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2 **Title:** Genome Sequences of eight Crimean-Congo hemorrhagic fever virus strains

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12 **Abstract:**

13 Crimean-Congo hemorrhagic fever virus (CCHFV) is a geographically widespread RNA virus with a high

14 degree genomic diversity that complicates sequence-based diagnostics. Here, we sequenced eight CCHFV

15 strains for improved assay design and deposition into FDA-ARGOS, the FDA's pathogen database for

16 diagnostic assay verification studies.

17

18 **Article Text:**

19 Crimean-Congo hemorrhagic fever virus (CCHFV) is a geographically widespread [1-6] and genetically

20 diverse virus [7-9]. Account for this genomic diversity is critical for efficacious diagnostic assay design,

21 as highlighted by Atkinson and colleagues [10]. Capturing appropriate viral diversity with sufficient

22 depth of coverage and completeness is required for inclusion in FDA-ARGOS, a database of regulatory-

23 grade genomes for clinically relevant pathogens maintained by the US Food and Drug Administration

24 (FDA). Here, we sequenced each genome segment of multiple CCHFV strains prepared by the Unified

25 Culture Collection (UCC) to improve our CCHFV assay and include in FDA-ARGOS.

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27 Total nucleic acids were acquired from the UCC for eight CCHFV strains including IbAr 10200 (UCC#
28 R4401), DAK8194 (UCC# R4416), SPU 128/81 (UCC# R4417), SPU 115/87 (UCC# R4448), UG 3010
29 (UCC# R4432), JD-206 (UCC# R4413), HY-13 (UCC# R4459), and Drosdov (UCC# R4405). Each
30 segment was amplified using a previously published protocol [7] with primers modified for Nextera-
31 based sequencing. Amplicon for each genome segment was gel-extracted, processed with the Nextera XT
32 kit (Illumina, San Diego, CA), and sequenced on the MiSeq Sequencer (Illumina).
33
34 Sequencing reads were analyzed using CLC Genomics Workbench (Qiagen, Valencia, CA). Reads were
35 trimmed for quality and to remove the internal L amplification primer sequences, *de novo* assembled, and
36 BLAST analyzed to identify the closest matching CCHFV sequence. Total reads were mapped again
37 against the virus-specific contigs to generate a final consensus sequence for each genome segment. For
38 JD-206, The L2 segment amplified poorly, resulting in an incomplete assembly. This segment was re-
39 amplified and sequenced using a sequence-optimized L2-F primer (5'-
40 GGAAGAGTTATACAACATAAGGC) modified for Nextera sequencing. The 5' end of the M segment
41 of JD-206 did not fully assemble, and Sanger sequencing data using the primer CCHF JD-206 M R (5'-
42 TTCCTCCATTGTGAGATGAAGC) was used to complete the assembly.
43
44 All segments had at least 100x coverage across the genome. Segments for IbAr 10200 (M segment),
45 Drosdov (M segment), SPU128/81 (M and S segments), UG3010 (L segment), and HY-13 (S segment)
46 had multiple nucleotide variants resulting in amino acid changes and/or in-frame deletions. Sequencing of
47 SPU 128/81 (L segment) and HY-13 (M segment) extended and completed the sequences already in
48 GenBank. Sequences for SPU 115/87 (all segments), the L segment for HY-13, and the L and M
49 segments of JD-206 have not been deposited into GenBank. All sequences were accessioned into
50 GenBank with the exception of those exactly matching sequences in GenBank. Sequencing reads for all
51 strains were deposited with NCBI Sequence Read Archive (SRA), and consensus sequences were
52 deposited into FDA-ARGOS as the assembly qualities met database requirements.

53

54 Overall, we generated 24 separate CCHFV genome segments from eight different strains. Six new
55 sequences having nonsynonymous variants or in-frame deletions were generated for genome segments
56 already within GenBank. Two segments in GenBank were extended to completion, and five novel
57 segment sequences were completed. Additionally, these data were collected in a manner amenable for
58 transition to the FDA-ARGOS database.

59

60 **Nucleotide Sequence Accession Numbers:**

61 Genome accession numbers to public databases are listed in Table 1.

62

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