Author’s response to reviews

Title: HPV testing for cervical cancer screening: technical improvement of laboratory logistics and good clinical performance of the cobas 6800 in comparison to the 4800 system.

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HPV testing for cervical cancer screening: technical improvement of laboratory logistics and good clinical performance of the cobas 6800 in comparison to the 4800 system.

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BMC Women's Health

Point-by-point response to Reviewers’ comments

Collette Bromhead (Reviewer 1):

Whilst this paper details an adequate comparative study between two molecular assays for HPV on liquid based cytology samples, the design of the trial and the samples tested are not sufficient to lead to the conclusion given that the 6800 cobas HPV assay is suitable for use in large centralised laboratories i operating in population based cervical cancer screening programs, particularly not in this setting of HPV Primary screening. There is no mention of comparison to the Meijer criteria. There is no consideration for prediction of clinical disease, ie CIN2, CIN3.
And there is no examination of the difference in viral load assessed between the two assays, which some of your discordant results suggest may be considerable. I agree your results show promise and that the utility of the new automated system in the clinical laboratory is a big advantage over the cobas 4800 system which is highly manual by comparison. A higher quality study would have made this paper of considerable significance to laboratory users around the world who are facing the transition to HPV Primary screening and the increasing workload that entails. Unfortunately it seems the 6800 assay is quite different to the 4800 HPV test that achieved FDA approval for HPV Primary Screening off the back of the large Athena Trial in the USA. More work is needed to validate this new assay.

We thank Dr. Bromhead for her comments and considerations. In reference to the specific points raised:

1- Design of the trial and the samples tested:

The aim of the trial was to compare the HPV results and the workflow of the cobas HR-HPV DNA assay performed on the cobas6800 to those obtained on the cobas4800. Qualitative results (overall and by HPV type), Ct values, clinical sensitivity and specificity, and intra- and inter-laboratory reproducibility were evaluated. All the samples were from women older than 30 years attending organized cervical cancer screening. The samples included consecutive as well as selected specimens in order reach a sufficient number of A) women with high-grade lesions, to evaluate clinical sensitivity and specificity, and 2) frequency of HPV-positivity for evaluating reproducibility. According to the results of the randomized clinical trials (reflected by the recommendations of the European guidelines), the clinical sensitivity and specificity requested for HPV testing as primary screening test in cervical screening are set in order to inform on the HPV-related oncogenic risk and not on the presence of any HPV infection. In other words, in this context the HPV test is not a test for detecting all the HPV infections, and the comparative evaluations between the two systems are carried out on these premises (see also the answer to point 4, “Difference in viral load”).

2- Meijer criteria:

The study has been designed and conducted according to the Meijer’s criteria (only for intra-laboratory reproducibility, due to logistical reasons, the final sample size was slightly smaller than the planned one, i.e., 460 instead of 500); this is now stated in the Methods section of the abstract (page 2, line 26), in the Methods (page 5, line 87) and in the Discussion (page 11, line 220).

3- Prediction of clinical disease:

The recommendations of the Meijer’s criteria on the evaluation of the clinical sensitivity and specificity are fulfilled, as already reported in page 9, lines 172-175 (it was previously reported also in former Table 3, that has been deleted, according to the suggestion of the Reviewer 2).
4- Difference in viral load:

The Ct values of the HPV channels were slightly lower for cobas6800 than for cobas4800. The Ct values are proportional to the amount of HPV DNA in the sample but this is not a quantitative measure of the viral load. Differences in semi-quantitative results in this setting are relevant only if they actually determine a change in the positive/negative result, i.e., if the value passes the threshold (this sentence is now reported in the Discussion at page 11, lines 229-231). The differences recorded between the two cobas systems are indeed small; accordingly to the considerations made at point 1, in the Discussion it is now stated that “Overall, the minor differences that emerged at the analytical level (i.e., Ct values) had no major impact on the clinical performance” (page 11, lines 228-229). Scatter plots of the Ct values for the three HPV channels displayed by cobas6800 in comparison to coba4800 and for samples assayed by cobas6800 for intra- and inter-reproducibility evaluation are now provided (Figure 1; page 10, lines 187-189).

5- Differences between cobas4800 and cobas6800:

The cobas4800 and cobas6800 HPV assays search for the same HPV types, use the same primers/probes, provide partial genotyping for HPV16 and HPV18 and are based on the same methodology (real-time PCR), with some differences in the Thermal Cycling (CT) profile since the assays on the cobas6800/8800 run on a universal thermal cycling profile to allow for mixed batching of different PCR tests (see Cobb B et al. Expert Rev Mol Diagn 2017;17:167-80, ref. 12 of our paper). This is now specified in the Methods section (page 7, lines 123-126). Another difference regards the elution volume that is 150 µl for cobas4800 and 50 µl for cobas6800/8800, out of a 400 µl processed aliquot, as already reported in the Methods section (page 7, lines 127-128); this implies that a higher amount of target sequences is analyzed by cobas6800 than by cobas4800. The HPV assay run on the cobas6800 system is therefore quite similar to the cobas4800 HPV assay, and the highly concordant results of our study demonstrate both clinical validation and reproducibility (a clinical trial is underway in the US to seek FDA approval). Moreover, the technical improvements of several instrumentation features displayed by the cobas6800 system translate into higher performance in workflow and laboratory productivity, that represent important determinants to guarantee the workload of a centralized laboratory.

Wen Chen (Reviewer 2):

This study evaluated the agreement of cobas4800 and cobas6800 for detecting HPV and performance of clinic sensitivity and specificity on cervical cancer screening. The intra-laboratory and inter-laboratory reproducibility for cobas6800 were studied, too. Several issues need to be clarified:

We thank Dr. Chen for his comments and considerations.
1. According to manuscript, the normal sample for cobas680 HPV test is around 6 months old but 3 years old for the abnormal. How long is the PreservCyt sample used for cobas4800 HPV test?

The reviewer is right; negative samples were tested within 6 months (as indicated by the manufacturer for PreservCyt samples, both for cobas4800 and cobas6800 systems) and positive samples up to 3 years after collection. It is important to note that testing for the present study of the samples stored for longer than 6 months were made at the same moment by both cobas4800 and cobas6800 systems. So samples’ storage time could not constitute a bias. Furthermore, testing after longer periods has been previously evaluated by others; consistent results have been obtained up to 2.5 years, as published by Agreda et al in 2013 (J Clin Microbiol 51:2702-2706); unpublished data on HPV retesting of archived ATHENA specimens (some up to 5 years old) performed within the Dual Stain (Cintec PLUS) study (Wright et al. Gynecol Oncol 2016) showed concordant results to the initial testing. In our study, the number of samples tested after more than 6 months is very small, being 25 overall. These samples have been re-tested by cobas4800 just before testing in cobas6800; all samples gave a valid result (1 was invalid only for the HPV channels previously negative) with overlapping qualitative results and Ct values, and were deemed suitable for inclusion in the study. This is now reported in the Methods (page 6, lines 110-113).

2. In view of 50 cycles for cobas4800 is applied. I am not sure if it is also 50 cycles for cobas6800. If yes, it is better to output all the ct value less than 50. Scatter plots of ct value can be draw for comparison between cobas4800 and cobas6800 by HPV16, HPV18 and Other types, which can facilitate evaluation of the impacts of storage time and the two technologies' difference in more details.

Regarding the Thermal Cycling (CT) profiles of the two systems, we know that they somehow differ because the assays on the cobas6800/8800 run on a universal thermal cycling profile to allow for mixed batching of different PCR tests (see Cobb B et al. Expert Rev Mol Diagn 2017;17:167-80, ref. 12 of our paper), but we don’t know more specific details. In both systems the Ct values of the positive samples are comprised in the 16-to-40 range. Scatter plots of the Ct values for the three HPV channels displayed by cobas4800 and cobas 6800 are now provided for all positive samples (Figure 1, panel A; page 10, lines 187-189).

3. Scatter plots of ct value are also needed for intra-lab agreement evaluation between cobas6800 HPV tests, inter-lab agreement evaluation.

The scatter plots of the Ct values for the three HPV channels obtained for samples assayed in cobas6800 for intra- and inter-reproducibility evaluation are now provided (Figure 1, panels B and C; page 10, lines 187-189).

4. Table3 is not necessary, suggest delete. Clinic sensitivity and specificity can be described in the manuscript.
Thank you for your suggestion; Table 3 has been deleted.