# Simultaneous detection of different Zika virus lineages via molecular computation in a point-of-care assay

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Supplementary Table S1. Primers used for single and multiplex Zika LAMP assays.

| | | |
|---|---|---|
| NS5 | NS5.BIP* | ATGCVCARATGTGGCAGCTYCTTCCCHGTTGGNACCCA |
| | NS5.B3 | GTCATCCARTCTCCRTTRCC |
| | NS5.F3 | ATCCATTGTGGTYCCYTGY |
| | NS5.FIP | GCHAGRCARGCAGTCTCMCGRGATGAAYTGATTGGCCGRGC |
| | NS5.LR | TGCTCCATCCYGCCCCYGGHGA |
| NS1 | NS1.F3 | GGGCCCAYCTGATYGAGAT |
| | NS1.B3 | TCCACATGTYTCCTCCACR |
| | NS1.FIP | GTGGMCCAGCTAAAGACTTGGGAATGGCCAAAGTCYCACAC |
| | NS1.BIP | ACAGRACYCAAATGAAAGGGCCGGGCATTCCTCAAAYCGRAT |
| | NS1.LF | ATGGCACAGTGARGAGCTYGAA |
| Capsid | Capsid.F3 | TGCTAAAACGCGGAGTAGC |
| | Capsid.B3 | TGGCAGCCAGRTCYTTCTTR |
| | Capsid.FIP | ACCATYCTRATGGGCCCATGCCCTTTGGRGGYTTGAAG |
| | Capsid.BIP | TCACGGCAATCAARCCATCACTAGCCTCTTTYTTCCCCACKG |
| | Capsid.LF | GGGTCTCATCAAYAGATGGGGTW |
| | Capsid.SF | CTTGGCAATTCTAGCCTTYTTGAGAT |
| NS3 | NS3.F3 | ACGAAGACCAYGCACAYTG |
| | NS3.B3 | ATTCCGGCAGATGCWACY |
| | NS3.FIP | TCGGCCTCAGGYCGATAGAGCAGCAAGAATGCTYCTTGACA |
| | NS3.BIP | AGCHGCCATTGAGGGAGAGTTCTAGGCCARCCARACRGGA |
| | NS3.LR | GAGGCTATGAGGCCATCYTGGAG |

* Mixed base identity: **V** = A,C,G; **R** = A,G; **Y** = C,T; **H** = A,C,T; **N** = A,T,C,G; **M** = A,C; **K** = G,T; **W** = A,T

Supplementary Table S2. Strand exchange probes used in this study.

| | | |
|---|---|---|
| Capsid OSD | Capsid.FAM | RCCCAGCAGRAGTCCGGCYGGCARCCT/56-FAM/ |
| | Capsid.Q | /5IABkFQ/AGGYTGCCRGCCGGACT/3InvdT/ |
| NS5 OSD | NS5.Q | RAGGTCYCTTCTRTGGAAGTC/3IABkFQ/ |
| | NS5.FAM | /56-FAM/GACTTCCAYAGAAGRGACCTYCGACTGATGGC/3InvdT/ |
| NS1 OSD | NS1.FAM | /56-FAM/CATGATMAGATCACTYTCTTCYAYTCCATCTGTCCACAAT/3InvdT/ |
| | NS1.Q | GGARTRGAAGARAGTGATCTKATCATG/3IABkFQ/ |
| NS3 OSD | NS3.FAM | GAGCAAAGGAAGACCTTYGTGGAACTCATGAARAGAGGAGA/36-FAM/ |
| | NS3.Q | /5IABkFQ/TCTCCTCTYTTCATGAGTTCCACRAAGGT/3InvdT/ |
| CAN3 2GO probe | CAN3.2GO.FAM | RCCCAGCAGRAGTCCGGCYGGCARCCTGATCC/36-FAM/ |
| | CAN3.2GO.Gate | GAGCAAAGGAAGACCTTYGTGGAACTCATGAARAGAGGAGAGGATCAGGYTGCCRGCCGGA /3InvdT/ |
| | CAN3.2GO.Q | /5IABkFQ/TCTCCTCTYTTCATGAGTTCCACRAAGGT/3InvdT/ |
| N5N1 2GO probe | N5N1.2GO.FAM | /56-FAM/CATGATMAGATCACTYTCTTCYAYTCCATCTGTCCACAAT/3InvdT/ |
| | N5N1.2GO.Gate | GGARTRGAAGARAGTGATCTKATCATGGACTTCCAYAGAAGRGACCTYCGACTGATGGC/3InvdT/ |
| | N5N1.2GO.Q | RAGGTCYCTTCTRTGGAAGTC/3IABkFQ/ |
| 4GO probe | 4GO.S1 | GTCYCTTCTRTGGAAGTCCTCCATGATMAGATCACTYTCTTCYAYTCCATCTGTCCACAAT/3InvdT/ |
| | 4GO.S2 | /56-FAM/CAGGGAGGAGGACTTCCAYAGAAGRGACCTYCGACTGATGGC/3InvdT/ |
| | 4GO.S3 | CAGCAGRAGTCCGGCYGGCARCCTGAGGAGGAGGGAG/3IABkFQ/ |
| | 4GO.S4 | GAGCAAAGGAAGACCTTYGTGGAACTCATGAARAGAGGAGACTCCTCAGGYTGCCRGCCGG/3InvdT/ |
| | 4GO.S5 | RAGTGATCTKATCATGTCCCTGCTCCCTTCTCCTCTYTTCATG/3InvdT/ |

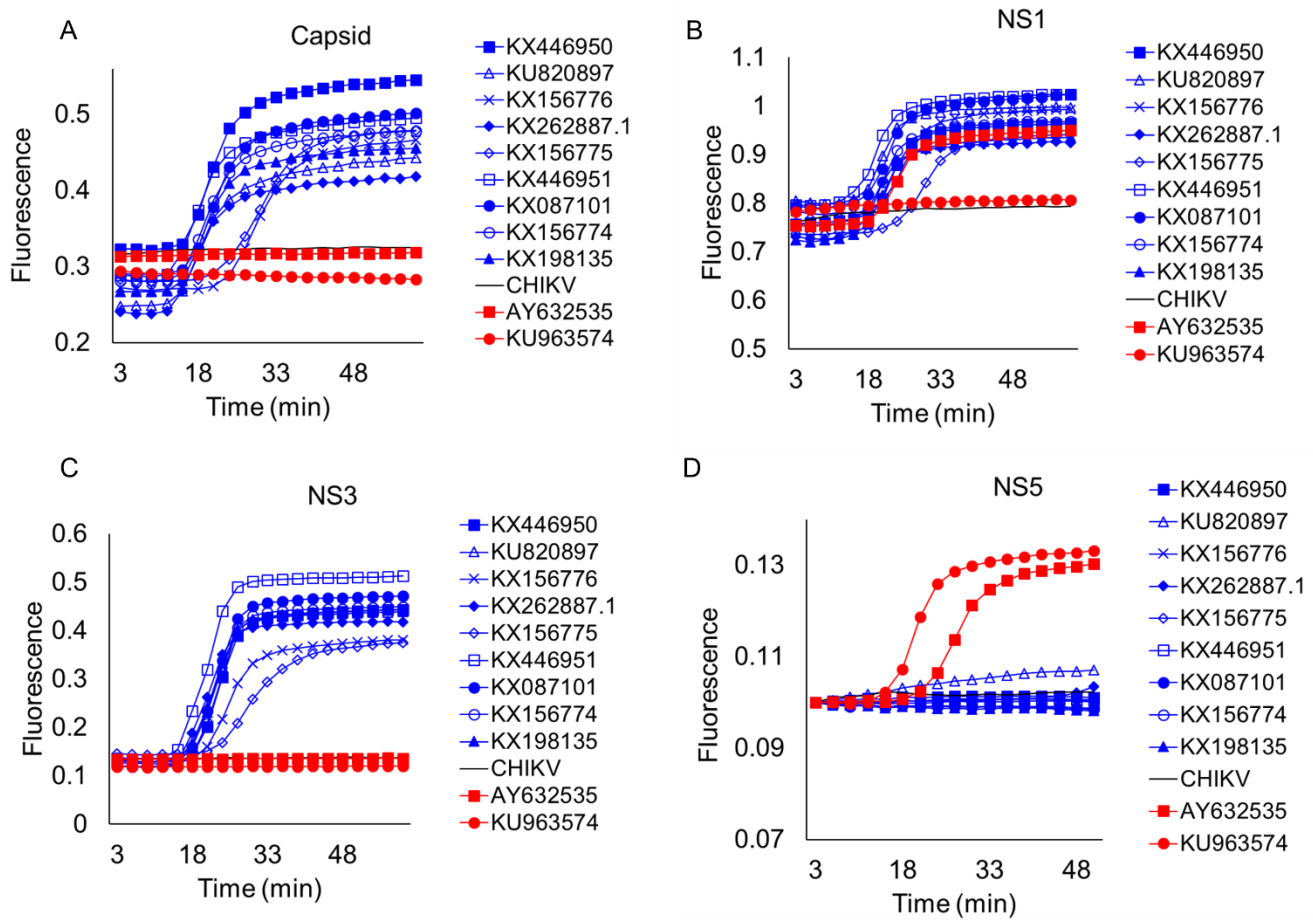Supplementary Table S3. LAMP synthetic template RNA sequences.

| Zika NS3 RNA template | GGGAGGCAGGAAUCCCAACAAACCUGGAGAUGAGUAUCUGUAUGGAGGUGGGGUGCGCAGAGACUGACGAAGACCAUGCACACUGGCUUGAAGCAAGAAUGCUCCUUGACAAUAUUUACCUCCAAGAUGGCCUCAUAGCCUCGCUCUAUCGACCUGAGGCCGACAAAGUAGCAGCCAUUGAGGGAGAGUUCAAGCUUAGGACGGAGCAAAGGAAGACCUUUGUGGAACUCAUGAAAAGAGGAGAUCUUCCUGUUUGGCUGGCCUAUCAGGUUGCAUCUGCCGGAAUAACCUACACAGAUAGAAGAUGGGUGCUUUGAUGGCACGACCAACAACACCAUAAUGGAAGACAGUGUGCGAAUUCUGCAGAUAUCCAUCACACUGGCGG |
|---|---|
| Zika NS1 RNA template | GGGCUGAAGAGGGCCCAUCUGAUCGAGAUGAAAACAUGUGAAUGGCCAAAGUCCCACACAUUGUGGACAGAUGGAAUAGAAGAGAGUGAUCUGAUCAUACCCAAGUCUUUAGCUGGGCCACUCAGCCAUCACAAUACCAGAGAGGGCUACAGGACCCAAAUGAAAGGGCCAUGGCACAGUGAAGAGCUUGAAAUUCGGUUUGAGGAAUGCCCAGGCACUAAGGUCCACGUGGAGGAAACAUGUGGAACAAGAGGACCAUCUCUGAGAUCAACCACUGCAAGCGGAAGGGUGAUCGAGGAAUGGUGCUGCAGGGAGUGCACAAUGCCCCCACUGUCGUUCCGGGCUAAAGAUGGCUGUUGGUAUGGAAUGGAGAUAAGGCCCAGGAAAGAACCAGAAAGCAACUUAGUAAGGUCAAUGGUGACUGCAGGAUCAAGAAUUCUGCAGAUAUCCAUCACACUGGCGG |
| Zika Capsid RNA template | GGGCGAGAGUUUCUGGUCAUGAAAAACCCAAAAAAGAAAUCCGGAGGAUUCCGGAUUGUCAAUAUGCUAAAAACGCGGAGUAGCCCGUGUGAGCCCCUUUGGGGGCUUGAAGAGGCUGCCAGCCGGACUUCUGCUGGGUCAUGGGCCCAUCAGGAUGGUCUUGGCAAUUCUAGCCUUUUUGAGAUUCACGGCAAUCAAGCCAUCACUGGGUCUCAUCAAUAGAUGGGGUUCAGUGGGGAAAAAAGAGGCUAUGGAAAUAAUAAAGAAGUUCAAGAAAGAUCUGGCUGCCAUGCUGAGAAUAAUCAAUGCUAGGAAGGAGAAGAAGAAUUCUGCAGAUAUCCAUCACACUGGCGG |
| Zika NS5 RNA template | GGUAGAUCCAUUGUGGUCCCUUGCCGCCACCAAGAUGAAUUGAUUGGCCGAGCCCGUGUAUCACCAGGGGCAGGAUGGAGCAUUCGGGAGACUGCCUGUCUAGCAAAAUCAUAUGCACAGAUGUGGCAGCUUCUUUACUUCCACAGAAGAGACCUUCGACUGAUGGCCAAUGCUAUUUGUUCGGCUGUGCCAGUUGACUGGGUACCAACCGGGAGAACCACCUGGUCAAUCCACGGAAAGGGAGAAUGGAUGACUACUGAGGACAUGCUCAUGGUGUGGAAUAGAGUGUGGAUUGAGGAGGAAUUCUGCAGAUAUCCAUCACACUGGCGGCCGCUCGAGC |

Supplementary Table S4. Zika NS2b qRT-PCR primers, TaqMan probe, and RNA template.
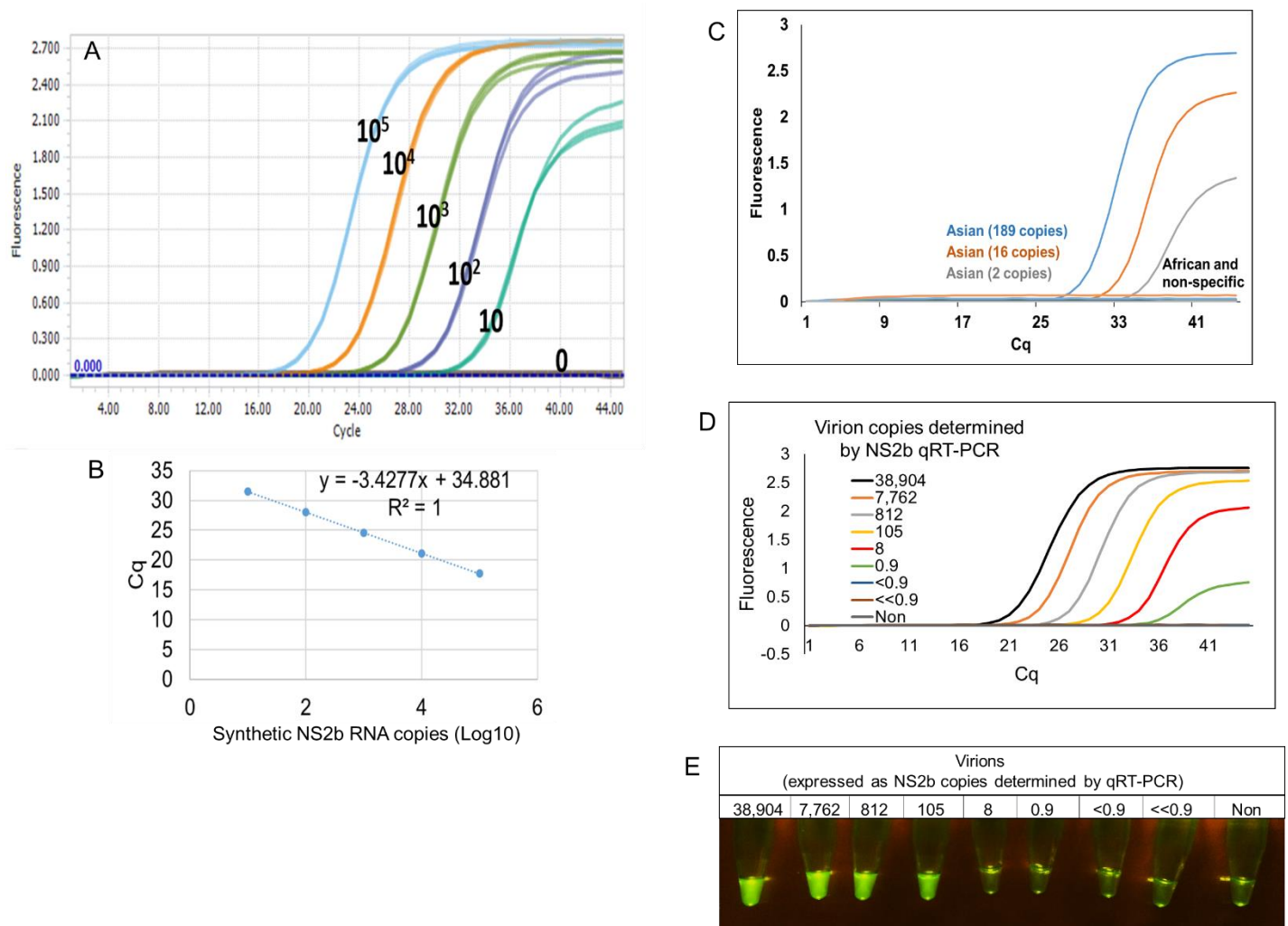
| Zika4481_F | CTGTGGCATGAACCCAATAG |
|---|---|
| Zika4552c_R | ATCCCATAGAGCACCACTCC |
| Zika4507cTqMFAM | /56-FAM/CCACGCTCCAGCTGCAAAGG/3IABkFQ/ |
| NS2b qRT-PCR RNA template | GGGAC CAUCUGUGGCAUGAACCCAA UAGCCAUACC CUUUGCAGCU GGAGCGUGGU ACGUGUAUGU GAAGACUGGAAAAAGGAGUG GUGCUCUAUG GGAUGUGCCU |

Supplementary Table S5. Viral genomic RNA samples used in this study.

| BEI Resources catalog number | GenBank Accession number | Description of material |
|---|---|---|
| NR-50369 | KX087101 | Zika virus -- Zika virus, PRVABC59, Heat-Inactivated |
| NR-50085 | AY632535 | Genomic RNA from Zika Virus, MR 766 -- Zika virus |
| NR-50086 | KU963574 | Genomic RNA from Zika Virus, IbH 30656 -- Zika virus |
| NR-50241 | KU820897 | Genomic RNA from Zika Virus, FLR -- Zika virus |
| NR-50283 | KX446950 | Genomic RNA from Zika Virus, MEX 2-81 -- Zika virus |
| NR-50284 | KX446951 | Genomic RNA from Zika Virus, MEX I-7 -- Zika virus |
| NR-50244 | KX087101 | Genomic RNA from Zika Virus, PRVABC59 -- Zika virus |
| NR-50358 | KX262887 | Genomic RNA from Zika Virus, R103451 -- Zika virus |
| NR-50328 | KX198135 | Genomic RNA from Zika Virus, H/PAN/2016/-259634 -- Zika virus |
| NR-50329 | KX156774 | Genomic RNA from Zika Virus, H/PAN/2015/CDC-259359 -- Zika virus |
| NR-50330 | KX156775 | Genomic RNA from Zika Virus, H/PAN/2015/CDC-259249 -- Zika virus |
| NR-50331 | KX156776 | Genomic RNA from Zika Virus, H/PAN/2015/CDC-259364 -- Zika virus |
| NR-50345 | AF192908.1 | Genomic RNA from Chikungunya Virus, 181/25 -- Chikungunya virus |
| NR-32847 | EU848545 | Dengue virus -- Dengue Virus Nucleic Acid Panel: Dengue Virus Type 1, Hawaii, Dengue Virus Type 2, New Guinea C, Dengue Virus Type 3, Philippines/H87/1956, Dengue Virus Type 4, H241 |

**Supplementary Figure 1.** Detection of Zika virus genomic RNA using non-degenerate primers in LAMP-OSD assays. Genomic RNA from CHIKV or Asian or African lineage ZIKV (indicated by their GenBank accession numbers) were used as templates for amplification assays for Zika virus capsid (panel A), NS1 (panel B), NS3 (panel C), and NS5 (panel D) genes. OSD fluorescence signals measured in real-time using LightCycler 96 real-time PCR machine are depicted as blue (Asian ZIKV), red (African ZIKV), and black (CHIKV) traces. Representative results from three replicate experiments are depicted.

**Supplementary Figure 2.** Estimation of template copies in Asian lineage ZIKV genomic RNA and ZIKV virions. (A and B) Standard curve analysis of NS2b TaqMan qRT-PCR assay using indicated copies of synthetic NS2b RNA template. (C) NS2b TaqMan qRT-PCR analysis of an Asian lineage ZIKV genomic RNA. Real-time amplification curves for different genomic RNA copies are depicted as blue (189 copies), orange (16 copies), and gray (2 copies) traces. Lower template copies or non-specific templates were not detected. Genome copy numbers were estimated using a standard curve analysis (B) of different amounts of synthetic NS2b RNA. (D) NS2b TaqMan qRT-PCR analysis of Asian lineage ZIKV virions. Template copies were estimated using standard curves depicted in panel B. (E) Detection limit of degenerate multiplex LAMP-2GO assays for Asian lineage ZIKV virions. ZIKV virion copies (as deduced by NS2b TaqMan qRT-PCR in panel B) in each reaction are indicated. 'Non': non-specific virions; $10^6$ TCID$_{50}$. Smartphone image was taken after 2 h of amplification.

**Supplementary Figure 3.** Detection of Zika virus infected mosquitoes using individual and multiplex degenerate LAMP assays. Zika virus infected ('Z') and uninfected ('U') *Aedes aegypti* mosquitoes were directly analyzed using degenerate NS1 and capsid LAMP-OSD assays or with multiplex LAMP-2GO ('4px 2GO') assays. Assay type is indicated at the bottom of each individual panel. As a positive control mosquitoes were tested using the *Ae. aegypti coi* LAMP-OSD assay. Smartphone images acquired after 2h of amplification are depicted. P: positive control; L+: mosquito analyte with LAMP primers; L-: mosquito analyte without LAMP primers; N: no template control.