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Using a commercial diagnostic assay requires compliance with the manufacturer's recommendations

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To the Editor:

We acknowledge the exhaustive overview on the Hepatitis B core-related Antigen (HBcrAg) performed by Adraneda and colleagues, recently published in Journal of Hepatology [1]. We congratulate the authors for covering a broad range of publications on this interesting new biomarker for the hepatitis B virus. We agree with the author's conclusion regarding the questionable utility of HBcrAg over HBeAg quantification in HBeAg positive patients, as the reactivity is predominantly due to HBeAg detection. This finding was recently confirmed by our work and in line with the report of Hong et al. [2,3]. Thus, restricting the use of this assay to HBeAg negative patients should be strongly recommended. Unfortunately, when targeting HBeAg negative cohorts, there is a risk of false positive results, as correctly stated by Adraneda et al. [1]. The specificity of this assay has not been extensively studied but data from Van Halewijn et al. on a small number of samples indicate a false positive rate of approximately 10% [4]. These results have also been observed in our laboratory (unpublished data). However, we are deeply concerned by the incorrect use of the Fujirebio HBcrAg reagent in numerous published works, which should therefore not have been taken into consideration in Adraneda's review. It is appalling that results are generated and published without following the manufacturer's recommendations. Indeed, in the original publication and assay notice, it is clearly indicated that the analytical sensitivity of the reagent is 3 log U/mL (1000 U/mL), with a dynamic range of 3 to 7 log U/mL [5]. Therefore, all publications of the Adraneda's review reporting values below 3 log U/mL should not be considered as they do not conform to the proper methodology. Considering values below 3 log U/mL incurs the risk of taking into account at least 10% of false positive results and unreliable quantifications out of the assay's linearity range. We believe all correlations that include HBcrAg values under 3 log U/mL are incorrect. Unfortunately, many references cited by Adraneda in the supplementary files, fall into this transgression. Not only does this issue represent a serious basic deviation from rigorous methodological procedures, it also introduces substantial bias in the correlation factors with other hepatitis B biomarkers and their subsequent interpretations. The consequences may be less obvious for studies aiming at defining a clinical threshold. Indeed, most defined thresholds reported in the review are above 3 log U/mL and false positive results or incorrect quantifications may not significantly modify the overall interpretation of these studies. Nevertheless, taking into account any value under 3 log U/mL displayed by the system, leads to absurd thresholds between 2 and 3 log U/mL (as found in ref. 16-table 3C or 25-table 3D), values that should not be interpreted.

In conclusion, the main principle to apply when using a biomarker is to conform to the assay specifications as recommended by the manufacturer. As many publications using HBcrAg quantification did not follow this basic rule, any summarizing work should apply a stringent selection process to convey an unbiased message not relying on skewed values.

This beforehand screening is mandatory to help clinicians identify a new biomarker based on robust data. While HBcrAg quantification may not be informative in HBeAg positive patients, its clinical value to characterize the HBeAg negative stages of HBV infection deserves further well-conducted studies.

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