

Rearing Field-Collected *Culex pipiens*

These guidelines lay out all the materials needed to rear mosquitoes collected as eggs from the field in a temporary colony so that they can be used for pesticide resistance testing. Additional resources for long term rearing are provided at the end of this document. These are general guidelines, so if these exact materials are not available, similar supplies can be substituted. Most of these supplies can be reused.

<i>Item</i>	<i>Number needed to rear ~1000 larvae</i>
Large (>50 oz) Plastic Tupperware® Trays with Lids	5
3 ml Plastic Pipettes	5
Water	1 L per tray
Fish Food Pellets	1 small bag
Ground Rabbit Pellets	1 small bag
Liver Powder	1 small bag
Small (3 – 4 oz) Plastic Cups	10
Large (~2 gallon) Paper Tub	5
Cotton Plugs	5
Fine Fabric Square (~1x1 ft) (ex: bridal organdy)	5
Large Rubber Bands	24
Plastic Wrap	1 roll
Sugar water (10% solution)	500 ml
Paper Towels	1 roll
Aspirators	2
Microscope (40 x magnification)	1

If your agency needs assistance in sourcing or buying supplies, please contact Dr. James Burtis at jb766@cornell.edu. We can provide limited rearing supplies to those who need them, or whose funding cannot be used to purchase some items.

Preparing Rearing Supplies

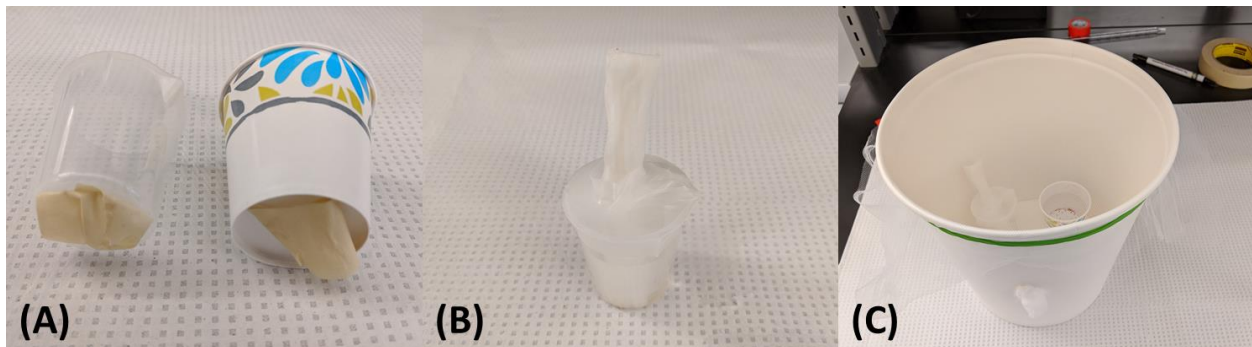
Before rearing mosquitoes, you will need to make some modifications to the supplies described above to make them appropriate for rearing.

- 1) Before cutting, lay out some of the **fine fabric** and make sure that the 1 x 1 ft squares will cover the tops of the **large tubs** that you are using. Some fabric must hang over the edges of the containers so that two rubber bands can be wrapped around it to keep mosquitoes from escaping.
- 2) You will also need to cut some fabric to top the **Large Plastic Tupperware® Trays**. This will vary depending on the tray you use, but it should be large enough so that there is an overhang where you can apply rubber bands. *Optional: You can also cut a hole in the top of the plastic Tupperware® and hot glue the fabric to it.*
- 3) Poke a hole in the side of the bucket so that the tip of your aspirator fits snugly into the hole. Do not make the hole too big or mosquitoes will be able to escape while you aspirate. Use the **cotton plug** to plug the hole. *Optional: You can also cut a larger hole in the side of the bucket and hot glue a fabric sleeve around the hole. It must be long enough so that it can be tied shut. This can make it easier to work in the buckets, but is generally not necessary for resistance bioassays.*
- 4) Cut the tips off the **plastic pipettes** so that the opening is slightly larger to avoid injuring larvae or pupae when transferring them between containers.
- 5) If DI water is not available, pour out 1 gallon of water in an open container and allow it to sit for 24 hours to dechlorinate.
- 6) The larval Culex food recipe is a slurry of 1:2:1 ratio of fish food: rabbit pellets: bovine liver powder. Crush or grind rabbit pellets and fish flakes. Combine 1 Tbs. **fish food**, 2 Tbs. **rabbit pellets**, and 1 Tbs. **liver powder** in a bowl and mix. The diet can be ground and mixed in a large batch ahead of time and stored in a container in a refrigerator.
- 7) Pour 2 Tbs of the dry diet mixture into a bottle and fill with 0.5 L of water. Shake slurry to mix thoroughly.

Rearing Instructions

- 1) Prior to collecting specimens from the field, you should identify good collection sites for *Cx. pipiens* in your jurisdiction. If you do not have a protocol established for collecting *Cx. pipiens* eggs from the field, NEVBD kits and guidelines are available on our website (<https://www.neregionalvectorcenter.com/resistance.php>).
- 2) Place the egg rafts into separate trays containing 1 L of water and about 1 Tbs of the Culex food you prepared. Allow to sit for between 1 – 3 days to hatch.

- 3) Once eggs have hatched, collect approximately five larvae from each egg raft to confirm their species identity using a [larval identification key](#).
- 4) Once identified, fill each tray with 1 L of water, add 1 Tbs of Culex food, and count 200 larvae into each tray using the pipette. Cover with fabric using rubber bands, or the modified top described above. Extra larvae should be killed by placing them in a freezer and storing for a minimum of 24 hours before discarding the container contents in the trash.
- 5) One day after pupation begins (day 5-10, depending on temperature), transfer pupae and 4th instar larvae from rearing trays to **small plastic cups** using pipettes. Place a small piece of tape on the bottom of the cups to keep them from tipping. Place the cups containing pupae into a large bucket, so that there are ~200 per bucket. *Optional: Male pupae are generally smaller and appear 1-2 days before females, pupae can be separated by sex during this stage in the rearing process and bucketed separately to streamline bioassays.*



Setting-up the rearing bucket (A) taping the bottom of the sugar and pupal cups, (B) making a wick for the sugar, and (C) placing them in a cloth covered rearing bucket.

- 6) Pour **10% sugar solution** into a second small cup. Tightly roll a paper towel the short way and dip both ends into the sugar solution. Leave the paper in the cup containing the sugar solution and wrap it with **plastic wrap** so that the paper towel is sticking out the top like a wick. This will draw sugar water up from the reservoir allowing mosquitoes to feed on it without drowning. *Optional: If attempting to rear a colony for multiple generations, you should keep some of the water with pupal casings in it as will encourage females to lay their eggs in the water.*
- 7) Allow adults to emerge (usually within 1 day). If using these mosquitoes for bioassays, test them at an age of 3 – 5 days.
- 8) Any extra mosquitoes not used in bioassays should be placed in a freezer for 24 hours before being discarded.

Additional Resources for Long-Term Colony Maintenance

BEI Resources

<https://www.beiresources.org/Catalog/VectorResources.aspx>

This website provides general guidelines for mosquito rearing in addition to live specimens. Registration is required to order specimens.

Rearing of Culex spp. and Aedes spp. Mosquitoes

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5654580/>

General guidelines for the establishment of Aedes and Culex mosquito colonies.