N O T E

An Inexpensive *In Vitro* Blood-Feeding System for Wild *Aedes albopictus* (Diptera: Culicidae)[1]

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The Asian tiger mosquito, *Aedes albopictus* (Skuse) (Diptera: Culicidae), was initially introduced and established in the United States in Houston, TX, in international shipments of used tires (Kuno 2012, J. Med. Entomol. 49:1163–1176). In less than 40 years, its range has expanded to include the Gulf States, midwestern states north to Lake Michigan, and the east coast as far north as Boston, MA (Hawley et al. 1987, Asian Sci. 236:1114–1116). *Aedes albopictus* are efficient vectors for human arboviral diseases such as dengue, Zika, chikungunya, West Nile, and yellow fever (Let et al. 2018, Int. J. Infect. Dis. 67:25–35). The species shows remarkable behavioral flexibility producing strains with enhanced preference for urban habitats and human hosts. Some introduced strains are adapted to survive in a temperate climate with the ability to diapause and produce desiccation-resistant eggs.

Limitations in resources are often impediments in maintaining mosquito colonies for research purposes. Herein is described a system, including in vitro blood-feeding, to provide a consistent supply of wild *Ae. albopictus* for research without the frequently prohibitive costs of maintaining laboratory colonies.

Oviposition containers and larval collection. Oviposition containers were 3.8-L plastic buckets (Encore Plastics, Huntington Beach, CA). Each lid was perforated with several 3-cm holes, and the containers were half filled with tap water to which approximately 1 ml pulverized fish food was added (TetraMin Tropical Flakes, Melle, Germany) (Fig. 1A). The modified lids slowed evaporation, reduced the quantity of leaves and debris falling into the container, and deterred most vertebrates from drinking the water. Containers were placed on the ground in a
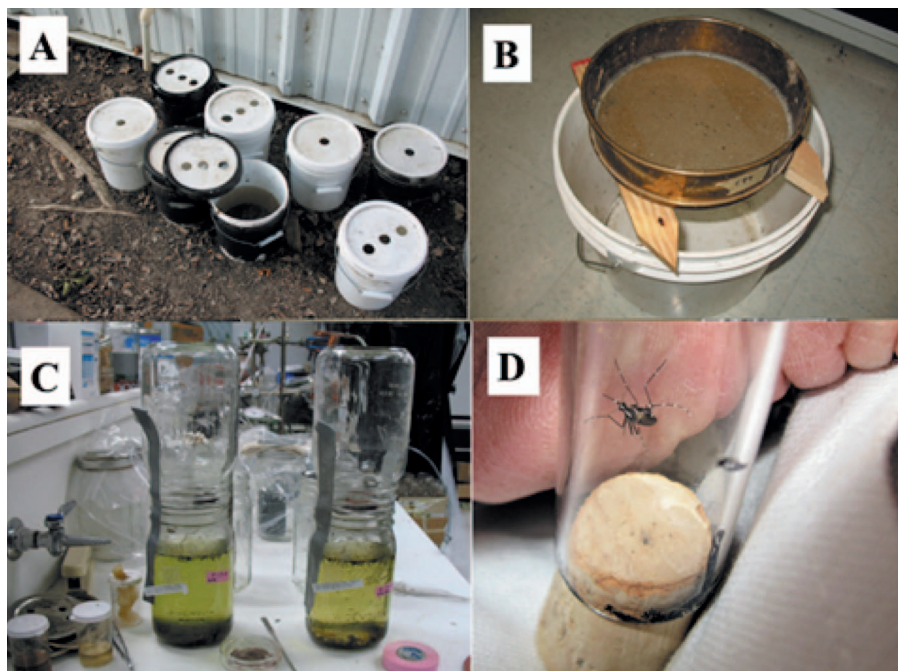
**Fig. 1. Collection and rearing containers for described methods of rearing
Aedes albopictus: (A) Buckets for wild *Ae. albopictus* oviposition; (B)
Sieve supported over larger bucket for larval collection; (C) Mason jar
larval rearing and emergence chambers; (D) Mosquito tube aspirator
holding engorged *Ae. albopictus*.**

shaded area (e.g., north side of a structure). Every 7 d, water in each container was
poured through a 425-μm No. 40 (Tyler equivalent 35 mesh) sieve supported above
a $\geq$ 9.5-L container (Fig. 1B). A wide-mouth 947-ml Mason jar (Jarden Home
Brands, Fishers, IN) was filled with sieved water collected in the larger container,
and the remaining sieved water was returned to the original oviposition container.
The sieve was then placed upside down into the empty larger collection container
and rinsed with the water collected in the Mason jar. This process rinsed mosquito
larvae and other debris trapped on the sieve into the collection container. The water,
larvae, and debris thus collected in the container was then poured back into the
Mason jar, which would become the laboratory rearing container. Each rearing
container was filled to within 3 cm of the top with deionized or tap water and then
covered with its flat lid secured with a screw-on ring. Multiple oviposition buckets
were often pooled (poured through sieve) depending on the numbers of mosquito
larvae collected and needed for studies.

   Larval rearing. Several ~2-cm holes were punched in one-half of the lid of each
rearing container. Paper toweling was folded and stapled to create a $2 \times 2 \times 0.5$ cm
pad that was soaked in 10% (w/v) sucrose and water solution and placed on the
other half of the lid. This provided a food source for emerging adult mosquitoes. The

flat lid (no lid ring) with holes and sucrose-soaked toweling was replaced on the rearing container Mason jar. Another Mason jar was inverted over the lid of the rearing container, thus, creating a chamber for emerging mosquitoes. The two jars were secured together with a single vertical strip of tape, which also acted as a hinge facilitating access to the rearing container (Fig. 1C). Larvae within the container were fed pulverized fish food (TetraMin Tropical Flakes fish food) ad libitum using a flat lab spatula. This also allowed for easy observation of mosquito larvae and emergence.

If larval predators (e.g., *Toxorhynchites* spp. [Diptera: Culicidae], copepods [Crustacea: Copepoda]) are detected in the rearing container's water, the contents of the rearing container can be poured into a tray to remove or separate the predators from the mosquito larvae. Water containing the mosquito larvae is then returned to the rearing container using a funnel. Oviposition and collection containers that produced the copepods should be cleaned or replaced.

Adult mosquitoes that accumulated in the upper emergence chamber may be contained as a cohort by sliding flat solid lids between the two jars to cap the lower and upper containers. If mosquito larvae remain in the rearing container, a lid with holes and fresh sucrose-soaked toweling is placed on the rearing container and a clean emergence container should be placed atop the lid to allow for continued collection of emerging adult mosquitoes. The sealed upper emergence chamber containing adult mosquitoes can be held or transferred, without anesthesia, for release into the in vitro feeding chamber (see below), or they can be frozen to kill and preserve the cohort.

In vitro blood feeding. An adult feeding chamber was constructed from a small mouth 236-ml Mason jar and an aluminum-framed cage (30.5 × 30.5 × 30.5 cm) (BioQuip Products, Rancho Dominguez, CA; Fig. 2A). The Mason jar was modified by grinding a 5-mm hole in the center of the base using a Dremel (Racine, WI) rotary tool with a silicone-carbide bit. This opening allowed for addition of blood, release of pressure during heating (e.g., $PV = nRT$), and insertion of a thermometer to monitor temperature during feeding (Fig. 2B). Parafilm M (American National Can, Chicago, IL) was stretched to double its length both horizontally and vertically (Note: warming the Parafilm in the palms of your hands facilitates stretching and might make the membrane more attractive; however, gloves can be worn if contamination of the Parafilm is of concern.). Stretched Parafilm was placed over the mouth of the jar and attached by carefully (so as not to perforate the Parafilm) screwing on a lid band (Fig. 2C). Citrated beef blood (1–3 ml), obtained from a slaughter house, was applied to the inner cover of the Parafilm using a syringe equipped with a 15-cm blunt-tipped Luer Lock device (C U Innovations, Chicago, IL). Larger quantities of blood applied to the Parafilm resulted in feeding problems possibly because of the settling and packing of red blood cells at the membrane surface.

A heating pad (Sunbeam [30.5 × 61.0 cm] with six heat settings, Jarden Consumer Solutions, Boca Raton, FL) with a no-shutoff option was wrapped around the 236-ml feeding chamber and secured with rubber bands (Fig. 2A, B). A precision instant-read pocket thermometer (Taylor, Oak Brook, IL) was inserted into the opening in the base of the jar (Fig. 2B). To prevent the inserted thermometer from puncturing the Parafilm membrane, a depth stop was created using masking
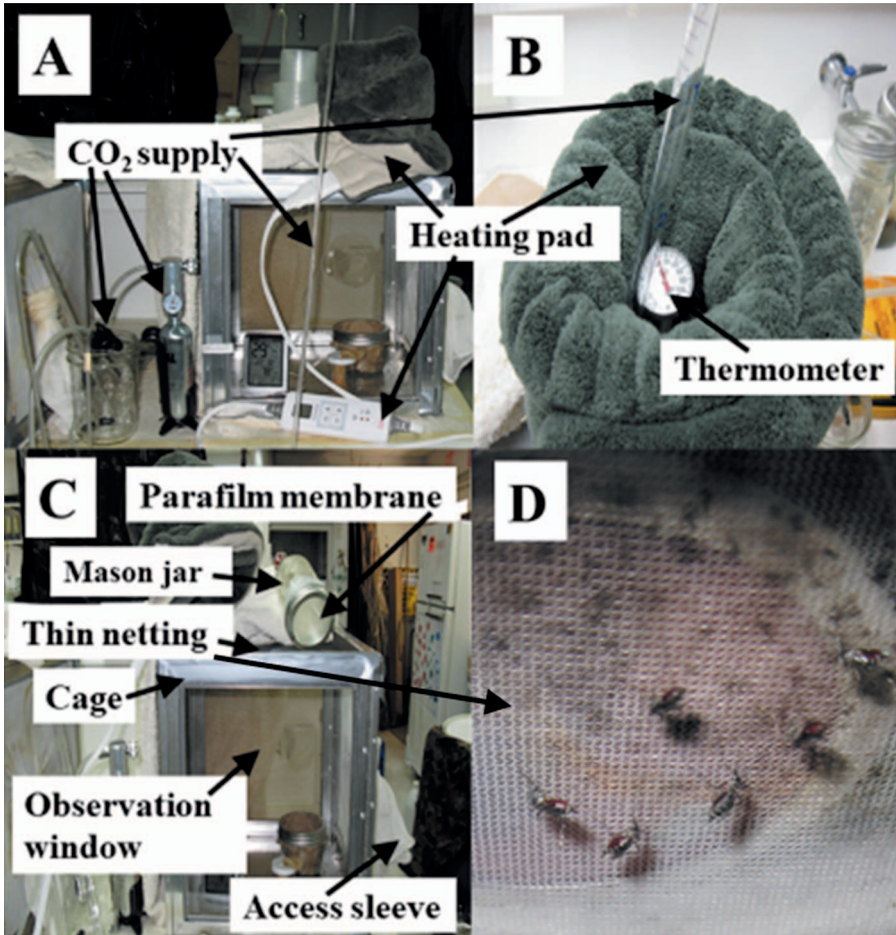
Fig. 2. (A) Feeding cage with CO_2 delivery system and heating pad to warm mason jar blood chamber, note the duct tape sealing the seams of the cage; **(B)** Thermometer indicating heating pad is on, the thick glass of mason jar is a great heat sink. Note the pipette receives the aquarium tubing for directed transfer of CO_2; **(C)** Stretched Parafilm membrane on mason jar blood chamber; **(D)** *Aedes albopictus* blood feeding through thin netting and stretched parafilm membrane.

tape at the appropriate height. Temperature within the chamber was then raised to ~37°C.

　　The jar was then inverted on the top of the cage to create the feeding chamber. The cage had a clear panel side for observation and photography (Fig. 2C); a stocking net functions as an access sleeve (Fig. 2C) into the cage, with the remaining sides composed of Lumite screen. The Lumite screen covering the top of the chamber (where the blood feeding device rests) was replaced with a thinner

Dacron chiffon netting (Fig. 2C, D; BioQuip Products) to facilitate blood feeding by adult mosquitoes. Duct tape was applied along the seams of the cage frame to prevent mosquitoes from escaping (Fig. 2A, C). In the absence of an environmental chamber or a humidity-controlled environment, draping a moist towel covered with a sheet of plastic around the cage elevates the humidity while the plastic sheet slows drying of the towel and, thus, reducing maintenance. A Fluval mini pressurized CO_2 kit (Rolf C. Hagen Corp., Mansfield, MA) was used to deliver CO_2 via tubing inserted into a pipette adjacent to the inverted jar on the cage (Fig. 2A, B).

Laboratory oviposition chamber. After being allowed to feed, engorged mosquitoes were individually removed from the cage using a glass tube aspirator and transferred to a laboratory oviposition chamber. Suction for the aspirator can be supplied by a standard vacuum cleaner, with vacuum strength controlled by incrementally clamping tubing so that plugging a vacuum bleed (hole) on a "Y" tube fitting with a finger allows controlled aspiration as an alternative to mouth aspiration.

A laboratory oviposition chamber was constructed with a 50-ml (15 dram) plastic snap cap vial lined with a paper towel strip and partially filled with several milliliters of water. The lined plastic vial was placed in a 473-ml wide-mouth Mason jar provisioned with a paper towel pad soaked in a 10% sucrose solution as previously described. Pulverized TetraMin Tropical Flakes fish food was added to the water in the vial.

Mosquito eggs, larvae, pupae, and adults can be counted and monitored in the vials. Deionized water can be added to the plastic vials as evaporation occurs, and sucrose solution can be supplemented as needed using a syringe equipped with a ~15-cm blunt-tip Luer Lock device. To prevent mosquito escape when adding water, a flat lid with a small hole for insertion of the Luer Lock device can be placed over the Mason jar lid. The latter is removed by sliding horizontally away from the jar leaving the lid with a small hole for access. Tape can be used to close the hole to prevent mosquito escape.

Observations. Approximately 30% of wild female *Ae. albopictus* collected became engorged using this method. Removal of the sucrose solution overnight appeared to improve blood feeding. Extending feeding time or beginning another cycle of heat and CO_2 after resting for 1 to 2 h also stimulated feeding. Similarly, feeding the next day with fresh blood stimulated some of the remaining mosquitoes to feed. With the blood chamber at ~37°C, it is the CO_2 (e.g., 8 bubbles/s = 480 bubbles/min = 30 ml/min) that promotes feeding in wild local *Ae. albopictus* (Kerrville, TX). A battery-powered electric fly electrocution racket is useful for quick elimination of the occasional escapee.

This is an inexpensive system that allows for collection, rearing, and in vitro feeding of wild *Ae. albopictus*. This system allows a small laboratory with few resources to produce abundant numbers of mosquitoes for quantifying and replicating treatments without the cost of purchasing expensive equipment, maintaining a rearing room, keeping vertebrates for blood meals, and maintaining compliance with Institutional Animal Care and Use Committee regulations and requirements. This system is ideal for feeding mosquitoes systemic insecticides, exposing to pathogens, evaluating repellents and vertical transmission, and investigating the effects of sublethal treatments, growth regulators, or other agents on the F_1 generation.