**WNV Surveillance Extraction Protocol: 6.22.17**

1. Verify pools and data correlate

*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.

*King Fisher RNA Extraction:*

Sample Plate Preparation: 190ul total volume. In 2.0ml deep 96 well plates.

Make **80x** master mix for **69** samples (69 samples + 3 controls +10% = 80)

1. Add 5ul Proteinase K to each well (400ul).
2. Add 5ul Mag-Bind particle beads to each well (400ul).
3. Add 60ul TNA Lysis Buffer to each well (4,800ul).
4. Add 70ul Isopropanol to each well (5,600ul).
5. No LPA needed.
6. **Vortex master mix thoroughly!** Add 140uL of master mix to each well.
7. Add 50ul of the sample supernatant to each well of RNA extraction plate.

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 nfH2O 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.