Author's response to reviews

Title: Comparison of CLART HPV2 genotyping assay to Linear Array and Hybrid Capture 2: a split-sample study

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COMPARISON OF CLART HPV2 GENOTYPING ASSAY TO LINEAR ARRAY AND HYBRID CAPTURE 2: A SPLIT-SAMPLE STUDY

Ditte Ejegod, Matejka Rebolj, Jesper Bonde

RESPONSE TO REVIEWERS

General response:

We thank the editors and reviewers for their comments. We addressed them point-by-point, as explained below. All changes in the manuscript are marked in gray.

Editorial requests:

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1) Please include e-mail addresses of each co-author on the title page.
2) Please rename contributor's section as "Authors’ contributions".
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Response: We included e-mail addresses and renamed the section as requested.

Reviewer:
CHUNXIA JING

Reviewer’s report:

This is very interesting topic, however, it need major compulsory revisions.
1# The title doesn’t cover the whole research contents: it just talk about comparison of three genotyping methods for HPV, but actually in full text, we also find the comparison for CIN detection.

Response: We changed the title to: Comparison of analytical and clinical
performance of CLART HPV2 genotyping assay to Linear Array and Hybrid Capture 2: a split-sample study.

2# In Statistical part, it is unclear about the criteria of detecting genotype in CLART method?
Response: To improve clarity, we added the following text:
The genotyping results were analyzed and reported automatically on the Clinical Array Reader (Genomica).

3# About relative prevalence, I don’t agree with the author. From Table, it is ratio of CLART vs LA.
Response: To avoid confusion, we added the following text:
The 95% CI for relative sensitivity and specificity, and for the relative prevalence (RP) of genotypes (CLART vs. LA), were calculated assuming that their logarithms were approximately normally distributed.

4# In the comparison of agreement between CLART and LA, it will be better to understand the consistence and difference in Table 2 as the following forma.

CLART
LA 16 18 31 33 .......
16
18
31
33
.....

AND

5# The similar revised should be done in Table 3.
Response: Our question was whether a particular genotype that was detected by CLART was also detected by LA. For this technical issue, we trust that the tabulation is sufficient as is. The tabulation proposed by the Reviewer would add information which other genotypes were also detected in samples with particular genotypes. This means that it would step into the area of HPV genotype epidemiology in the studied population, which was not the aim of this publication. We addressed this question previously in a general screening population from the same catchment area, see Goldman et al. Vaccine 2013.

6# Some important comparisons in Table 1 should be analyzed using statistical methods and tell the reader whether there are differences among different variables (age, cytology..) or not.
Response: We added the comparisons in Table 1. We added the following text:
- in Statistical analysis: Differences in the distribution of women’s characteristics for the three assays were calculated using the #2 distribution.
- In Results: On CLART and LA, the proportion of high-risk genotypes decreased by age and increased by the severity of the cytologic interpretation; on HC2, the trends were not statistically significant. The differences between CLART and LA were not statistically significant. Between CLART and HC2, some differences were seen, particularly by age where more women aged #30 years had high-risk HPV genotypes detected on HC2 than on CLART. The differences in the distribution of test results in women lost to follow-up were not statistically significant.

7#What are significances of data from both Table4 and Table5?
Response: 95% CI for differences were reported in the text under Results; although by request of Reviewer #2 this section has now been revised to present more detail (see below).

8#In line 239-242, how the author analyses the se of CLART, LA and HC2?
Response: As explained in the original text of Statistical analysis, clinical sensitivity of each assay was calculated as the proportion of high-grade CIN with a positive test result on the assay; clinical specificity was calculated as the proportion of women testing negative among those without high-grade CIN.

9#Why in your study, you use Surepath samples? Do you have any test results in general population?
Response: We used SurePath samples because only this cytology medium is used in the catchment area of our laboratory. Only one Danish laboratory (covering about 20% of the population) is currently using ThinPrep. Unfortunately, we do not yet have data for the general population comparing CLART to both HC2 and LA. However, a comparison between CLART and HC2 in a general population from the same catchment area was reported by Bonde et al. BMC Inf Dis 2014.

Reviewer:
Darrel Cook
Reviewer’s report:
This article compares the performance of two commercial HPV genotyping assays, CLART and Linear Array, against each other and the well-validated HC2 high-risk HPV screening assay, in a population of ~400 Danish women with abnormal cervical cytology. The article is clearly written, the research questions are well-defined and the data are sound. The findings of this study are similar to other reports in the literature with respect to these genotyping assays. The estimation of sensitivity and specificity of all three assays for CIN2 or greater diagnoses obtained over an average 17 month follow-up period is a strength of
this paper, as many similar studies examine only analytical comparisons between assays.

Response: We thank the Reviewer for his positive evaluation.

My major concern is related to the methodology description as noted below.

Major compulsory revision:

The authors state on several occasions that results were not statistically significantly different (e.g., lines 221, 224, 243, 246, 256, 262), but there is no description of the statistical methods used to arrive at these conclusions. The methods section should be updated to include the statistical tests/methods used. It would also be helpful to include the relevant p values in the results section.

Response: All statistical methods were explained in the original text in Statistical analysis. To avoid misunderstanding and add the requested information on statistical significance, we added the following text:

This was similar in 125 women with #CIN2 (treatment threshold in Denmark), with CLART detecting statistically significantly fewer HPV 45 infections than LA, RP: 0.35 (95% CI: 0.14-0.87; Table 3). CLART found single HPV infections in 130 (32%), and multiple infections in 235 (59%) women (Table 4). For LA, this was the case in 121 (30%) and 259 (65%), respectively. These differences were not statistically significant, RP for single infections: 1.07 (95% CI: 0.87-1.32). The # was 0.64, with an overall agreement of 81% (95% CI: 77-85). For detecting high- and low-risk infections (Table 5), the # was 0.76, with an overall agreement of 92% (95% CI: 88-94), and positive agreement (for detecting at least one high-risk genotype) of 92% (95% CI: 89-95). The differences in detecting high-risk infections overall (for detecting at least one high-risk genotype) were not significant, RP: 0.95 (95% CI: 0.89-1.02).

CLART detected 116 of 125 #CIN2 (sensitivity: 93%, 95% CI: 87-97), and 84 of 90 #CIN3 (sensitivity: 93%, 95% CI: 86-98; Table 7). LA detected 120 #CIN2 (sensitivity: 96%, 95% CI: 91-99) and 87 #CIN3 (敏感性: 97%, 95% CI: 91-99). HC2 detected 123 #CIN2 and 89 #CIN3, sensitivity 98% (95% CI: 94-100), and 99% (95% CI: 94-100), respectively. These differences, assessed through relative sensitivity (Table 7), were not statistically significant. Three women with cervical cancer tested positive for high-risk HPV on all three assays. The fourth woman tested negative on CLART, and positive on LA (genotype 39) and HC2. Given that all women had cytological abnormalities, the specificity of all three assays was low, but significantly higher (assessed through relative specificity) for CLART (30%, 95% CI: 25-36, for #CIN2) and LA (26%, 95% CI: 21-32) than for HC2 (17%, 95% CI: 12-22).

Minor essential revision:

In line 116, it is stated that HC2 negative ASCUS cases are routinely downgraded to normal cytology. Later, in line 121 it is stated that women with persistent ASCUS are referred to colposcopy. This should be clarified. Does persistent ASCUS refer only to those HC2 positive and persistent? How is
persistent ASCUS defined if an initial HC2 negative ASCUS smear is downgraded to normal and a later ASCUS result is obtained?

Response: We apologize for being unclear. Persistent ASCUS refers only to women below age 30, as explained in the revised text:

Women with HC2-positive ASCUS were referred for colposcopy, as were women with high-grade squamous intraepithelial lesions (HSIL), atypical squamous cells – cannot exclude HSIL (ASC-H), atypical glandular cells (AGC), adenocarcinoma in situ (AIS), cytological squamous carcinoma, and women with persistent ASCUS at age <30 years or low-grade squamous intraepithelial lesions (LSIL).