Reviewer's report

Title: Human Papillomavirus Testing by Novel FTA Card vs. Liquid-state Medium: Accuracy Comparison of Three Assays in a Population-based Cervical Cancer Screening Study

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Reviewer: Bart Hesselink

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Wang et al. describe a comparison between the FTA card and liquid-based cytology medium for HPV detection by three different HPV detection assays (HC2, careHPV, and COBAS4800). They show that the CIN2+ sensitivity by HPV testing is comparable for the two specimen storage methods, whereas the specificity for CIN2+ shows more variation between the methods and the HPV assays. Enclosed are my comments.

Major Compulsory Revisions

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.

Please also consider the items below in your response.

- 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.

- 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.

2) 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.

3) 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.

4) 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.

5) Include 95% confidence intervals for agreements results in line 197.

6) 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.

7) 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.

8) Discussion section is too long. Please shorten.

9) 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.

10) Since this study did not include self-sampling tune down statement in line 304-305.

11) 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.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests.