Could Droplet Digital PCR Be Used Instead of Real-Time PCR for Quantitative Detection of the Hepatitis B Virus Genome in Plasma?

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Droplet digital PCR (ddPCR), which has been recently developed to provide an absolute quantitation of target molecules without relying on the use of standard curves (1), might be an interesting alternative to conventional real-time PCR assays used for viral load (VL) determination (2, 3).

According to European guidelines, the goal of hepatitis B virus (HBV) infection treatment in chronic carriers is to reduce the level of HBV DNA to below 10 to 20 IU/ml (4). This threshold has been established on the basis of the lowest detection limits of currently available assays. However, a method able to accurately quantify viral replication with an improved sensitivity would certainly be relevant to precisely document viral clearance.

To assess the capacity of ddPCR to detect low levels of HBV DNA in comparison with real-time PCRs, we submitted 29 samples with low HBV DNA levels retrieved from the French HBV blood donor survey data bank (5) to this method (ddPCR; Bio-Rad, Marnes la Coquette, France). Among these samples tested with the High Pure System/COBAS TaqMan HBV assay (Roche Diagnostics, Meylan, France; limit of quantification of 6 IU/ml), 17 were detectable with VLs below 6 IU/ml, and 12 had VLs that ranged from 6 to 15 IU/ml.

All samples were also tested with Abbott HBV RealTime assay (Abbott Molecular, Rungis, France; range of quantification from 10 to 1 billion IU/ml) and a quantitative in-house real-time PCR (qHPCR) with a limit of detection of 20 IU/ml (6). A sample with 5×10^6 IU/ml HBV DNA (Abbott Molecular) was used as a positive control.

The ddPCR reaction consisted of a mix provided by Bio-Rad, HBV primers/probe used for qHPCR with an adapted annealing temperature procedure (6), and nucleic acid purified with an Abbott M2000sp system.

Each sample was tested in duplicate in the same run with a QX100 droplet digital PCR system (Bio-Rad). The results expressed as copies were converted into IU, considering 3.5 copies as 1 IU, according to the conversion factor recommended in the Abbott procedure.

Among the 29 tested samples, 93.1%, 72.4%, and 48.3% were HBV DNA positive, with mean VLs for positive samples at 21.1 ($n =$

27), 30.5 ($n = 21$), and 13.9 ($n = 14$) IU/ml for Abbott, qHPCR, and ddPCR assays, respectively (Table 1). The comparison of qHPCR and ddPCR, which were based on the same PCR, of the 11 samples which were positive with both methods shows a slight difference ($P = 0.07$) between the mean VLs (36.9 and 11.6 IU/ml).

Of note, this relative underquantification using ddPCR was also observed on the positive control with a mean value of 2.7×10^5 IU/ml, while expected at 5×10^6 IU/ml. As previously described for cytomegalovirus (CMV) (7), even though carried out on a limited number of samples, our study shows that real-time PCR assays used for low HBV DNA levels remains more sensitive than ddPCR. Hence, as recently reported (8), optimization of this method is still needed, especially to delineate between positive and negative results and to accurately calculate the copy numbers, particularly in samples with very low levels of target DNA molecules. In addition, further studies on clinical samples will certainly help define the practical utility of this approach.

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TABLE 1 Comparison of three assays for HBV DNA detection and quantification

Assay	Cobas Taq Man HBV ^a					
	<6 IU/ml (17 samples)		6–15 IU/ml (12 samples)		Total (29 samples)	
	No. (%) positive	Mean VL (range) in IU/ml	No. (%) positive	Mean VL (range) in IU/ml	No. (%) positive	Mean VL (range) in IU/ml
Real Time Abbott	15 (88.2)	15.4 (10–24)	12 (100)	25.7 (10–46)	27 ^b (93.1)	21.1 (10–46)
qHPCR	11 (64.7)	26.6 (4–52)	10 (83.3)	34.8 (6–77)	21 (72.4)	30.5 (4–77)
ddPCR	6 (35.3)	12 (8.6–18)	8 (66.7)	15.3 (7.7–40.4)	14 (48.3)	13.9 ^b (7.7–40.4)

^a Proportions of positive samples and mean VLs were compared using Chi-square test and Student test, respectively.

^b Significant difference ($P < 0.05$).

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