Electronic Supplementary Information

A rapid water bath PCR combined with lateral flow assay for the simultaneous detection of SARS-CoV-2 and influenza B virus

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1. The assembly of DNA fragments in vitro.

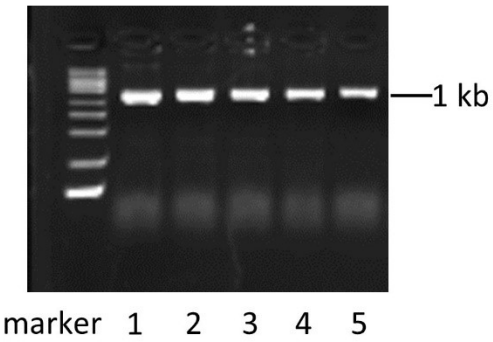

Figure S1. The image of agarose gel electrophoresis indicated that DNA fragments with length of 1 kb could be assembled in vitro by water bath PCR. Electrophoretogram of 1-5: DNA fragments with length of 1 kb.

2. Determination of the shortest incubation time in each water bath.

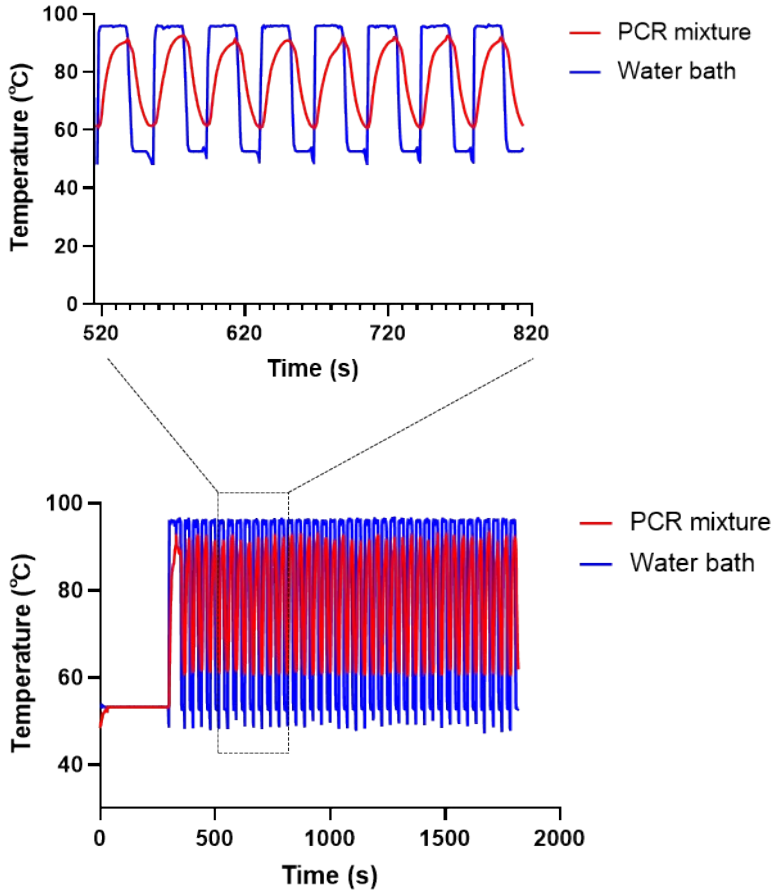

Figure S2. Temperature profiles of the PCR mixture and water bath in an entire PCR protocol. Temperatures were recorded by two thermocouples fixed inside and outside the reaction tube.

3. Optimization of the concentration of the used SA-QBs.

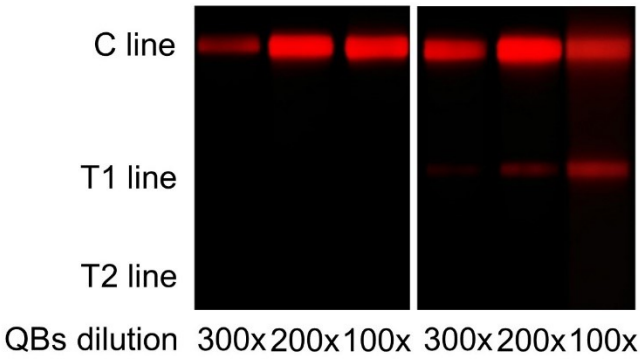

Figure S3. The SARS-CoV-2 negative results and positive results using 300, 200, and 100 times diluted SA-QBs.

4. Optimization of the concentration of capture antibodies.

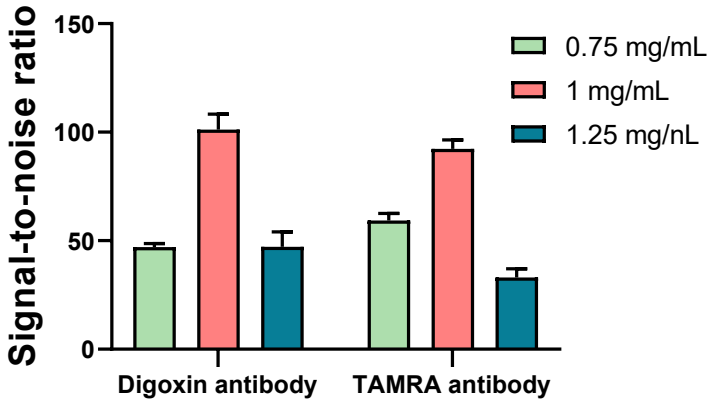

Figure S4. The signal-to-noise ratio of positive results (10^4 copies/μL of SARS-CoV-2 and IBV) to negative control at different concentrations of digoxin antibody and TAMRA antibody.

5. Agarose gel electrophoresis of PCR products.

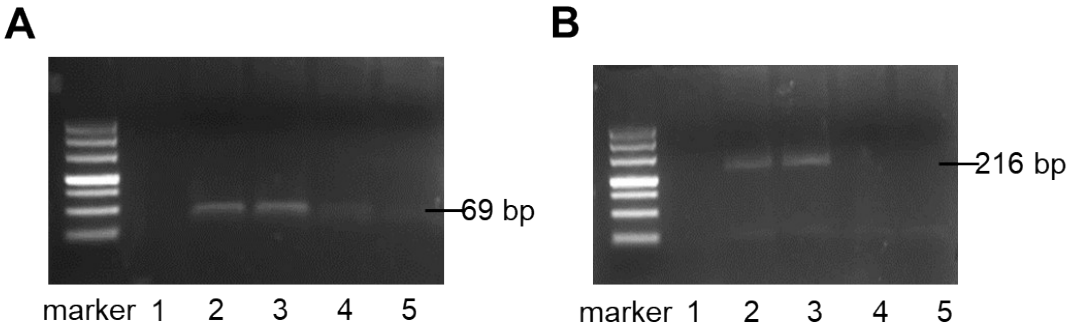

Figure S5. Images of agarose gel electrophoresis at different concentrations of (A) SARS-CoV-2 and (B) IBV in the range of 10^2–10^5 copies/μL. In each electrophoretogram, 1: negative control, 2–5: 10^5–10^2 copies/μL.

6. The nucleic acid template of the influenza B virus and SARS-CoV-2.

IBV NS1 Gene:

TAAAGGATGAAGTAAACACTCAGAAAGAGGGGAAATTCCGTTTGACA
ATAAAAAGGGATATACGTAATGTGTTGTCCTTGAGAGTGTTGGTGAACGG
AACCTTCCTCAAGCACCCTAATGGAGACAGTCCTTATCMACTCTICATAGA
TTGAATGCATATGACCAGAATGGAGGGCTTGTTGCTAAACTTGTTGCTACT
GATGATCTTACAGTGGAGGATGAAAAAGMTGGCCATCGGATCCTCAACTC
ACTCTTCGAGCGTTTTGATGAAGGACATTCAAAGCCAATTCGAGCAGCTG
AAACTGCGGTGGGAGTCTTA
TCCCAATTTGGTCAAGAGCACCGATTATCACCAGA

SARS-CoV-2 N gene:

ATGTCTGATAATGGACCCCAAAATCAGCGAAATGCACCCCGCATTACG
TTTGGTGGACCCTCAGATTCAACTGGCAGTAACCAGAATGGAGAACGCAG
TGGGGCGCGATCAAAACAACGTCGGCCCCAAGGTTTACCCAATAATACTG
CGTCTTGGTTCACCGCTCTCACTCAACATGGCAAGGAAGACCTTAAATTCC
CTCGAGGACAAGGCGTTCCAATTAACACCAATAGCAGTCCAGATGACCAA
ATTGGCTACTACCGAAGAGCTACCAGACGAATTCGTGGTGGTGACGGTAA
AATGAAAGATCTCAGTCCAAGATGGTATTTCTACTACCTAGGAACTGGGC
CAGAAGCTGGACTTCCCTATGGTGCTAACAAAGACGGCATCATATGGGTT
GCAACTGAGGGAGCCTTGAATACACCAAAAGATCACATTGGCACCCGCAA
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GGTGCCATCAAATTGGATGACAAAGATCCAAATTTCAAAGATCAAGTCAT
TTTGCTGAATAAGCATATTGACGCATACAAAACATTCCCACCAACAGAGC
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TTGCAACAATCCATGAGCAGTGCTGACTCAACTCAGGCCTAA