Author's response to reviews

Title: Comparison of the performance of carcinogenic HPV typing of the Roche Linear Array and Qiagen LiquiChip HPV assays

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Author's response to reviews: see over
Dear Editor,

We would like to thank you for giving us the opportunity to review our manuscript. You will find hereafter a point-by-point answer to reviewers’ comments, and a revised version of our manuscript “Comparison of the performance of carcinogenic HPV typing of the Roche Linear Array and Qiagen LiquiChip® HPV assays”.

We hope that our revised version will be suitable for a publication in BMC Infectious Diseases.

Best Regards,

Guillaume PENARANDA
Reviewer's report
Reviewer: Martin Steinau

Reviewer's report:
This manuscript describes the performance of the Qiagen LQ HPV genotyping test with respect to its clinical sensitivity for CIN2+ and analytically in comparison to the Roche Linear Array. The materials and methods are adequately described and the analyses appear to be satisfactory.

The following issues should be addressed in a Revision:

The sample population was recruited from referral colposcopy clinics and implies that the study addresses the clinical utility of this HPV genotyping test specifically for triaging women with abnormal pap test. The authors should state this clearly in the Background and possibly even in the abstract.

Authors: we considered this remark and added it in the Abstract and in the Background section

Abstract - Study Design: “The study population comprised 311 ASCUS+ women with abnormal pap test,…”

Manuscript – Last sentence of Background section: “However, no clinical validation has been performed in women aged >18 years old with abnormal pap test.”

Positive predictive value is stated as a major evaluation criteria under the Objectives, but not calculated and not taken into consideration in the discussion or conclusion. It looks like this value should be in table 2, but got omitted for some reason.

Authors: We agree with the reviewer and decided to add positive predictive value and specificity in the Table 2.

The discussion of type specific discrepancies needs improvement. The meaning of the sentence “All the cases where the listed HPV types were detected by Qiagen LQ … “ (p. 8, 199) is unclear.

Authors: we agree with the reviewer and the discussion was modified accordingly.

“LA and LQ tests were concordant in detecting the majority of HR-HPV genotypes except HPV35, HPV53, HPV59, and HPV73. The three prevalent genotypes, HPV16, HPV18, and HPV31, were found with a high concordance between LA and LQ: kappa 0.93, 0.83, and 0.91 respectively. These findings corroborate those reported by Godinez et al. [16].”

The referenced overestimation of HPV by Linear Array due to cross hybridization (p. 8, 202) is specific to HPV type 52 and may not explain the higher detection rates for other types.

Authors: We agree with the reviewer as Godinez et al reported overestimation of LA for HPV52 and HPV53. Anyhow we hypothesize it might be a reason why LA and LQ are different for few HPV types (35, 53, 59, and 73). We decided to clarify the sentence: “Godinez et al reported overestimation of LA for HPV52 and HPV53 because of cross-reactivity with other probes. We think it might explain the discrepancies between LA and LQ in detecting HPV35, HPV53, HPV59, and HPV73.”

One would expect that utility of type specific information (i.e. HPV16, 18) and clinical performance as illustrated in figure 1 and 2 would be discussed at some point.
Authors: we agree with the reviewer and figure 1 and 2 are now discussed.

The study population is relatively low for clinical evaluation and should be mentioned as a limitation.

Authors: This study is very specific according to its eligibility criteria. We do think that a cohort of 311 women is high enough in order to compare two tests. We decided to perform a multicenter study based in three European countries in order to obtain a precise screenshot of HPV prevalence according to two commercially available HPV tests. Anyhow we considered adding a sentence in the discussion taking into account this remark: “A limitation of this study might be its relative low number of patients included: the inclusion criteria of this study are specifics, thus it is not simple to include many more patients in a short time period.”
Reviewer's report
Reviewer: Magdalena Grce
Reviewer's report:
The revised manuscript is presented more clearly now, however there are still some issues to be clarified:

- How and where the histology was performed? I guess in respective collaborating centres but it has to be specified.
Authors: This was specified in Patients section

- The median age of 36 (18-79) of the study population is close to 40 therefore delete the sentence in line 83-84. Table 1 should be completed accordingly, add or replace mean age by median age.
Authors: Table 1 mean age was replaced by median age.
We think we should not delete sentence in line 83-84 because its an important issue. We performed our study in women aged over 18 whereas Godinez et al. performed their study in women aged over 40. Even if the median age of our study population is 36, it means that 50% are under 36, and 50% are over.

- Line 80: replace HRHPV by HR-HPV
Authors: ok

- Although the authors made an effort to present the discrepancies between tests they did not provide a plausible explanation for such extreme differences, they gave just hypothesis. In addition, the authors are comparing their findings by those of Godinez et al 2011, that are not quite similar, as they state, regarding discrepant findings between test. The only solution to clarify this issue is by using an alternative more specific genotyping test, i.e. type specific PCR for at least HPV types 35, 53, 59 and 73, or PGMY PCR product sequencing (not suitable for multiple HPV infection that are apparently very common in ASCUS specimens) that the authors should do and as such bring something novel in this study.
Authors: This remark was considered. The following sentences were added in Results section: “In house RT PCR test was performed on LA and LQ discordant cases. Among the 22 discordant cases, 21 were HR-HPV positive by LA and one was HR-HPV positive by LQ. Among the 21 HR-HPV positive by LA, nine were HR-HPV positive by RT-PCR (all HR-HPV types detected by RT-PCR were concordant with LA), and 12 were RT-PCR negative (and thus concordant with LQ). The case that was HR-HPV positive by LQ and negative by LA was also HR-HPV positive by RT-PCR (the same HR-HPV type was detected between LQ and RT-PCR). Thus among the 22 LA and LQ discordant cases, in house RT-PCR was in accordance with LA in nine cases, and with LQ in 13 cases.”

- The authors did not show the concordance of multiple HPV infections between tests. From these findings one can speculated about the cross-reactivity of particular probes, but not otherwise. Still the extreme differences have to be elucidated as mentioned above.
Authors: We agree with this remark and added the analysis of mixed genotypes in Table 3 and in Results section.

- Lines 184-185: Avoid repeating, so delete the first sentence of the discussion. 
  Authors: ok

- Lines 190-191: Start the sentence “HPV negative results using Roche LA assay were found in 4 (1%) cases, compared with 24 (8%) for Qiagen LQ assay.” With “Although all samples were HCII positive,... “ Only from this finding the author should be more critical toward the LQ test and not vice versa. 
  Authors: ok

- Lines 191-194: This sentence is unclear and should be rephrased. 
  Authors: Sentence was rephrased: “Among the 21 HPV positive cases detected by LA but not by LQ, there were nine cases in which LA detected HR-HPV types LQ was not designed for.”

- Lines 64 and 205: replace then by than. – The whole manuscript should be revised by a native English speaker. 
  Authors: ok

- Line 219 and elsewhere: Use the LA and LQ abbreviation all along the manuscript without additional specification. 
  Authors: Remark was considered

- Line 225: replace RLB by LA. RLB abbreviation is not necessary in this study. 
  Authors: ok

- Table 1: add or replace mean age by median age. How many cancer cases in each group there is if the percentage is negative? If there is one cancer then the percentage is 0.3. The tile should be Key characteristics of the study population. Insert a row with the HCII results. 
  Authors: Mean age was replaced by median age. The percentage of cancer was not negative but lower than 1%. We indicated (<1%) in the Table. Title was changed according to reviewer remark. We did not insert HCII results as they were all HPV positive, as indicated in patient criteria for eligibility.

- Table 2: Delete the 5th and 6th empty columns or insert Specificity (%) and PPV (%) values. 
  Authors: We added specificities and positive predictive values in Table 2.
- Insert a new Table showing multiple HPV infections found by each test.
Authors: We considered adding a row in Table 3 showing the number of mixed genotypes detected by each tests, and comparing LA and LQ accordingly.

- The legend of Fig. 1 and Fig. 2 should be identical, with or without lines.
Authors: Figure 2 was deleted from the manuscript.

- Figure 2: Shouldn’t the second columns correspond to HPV16-/HPV18+ instead of HPV16-/HPV18-, or is it HPV16-/HPV18-/other HR-HPV+? Anyhow, it is not clear what “various combination of HPV types” stand for? It should be clarified. In addition, as there is no statistical difference between CIN 2+ and CIN 3+, the A and B should be combined into one figure or simply deleted as Figure 1 is sufficiently showing the contribution of particular HR-HPV types.
Authors: We decided to clarify results shown in Figure 1 and 2. Figure 1 shows the absolute risk of CIN2+ (and CIN3 for figure1b), and Figure 2 shows the relative risk of CIN2+ (and CIN3 for figure 2b). Absolute risk is the risk of CIN2+ (or CIN3) when being HPVX without considering other HPV types. Relative risk is the risk of CIN2+ (or CIN3) when being positive or negative on two different HPV types. Changes were made in the manuscript.

- Competing interests: The authors declared no conflict of interest, while Qiagen is providing the reagents for this study, therefore, the authors are supported by Qiagen to perform this study and they should declare it as such.
Authors: Qiagen provided the reagents to perform this study, but we did not receive any funds by Qiagen or any other.