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LETTER TO EDITOR

Borderline Results of Diagnostic Real-Time PCR Assay for Hepatitis B Virus DNA

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The National Institute of Health's (NIH) workshop, "Management of Hepatitis B," has proposed the HBV DNA level of 10^5 copies/ml (20,000 IU/mL) as the point of differentiation of chronic hepatitis B from the inactive carrier state [(1)]. Optimal management of chronic hepatitis B requires the use of PCR assays to establish a baseline HBV DNA level and then the use of PCR assay antiviral therapy for monitoring the response and viral rebound associated with viral resistance [(2)]. Although accepted by most authors, some authors have challenged this opinion [(3)]. Quantitative real-time PCR is a reproducible and accurate method for viral load measurement with a very large dynamic range of starting-target molecules (10–10^8). Determination of starting-target molecules is usually accomplished by fitting a regression line to the mean CT (threshold cycle) values obtained for each standard deviation [(4)]. As with other laboratory tests, some variation is expected to occur in real-time PCR results. There are many sources of variability: DNA extraction, pipetting, thermal variation, fluorescent noise, and the regression [(5)], to name just a few. This variation can be expressed as a confidence interval of the result, which is calculated based on the desired confidence level (Type I error) and the coefficient of variation (CV) of the test at the result point [(6)]. When the boundaries of the confidence interval violate a cutoff value or decision point, the test is interpreted as borderline. In these cases the outcome of a repeated test can be precisely calculated statistically using the CV of the test at that point. For example, given a decision point of 20,000 IU/mL for antiviral treatment, a value of 15,000 IU/mL with a confidence interval of 13,000–17,000 IU/mL has a different meaning than a value of 15,000 IU/mL, with a confidence interval of 9,000–21,000 IU/mL (Fig. 1). Moreover a change in viral load from 21,000 IU/

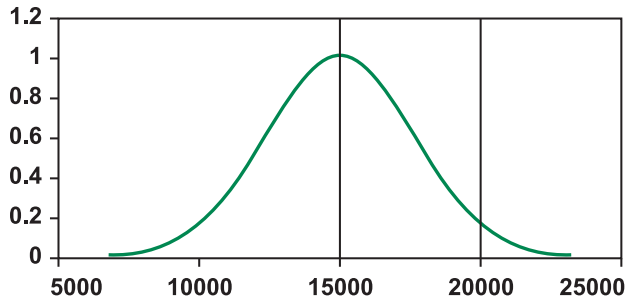

Figure 1. The confidence interval violates the cutoff point. Although the result is considered borderline, the assay result is much lower than the cutoff point. The appropriateness of categorizing the patient as low titer is computed by the area under the Gaussian curve, on the side of the mean.

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mL to 19,000 IU/mL may not necessarily indicate a change in the patient's status when the range in the confidence interval is 2000 IU/mL. Switching from a manual to an automated method usually lowers the variation in the test and generates less borderline results. However, the problem of borderline results cannot be eliminated altogether. In this study, the variability of a real-time PCR assay for HBV-DNA at the cutoff of 20,000 IU/mL was computed, and the magnitude of borderline results was determined accordingly.

Quantitative real-time PCR is routinely performed in our laboratory for HBV DNA upon request. The standard deviation and CV of the test at 20,000 IU/ml were determined by assaying one sample in 6 different runs using a Qiagen commercial HBV kit, and the values were 3187 IU/ml and 15%, respectively. Twenty-nine patients were chosen and the relevant data were retrieved from the computer system. The patients were categorized into low- and high-titer groups, with 20,000 IU/mL as the cutoff point. Confidence intervals and the rate of violation of cutoff points were calculated using a statistical package (Microsoft Excel 2007). The categorization of the patients was done based on the assay value and the cutoff point (20,000 IU/mL). When the confidence interval violated the cutoff value, the appropriateness of the categorization was computed by the area under the Gaussian curve, on the side of the mean (Fig. 2).

Twenty-one patients (72%) were male, and the rest (28%) were female. Their ages ranged from 17 to 75 years with a mean and standard deviation of 40.67 and 15.5 years, respectively. The results ranged from undetectable to 181,415,287 IU/mL. Three out of 29 participants (10%) were found to violate the cutoff value of 20,000 IU/mL (Table 1). Although the confidence interval of the assay results in Case 1 violated the cutoff value, the probability of this result being low titer was about 97%, which was still quite high and thus this patient could be confidently categorized as low titer. The two other values were considered borderline results.

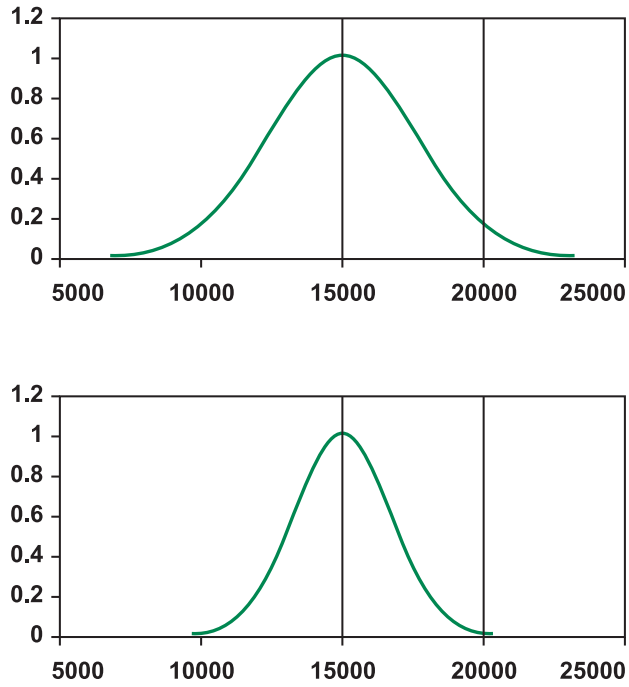

Figure 2. Two different assay results with the same value (1500 IU/ml) but which should be interpreted differently due to different CVs. In the upper pane, the area under the curve on the right side of the cutoff point (gray area) is more than 5%, and therefore this result is considered borderline. This is not the case, however, for the result in the lower pane, which has a lower CV.

Most laboratories do not include confidence intervals in their final reports; consequently, the clinician is not able to distinguish between definite and borderline results. When uncertain, a number of clinicians repeat the test to find out whether the result is borderline or not. This is costly, time consuming, unnecessary, and ultimately does

Table 1. Patients' results with confidence intervals violating the cutoff point of 20,000 IU/mL. The first result (No. 1) is categorized as low titer because the result is lower than the cutoff point. The appropriateness of this categorization is about 97%, which is quite high. Two other results are categorized as high titer, but the degree of certainty is lower than the first one.

No.	ID	Result (IU/mL)	Confidence Interval (IU/mL)	Appropriateness[1]
1	127	7,427	1991–21711	97%
2	138	24,420	18865–31612	65%
3	143	14,811	5107–42957	75%

[1] The area under the Gaussian curve, on the side of the mean.

not have to be done if the confidence interval is provided. Additionally, the outcome of the repeated test can be more precisely calculated statistically using the CV of the test at the decision point. Therefore, repeating the test would be a waste of money and time. The option that exists is to have the technician examine the result, use the CV to predict the appropriateness of the categorization, and mark the result with a comment (*e.g.*, "Although the confidence interval for the result from this patient violates the cutoff value, the CV suggests that this result is best categorized as . . .").

A drawback of the current study is the limited number of samples used to calculate the standard deviations. Therefore, the CV might not represent all sources of assay variability. However, the idea is still conveyed. In conclusion, reporting the confidence interval along with the result value seems logical and might assist clinicians in distinguishing between definite and borderline results. In a truly borderline case, the appropriateness of the categorization can be predicted using the assay's CV. These two considerations ought to lead to a reduction in test reordering.

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