



Bottle bioassay shipping protocol

1. Collect 4th instar larvae/pupae from sources likely to produce *Culex quinquefasciatus*.
 - a. Collect a minimum of 200 mosquitoes from one source, preferably 400+. If fewer than 200 mosquitoes are collected at one site, you can pool collections from multiple sites.
 - b. If the majority of collected mosquitoes are 4th instars, retrieve up to 5-gallons of water from the source to be used later in the insectary. This is not necessary if mosquitoes are majority pupae.
2. Confirm that collected larvae are *Cx. quinquefasciatus* under a microscope.
3. Up to 200 4th instar larvae should be kept in a tray with ¾ inch water collected from the same source. Adding larvae beyond 200 may stunt growth and physiology. Additional trays can be used for collections of more than 200 individuals.
4. If the majority of collected mosquitoes are pupae, or as 4th instars begin to pupate, place pupae in cups of water within adult observation cages.
 - a. Observation cages can contain thousands of adults, unlike larval trays.
5. Fill out columns A through F of the attached spreadsheet (IR data.xlsx).
 - a. Note: 'Pooled Collection' in column B indicates pooling multiple sites.
6. Write parish of origin and the unique collection ID # (Column C in IR data.xlsx) on cage.
7. Check observation cages daily for adult emergence.
 - a. Write date of first adult emergence on cage.
8. In each cage, place cotton coils in flasks saturated with a 10% sucrose solution.
9. After the majority of pupae have eclosed, approximate if there are enough female mosquitoes for a bottle test, bearing in mind a minimum of 100 are needed to perform a bottle test, preferably more.
10. Prior to transfer to LSU, remove pupal cups and sucrose solutions from cages.
11. Keep cages out of direct sunlight to the best of your ability en route.
 - a. If traveling longer than 1 hr in hot weather, place cages in an ice-chest or Styrofoam box alongside ice packs wrapped in newspaper.
12. Deliver cage to LSU, and retrieve a replacement cage for next shipment.