***Culex pipiens* Collection and Submission Guidelines**

*** If you are using a collection protocol specific to your agency, please use these guidelines for shipping instructions.**

Item	***Number included in kit***
White Ladle	1
Mesh Dippers (stick and screen mesh)	2
Styrofoam Container	1
Ice Packs	5

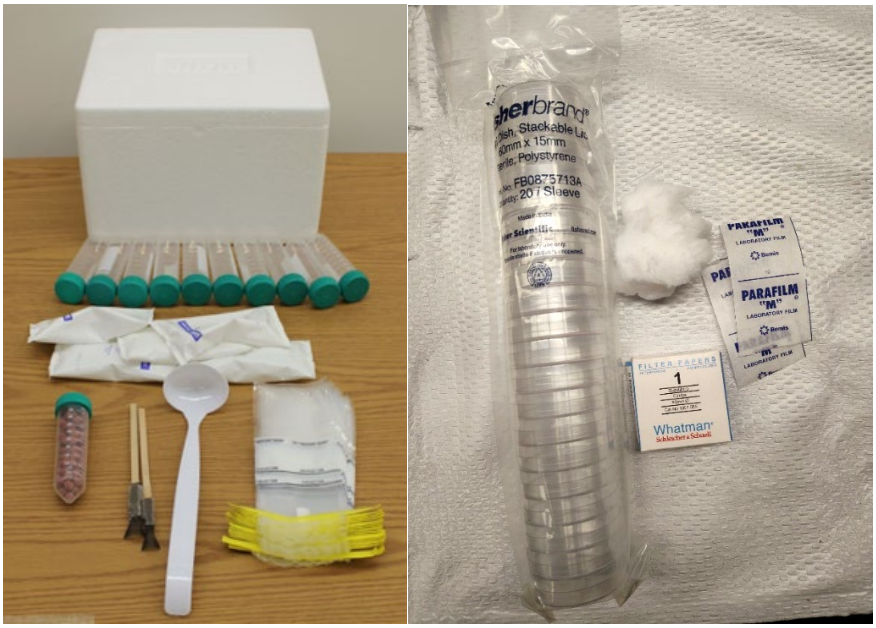
For shipping larvae:

Falcon Tubes (one to be used per egg raft)	10
Whirl-Pak Bags	20
Fish food tube	1
Ice barrier (cardboard)	1

Larval Identification Key

For shipping egg rafts:

Small plastic petri dishes (one to be used per egg raft)	15
Filter paper	30
Cotton pads	15
Parafilm	15



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. They can be found in a range of containers that hold water, and are 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 on **pages 6-7**. Wait to sample until July through August to increase your probability of obtaining *Cx. pipiens* egg rafts. Earlier sampling may be suitable along the southern border of the Northeast region.

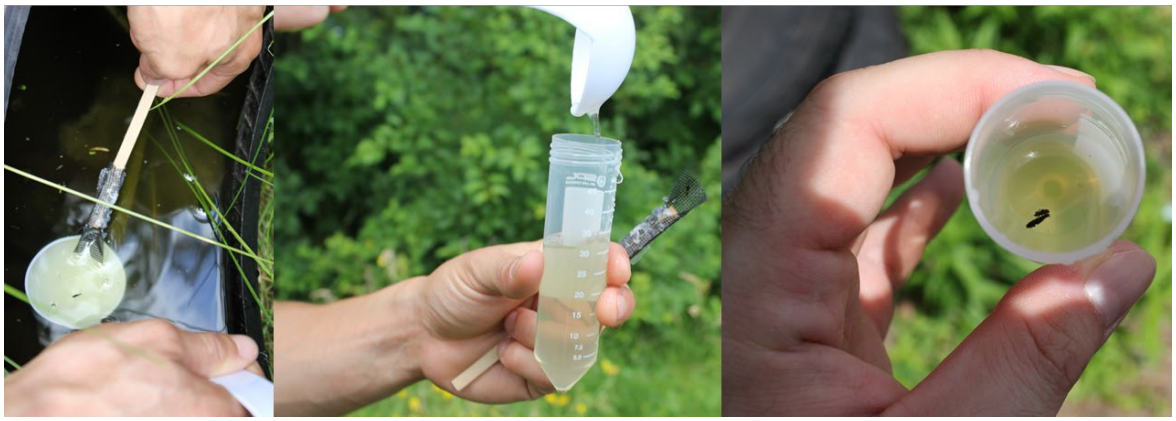
Egg rafts with adult Cx. pipiens for scale

1) Before sampling, pour about 1 gallon of water into an open container and leave it uncovered for 24 hours to allow it to dechlorinate. Find a pool of stagnant water that has larvae in it or place a bucket containing water in an area where routine mosquito collections are performed. Water infused with hay and/or chicken fertilizer can be used to better attract *Culex* females.

2) When ready to search for egg rafts, pour water from the collection site into one of the provided **falcon tubes** using the **white ladle** to at least 50% full. Make sure you do not transfer any larvae into the tube with the water. Search the standing water for egg rafts by slowly dipping the white ladle into the water and gently 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.

3) Once an egg raft is identified, use the **mesh egg dipper** to gently lift the raft out of the water and place it into the falcon tube. If the egg raft sticks to the mesh, 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. Collect a minimum of two egg rafts per larval assay and at least four egg rafts per adult assay requested. **If possible, it is preferable to collect egg rafts from many females to provide more accurate resistance data for your local population of mosquitoes.** Place caps on falcon tubes and keep upright during transit.

(A) (B) (C)



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.

4) If hatching and identifying larvae, proceed to the ***Culex* Larvae Packing Procedure (below).** If shipping eggs immediately after collection and prior to hatching, skip to the ***Culex* Egg Raft Packing Procedure (page 4).** Shipping egg rafts is best if collection timing would result in larvae maturing too much before weekday shipping is possible, i.e. if egg rafts are collected on a Wednesday or Thursday. Wait to collect and submit egg rafts until a point in the summer season when there are known to be *Culex pipiens* at your collection site to decrease the chance of submitting only *Culex restuans* egg rafts.

Option 1: *Culex* Larvae Packing Procedure

1) **Make sure there is only a single egg raft in each falcon tube** so that once the larvae emerge (typically in 24-48 hours) each raft can be identified to species individually. Loosen caps slightly to allow airflow while waiting for hatching and keep falcon tubes upright.

2) After hatching, only 2 – 3 larvae per raft need to be identified to confirm the species. They should be chilled in ice water or placed in 70% ethanol prior to examination to immobilize them. The **Larval Identification Key** can be used to determine the species of each egg raft. **The key to identify 2nd instar *Culex* can be found on pages 6-7 at the end of this document.**

3) Once ready to ship, pour the contents of the falcon tube, including the larvae, into a **whirl-pak bag** and fill the bag ~75% full of purified water and a small amount of **fish food** (~1/4 of a pellet). Make sure there is a small air pocket at the top of the bag to allow hatched larvae to breath in transit. Tightly seal the whirl-pak and enclose it within a second whirl-pak.

4) Place the bags into the **styrofoam cooler** with frozen **ice packs**. Separate the bags from the ice packs using the provided cardboard divider to prevent direct contact between the bags and ice packs. Place cooler inside cardboard box and tape closed.

(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.

Option 2: *Culex* Egg Raft Packing Procedure

1. Place a slightly damp **cotton pad** in the bottom of one of the **small plastic petri dishes**. Squeeze excess water out of the cotton pad - it should be damp to the touch but not dripping. Cover the damp cotton pad with **filter paper**.

2. Use the **mesh dipper** to carefully transfer one egg raft from a falcon tube onto the filter paper. Eggs may hatch in transit so it is important to **place only one egg raft into each petri dish so that rafts can be identified to species separately.**

(A) (B) (C)

(A) Place cotton in petri dish (B) place filter paper on top (C) scoop one egg raft onto paper.

3. Place another filter paper on top of the egg raft and place the lid on the petri dish. Wrap the entire closed petri dish sandwich in **parafilm**. Write the specific collection location, date, and collecting agency on a piece of tape and stick to the top of the petri dish. Repeat with remaining egg rafts.

(A) **(B)** **(C)**

(A) Place a second filter paper over egg raft and cover dish (B) wrap outside of dish with parafilm (C) label with sample information.

4. Place frozen **ice packs** into the **styrofoam cooler**, followed by the provided **cardboard divider**. Place the petri dishes on top of the cardboard. If there is extra space inside the cooler, add packing material before closing the lid to keep petri dishes from moving around in transit. Place cooler inside cardboard box and tape closed.

***Culex* shipping procedure**

1) Fill out the electronic **Specimen Submission Form** on our website at https://www.neregionalvectorcenter.com/resistance.

2) Ship to the address below **overnight Monday-Thursday** to ensure that packages do not arrive during the weekend when mail cannot be delivered to our lab. Once you have received a tracking number, please email it to us at **pesticide@cornell.edu**.

Primary Address
Harrington Lab
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 pesticide@cornell.edu. Please note, we cannot support shipping for all agencies, so please only request a Fedex number if necessary.***

CULEX

1. Siphon (S) with three or more long, single, irregularly placed setae (Fig. 106); antenna nearly uniform in shape with seta 1-A attached near middle of shaft (Fig. 107) . . . . . ***Culex restuans***

Siphon with three or more pairs of branched setae (Fig. 108); antenna constricted distally with seta 1-A attached near outer third of shaft (Fig. 109) 2

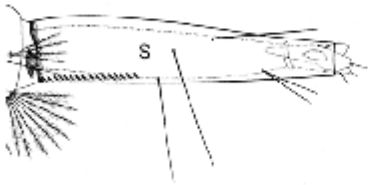


Fig. 106

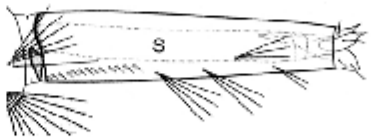


Fig. 108

Fig. 107

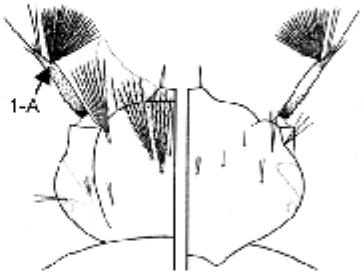


Fig. 109

2. Head hair 6 single or double (Fig. 110) 3

Head hair 6 with three or more branches (Fig. 111) 4

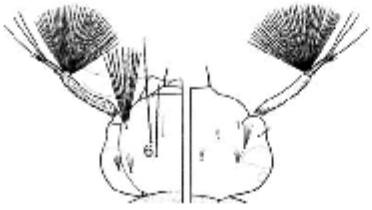


Fig. 110

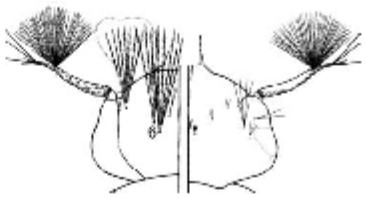


Fig. 111

3. Head hairs 5 and 6 long and about equal in length (Fig. 112); comb scale without long median spine (Fig. 113) . . . . . ***Culex territans***

Head hair 5 at most half the length of 6, multibranched (Fig. 114); comb scale with long median spine (Fig. 115) . . . . . ***Culex erraticus***

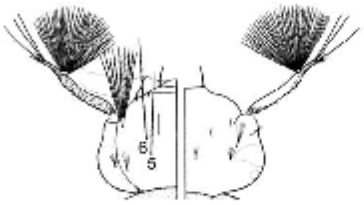


Fig. 112

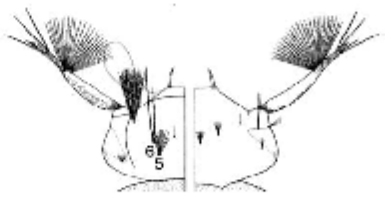


Fig. 114

Fig. 113

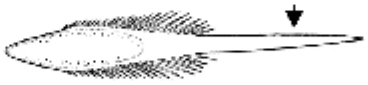


Fig. 115

4. Siphon (S) short and slightly convex, less than six times as long as wide (measured at base) (Fig. 116) . . . . . ***Culex pipiens***

Siphon narrow, seven to eight times as long as wide (Fig. 117) . . . . ***Culex salinarius***

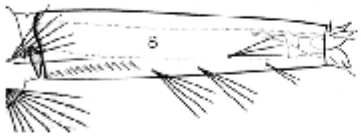


Fig. 116

Fig. 117

Source: Andreadis, Thomas and Shepard. 2005. Identification guide to the mosquitoes of Connecticut. The Connecticut Agricultural Experiment Station Bulletin No. 966