**WNV Surveillance Extraction Protocol: 7.22.21**

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 pipeter to each tube

(make sure to aliquot mosquito diluent into 50 mL conical(s) in BSC).

1. Place in Tissue homogenizer @ 24 Hz for 60 sec.
2. Centrifuge on desktop centrifuge at max speed for 5 minutes.
3. 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 **220x** master mix for **202** samples (202 samples + 9 controls +4% = 220)

1. Add 5ul Proteinase K to each well (**1,100uL**).
2. Add 5ul Mag-Bind particle beads to each well (**1,100uL**).
3. Add 60ul TNA Lysis Buffer to each well (**13,200uL**).
4. Add 70ul Isopropanol to each well (**15,400uL**).
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

**(Note: remove PCR-standard and master-mix from cold storage to thaw before this step)**

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.