

## PA Dept of Environmental Protection Lab Operations - Mike Hutchinson

- a) What happens to PA mosquitoes when they get to the lab?
    - i) Receiving process
      - (1) Samples in barcoded bottles
      - (2) Box is bar-coded
      - (3) Samples shipped on dry ice
    - ii) Receiving screen
      - (1) Barcode data are uploaded to database
      - (2) Field info uploaded by surveillance person
      - (3) Database flags samples from
        - (a) Positive counties
        - (b) Counties with large numbers of vectors submitted
    - iii) Samples placed into ULV freezer
    - iv) Cold chain maintained
    - v) Identification process
      - (1) Include non-*Culex* species when a county is positive
      - (2) Drop down menus for entering ID info
      - (3) Check boxes to indicate mosquitoes kept for side projects
      - (4) Quality control pop-ups included to let interns know to consult a taxonomist about the ID
      - (5) Samples placed into barcoded pool vials that correspond with original barcode
    - vi) Testing process
      - (1) 90 pools per test run
      - (2) Molecular testing
      - (3) BSL-3 lab
        - (a) Work under biosafety cabinet maintaining the cold chain
        - (b) Place BBs
        - (c) Vortex vial
        - (d) Add buffer solution
        - (e) Re-vortex
        - (f) Centrifuge
        - (g) Transfer to 96-well block containing lyses solution
        - (h) Extract RNA
        - (i) RT-PCR
          - (i) Prepare master mix, probes, and primers in new well plate
          - (ii) Add RNA samples to new well plate
          - (iii) Add controls
          - (iv) Taqman - ~30 minutes
            1. Amplification
              - a. Polymerization
              - b. Strand displacement
              - c. Cleavage
              - d. Polymerization complete
            2. Results in graph form
- b) Other projects

- i) Test pools for other less common viruses
- ii) Molecular ID of blood meals
- iii) Molecular ID of specific mosquitoes species
  - (1) Spot check on *Culex* spp ID
  - (2) Differentiate members of *Anopheles* complexes
- iv) Collaborate with other researcher on a variety of projects
- v) Photographic key of mosquitoes
- vi) Lots of black fly work