

Culex pipiens Collection and Submission Guidelines

*** If you are using a collection protocol that is already established by your agency please use [these guidelines](#) instead for shipping instructions.**

<u>Item</u>	<u>Number included in kit</u>
Falcon Tubes (one to be used per egg raft)	10
White Ladle	1
Mesh Dippers (stick and screen mesh)	2
Whirl-Pak Bags	20
Ice Packs	5
Styrofoam Container	1
Falcon tube fish food	1

Larval Identification Key



Materials included in the kit

Specimen Collection Procedure

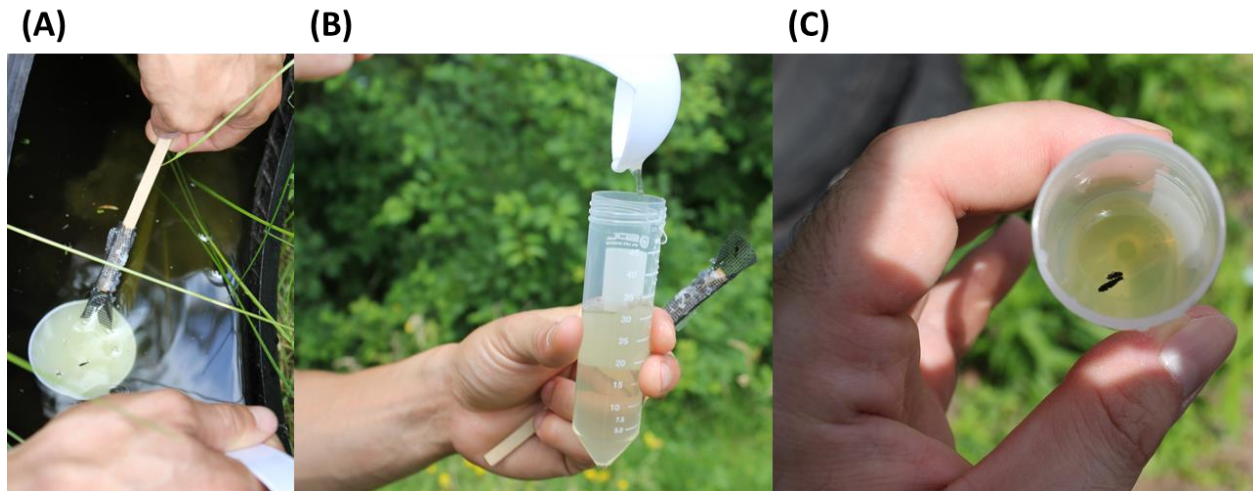
Culex pipiens larvae and egg rafts are often found in standing water and the species prefers polluted water with high levels of organic content. *Cx. pipiens* can be found in a range of containers that hold water, and is often associated with tire piles, catch basins, and storm drains. Another species, *Cx. restuans*, is often found alongside *Cx. pipiens*. These species are difficult to differentiate as adults, but the larvae can be identified to species using the key posted [here](#).

- 1) Wait to sample until July – September to increase your probability of obtaining *Cx. pipiens* egg rafts. Earlier times may be suitable along the southern border of our region.



Egg rafts with adult Cx. pipiens for scale

- 2) Before sampling, pour out about 1 gallon of water and leave it in an open container for 25 hours to allow it to dechlorinate.
- 3) Find a pool of stagnant water that has larvae in it and pour some of the water into one of the provided **falcon tubes** using the **White ladle** at least 50% full. Make sure you do not transfer any larvae into the tube with the water.
- 4) Search the standing water within the container for egg rafts by slowly dipping the white ladle into the water and moving it around just under the water. The white ladle will provide a contrast with the dark egg raft allowing you to see it more easily than you would against the dark background of the container.
- 5) Once an egg raft is identified use the **mesh egg lifter** to gently lift the raft out of the container and place it into one of the falcon tubes containing water. If the egg raft sticks, gently spin the dipper in the water until the raft becomes dislodged. Egg rafts that have already hatched will often look like the eggs are breaking apart from each other.



Collecting *Cx. pipiens* egg rafts (A) searching with white ladle, (B) adding water from collection site into the falcon tube, and (C) placing the egg raft into the tube

- 6) Only place a single egg raft into each falcon tube so that once the larvae emerge (~24 hours) each raft can be identified to species individually. After hatching, only 2 – 3 larvae per raft need to be examined and they should be chilled in ice water or placed in 70% ethanol prior to examination to immobilize them. If collecting more than one egg raft prepare another falcon tube and keep tubes containing egg rafts upright. The [Larval Identification Key](#) can be used to determine the species of each egg raft. The features used to differentiate 4th instar *Culex* larvae are also present in 1st instar larvae. If you are lacking equipment or training, skip this step, submit the specimens to us and they will be identified once they arrive.
- 7) ***If unable to send larvae immediately after egg rafts hatch***, pour ~1/4 of the water out of the **bottle of water** and transfer the larvae to bottle and add a tablet of **fish food**. Keep the cap of the bottle off so that air can get in. This will reduce density-related mortality and keep the larvae alive for 2 – 3 days so that they can be shipped. If possible, do not place more than 100 larvae in the water bottle. If you must use higher densities, then send the larvae as quickly as possible.
- 8) Once you are ready to ship the larvae, pour the contents of the falcon tube, including the larvae, onto a **whirl-pak bag** and fill the bag ~75% full of water and a small amount of **fish food** (~1/4 a pellet). Make sure there is a small air pocket enclosed in the bag to allow any hatched larvae to breath in transit. Tightly seal the whirl-pak enclose it within a second whirl-pak. Allow the egg rafts to sit at room temperature and hatch into larvae (usually 1 – 2 days).

- 9) Once the larvae have emerged place the bags into the **Styrofoam container** with **ice packs** separated by paper towels to prevent direct contact between the bags and ice packs. Fill out the **Specimen Submission Form** on our website (<https://www.neregionalvectorcenter.com/resistance>) and send it to James Burtis jb766@cornell.edu to notify us of the impending delivery. Please also print out a copy and include in the package so that the label can be matched once it arrives at Cornell University.

(A)



(B)



Ship the culex (A) place ice packs in the bottom of the Styrofoam container and cover them with paper towels and (B) place whirl pak bags in container

Please send the package overnight* to the address below:

Primary Address

James Burtis
Cornell University
129 Garden Ave
3131 Comstock Hall
Ithaca, NY 14853

** If you require a fedex number to send the package overnight, one may be obtained by contacting James Burtis (jb766@cornell.edu). Please note, we cannot support shipping for all agencies, so please only request a fedex number if necessary.*