# DEVELOPMENT AND VALIDATION OF A FIELD SEQUENCING PROTOCOL FOR RIFT VALLEY FEVER VIRUS (RVFV) USING MinION

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Rift Valley fever (RVF) is a mosquito-borne viral zoonosis endemic across numerous African countries and the Arabian Peninsula. The RVF virus predominantly targets domestic livestock such as sheep, goats, cattle, and camels, leading to elevated rates of neonatal mortality and abortion. Human infection manifests with a wide variety of clinical outcomes, ranging from mild febrile illness to eye disease, encephalitis and severe hemorrhagic diatheses. RVF is included in the Category A list according to Regulation (EU) 2016/429 on Animal Health Law. This designation underscores the severe threat it poses to animal health and emphasizes the need for stringent control measures to prevent its spread. RVF is also prioritized by the World Health Organization for urgent research and development of diagnostic methods aimed at early detection and characterization of the viral strains. Given the challenges of accessing areas where RVF virus (RVFV) normally circulates and the difficulty in sending samples for analysis, the possibility of conducting field diagnoses becomes crucial.This study describes the development and validation of an amplicon-based sequencing method to be used in the field by portable MinION sequencer. Serial dilutions of RVFV Namibia 2010 strain (acc.no. MT561459, MT561460 and MT561461) were processed in triplicate to evaluate the detection limit (LOD) of the RVFV sequencing protocols under optimal conditions. To create a dataset of field samples, the RVFV Namibia 2010 strain was spiked-in the ovine serum samples. Total RNA was purified using Quick-RNA miniprep kit (Zymo research) and then reverse-transcribed using random hexamers and Superscript IV Reverse Transcriptase enzyme (ThermoFisher Scientific, Waltham, MA, USA). cDNA amplification was performed using two non-overlapping pools targeting the three genome segments of RVFV and Q5® High-Fidelity 2X Master Mix (New England Biolabs). The amplicons of 400 bp in length were then used as input for library preparation by Rapid PCR Barcoding Kit 24 V14 (SQK-RPB114.24, Oxford Nanopore, Littlemore, Oxford, UK). The libraries pool was then loaded onto flowcell R9.4.1 (FLO-MIN106). The run parameters including the duration time (24 h) and basecaller model (Fast basecalling) were set-up, operating onto MinKNOW software. Fastq WIMP analysis was launched on EPI2ME platform to perform real time taxonomic classification of the fastq files. Mapping of the trimmed reads were performed using Minimap2-2.28 (r1209) and the horizontal (Hcov) and vertical (Vcov) coverage values of the consensus sequences obtained for each genome segments were calculated. These amplicon-based sequencing protocols allow us to perform detection and characterization of the RVFV genome constellation in the simulated field samples. We obtained consensus sequences with horizontal coverage >92% for all three segments from samples with real time Ct value of 30. Further analyses are needed to validate this protocol on different specimens (e.g., animal tissues, mosquitos' samples). Genomic surveillance of virus evolution is crucial for developing effective intervention strategies. The amplicon-based protocol for Rift Valley fever virus (RVFV) sequencing by MinION holds promise for widespread use, both in the field and in laboratories. This is especially significant for low and middle-income countries, where resources may be limited.