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**Supplemental materials**

**The infection kinetics and transmission potential of two Guaico Culex viruses in *Culex quinquefasciatus* mosquitoes**

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**Tables**

Table S1. Primers and probes used in RT‒qPCR assay.

|  |  |  |
| --- | --- | --- |
| Target | Primer/Probe | Sequence (5′ –3′) |
| Segment1 | S1-Fwd | ATGGAATGGGTGCGCTTTAC |
| S1-Rev | TGGTTCTGATTGAGCGGGAT |
| S1-Probe | FAM- CGCTCCGCTCACTCCAGAACATCA-BHQ1 |
| Segment2 | S2-Fwd | CCACCCTGACTTTCTAACGGA |
| S2-Rev | ACGGGTATTTCCGCATACAGG |
| S2-Probe | FAM-ACGGAACCACGCCAAGCTTCATCCACC-BHQ1 |
| Segment3 | S3-Fwd | TCCCTGTCCTCCTAACCCTATT |
| S3-Rev | ATTGACTGTTGAGGATCGGAGT |
| S3-Probe | FAM-AATGTGCAGATCTGGAACTCGCTGATG-BHQ1 |
| Segment4 | S4-Fwd | GGGCGACGAAATGAAGGC |
| S4-Rev | GTAGGTGCGGTTGTTGAAGC |
| S4-Probe | FAM-TAGACGGAGACAGCGAGAGCGG-BHQ1 |
| Segment5 | S5-Fwd | GAAAGCCATCCCTCATGCTG |
| S5-Rev | ACCTTACCACGAAGCAGACA |
| S5-Probe | FAM-CGGTCCCGACAAAGCTACCTGGT-BHQ1 |
| RPL8 | RPL8-Fwd | AGTTCAAGCTCCGCAAGCA |
| RPL8-Rev | CACGAACTGGCCGGTGTAC |

**Supplementary figure**



**Supplementary Figure S1.** Log-linear regressions fit to the standard curves for the GCXV-LO35 Segment 1–5 detection assays.Determination of viral RNA copies of target segment 1 (S1) to segment 5 (S5). The number of CP cycles versus the number of log viral RNA copies was plotted to calculate the slope. The corresponding number of virus-specific RNA copies was calculated according to the following equation: The detection limit is indicated by red dashed lines (Ct = 35). The linear equation and R2 values for the linear fit to each set of data are shown.