Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supplementary information

Figure S1 Interpretation of HBV Cas13a-SHERLOCK lateral flow results

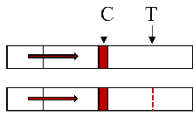
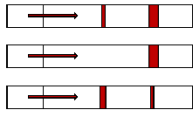
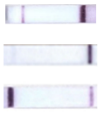
Result	Schematic	Example	Interpretation
Negative			Negative strips show no signal at the test line. Previous research has shown that negative strips left at room temperature for more than 10 minutes may show a fainter signal than a real positive signal at the test line. Because the lateral flow test strip technique in our study required 30 minutes of incubation in a metal bath at 37 °C before the test, a weak signal may be noticed on test lines with negative strips.
Positive			A signal will be present at the test line for a strip that displays a positive result. The control line may or may not also exhibit a signal, depending on the degree of collateral cleavage of reporter molecules. When the reporter molecule is entirely cleaved, the control line will not display a signal.

Figure S2 ROC curve of Cas13a-SHERLOCK assay

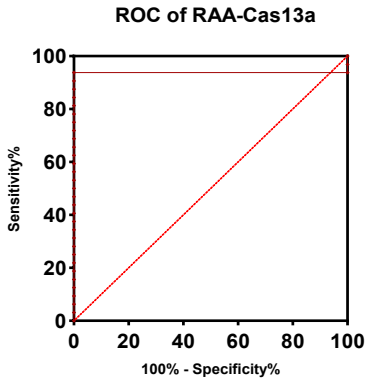


Table S1. Sequences involved in this study

HBV standard plasmid synthesis

Name	Sequence (5'-3')
Target dsDNA	GGAAGTTGGGGTACTTTACCACAGGAACATATTGTACACAAAATCAAG
HBV	CAATGTTTTCGAAAATTGCCTGTAAATAGACCTATTGATTGGAAAGTAT
polymerase	GTCAAAGAATTGTGGGTCTTTTGGGTTTTGCTGCCCCTTTTACACAATGT
coding region	GGATATCCTGCTTTGCTGCCTTTATATGCATGTATACAATCCAAGCAGG
(MN683731)	CTTTCACTTTCTCGCCAACTTACAAGGCCTTTCTGTGTAAACAATATCTG
genotype: C/D	AACCTTTACCCCGTTGCCCGGCAACGGTCAGGTCTCTGCCAAGTGTTTG
recombinant	CTGACGCAACCCCCACTGGATGGGGCTTGGCCATAGGCCATCGGCGCA
	TGCGTGGAACCTTTGTGGCTCCTCTGCCGATCCATACTGCGGAAC

The standard plasmid was cloned from the above sequence into the PUC57 vector plasmid, which was manufactured by Sangon Biotech (Shanghai).

crRNAs

crRNA1	GAAAUUAAUACGACUCACUAUAGGGGAUUUAGACUACCCCAAAAA
	CGAAGGGGACUAAAACGAAAGGCCUUGUAAGUUGGCGAGAAAGU
crRNA2	GAAAUUAAUACGACUCACUAUAGGGGAUUUAGACUACCCCAAAAA
	CGAAGGGGACUAAAACGUGGGGGUUGCGUCAGCAAACACUUGGC
T7 primer	GAAATTAATACGACTCACTATAGGG
Template crRNA1	ACTTTCTCGCCAACTTACAAGGCCTTTCGTTTTAGTCCCCTTCGTTTT
	TGGGGTAGTCTAAATCCCCTATAGTGAGTCGTATTAATTTC
Template crRNA2	GCCAAGTGTTTGCTGACGCAACCCCCACGTTTTAGTCCCCTTCGTTTT
	TGGGGTAGTCTAAATCCCCTATAGTGAGTCGTATTAATTTC

RAA primers

Primers	Sequence (5'-3')
F1	TAATACGACTCACTATAGGGTGTTACAGGCGGGGTTTTTCTTGTTGACA
R1	ATGATAAAACGCCGCAGACACATCCAGCGATA
F2	TAATACGACTCACTATAGGGTATTGATTGGAAAGTMTGTCAAMGAATTGTG
	GG
R2	CCAGTGGGGGTTGCGTCAGCAAACACTTGGCA
F3	TAATACGACTCACTATAGGGTGAACCTTTACCCCGTTGCYCGGCAA
R3	GCTAGGAGTTCCGCAGTATGGATCGGCAGAGGAG
F4	TAATACGACTCACTATAGGGTGCCAAGTGTTTGCTGACGCAACCCCCACTGG
R4	TACGTCCCGTCGGCGCTGAATCCCGCG
F5	TAATACGACTCACTATAGGGGTCCTTTGTYTACGTCCCGTCGGCGCTGA
R5	CACGTGCAGAGGTGAAGCGAAGTGCACACGG

ssRNA reporter

Fluorescent reporter	5′-/6-FAM/UUUUUU/BHO/-3′
Biotin reporter	5′-/6-FITC/UUUUUU/Biotin/-3′

In vitro transcription (IVT) steps of crRNA

I Synthesis of double-stranded crRNA

Table S2 First add to the PCR tube according to the system：

Component	Volume to add (μL)
crRNA template (100 μM)	1
T7 primer (100 μM)	1
RNase-Free Water	8
Total	10

Next, anneal the crRNA template and T7 primers by performing a 10 min denaturation. The reaction was then slowly cooled to 4°C in a PCR thermocycler.

II T7 transcription of crRNA

Table S3 The above products were mixed in the following system:

Component	Volume to add (μL)
Annealing reaction	10
NTP buffer mix (20 mM each)	10
T7 RNA polymerase mix	2
RNase-Free Water	17
Total	39

The HiScribe™ T7 Quick High Yield RNA Synthesis Kit (NEB, E2050S) was mixed according to the above system and placed in a 37°C incubator for 10 hours.

III Purification of crRNA

The above T7 transcript product was purified by using Monarch® RNA Cleanup Kit (10 μg) (NEB, T2030L) and following the instructions strictly.

Table S4 Information on purchased reagents

Name	Company and item number
LwaCas13a protein (5μM)	GUANGZHOU BIO-LIFESCI, M20202
HiScribe™ T7 Quick High Yield RNA Synthesis Kit	NEB, E2050S
Monarch® RNA Cleanup Kit (10 μg)	NEB, T2030L
RNase-Free Water	CWBIO, CW0612
Milenia HybriDetect 1	Milenia biotec, MGHD1
RNase Inhibitor, Murine (40000 units/ml)	NEB, M0314L
Reporter	Shanghai Sangon Biotech
HBV Nucleic Acid Assay Kit (Prominence HBV DNA)	Sansure Biotech
T7 RNA Polymerase (50000 units/ml)	NEB, M0251L
$MgCl_2$ (1 M)	Thermo Fisher, AM9530G
rNTP (25 mM)	NEB, N0466L
Recombinase-Aid Amplification Kit	Hangzhou ZC Biotech, S001ZC
HiPure Viral DNA Kits	Magen Biotech

Table S5 RAA reaction systems

Recombinase-aided amplification (RAA) is an isothermal nucleic acid rapid amplification technology that varies from RPA in that it employs recombinases derived from bacteria or fungi. Under isothermal conditions (37℃-42℃), the recombinase UvsX and recombinase mediator protein UvsY can bind firmly to the primers, forming an enzyme-DNA complex. Once the primer locates on the homologous sequence, a chain exchange reaction occurs, triggering DNA synthesis and chain extension by the DNA polymerase and exponentially expanding the target region on the template. This method has the merits of ultra-sensitivity, specificity, rapidity, and simplicity.

The RAA reaction system is listed below:

Component	Volume to add (μL)
A buffer (20% polyethylene glycol)	41.5
B buffer (280 mM magnesium acetate)	2.5
Forward Primer (10 Mm)	2
Reverse Primer (10 μM)	2
HBV DNA	2
Total	50

RAA reaction condition is 37℃, after the reaction is finished, store on ice

Table S6 Reaction system of Cas13a- SHERLOCK

Component	Volume to add (μL)
RAA amplification product	5-10
10x reaction buffer	2.5
crRNA (30ng/μL)	1
$MgCl_2$ (1M)	0.25
rNTP	1.5
RNase inhibitor (40000 units/ml)	1
T7 RNA Polymerase (50000 units/ml)	1
Cas13a (1μM)	2
Fluorescent reporter, 10μM or biotin reporter, 1μM	1
RNase-Free Water	Up to 25

The fluorescence assay was performed by mixing the above system and then performing a fluorescence collection assay. However, the lateral flow test strip (Milenia HybriDetect 1, TwistDx) need to be incubated for 30 min at 37°C in a warm metal bath, and then 50 μL of HybriDetect assay buffer is pipetted and mixed to reach the required volume of lateral flow test strip. After 2-3 minutes, the results can be visible to the naked eye.

Table S7 Results of qPCR for 74 clinical serum samples

qPCR test positive sample no.	HBV viral load (IU/mL)	qPCR test negative sample no.	HBV viral load (IU/mL)
1	2.33E+2	1	<30
2	1.96E+6	2	<30
3	5.42E+6	3	<30
4	3.84E+4	4	<30
5	3.61E+7	5	<30
6	3.53E+4	6	<30
7	5.36E+7	7	<30

8	4.42E+7	8	<30
9	1.16E+2	9	<30
10	1.40E+4	10	<30
11	6.10E+6	11	<30
12	9.85E+4	12	<30
13	4.98E+6	13	<30
14	3.46E+7	14	<30
15	8.10E+5	15	<30
16	2.71E+7	16	<30
17	2.71E+3	17	<30
18	4.08E+3	18	<30
19	6.83E+3	19	<30
20	1.06E+3	20	<30
21	3.47E+2	21	<30
22	4.79E+2	22	<30
23	7.47E+2	23	<30
24	2.04E+2	24	<30
25	2.14E+2	25	<30
26	6.80E+2	26	<30
27	2.31E+4	27	<30
28	8.63E+4	28	<30
29	2.13E+3	29	<30
30	7.17E+6	30	<30
31	1.37E+2	31	<30
32	2.23E+2	32	<30
□	□	33	<30
□	□	34	<30
□	□	35	<30
□	□	36	<30
□	□	37	<30
□	□	38	<30
□	□	39	<30
□	□	40	<30
□	□	41	<30
□	□	42	<30