

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