**WNV Surveillance Extraction Protocol: 6.25.15**

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.
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 143x master mix for 130 samples)

1. Add 5ul Proteinase K to each well (390ul).
2. Add 5ul Mag-Bind particle beads to each well (390ul).
3. Add 60ul TNA Lysis Buffer to each well (4,680ul).
4. Add 70ul Isopropanol to each well (5,460ul).
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.