INNOVATING CROSS-DOMAIN SOLUTIONS TO DETECT EMERGING BIOLOGICAL THREATS

Novel Taqman Assay For The Detection Of Mayaro Virus Circulation In South America

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United States (U.S.) forces deployed around the world are at risk of contracting acute febrile illness (AFI), including that caused by novel zoonotic viruses. In fact, the vast majority of viruses are generally thought to be as yet undiscovered, so human infections with vector-borne pathogens often present as undifferentiated AFI. Sequencing-based pathogen discovery is more successful when samples are appropriately triaged by adequately screening for known pathogens upfront. Therefore, surveillance for AFI pathogens necessitates sensitive and robust pathogen detection reagents. Recently, we performed viral whole genome sequencing using selected samples from AFI cases received from NAMRU-SOUTH, located in Lima, Peru, that allowed us to characterize the genomes of novel viruses circulating in South America. Among them, we obtained 11 coding complete genomes of Mayaro virus, a mosquito-borne zoonotic virus causing AFI that is endemic to parts of Central and South America. In order to create an improved PCR assay for MAYV detection, we used the 11 coding complete MAYV genome sequences as well as the MAYV reference sequence (MAYV_NC_003417.1) to design highly-specific amplification primers using Primer-Blast. Conserved viral sequences were targeted while minimizing host and off-target amplification. Among the suggested forward and reverse amplification primers generated, the set with the best thermodynamic values, producing an amplicon of size 100-300 and containing a conserved intervening sequence that could serve as the target for TaqMan probes was selected. Using this set for detection of MAYV, we expect a 164 bp amplicon targeting the 5’ end of the polyprotein MAYVgp3 (capsid). We visualized sequence conservation using Clustal Omega Multiple Sequence Aligner and determined that both the forward and reverse primers have 100% sequence identity with all 11 of the MAYV genomes sequenced at BDRD as well as with the MAYV reference genome and that the primer sequences are not conserved for other species of the same viral genus (alphaviruses), thus ensuring high specificity. This sequencing conservation of 100% for MAYV also applies to the TaqMan probe itself, thus making unnecessary the use of degenerate sequences to cover all the targeted viruses. We will transition this surveillance assay to NAMRU-SOUTH to help improve the triage of samples for sequencing by detecting more known pathogens and reducing the number of negatives.