

Rearing Field-Collected *Aedes albopictus*

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 bioassays. 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
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
Ziploc® Bags	1 pack
Whirl-Pak® Bags	1 pack
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.

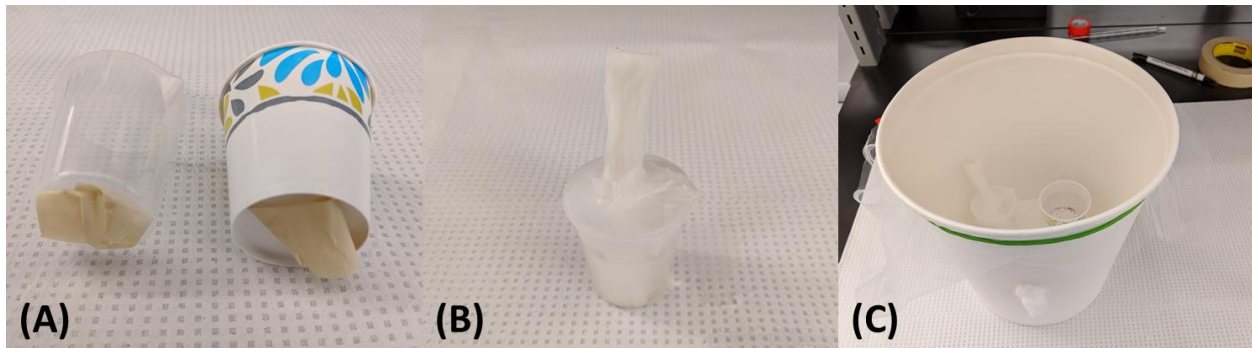
- 1) Before cutting, lay out some of the **fine fabric** and make sure that the 1 x 1 ft squares will cover the tops of the **large tubs** that you are using. Some fabric must hang over the edges of the containers so that two rubber bands can be wrapped around it to keep mosquitoes from escaping.
- 2) 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.

Rearing Instructions

- 1) Prior to collecting specimens from the field, you should identify good collection sites for *Ae. albopictus* in your jurisdiction. If you do not have a protocol established for collecting *Ae. albopictus* eggs from the field, NEVBD kits and guidelines are available on our website (<https://www.neregionalvectorcenter.com/resistance.php>). Make sure to write species, collection site, and date on papers deployed in the field.
- 2) Using a microscope with 40x magnification, eggs collected from the field can be identified using [this](#) guide. Any eggs that are not *Ae. albopictus* can be punctured with a pin so that they do not hatch. *After hatching, you should still identify a subset of the adults or larvae after hatching to ensure that the eggs were processed thoroughly.*
- 3) Allow the egg sheet to dry overnight in a **plastic tray**. In order to stop the eggs from over-drying, do not let the egg sheet to become dry completely. Allow the sheet to remain slightly wet. Gently roll up the egg papers and put into an open **Whirl-Pak® bag**. Store in a labeled **Ziploc® bag** with a wet paper towel or sponge. The eggs must be

conditioned (to embryonate) at high humidity in the Ziploc® for at least 3 days before they will hatch.

- 4) To hatch eggs, place egg sheets in a plastic tray that contains 1 L of water and a pellet of crushed fish food. Leave in the water for 24 hours. **Optional:** *If a vacuum pump and vacuum flask are available, you can fill the flask 1/3 of the way with water and let the egg paper soak for at least 30 minutes. Then connect the flask to the vacuum pump and turn it on for 30 minutes. After 30 minutes, turn vacuum off and allow the eggs sit for another 10 minutes before disconnecting the flask from the vacuum. Place a pinch of fish food in flask and hold overnight until larvae are large enough to count. This will help synchronize your hatch so that all the larvae are the same age.*



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.

- 5) After 24 hours, fill each tray with 1 L of water, count 200 larvae into each tray using the pipette, and add four pellets of fish food. Cover with fabric using rubber bands, or the modified top described above. Extra larvae and eggs 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. **Optional:** *If attempting to rear a colony for multiple generations, you should keep some of the larval water as they do not lay well in clean water.*
- 6) One day after pupation begins (day 5-10, depending on temperature), transfer pupae 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.*
- 7) 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.
- 8) Allow adults to emerge (usually within 1 day). If using these mosquitoes for bioassays, test them at an age of 3 – 5 days.

- 9) 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.