**q-RT PCR Protocol for WNV Surveillance Week 28 – 7.14.22**

1. Thaw 4x (per 96 well plate) 27x WNV q-RT PCR Master Mix on Ice.
2. Prepare standards by adding 45 uL of dilution solution (5% BSA in nf-H2O) to seven tubes of an Program

8-tube PCR strip. Retrieve one 1.00E+08 WNV standard from -80, thaw on ice, vortex and spin 1. 15m at 50˚C

down thoroughly, and then make a tenfold dilution series from 1.00E+07 through 1.00E+01 by 2. 2m at 95˚C

taking 5 uL from the standard (and so on) across the strip. Mix ~20 times with pipette between 3. 15s at 95˚C

each transfer step. Hold standard dilutions on ice. 4. 1m at 60˚C

1. Add **27 uL** of Reverse transcriptase to 27x master mix (per 2018 Invit. superscript recipe) CAPTURE
2. Place PCR plates on frozen plate rack. 5. Goto cycle 3 39X
3. Add 15ul of complete master mix per well (mix with pipette and add individually). (No Passive Ref/ROX)
4. Add 5ul of RNA extract to wells (use multichannel pipettor).
5. Seal plate and run using WNV template:

Program

1. 15 m at 50˚ C

2. 2 min at 95˚ C

3. 15 s at 95˚ C

4. 1 m at 60˚ C

CAPTURE

5. Repeat cycles 3-4 39X

(No passive reference/ROX)