**WNV Surveillance Extraction Protocol Week 28: 7.14.16**

1. Verify pools and data correlate ( 9166-9292 ) 127 total

Mosquito Homogenizing:

1. Do 48x samples at a time.
2. Add 1x steel BB to each tube.
3. Add 1.0ml of mosquito diluent with the repeat pippeter to each tube.
4. Place in Tissue homogenizer @ 24 Hz for 60 sec.
5. Centrifuge on desktop centrifuge at max speed for 5 minutes.
6. Refrigerate at 4 C until ready to extract.
7. Add 50ul of supernatant to RNA extraction plate.

King Fisher RNA Extraction:

Sample Plate Preparation: 190ul (191ul if LPA used) total volume. In 2.0ml deep 96 well plates.

(Make 140 x master mix for 127 samples)

1. Add 5ul Proteinase K to each well ( **700 ul).**
2. Add 5ul Mag-Bind particle beads to each well ( 700 **ul**).
3. Add 60ul TNA Lysis Buffer to each well **( 8400 ul**).
4. Add 70ul Isopropanol to each well **( 9800 ul**).
5. No LPA needed.
6. Add 50ul the **Sample** to each well.

VBH Plate Preparation: In 2.0ml deep 96 well plates.

1. Add 200ul of VBH Buffer to each well.

Spr-1 & SPR-2 Plate Preparation: In 2.0ml deep 96 well plates.

1. Add 200ul SPR Buffer to each well

Elution Plate Preparation: In short 96 well elution plates

1. Add 50ul H2O to each well.
2. No LPA needed due to high concentration of RNA.

Tip Plate Preparation: In 2.0ml deep 96 well plates.

1. Add tip comb to tip plate.

Start Fisher Protocol from Lab PC.

Run WNV Surveillance q-RT PCR as previously described.