Reviewer’s report

Title: Dynamic comparison between Daan real-time PCR and Cobas TaqMan for quantification of HBV DNA levels in patients with CHB

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Reviewer: Bruno Pozzetto

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This paper presents an interesting comparison between two techniques for quantitating HBV DNA in serum samples of patients with chronic hepatitis. The authors conclude that the Daan test is less sensitive than the Cobas Taqman assay and, consequently less adapted for the surveillance of patients under antiviral treatment.

The paper is easy to read and the conclusions are interesting from a practical point of view. However, it suffers of major methodological limitations that are detailed just after.

1) The design of the study is not clear. The number of centers participating to the study together with the number of patients included in each center must be mentioned in the Methods section and not in the Results. It is not clear to identify which patients received lamivudine-adenovir (an association not recommended by American and European guidelines) or entecavir? How many subjects were treated by each regimen? On which criteria? Were they randomised? Did this factor influence the response at 24 weeks after the beginning of the treatment? Another important point concerning the design of the study regards the realisation of the two tests. Were they performed simultaneously and retrospectively on the 134 sera by both tests in a same center? Were the two tests performed on a routine basis in each center at the time of sampling? Were paired sera tested simultaneously? All of these strategies are not equivalent and can influence the global comparison of the results.

2) The analysis of the Results as described in Table 2 is not pertinent. The reviewer does not understand the justification of the four categories proposed for comparing the patients’ viral loads by the two tests, notably regarding the limits of 7 and 3 log10 IU/ml. Why was the Cobas Taqman chosen as the gold standard for this categorisation? From a methodological point of view, the two series of 67 serums from the same patients cannot be considered as independent results and thus cannot be simply added. It would have been better to analyse the average drop in viral load from the 33 patients who exhibited a detectable viral load by both tests between D0 and W24.

3) The analysis of undetectable results is not satisfying. A sample positive under the limit of 100 or 10 IU/ml according to the test has not the same value than a sample totally negative. It would be interesting to precise how many samples...
noted “undetectable” by the Daan test but positive by the Cobas Taqman assay were detected under the limit of 100 IU/ml by the Daan test.

4) For Figure 1, the negative results must be removed. The correlation can only be performed on samples tested positive by both techniques. Again, it would be more useful to correlate individually the drop in viral load from the 33 patients who exhibited a detectable viral load by both tests between D0 and W24.

5) The numbers of patients with total, partial or absence of virological response after treatment according to both tests, which are presented in the Discussion section, must be reintegrated in the Results. For categorising the patients, it could be more pertinent to consider patients with a positive PCR signal under the threshold of quantitation as partial responders.

6) Concerning the agreement analyses performed by Bland-Altman plots, the reviewed cannot see any difference between the set of results at baseline and at week 24. Despite a translation in results at baseline, probably due to the better sensitivity of the Cobas Taqman assay, the agreement between the two tests was satisfying for the two sets of results. Figures 2 and 3 must be incorporated in a single figure with two panels.

7) The Discussion section must be rewritten in the light of the new analysis of results as suggested above. A cost-effective analysis must also be performed. If the Daan test is much more cheaper than the Cobas Taqman one, it could be cost-effective to use the Daan test for screening the patients and to retest paired sera by Cobas Taqman from the sole patients showing a negative result (neither quantified nor detected) by the Daan test. The summary must be corrected accordingly.

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

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.