, in terms of how this responds to storage conditions?

Re: According to reviewer’s comment, we have added the stability data in the introduction section:” The current report found that genomic DNA stored on FTA cards at room temperature for more than 17.5 years still can be successfully
Comment 1.4#
Please note whether the companies participated in developing testing protocols for the FTA protocol, or whether this was in house development.

Re: According to reviewer’s comment, we have added this information in the method section with the following revision:” In order to acquire high accuracy, CICAMS performed several pilots with the help of GE Healthcare to develop testing protocols of FTA card-based sample for three HPV assays.”

Comment 1.5#
Table 2: Are the HPV positive findings concordant? Do the methods and tests find the same women positive?

Re: Thanks for reviewer’s comment. The HPV positive findings were not concordant. We have added HPV prevalence data in Table 2 in the result section with the following revision:” HPV prevalence and accuracies of detecting CIN2+ lesions for six combinations of two sampling mediums with three HPV testing methods are shown in Table2. The liquid sample showed comparable HPV positivity among HC2 (30.4%), careHPV™ (28.8%) and Cobas®4800 test (32.1%). FTA-based sample showed a lower HPV positivity than that of DCM medium for HC2 (22.8%) and careHPV™ test (23.9%), but a higher HPV positivity for Cobas®4800 test (39.4%).” We also added explanations in the discussion section:” FTA card-based sample was expected to be eluted to comparable HPV concentration as that of DCM preservative solution for HC2 and careHPV™ assay. However, concentration discrepancy still existed when transferring sample from solid to liquid, which resulting in a relatively lower HPV positivity and a higher specificity for FTA card-based sample.” For the Cobas®4800 test:” As the manufacturer didn’t have instructions for DCM-based sample detection, CICAMS developed the workflow according to several small pilot studies. Specifically, samples using for PCR amplification from FTA card actually had a much higher HPV concentration than that of DCM preservative solution (3.6% vs. 0.5%) to acquire a high sensitivity (91.7%). As a result, a higher HPV positivity and a lower specificity were found for FTA card-based sample than that of DCM sample.”

Comment 1.6#
Figure 2 makes very little sense in this respect. I suspect that the sample size is insufficient to show potential differences in the methodology.

Re: We agree with the reviewer that Figure 2 makes little sense, and have deleted Figure 2 in the manuscript.

Comment 1.7#:
The concept of looking to alternative sampling strategies for primary HPV screening is very interesting, however, this study would benefit by being re-written for what it is, a pilot study, rather than to attempt to show proficiency of this alternative sampling strategy. Moreover, the title indicates that it is a population based study, whereas in my opinion this is a small size, proof-of-concept, split sample study, and should be correctly addressed as such.

**Re: according to the reviewer’s suggestion, we have revised the title as:” Feasibility and Accuracy Evaluation of Three Human Papillomavirus Assays for FTA Card-based Sampling: a Pilot Study in Cervical Cancer Screening” and revised the manuscript as a pilot study accordingly.**
Reviewer: Bart Hesselink

Major Compulsory Revisions
Comment 2.1:
This reviewer raises concerns regarding the gold-standard of this study, which is colposcopy-directed biopsy, against which the sampling types and HPV assays are validated. Currently information is missing to judge whether colposcopy-directed biopsy potentially missed high-grade lesions. This is important because large high-grade lesions have high viral loads. If a bias in colposcopy is present towards large high-grade lesions, and as a consequence not biopsying the somewhat smaller and more difficult to detect high-grade, performance characteristics like sensitivity and specificity could be overestimated.

Re: Thanks for reviewer’s comment. We added the detailed information of our study procedures for screening tests and colposcopy examination to make it clear in the method section:” After a socio-demographic questionnaire survey, each woman provided a self-collected and two clinician-collected specimens [13, 14]. The self-collected and one clinician-collected specimen were tested by careHPV™ and Hybrid Capture 2 (Qiagen, Gaithersburg, MD, USA); the other clinician-collected specimen was tested for HPV16/18/45 E6 protein by OncoE6™ Cervical test (Arbor Vita Corporation, Sunnyvale, CA, USA). After sample collection, women were screened by visual inspection with acetic acid (VIA). One or two weeks later, 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) and approximately 10% random sample of women who tested negative for all screening tests (screen-negative women) were called back to undergo a second VIA and a rigorous colposcopic evaluation. All colposcopically detected abnormalities (acetowhite lesions) were biopsied. If the colposcopic examination showed no lesion in a quadrant but any of the screen results was positive, a random biopsy was obtained at the squamocolumnar junction in that quadrant at 2, 4, 8, or 10 o’clock. An ECC was performed after the cervical biopsies. Women who screen negative and were without any colposcopic indications of abnormality were not undergo colposcopically-directed biopsies.” According to our rigorous colposcopic evaluation and the microbiopsy protocol, the number of missed lesions should be very little.

Comment 2.2:
The authors must specify how lesions were visualized during colposcopy. Was acetic acid applied to all women? If so, please explain the discrepancy between VIA and colposcopy, because the positivity rate of VIA in the visit the week before was extremely low.

Re: Thanks for reviewer’s comment. We have added the detailed information of colposcopy in the manuscript as Response to comment 1# addressed. Yes, acetic
acid was applied to all women both for colposcopy examination and VIA. There were several reasons for the discrepancy between VIA and colposcopy: First, the LCMCCSS study was designed to have twice VIA. The first VIA was used in the primary screening without prior knowledge of the screening results. One week later, the second VIA was performed before the colposcopy and used for triage as it would be used in a real-world setting in which the person performing the VIA would know that a woman was HPV positive (“informed screening”). According to our data, knowing the prior HPV screening result had significantly impact on the clinical performance of VIA. The sensitivity increased from 8.3% of 1st VIA to 38.5% of 2nd VIA, and was identical to the sensitivity of colposcopy (38.4%). Although the sensitivity is not very high, with the rigorous colposcopy microbiopsy protocol, the number of missed lesions should be very little in this study. Second, the low sensitivity of 1st VIA was partly due to the small sample size. According to our result from the whole LCMCCSS study (n=7500), the sensitivity of 1st VIA is 36.8%, and the 2nd VIA is 46%, which gained a comparable sensitivity with colposcopy (55%)(Qiao2014, IJC). All the sensitivities of the large population-based study were higher than our small study. These data provides evidence that training is needed to improve the skill of local clinicians in rural China, which also points out the importance of objective HPV testing.

Comment 2.3#: The authors must specify the referral numbers for inclusion in the study. How many were HPV+, VIA+, and the number of randomly selected HPV-/VIA samples. How did the HPV positivity relate to that of the next visit only one week later of the same woman. Please provide explanation when HPV-positivity differs significantly between the 2 time points, given the fact that these are only 1 week apart.

Re: According to reviewer’s comment, we have added the referral numbers in the method with the following revision:”Specifically, the population included 207 women who were HPV positive, 17 women who were VIA positive (including 10 HPV positive women) and 182 randomly selected normal women.” As the primary screening tests included five liquid samples (HPV16/18/45 E6 test, careHPy™ and HC2 for both self- and clinician-collected samples), the total HPV positivity (52.3%) is higher than our study which only based on clinician-collected samples (ranges from 22.8% to 39.4 % for three HPV assays). However, if we compare the HPV positivity of clinician-collected samples at two time points, the HPV positivity is quite comparable for different HPV testing assays (HC2: 32.6% vs. 30.4%; careHPy™: 30.8% vs. 28.8%).

Comment 2.4#: For all three HPV assays a 2x2 table must be included in the manuscript displaying the results on the FTA relative to that of the LBC sample. This helps the reader to better understand the performance and discrepancies between the two specimen storage methods.
Re: Per reviewer’s suggestion, we have added the 2x2 table as Table 4 in the manuscript displaying the results on the FTA relative to that of the LBC sample for all three HPV assays.

Comment 2.5#:
Literature shows that HC2 has a higher performance compared to careHPV for detection of high-grade CIN. This study shows an identical performance. Please explain this apparent discrepancy.

Re: Thanks for the reviewer’s comment. We agree with the reviewer that HC2 has a higher performance compared to careHPV™. Although the sample size of our study is small, our data still showed that HC2 had a significantly higher sensitivity than that of careHPV™ for detection of high-grade CIN (careHPV™ vs. HC2: 83.3% vs. 91.7%). We stated this in the discussion section with the following sentences:” careHPV™ demonstrated a relatively lower sensitivity than that of HC2, both in our study (careHPV™ vs. HC2: 83.3% vs. 91.7%) and in previous studies (careHPV™ vs. HC2: 90.0% vs. 96.3%)[16, 20]. However, considering the simplicity of sample transportation and storage, lower cost and simpler administration, it is nonetheless recognized as a promising tool for future large scale cervical cancer screening projects, especially in low resource areas.”

Comment 2.6#:
Please explain why different numbers of discs were used for the careHPV compared to the HC2/COBAS4800? Explain how this specific ratio (6 vs 9) was selected.

Re: Thanks for reviewer’s comment. There were two reasons. First, HC2 and Cobas®4800 test used the same buffer but careHPV™ used different one. Second, three HPV testing assays required different testing concentration. Therefore, different punches were used for different assays. We have added the explanation in the method section with the following revision:” In order to acquire high accuracy, CICAMS performed several pilots with the help of GE Healthcare to develop testing protocols of FTA card-based sample for three HPV assays. FTA cards were punched using a specifically designed sterilized perforator (3-mm Harris Uni-Core device; Whatman, GE Healthcare, Buckinghamshire, UK). As HC2 and Cobas®4800 test used the same buffer but careHPV™ used different one, HC2 and Cobas®4800 tests shared 9 punched disks but careHPV™ test used another 6 disks to get the required testing concentration for different manufacturers of HPV testing assay.”

Comment 2.7#:
Include 95% confidence intervals for agreements results in line 197.

Re: Per reviewer’s suggestion, we have added the 95% confidence intervals for agreements in the manuscript.
Comment 2.8#
Please explain the higher specificity for COBAS in the liquid group versus the FTA group (Table 2), whereas for careHPV and HC2 this is the other way around.

Re: Thanks for reviewer’s suggestion. To better explain the data of specificity, we added HPV prevalence in Table 2 and the following sentences in the result section:”
HPV prevalence and accuracies of detecting CIN2+ lesions for six combinations of two sampling mediums with three HPV testing methods are shown in Table2. The liquid sample showed comparable HPV positivity among HC2 (30.4%), careHPV\textsuperscript{TM} (28.8%) and Cobas\textsuperscript{®}4800 test (32.1%). FTA-based sample showed a lower HPV positivity than that of DCM medium for HC2 (22.8%) and careHPV\textsuperscript{TM} test (23.9%), but a higher HPV positivity for Cobas\textsuperscript{®}4800 test (39.4%).” We also added the corresponding explanations in the discussion section with the following revision:”
FTA card-based sample was expected to be eluted to comparable HPV concentration as that of DCM preservative solution for HC2 and careHPV\textsuperscript{TM} assay. However, concentration discrepancy still existed when transferring sample from solid to liquid, which resulting in a relatively lower HPV positivity and a higher specificity for FTA card-based sample.” For the result of Cobas\textsuperscript{®}4800 test, we also added explanation in the discussion section:” As the manufacturer didn’t have instructions for DCM-based sample detection, CICAMS developed the workflow according to several small pilot studies. Specifically, samples using for PCR amplification from FTA card actually had a much higher HPV concentration than that of DCM preservative solution (3.6% vs. 0.5%) to acquire a high sensitivity. As a result, a higher HPV positivity and a lower specificity were found for FTA card-based sample than that of DCM sample. Further study is needed to optimize the workflow of Cobas\textsuperscript{®}4800 test for FTA card-based sample to reduce the false positive and improve the specificity.”

Comment 2.9#
The ROC curves with AUC values should be omitted from the manuscript. They are a repeat of the data in Table 2, and, in addition, the ROC curve are more commonly used to investigate different threshold settings of a markers/situation. Now only one point estimate is included.

Re: we agree with the reviewer’s comments, and deleted the ROC curve in the manuscript.

Comment 2.10#
Discussion section is too long. Please shorten.

Re: According to the reviewer's comment, we have rewritten the discussion section.

Comment 2.11#
Sentence “In theory…. (10% vs 7.2%).” line 239-242 must be omitted. Since human
DNA was not quantified between the FTA and the LBC. This is speculation.

Re: We agree with the reviewer’s comment, and deleted these sentences in the manuscript.

Comment 2.12#:
Since this study did not include self-sampling tune down statement in line 304-305.

Re: According to the reviewer’s comment, we rephrased the sentences in the following revision: “Our previous study showed that the agreement between self-collected and clinician-collected specimens on FTA card was very good and the acceptability of FTA card-based self-collection was pretty high [5, 6]. Therefore, future study should focus on the feasibility and accuracy evaluation of FTA-card based self-sampling in cervical cancer screening. There is new possibility that in the future women can self-sample at home, and mail the sample to the regional central laboratory for sample analysis. Doctors would only call back those with positive result for colposcopy examination. Consequently, limited healthcare resources could be centralized to high-risk populations and maximally increases the coverage of the current screening initiative.”

Comment 2.13#:
Explain the term ‘workflow’ in line 317 and line 324. What needs to be optimized as there is no previous mention in the material& methods or results section that the workflow is not optimal.

Re: According to the reviewer’s comment, we have added explanation in the discussion section with the following revision:” As the manufacturer didn’t have instructions for DCM-based sample detection, CICAMS developed the workflow according to several small pilot studies. Specifically, samples using for PCR amplification from FTA card actually had a much higher HPV concentration than that of DCM preservative solution (3.6% vs. 0.5%) to acquire a high sensitivity. As a result, a higher HPV positivity and a lower specificity were found for FTA card-based sample than that of DCM sample. Further study is needed to optimize the workflow of Cobas® 4800 test for FTA card-based sample to reduce the false positive and improve the specificity.”
Reviewer: Adriaan Dr van den Brule

Major Compulsory Revisions:
Comment 3.1#:
Authors use first DCM followed by second scrape for FTA use. Although they mention this as a limitation of this study, they might discuss this in more detail what the potential consequence might be. In my opinion the FTA results are potentially negatively influenced and the FTA results might even be better?

Re: We agree with the reviewer’s comment and added the following sentences in the study limitation part of the discussion section: “Fourth, we didn’t consider the sequence of sample collection. Taking two samples with a short time interval might have a negative impact on the accuracy of the second sample. However, the second sample collected on FTA card showed comparable accuracy of detecting CIN2+ with the first one, indicating a good capacity of sample capture for the FTA card. Future study should further evaluate the accuracy of these two sampling medium in a random sampling sequence to avoid any potential bias.”

Comment 3.2#:
FTA procedure is described in detail: please also briefly describe the DCM procedure as well as HC2, care HPV and Roche assay. In addition, the authors use 10x diluted DCM sample for Roche assay, and it is unclear to me why this was done. This might be a consequence of the lack of describing the procedures (in brief).

Re: According to the reviewer’s suggestion, we have added detailed information for DCM samples in the “Liquid Sample Detection” section with the following revision:”

HC2 is the first HPV DNA testing method approved by the United States Food and Drug Administration for clinical use which utilizes hybrid capture technology to detect 13 carcinogenic HPV genotypes. 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. careHPV™ is a newly-developed and promising screening system targeting low resource areas. Its workflow is similar but simpler than the HC2 test with a lower cost and simpler administration. careHPV™ test was done according to the manufacturer’s instructions as previously described [16]. A signal strength of 1.0 relative light units per positive control (rlu/pc) or greater was considered positive for both tests. The Cobas® 4800 test (Roche Molecular Systems, Pleasanton, CA) is a widely-used, PCR-based testing method with good clinical implications [17,18]. It can detect 14 types of high-risk HPV, and requires lower DNA copies for a positive result than that of the HC2 test. As the manufacturer didn’t have instructions for DCM-based sample detection, CICAMS developed the workflow according to several small pilot studies. DCM preservative solution was diluted 10 times with sterilized PBS buffer referring to careHPV™
which had approved instruction for DCM samples. Thereafter, DNA extraction and PCR amplification were performed according to the manufacturer’s instructions.”

Comment 3.3#:
Why were different punches used in FTA for careHPV and the other methods? Please describe the possible consequences of this for the comparative results.

Re: Thanks for reviewer’s comment. There were two reasons. First, HC2 and Cobas® 4800 test used the same buffer but careHPV™ used different one. Second, three HPV testing assays required different testing concentration. Therefore, different punches were used for different assays. We have added the explanations in “FTA Card Sample Detection” section with the following revision:” In order to acquire high accuracy, CICAMS performed several pilots with the help of GE Healthcare to develop testing protocols of FTA card-based sample for three HPV assays. FTA cards were punched using a specifically designed sterilized perforator (3-mm Harris Uni-Core device; Whatman, GE Healthcare, Buckinghamshire, UK). As HC2 and Cobas® 4800 test used the same buffer but careHPV™ used different one, HC2 and Cobas® 4800 tests shared 9 punched disks but careHPV™ test used another 6 disks to get the required testing concentration for different manufacturers of HPV testing assay.”

Comment 3.4#:
Why was no cytology performed? Is this no routine in China? Please discuss this as well.

Re: Thanks for reviewer’s comment. In developing countries like China where appropriate infrastructure is not achievable, screening procedure by gynecologists and cytologists were generally inefficient and unworkable. Expanding the coverage of objective HPV testing methods is a more expeditious and effective way for large-scale of cervical cancer screening. The government now is putting their efforts on changing the screening strategy from the subjective Pap or VIA to objective HPV testing for national cervical cancer screening as well. Therefore, the purpose of this study is evaluate whether the FTA card have comparable characteristics with the liquid sampling medium and whether it could be combined with three common HPV testing assays. As a result, we didn’t include cytology in this study. We have provided the explanation in the introduction section.

Comment 3.5#:
Discussion should be more focused and better organized based on the aims of the study.

Re: According to the reviewer’s comments, we rewrote the discussion section.

Comment 3.6#: 
Although it is of importance to study the clinical relevant performances if each test combination, no HPV prevalence is provided. So please include the HPV prevalence for each method and collection medium. Also discuss this difference in HPV detection in relation to clinical sensitivities observed. The calculations of PPV and NPV are unclear to me as shown in table 2. Could there be a bias in results due to the fact that besides HPV positive women only a random group of HPV negative women are followed up for colposcopy and biopsy based histology?

Re: Thanks for reviewer’s suggestion. We have added the HPV prevalence for each method and collection medium in Table 2 and description in the result section with the following revision:” The liquid sample showed comparable HPV positivity among HC2 (30.4%), careHPV™ (28.8%) and Cobas®4800 test (32.1%). FTA-based sample showed a lower HPV positivity than that of DCM medium for HC2 (22.8%) and careHPV™ test (23.9%), but a higher HPV positivity for Cobas®4800 test (39.4%). As a result, the FTA card demonstrated a much higher specificity for HC2 test than that of DCM medium (FTA vs. Liquid: 79.5% vs. 71.6%, P=0.015), but a relative lower specificity for Cobas®4800 test (FTA vs. Liquid: 62.4% vs. 69.9%, P=0.032). For the careHPV™ test, two sampling methods demonstrated comparable specificity (FTA vs. Liquid: 78.1% vs. 73.0%, P>0.05). For the other accuracy parameters, FTA and the conventional DCM sampling medium got comparable results.”

We agree with the reviewer that HPV positivity has great impact on the sensitivity, specificity, PPV and NPV. Therefore, we added explanations in the discussion section for HC2 and careHPV™:” FTA card-based sample was expected to be eluted to comparable HPV concentration as that of DCM preservative solution for HC2 and careHPV™ assay. However, concentration discrepancy still existed when transferring sample from solid to liquid, which resulting in a relatively lower HPV positivity and a higher specificity for FTA card-based sample.”

For Cobas®4800 test:” As the manufacturer didn’t have instructions for DCM-based sample detection, CICAMS developed the workflow according to several small pilot studies. Specifically, samples using for PCR amplification from FTA card actually had a much higher HPV concentration than that of DCM preservative solution (3.6% vs. 0.5%) to acquire a high sensitivity. As a result, a higher HPV positivity and a lower specificity were found for FTA card-based sample than that of DCM sample. Further study is needed to optimize the workflow of Cobas®4800 test for FTA card-based sample to reduce the false positive and improve the specificity.”

We also added the following sentences in the limitation section to explain the high HPV positivity:” As our study was based on referral population that was triaged for colposcopy, a higher HPV prevalence was found in this group than the general population, which also influenced the accuracy parameters of detecting CIN2+
lesions."

Comment 3.7#:
It seems to me that the FTA procedure is quite laborious; the advantage in transport seems to be largely compensated by the laborious lab procedure? Please address this in the discussion.

Re: Thanks for reviewer’s comment. This study explored experimental workflows for the careHPV™ and Cobas® 4800 tests and optimized the current workflow for the HC2 test. To provide scientific data for future laboratory investigations and repeat of the experiments, we made detailed description of the experimental procedures for FTA card. Lots of semi-automatic devices in this study such as perforator could be improved to automatic devices in future study, which will greatly save the human resources. According to reviewer’s suggestion, we added prerequisites for future population-based implementation of the FTA card in the discussion section:” This work also draws a promising picture for future large-scale cervical cancer screening project. As dried material on a solid carrier is neither hazardous nor inflammable, applying genital self-samples on FTA card can solve storage and transportation problems encountered in developing areas. With further optimization and automation of the experimental procedures, the FTA card in combination with careHPV™ could be used for cervical cancer screening in remote areas, and FTA card in combination with HC2 or Cobas® 4800 could be used in metropolitan areas.”

Comment 3.8#:
Please explain the higher specificity of the FTA? Is this due to removal of blood during processsing the sample?

Re: Thanks for reviewer’s comment. As response to Comment 6# addressed, the higher specificity of FTA card was due to the lower HPV positivity. FTA card-based sample was expected to be eluted to comparable HPV concentration as that of DCM preservative solution for HC2 and careHPV™ assay. However, concentration discrepancy still existed when transferring sample from solid to liquid, which resulting in a relatively lower HPV positivity. We have added the corresponding explanations in the discussion section.

Minor comments;
Comment 3.9#:
Describe company names and places in standard format throughout.

Re: Thanks for the reviewer’s comment. We have revised the company names and places in standard format throughout the manuscript.

Comment 3.10#:
Check English grammar and spelling throughout

*Re: Thanks for the reviewer’s comment. We have checked English grammar and spelling throughout the manuscript.*

Comment 3.11#:
Discussion: reduce and rewrite the passage where Dutch FTA results are compared to Chinese data.

*Re: According to the reviewer’s comment, we re-wrote the passage where Dutch FTA results are compared to Chinese data.*

Comment 3.12#:
Line 236-237: unclear, please rewrite

*Re: According to the reviewer’s comment, we deleted this sentence.*

Comment 3.13#:
.....hypothetical sample utilized...: what do authors mean with this? Please clarify in the text.

*Re: According to the reviewer’s comment, we rephrased this sentence with the following revision:*”As the manufacturer didn’t have instructions for DCM-based sample detection, CICAMS developed the workflow according to several small pilot studies. Specifically, samples using for PCR amplification from FTA card actually had a much higher HPV concentration than that of DCM preservative solution (3.6% vs. 0.5%) to acquire a high sensitivity.”