**q-RT PCR Protocol for WNV Surveillance Week 25 – 6.24.21**

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 8-tube PCR strip. Retrieve one 1.00E+08 WNV standard from -80, thaw on ice, vortex and spin down thoroughly, and then make a tenfold dilution series from 1.00E+07 through 1.00E+01 by taking 5 uL from the standard (and so on) across the strip. Mix ~20 times with pipette between each transfer step. Hold standard dilutions on ice.
3. Add **27 uL** of Reverse transcriptase to 27x master mix (per 2018 Invit. superscript recipe)
4. Place PCR plates on frozen plate rack.
5. Add 15ul of complete master mix per well (mix with pipette and add individually).
6. Add 5ul of RNA extract to wells (use multichannel pipettor).
7. 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)