Complete Genome Sequences of Zika Virus Strains Used for the Formulation of CBER/FDA RNA Reference Reagents and Lot Release Panels for Nucleic Acid Technology Testing

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ABSTRACT We report here the complete genome sequences of two Zika virus strains (FSS13025 and PRVABC59) used for formulation of CBER/FDA RNA reference reagents and lot release panels for use with nucleic acid technology (NAT) testing.

Zika virus (ZIKV) is a positive-stranded RNA virus (genus Flavivirus, family Flaviviridae) (1) spread by Aedes sp. mosquitoes (2), and it can also be transmitted from human to human by sexual contact during pregnancy, and by blood transfusion (3–10).

Most human ZIKV infections are asymptomatic (>80%), and clinical cases present mild symptoms or influenza-like illness accompanied by conjunctivitis, skin rash, fatigue, and joint pain (11). ZIKV infection can cause Guillain-Barré syndrome in adults, and infection during pregnancy may cause fetal congenital Zika syndrome, characterized by microcephaly and other brain anomalies, ocular defects, loss of hearing, and muscle and joint issues (3–5, 12–20).

Discovered in Africa in 1947, ZIKV spread to Asia (21–25), the Pacific Islands (26–29), and in 2015 to the Americas (30–32). Zikadisease became a U.S. national threat in 2016, with 4,897 travel-related cases, 224 local mosquito-transmitted cases, and 47 cases acquired through other routes, including sexual transmission (33).

In 2016, a panel of CBER/FDA reference reagents was prepared using two ZIKV strains, FSS13025 and PRVABC59. This material has been used to assist in the development of nucleic acid technology (NAT) assays for blood screening and to support the regulatory evaluation and lot release of licensed and investigational ZIKV NAT assays. Currently, there is one FDA-approved NAT blood screening assay for ZIKV (34).

We report here the complete genome sequences of the following two strains used to produce the CBER/FDA ZIKV RNA reference reagents: FSS13025 (Cambodia, 2010, kindly provided by N. Vasilakis, University of Texas Medical Branch) and PRVABC59 (Puerto Rico, 2015, kindly provided by B. Johnson, CDC). Both strains were provided as supernatants from passage 3 in Vero cell culture and subjected to an additional passage in Vero cells at a multiplicity of infection (MOI) of 0.01, and clarified supernatants were used for total RNA extraction using the QIAamp viral RNA minikit (Qiagen). Overlapping PCR amplicons covering the entire genome were produced with the Qiagen OneStep kit using a set of ZIKV-specific primers (primer sequences are available upon request). A genome cyclization technique was employed to amplify noncoding regions (NCRs) of the viral genome. The assembly and sequence analyses were performed using Vector NTI version 11.5 (Invitrogen) and the Sequencher software (Gene Codes).

The full-length genomes of the FSS13025 and PRVABC59 strains are 10,807 nucleotides (nt) each and contain a single 10,272-nt open reading frame (ORF) flanked by a 107-nt 5′ NCR and a 429-nt 3′ NCR. The ORF encodes a 3,423-amino-acid (aa) polypro-
tein cleaved into the following 10 proteins by the viral and cellular proteases (35): C (122 aa), prM (168 aa), E (504 aa), NS1 (352 aa), NS2A (226 aa), NS2B (130 aa), NS3 (617 aa), NS4A (150 aa), NS4B (251 aa), and NS5 (903 aa). We found no differences between the nucleotide and amino acid sequences of the original isolates (GenBank accession numbers KU955593 and KX377337) and those of the FSS13025 and PRVABC59 isolates cultivated and sequenced in our laboratory.

**Accession number(s).** The complete sequences of the ZIKV FSS13025 and PRVABC59 strains used for the formulation of CBER/FDA RNA reference reagents and lot release panels have been submitted to GenBank under the accession numbers MH158236 and MH158237, respectively.

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The findings and conclusions in this article are an informal communication and represent the authors’ own best judgment. These comments do not bind or obligate the FDA.

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