Supplementary Information

Sequence-encoded Quantitative Invader assay enables highly sensitive hepatitis B virus DNA quantification in a single tube without the use of a calibration curve

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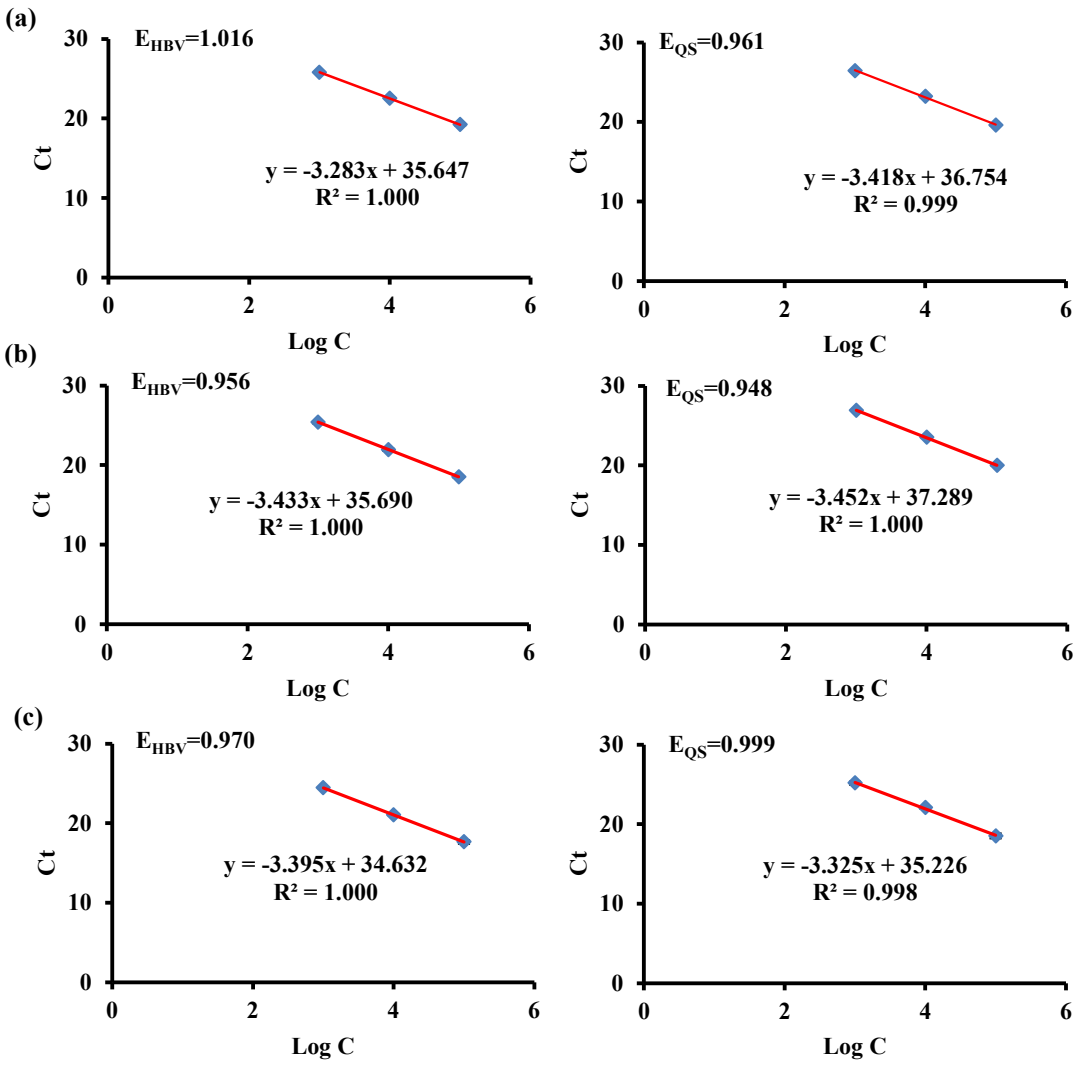

Fig. S1 Amplification efficiencies of HBV-DNA (left-side) and QS-DNA (right-side) in a 20-μL reaction system by amplifying the S gene (**a**), C gene (**b**) and simultaneous amplifying multiple genes (**c**). N=3.

5’- CTG GAT GTG TCT GCG GCG TTT TAT CAT CTT CCT CTT CAT CCT GCT GCT
ATG CCT CAT CTA CTT CTT GGT TGT TCT GGA CTA TCA AGG TAT GTT GCC
CGT TTG TCC TCT AGG AGG CTG TAG GCA TAA ATT GGT CTG TTC ACC AGC
AGC ATG CAA CTT TTT CAC CTC TCC GTA ATC TTC TCT TGT TCA TGT CCT
ACT GTT CAA GCC TCC AAG CTG TGC CTT GGG TGG CTT TG -3’

Fig. S2 Sequence of the artificially designed quantitative-standard. The underlined sequences were identical or reverse complementary to the DP-QS probe. The boxed bases were altered based on the HBV-DNA template according to Watson-Crick base pairing.

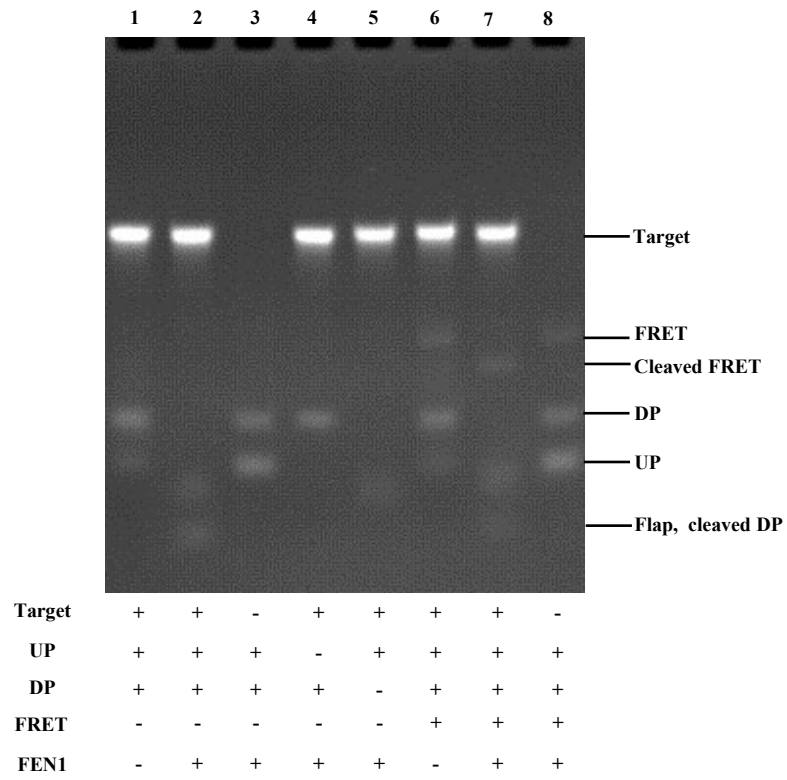

Fig. S3 Electrophoregram of products from the invasive reaction with various components. Lane 1: invasive reaction without FEN1, lane 2: normal invasive reaction, lane 3-5: invasive reaction without target, UP and DP, respectively, lane 6: cascade invasive reactions without FEN1, lane 7: normal cascade invasive reactions, lane 8: cascade invasive reactions without target.

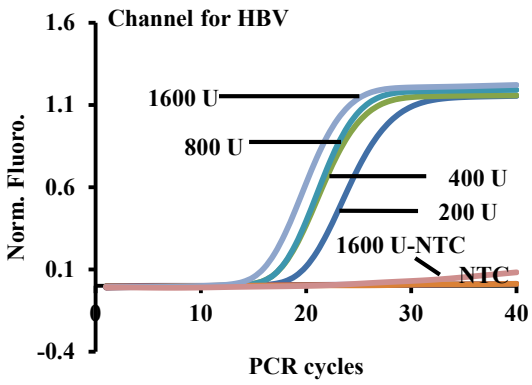

Fig. S4 Amplification profiles of 1×10^3 copies of HBV-DNA in a 20-μL reaction system by adding different amounts of FEN1 (200 U, 400 U, 800 U and 1600 U). NTC was no target control, the reaction without a target.

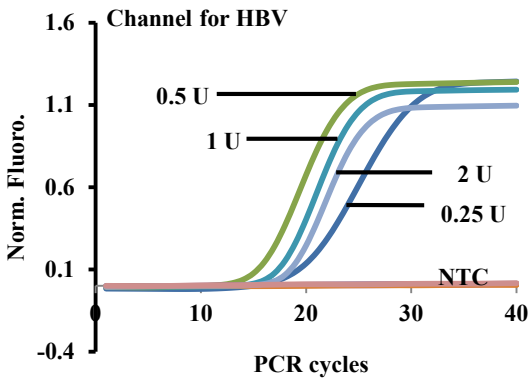

Fig. S5 Amplification profiles of 1×10^3 copies of HBV-DNA in a 20-μL reaction system by adding different amounts of *Taq* DNA polymerase (0.25 U, 0.5 U, 1 U and 2 U). NTC was no target control, the reaction without a target.

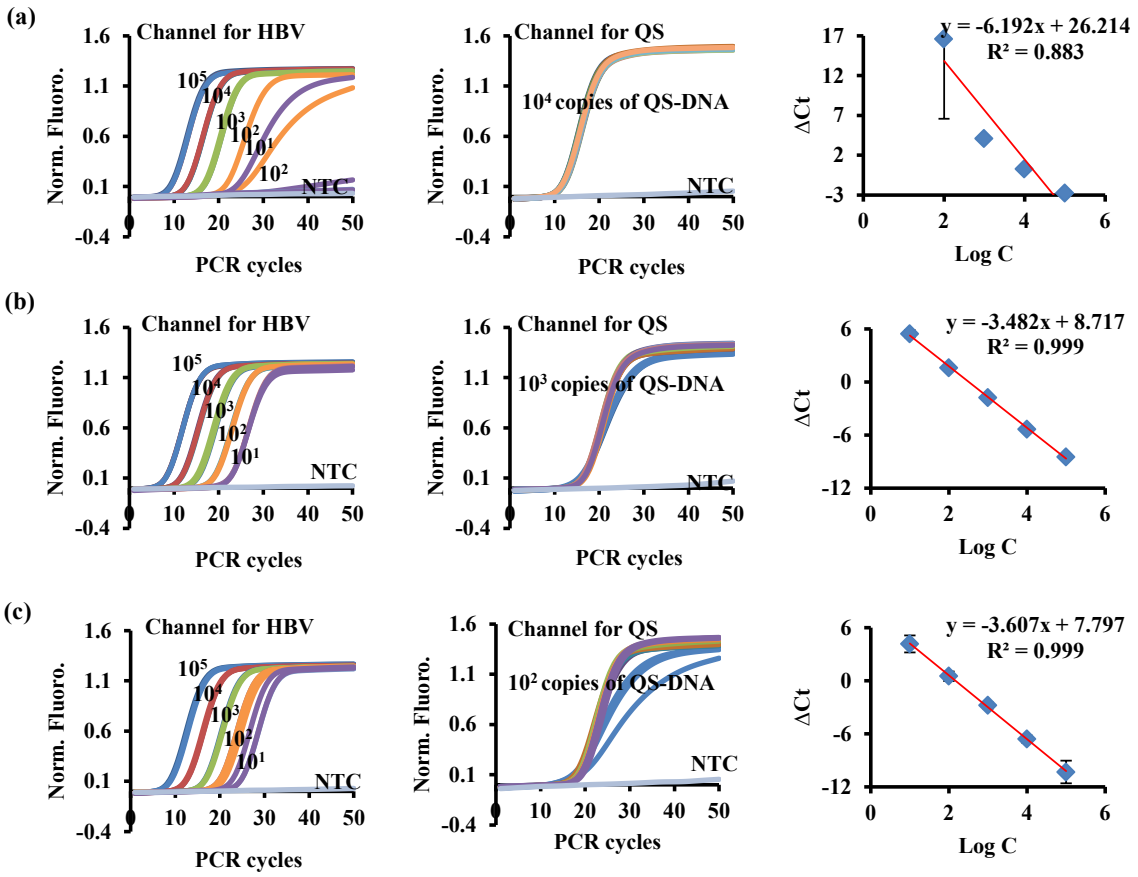

Fig. S6 Amplification profiles from the channel for HBV-DNA (left side) and the channel for QS-DNA (middle) by individually spiking 1×10^4 (**a**), 1×10^3 (**b**), and 1×10^2 (**c**) copies of QS-DNA into the tubes with HBV-DNA ranging from 10 to 1×10^5 copies in a 20-μL reaction system, and the quantitative relationship between ΔCt and the Log C (right side). NTC was no target control, the reaction without a target. N=3.

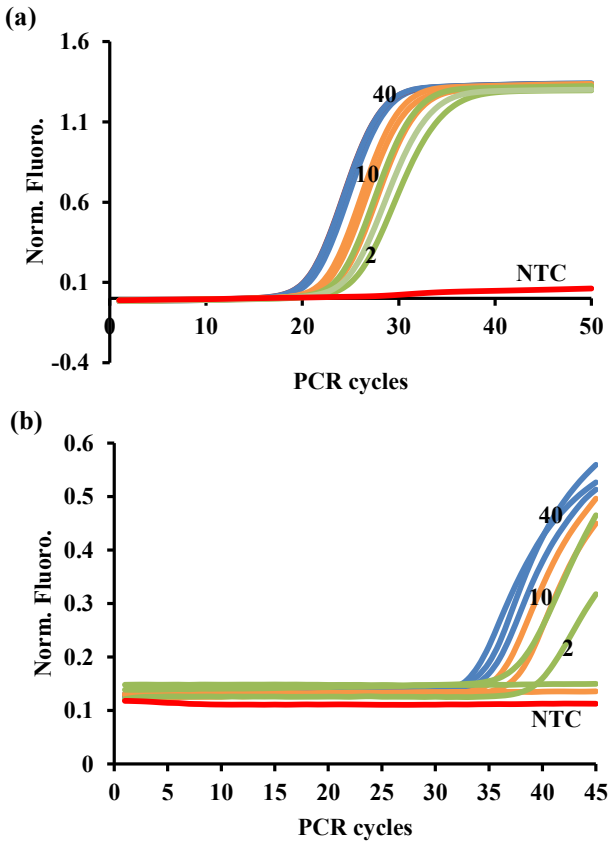

Fig. S7 Amplification profiles of sQ-Invader (**a**) and TaqMan probe-based qPCR (**b**) for detecting HBV-DNA ranging from 2 to 40 copies per reaction. NTC was no target control, the reaction without a target. N=3.

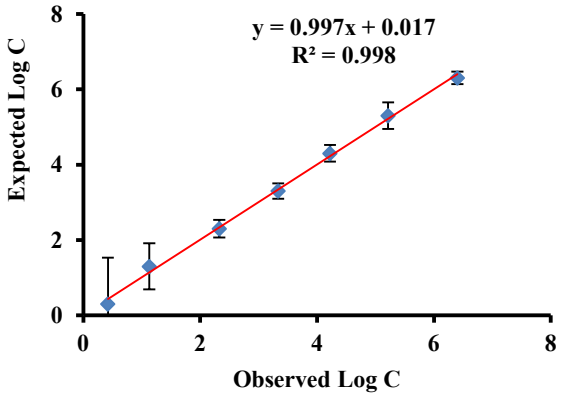

Fig. S8 Relationship between the expected concentrations and the observed concentrations of HBV-DNA by using Equation (6) for quantification. N=3.

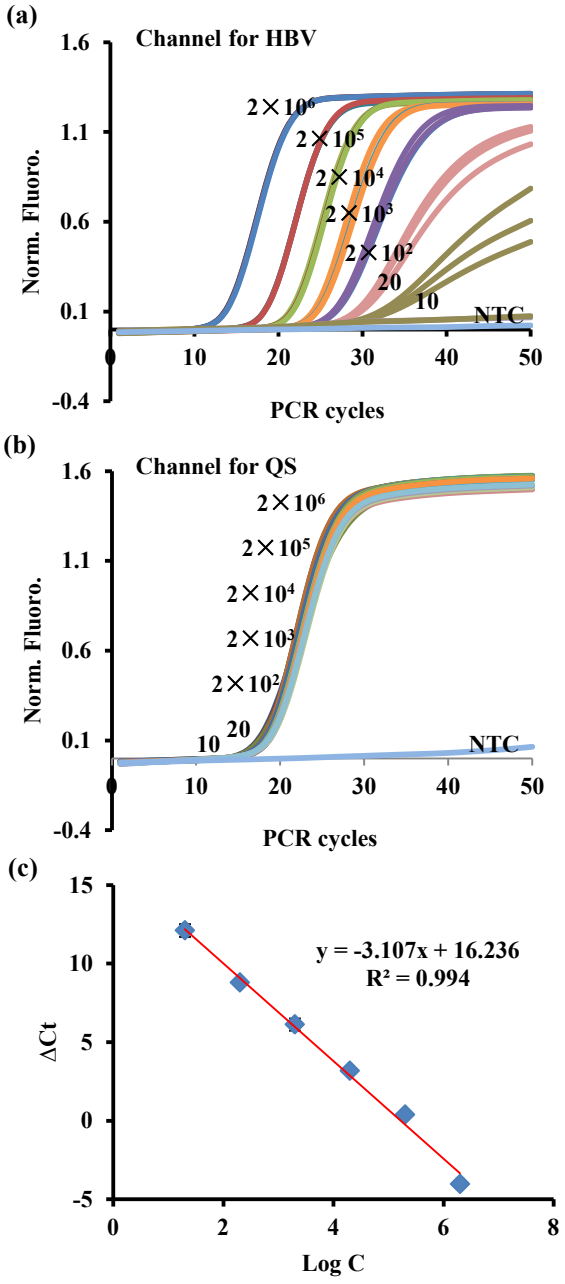

Fig. S9 Amplification profiles from the channel for HBV-DNA (**a**) and the channel for QS-DNA (**b**) by spiking 4×10^3 copies of QS-DNA into the serum with HBV-DNA ranging from 10 to 2×10^6 IU mL^{-1} before extraction, and the quantitative relationship between the serum HBV loads (log C) and ΔCt (**c**). ΔCt was defined as the difference between the Ct values of HBV-DNA and QS-DNA in the reaction system. NTC was no target control, the reaction without a target. N=3.

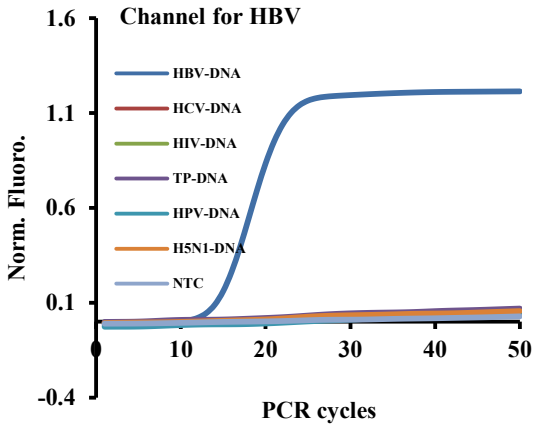

Fig. S10 Amplification profiles from the channel for HBV-DNA by spiking 1×10^4 copies of various plasmid DNAs of other virus genes into the HBV-negative human serum, including HPV, HCV, HIV, TP and H5N1. NTC was no target control, the reaction without a target.

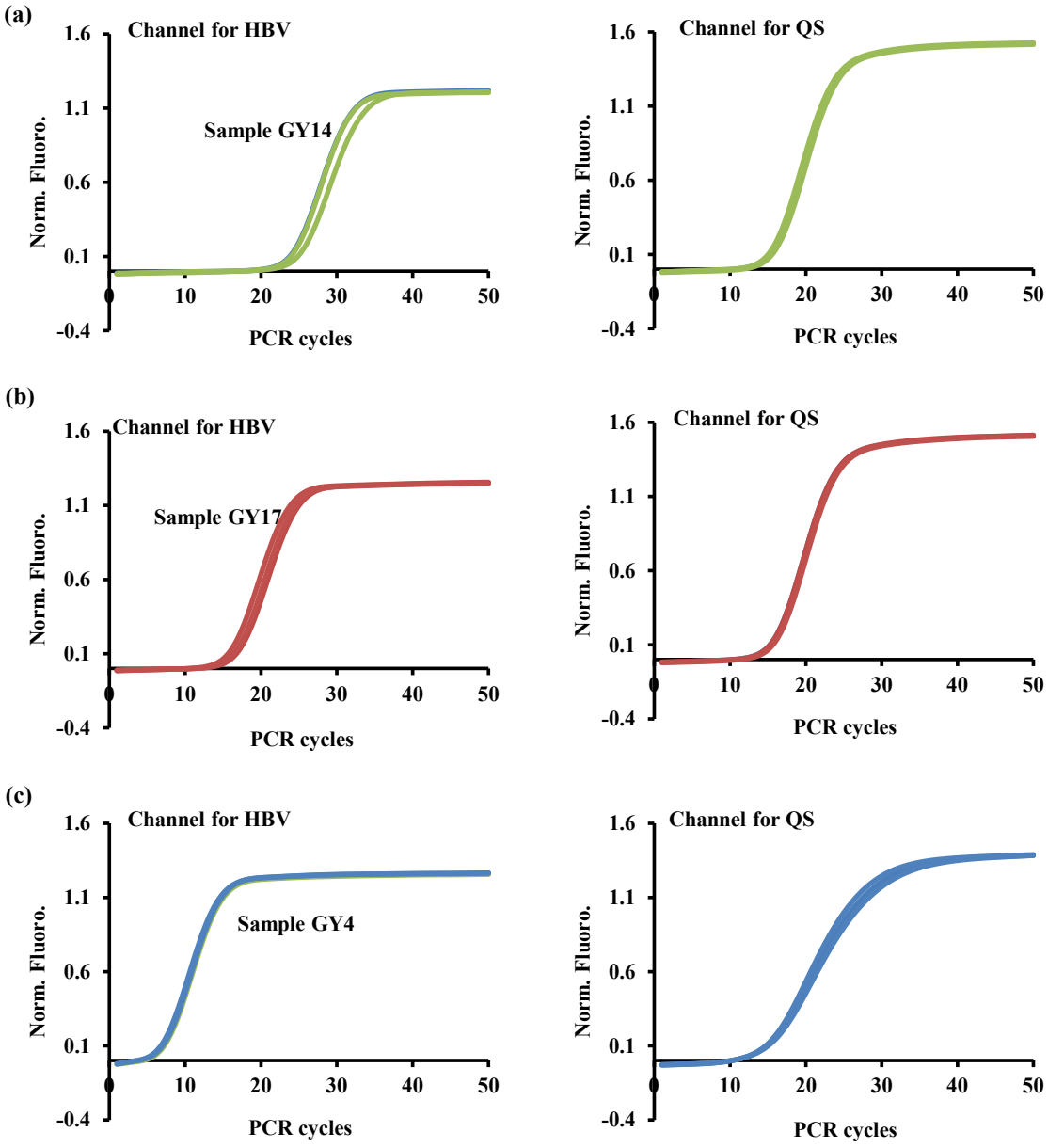

Fig. S11 Amplification profiles from the channel for HBV-DNA (left side) and the channel for QS-DNA (right side) of clinical samples with relative low (**a**), middle (**b**) and high (**c**) viral load in the reaction system. Sample GY 14 was 1.26×10^{2} IU mL^{-1}, Sample GY 17 was 1.69×10^{5} IU mL^{-1} and Sample GY 4 was 1.68×10^{8} IU mL^{-1}. N=3.

Table S1. Calculated E values in five separate tests

Test*	E
1	0.993
2	1.079
3	1.016
4	1.085
5	0.990

Note: *Test 1 and 2 were operated two years ago; test 3, 4 and 5 were operated recently.

Table S2. Precision of intra-assay for quantifying HBV-DNA

$C_{expected}$	1st day		2nd day	
(IU mL^{-1})	$C_{measured}$ (IU mL^{-1})	Log $C_{measured}$ (CV, %)	$C_{measured}$ (IU mL^{-1})	Log $C_{measured}$ (CV, %)
2×10^4	1.7×10^4	4.22 (2.3)	1.2×10^4	4.07 (1.9)
2×10^3	1.6×10^3	3.20 (1.6)	1.4×10^3	3.15 (1.7)
2×10^2	2.5×10^2	2.38 (3.8)	4.5×10^2	2.65 (0.9)
$C_{expected}$	3rd day		4th day	
(IU mL^{-1})	$C_{measured}$ (IU mL^{-1})	Log $C_{measured}$ (CV, %)	$C_{measured}$ (IU mL^{-1})	Log $C_{measured}$ (CV, %)
2×10^4	1.2×10^4	4.07 (2.1)	1.1×10^4	4.04 (0.64)
2×10^3	1.2×10^3	3.04 (4.2)	2.5×10^3	3.39 (2.3)
2×10^2	4.3×10^2	2.62 (3.3)	4.4×10^2	2.64 (2.9)

Table S3. Precision of inter-assay for quantifying HBV-DNA

$C_{expected}$ (IU mL^{-1})	$C_{measured}$ (IU mL^{-1})	Log $C_{measured}$ (CV, %)
2×10^4	1.3×10^4	4.10 (2.5)
2×10^3	1.7×10^3	3.20 (4.7)
2×10^2	3.9×10^2	2.57 (5.2)

Table S4. Ct values of samples tested by sQ-Invader and TaqMan probe-based qPCR

Sample*	sQ-Invader (SD)	qPCR (SD)
S1	17.12 (0.20)	27.18 (0.04)
S2	13.53 (0.06)	24.99 (0.03)
S3	8.17 (0.18)	18.38 (0.11)
S4	5.82 (0.22)	15.09 (0.01)
S5	11.06 (0.13)	21.72 (0.01)
S6	23.60 (0.64)	35.04 (0.16)
S7	19.00 (0.32)	31.04 (0.19)
S8	15.11 (0.49)	26.01 (0.03)
S9	9.67 (0.28)	20.965 (0.05)
S10	17.64 (0.51)	28.68 (0.10)
S11	12.18 (0.04)	22.02 (0.02)
S12	3.59 (0.12)	12.18 (0.06)
S13	6.90 (0.01)	16.67 (0.17)
S14	22.70 (0.68)	31.66 (0.16)
S15	16.79 (0.21)	27.70 (0.10)
S16	22.50 (0.34)	33.55 (0.65)
S17	6.02 (0.08)	16.405 (0.02)

Note: *Each sample was tested for three times.