Electronic Supplementary Material

A dual-labeled fluorescence quenching lateral flow assay based on one-pot enzyme-free isothermal cascade amplification for rapid and ultrasensitive detection of pathogen

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Experimental section

Table S1 Sequences of CHA-HCR probes

probe	Target sequence (5'-3')
ORF1ab	CCTAAAAGCATCCTCCGAGATCC
CHA-H1	AAAAAAAAAAAAAAAAAGCATCCTCCGAGATCCCTT CATCTTCATGGATCTCGGAGGATGCTTTTAGG
CHA-H2	CTTGCTCTTCATCTTCATTTTAGGTCAGCATCCTCCGA GATCCATGAAGATGAAGGGATCTCGGA
HCR-trigger	GCTCTTCATCTTCATTTTAGGTC
HCR-H1	CATCTTCATTTTAGGTCCAAAGTGACCTAAAATGAAG ATGAAGAGC
HCR-H2	ACTTTGGACCTAAAATGAAGATGGCTCTTCATCTTCA TTTTAGGTC
biotin-HCR-H1	biotin- CATCTTCATTTTAGGTCCAAAGTGACCTAAAATGAAG ATGAAGAGC
biotin-HCR-H2	biotin- ACTTTGGACCTAAAATGAAGATGGCTCTTCATCTTCA TTTTAGGTC
Mis 1	CCTACAAGCATCCTCCGAGATCC
Mis 2	CCTACAAGCATCCTCCAAGATCC
Mis 3	CCTACAAGTATCCTCCAAGATCC
Mis 4	CCTACAAGTATCCGCCAAGATCC
T line	AACCCACTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTT

Table S2 Determination of the optimal streptavidin concentration by visual observation

Number	AuNPs（μL）	streptavidin（μg）	10 %NaCl (μL)	Color change
1	1000	0	100	Blue-black
2	1000	5	100	Blue-black
3	1000	10	100	Blue-black
4	1000	15	100	blue-violet
5	1000	20	100	blue-violet
6	1000	25	100	purplish red
7	**1000**	**30***	**100**	**red**
8	1000	35	100	red
9	1000	40	100	red

* Shows optimal streptavidin concentration

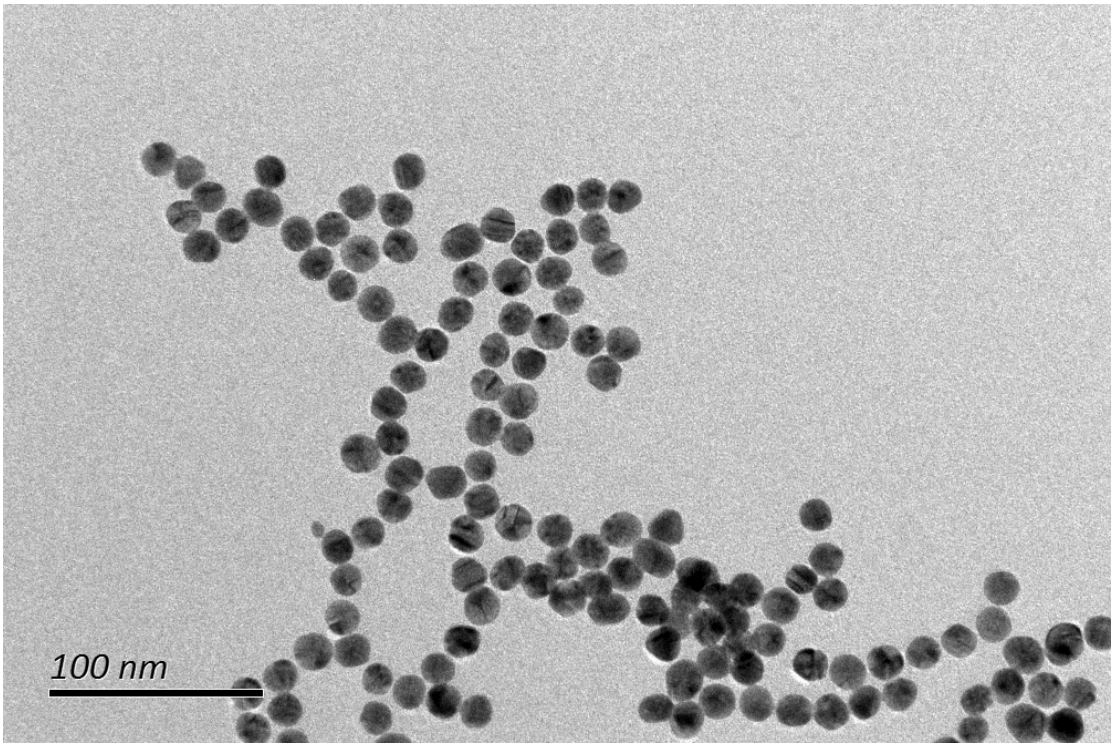

Figure S1. TEM image of colloidal gold nanoparticles

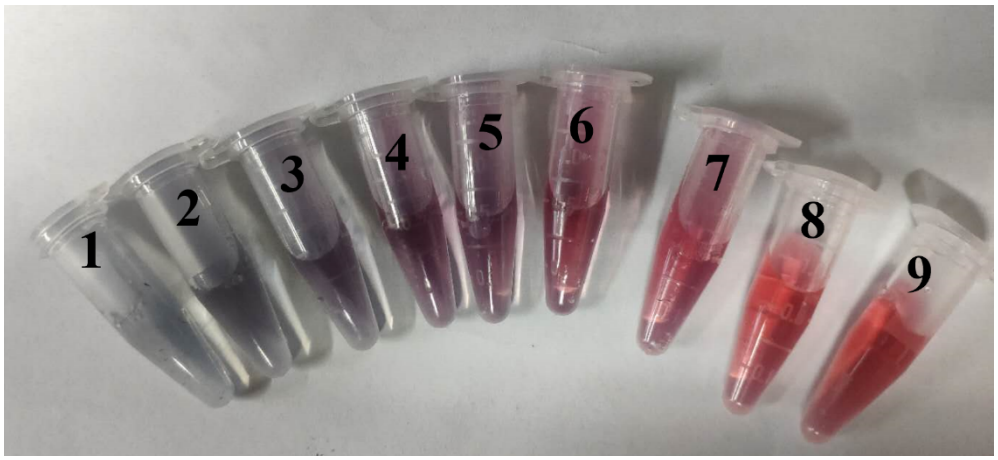

Figure. S2 Determination of the optimal SA concentration by visual observation

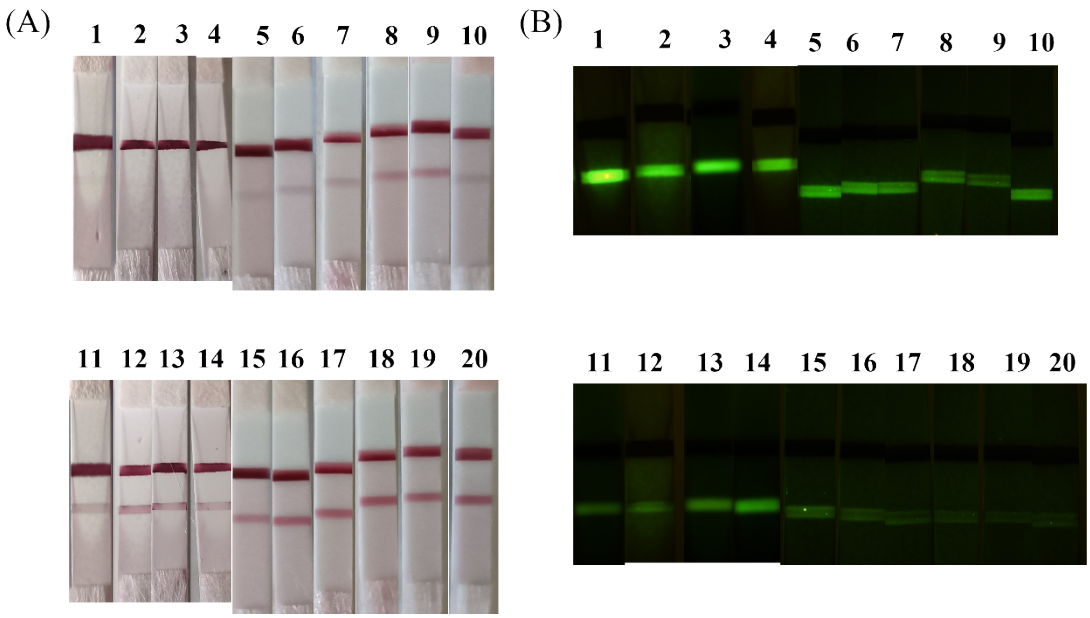

Figure. S3 Result of different ratio of CHA and HCR probes on test strips.

A. Visualization results. Strips 1-10: negative groups (0.1:1, 0.3:1, 0.5:1, 0.7:1, 0.3:0.5, 0.3:1, 0.3:1.5, 0.3:2, 0.3:2.5, 0.3:3). Strips 11-20: positive groups (0.1:1, 0.3:1, 0.5:1, 0.7:1, 0.3:0.5, 0.3:1, 0.3:1.5, 0.3:2, 0.3:2.5, 0.3:3). B. Fluorescence result. Strips 1-10: negative groups (0.1:1, 0.3:1, 0.5:1, 0.7:1, 0.3:0.5, 0.3:1, 0.3:1.5, 0.3:2, 0.3:2.5, 0.3:3). Strips 11-20: positive groups (0.1:1, 0.3:1, 0.5:1, 0.7:1, 0.3:0.5, 0.3:1, 0.3:1.5, 0.3:2, 0.3:2.5, 0.3:3).

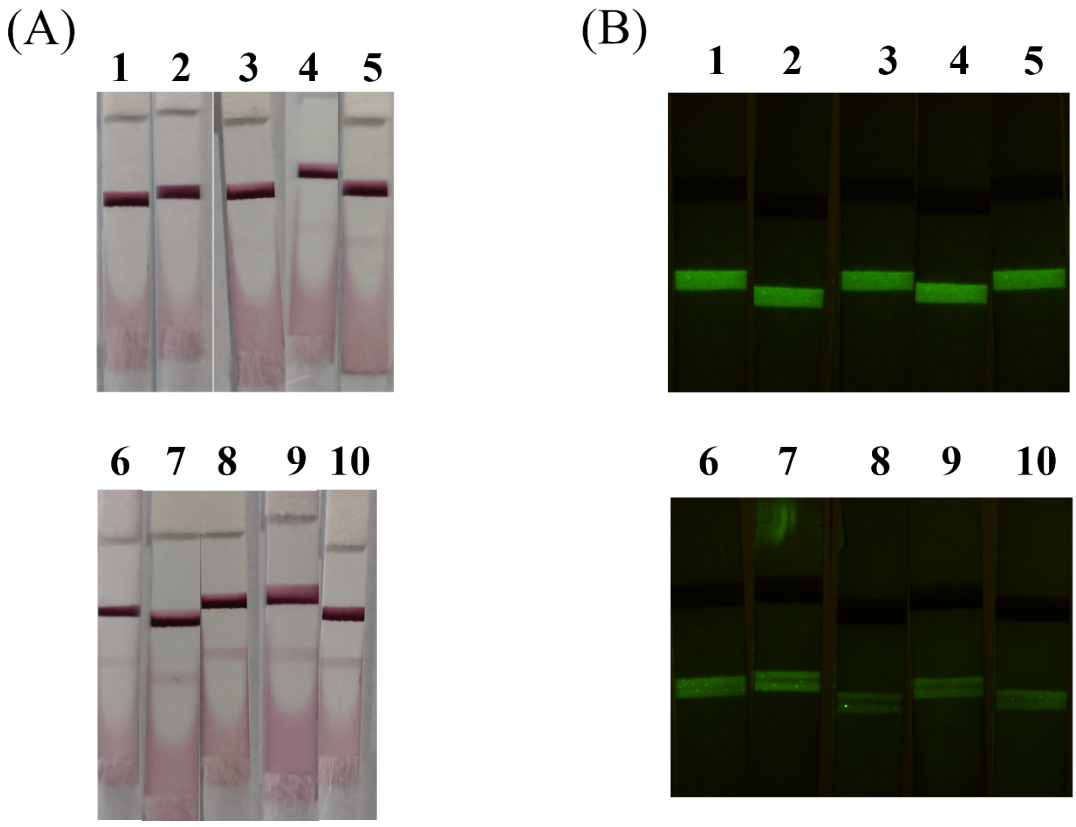

Figure. S4 Result of different ratio of HCR modified/unmodified probes on test strips.

A. Visualization results. Strips 1-5: negative groups (10:0, 9:1, 8:2, 7:3, 6:4, 5:5). Strips 6-10: positive groups (10:0, 9:1, 8:2, 7:3, 6:4, 5:5). B. Fluorescence result. Strips 1-5: negative groups (10:0, 9:1, 8:2, 7:3, 6:4, 5:5). Strips 6-10: positive groups (10:0, 9:1, 8:2, 7:3, 6:4, 5:5).

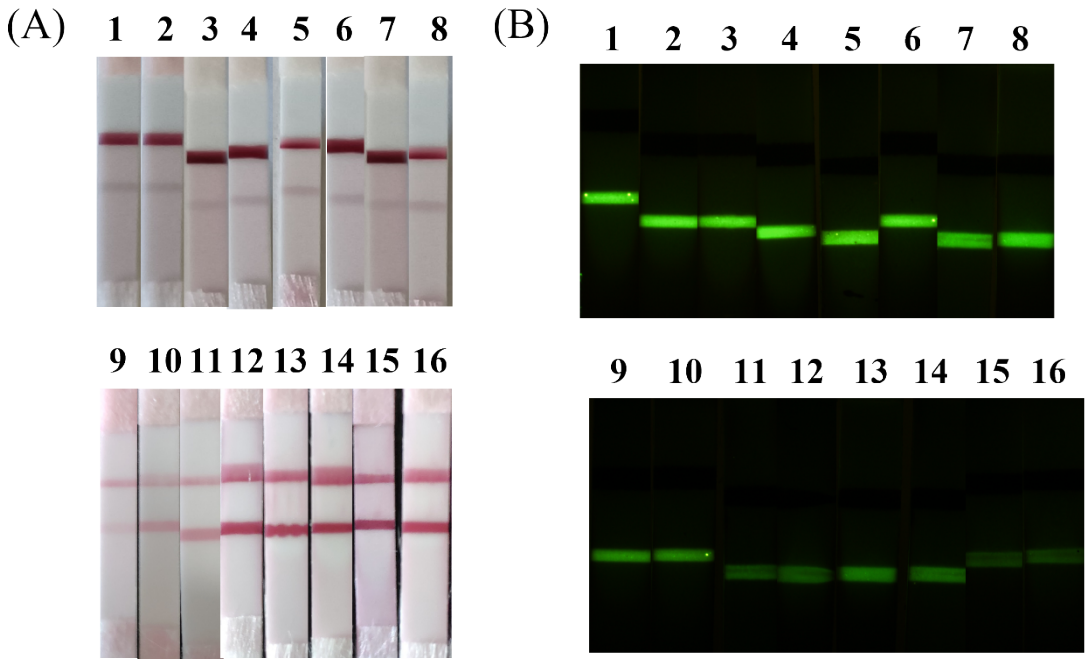

Figure. S5 Result of CHA-HCR time on test strips.

A. Visualization results. Strips 1-8: negative groups (0 min, 5 min, 10 min, 15 min, 30 min, 60 min, 90 min, 120 min). Strips 9-16: positive groups (0 min, 5 min, 10 min, 15 min, 30 min, 60 min, 90 min, 120 min). B. Fluorescence result. Strips 1-8: negative groups (0 min, 5 min, 10 min, 15 min, 30 min, 60 min, 90 min, 120 min). Strips 9-16: positive groups (0 min, 5 min, 10 min, 15 min, 30 min, 60 min, 90 min, 120 min).

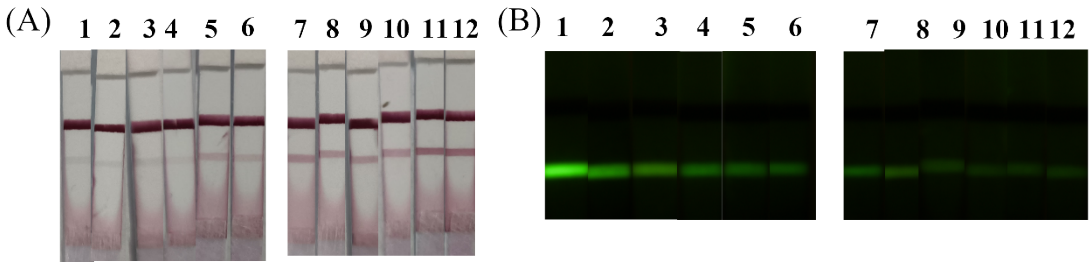

Figure. S6 Result of ion concentration on test strips.

A. Visualization results. Strips 1-6: negative groups (0 mM, 300 mM, 600 mM, 900 mM, 1200 mM, 1500 mM). Strips 7-12: positive groups(0 mM, 300 mM, 600 mM, 900 mM, 1200 mM, 1500 mM). B. Fluorescence result. Strips 1-6: negative groups (0 mM, 300 mM, 600 mM, 900 mM, 1200 mM, 1500 mM). Strips 7-12: positive groups(0 mM, 300 mM, 600 mM, 900 mM, 1200 mM, 1500 mM).

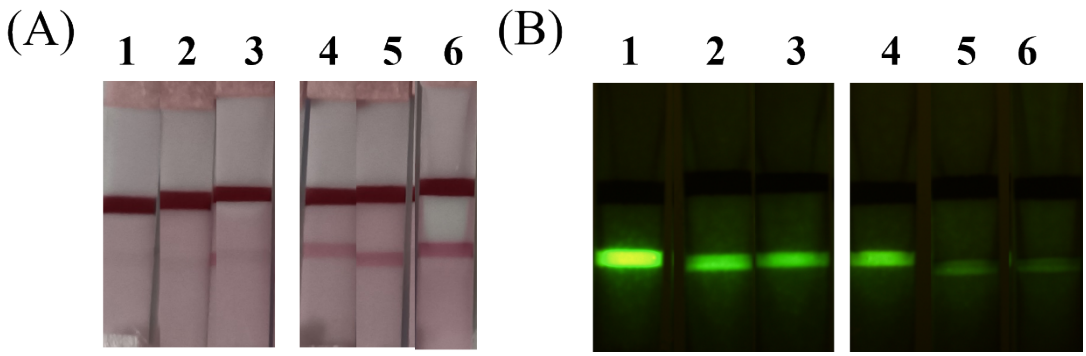

Figure. S7 Result of T line concentration on test strips.

A. Visualization results. Strips 1-3: negative groups (1 OD, 2.5OD, 5OD). Strips 4-6: positive groups (1 OD, 2.5OD, 5OD). B. Fluorescence result. Strips 1-3: negative groups (1 OD, 2.5OD, 5OD). Strips 4-6: positive groups (1 OD, 2.5OD, 5OD).

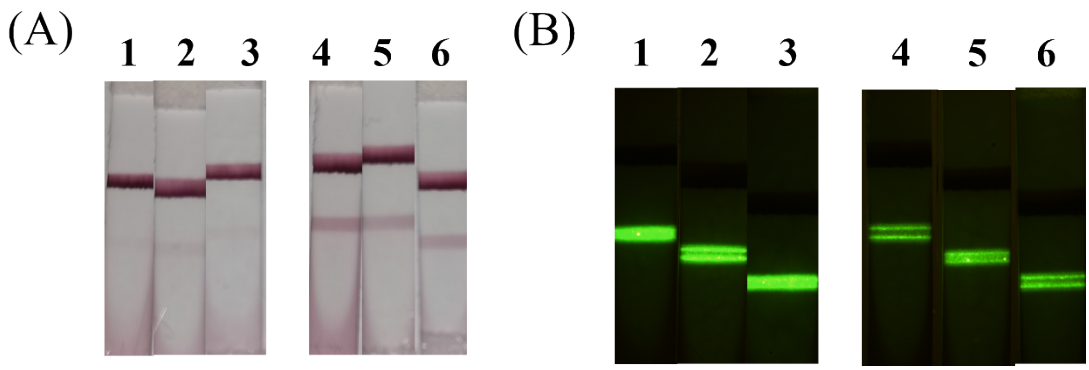

Figure. S8 Result of running buffer type on test strips.

A. Visualization results. Strips 1-3: negative groups (PBS, SSC, Tris). Strips 4-6: positive groups (PBS, SSC, Tris). B. Fluorescence result. Strips 1-3: negative groups (PBS, SSC, Tris). Strips 4-6: positive groups (PBS, SSC, Tris).

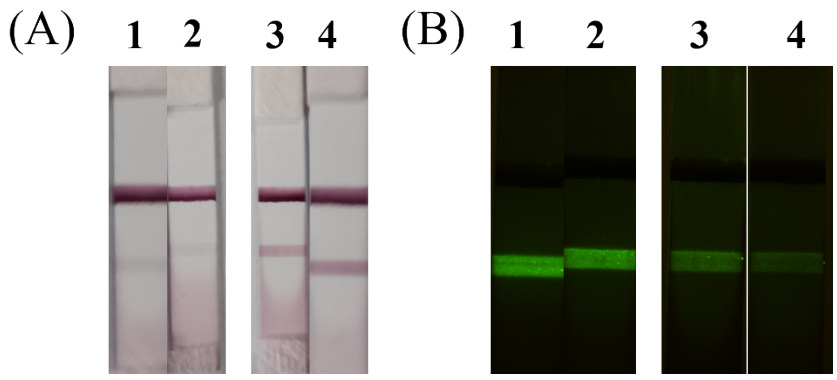

Figure. S9 Result of surfactant type on test strips.

A. Visualization results. Strips 1, 2: negative groups (Triton X100, Tween). Strips 3, 4: positive groups (Triton X100, Tween). B. Fluorescence result. Strips 1, 2: negative groups (Triton X100, Tween). Strips 3, 4: positive groups (Triton X100, Tween).

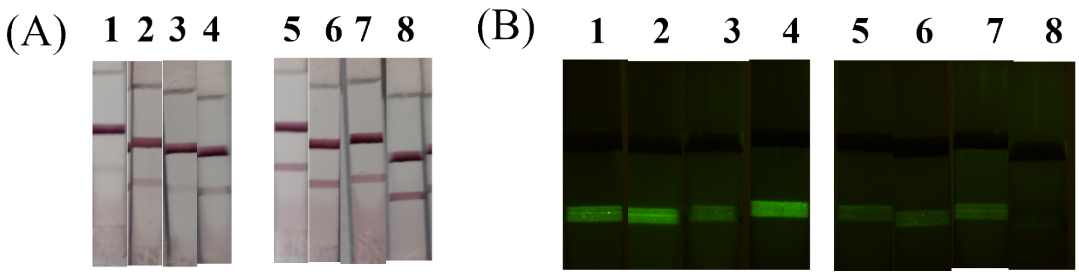

Figure. S10 Result of surfactant concentration on test strips.

A. Visualization results. Strips 1-4: negative groups (0.5%, 1.0%, 5%, 10%). Strips 5-8: positive groups (0.5%, 1.0%, 5%, 10%). B. Fluorescence result. Strips 1-4: negative groups (0.5%, 1.0%, 5%, 10%). Strips 5-8: positive groups (0.5%, 1.0%, 5%, 10%).

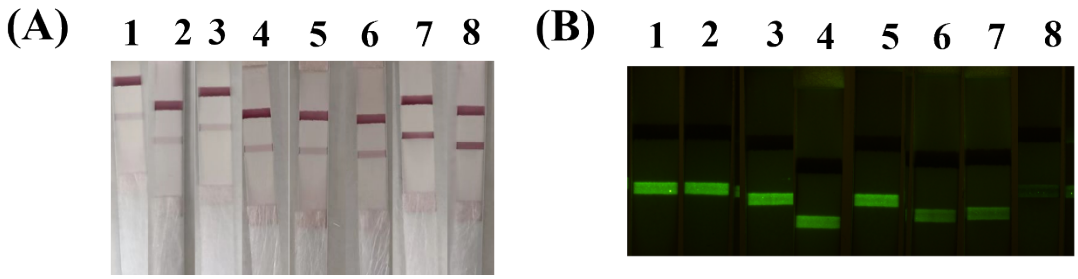

Figure. S11 Result of Sensitivity of CHA-HCR-LFA in reaction buffer.

A. Visualization results. Strips 1-8: different concentration of SARS-CoV-2 (0 pM, 10 pM, 50 pM, 100 pM, 500 pM, 1 nM, 5 nM, 10 nM, 50 nM). B. Fluorescence result. Strips 1-8: different concentration of SARS-CoV-2 (0 pM, 10 pM, 50 pM, 100 pM, 500 pM, 1 nM, 5 nM, 10 nM, 50 nM).

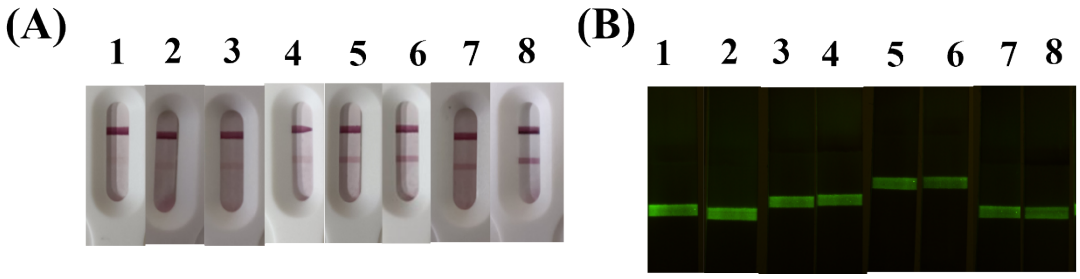

Figure. S11 Result of Sensitivity of CHA-HCR-LFA in serum matrix.

A. Visualization results. Strips 1-8: different concentration of SARS-CoV-2 (0 pM, 10 pM, 50 pM, 100 pM, 500 pM, 1 nM, 5 nM, 10 nM, 50 nM). B. Fluorescence result. Strips 1-8: different concentration of SARS-CoV-2 (0 pM, 10 pM, 50 pM, 100 pM, 500 pM, 1 nM, 5 nM, 10 nM, 50 nM).