

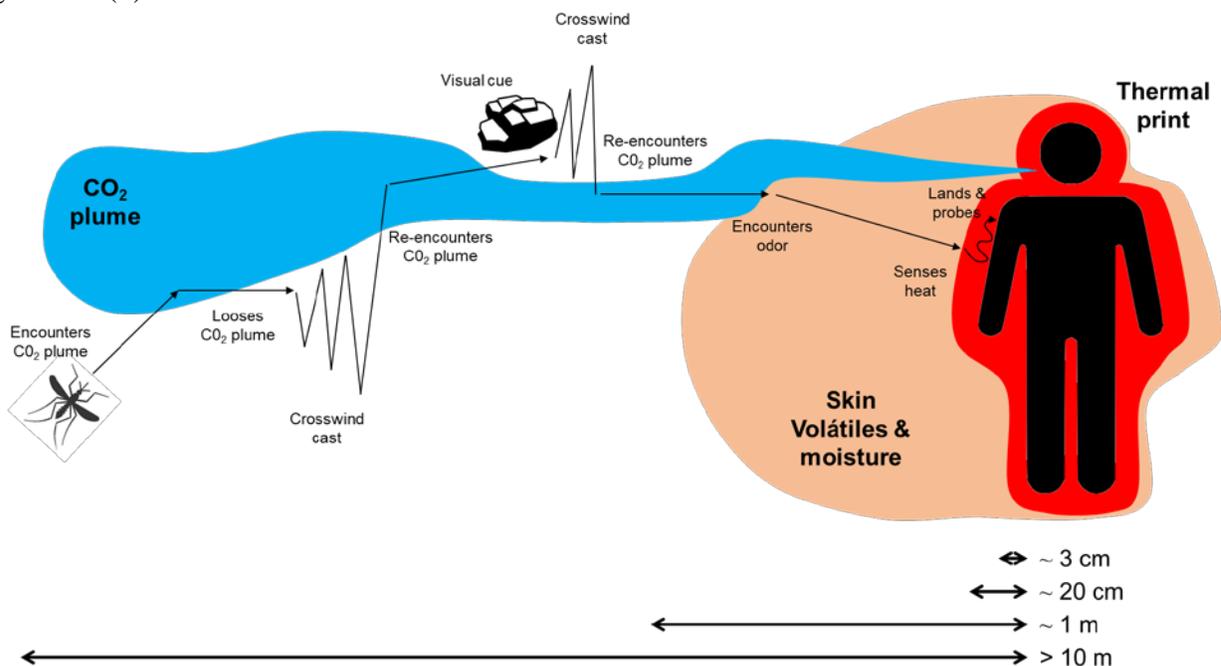
## Yoy mosquito trap construction.

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This protocol describes detailed instructions into the construction of the Yoy (Tenek Amerindian word for mosquito) trap developed by the Viral & Human Genomics Laboratory for use in vector and arbovirus surveillance. The trap incorporates different strategies to attract, confine and maintain live mosquitoes for up to 24 hours on field and for up to 72 hours within lab premises. The trap was designed to be constructed from recycled materials to improve adoption, lower costs and for use in resource limited settings. The trap uses dry ice generated CO<sub>2</sub> as a long-distance attractant to lure mosquitos from >10 meters distance and provides additional stimuli to mosquitos (odour volatiles and humidity) to further attract mosquitoes in the vicinity of 1 meter. Mosquitoes are entrapped by a conical funnel and their escape is prevented through the use of transparent plastic container as the main collection vessel (which allows ambient light to distract mosquitoes from the funnel exit). Once trapped, the mosquitoes are provided free access to a honey-impregnated FTA bait card as an alternate food source, thereby maintaining viable mosquitoes during the night and for up to 72 hours. Mosquitoes probing the honey-impregnated FTA card regurgitate saliva during the process and deposit viral nucleic acids which have been shown to be preserved for up to 7 days in the FTA card (1–3). Trials carried out at our lab have demonstrated that more than 50% of the captured mosquito population probes the card and feeds daily during the first 3 days.

### Trap operating principle

When mosquitoes encounter a CO<sub>2</sub> plume they will attempt to follow-it up-wind in the search of odour, humidity, and thermal cues indicative of a suitable host animal (see figure below adapted from van Breugel et al. (4)).





If the plume is lost during their approach, mosquitoes will cast (up-wind perpendicular zig-zag flight pattern) to re-engage the CO<sub>2</sub> cue. Upon re-engaging the CO<sub>2</sub> plume mosquitoes might use visual cues to find their meal. If the visual cues lead to objects not providing additional cues (such as skin volatiles, moisture, and heat) they will again cast in the search of the CO<sub>2</sub> plume. If the plume is successfully tracked, mosquitoes will sense the presence of skin volatiles and continue tracking these in search of moisture and thermal cues. Once found, mosquitoes will land and probe the animal to attain a blood-meal.

### Materials needed per trap

- 10 litre polyethylene terephthalate (PETE) water jug (recycled) with screw-on cap.
- Two 2 litre PETE soft-drink bottle (recycled) without screw top.
- 60 cm of ½ inch (1.27 cm) diameter 315 PS1 (1.57 mm thick) polyvinyl chloride (PVC) pipe.
- 50 ml bottle and screw-on cap.
- 15 ml polypropylene conical centrifuge tube with screw top.
- 50 ml polypropylene conical centrifuge tube without top.
- 0.6 ml polypropylene microcentrifuge tube.
- 2 ml polystyrene or polypropylene serological pipette.
- 3 x 3 cm square of plastic mosquito mesh.
- 42 x 27 cm sheet of plastic mosquito mesh.
- 50 mg Octenol piece (Flowtron® MA-1000-6 Octenol Mosquito Attractant Cartridges).
- Two 15 x 15 cm gauze pads.
- 2 kgs of pelleted dry-ice (solid CO<sub>2</sub>).
- 50 ml natural honey.
- Sachet of powdered green food colouring.
- 100 ml of tap water.
- Enough sponge foam to fill 40 ml bottle.

### Equipment needed

- 1 roll of PVC electrician's tape.
- Dremel® (model 4300, Robert Bosch GmbH, Wisconsin, United States) variable speed rotary tool.
- Dremel® router carbide engraving bits with 3mm shank.
- Dremel® 2 mm wide drill bit.
- Scissors.
- Electrical drill with 5 mm, 8 mm, and 10 mm drill bit.



- X-Acto® cutter.
- Low profile plastic bag heat sealer having at least 20 cm long sealing track.



Dremel rotary tool



Dremel carving bits



Plastic bag sealer

## Construction

1. Main mosquito collection vessel.
  - a. Use scissors to remove lowest 4 cm of 10 litre water jug (see step #1 on the construction diagram provided below).
  - b. Discard lower portion of water jug.
  - c. Drill a 5 mm hole on opposite sides of the water jug on the highest part of flat sides (before the plastic jug begins to curve towards the top). See step #2 on the construction diagram).
  - d. Use the Dremel rotary tool and carving bit to make a hole in the 10 litre water jug cap barely large enough to tightly secure the top of a 40 ml bottle cap (step #3 on the construction diagram).
  - e. Print out the plastic wire mesh funnel template provided at our website ([http://www.genomica.uaslp.mx/Protocolos/ARBO\\_Yoy\\_funnel\\_template.pdf](http://www.genomica.uaslp.mx/Protocolos/ARBO_Yoy_funnel_template.pdf)) at the required size so that dimension marks are printed at the correct size or scale template to fit your custom water jug.
  - f. Cut out the 42 x 27 cm sheet of plastic mosquito mesh according to the template and fold into funnel (step #4 on the construction diagram).
  - g. Use the plastic bag sealer to fuse plastic mesh to maintain funnel shape.
  - h. Use PVC electrician's tape to fix funnel to bottom of 10 litre water jug (step #5 on the construction diagram).



## 2. FTA card holding vessel.

- a. Use the Dremel rotary tool and carving bit to make a 2.5 cm hole in the centre of 40 ml bottle's cap (stay at least 2 mm from rim of cap), step #6 on the construction diagram.
- b. Cut out a 2.8 cm diameter circle from card stock and make sure the card will remain secured between 40 ml bottle mouth and its screw top cap (step # 7 on construction diagram).

NOTE: This 2.8 cm diameter circle will serve as a template for cutting FTA cards further on.

## 3. CO<sub>2</sub> delivery system.

- a. Using scissors cut one of the 2 litre PETE soft-drink bottle bottoms approximately 2/3 of the way from mouth (step #8 on construction diagram).

NOTE: This will serve as a funnel to fill the other 2 litre PETE soft-drink bottle with dry-ice pellets using a "bottle connector" made by cutting the conical bottom of a 50 ml conical centrifuge tube.

- b. Cut a 2 ml plastic serological pipette to a length capable of traversing the 10 litre main mosquito collection vessel through the two 5 mm holes previously drilled into it leaving at least 2.5 cm overhang on both sides. Place enough turns of PVC electrician's tape on one of the ends to prevent pipette from slipping into main mosquito collection vessel (step #9 on construction diagram).
- c. Using the Dremel rotary tool create 1 cm<sup>2</sup> notches on the bottom end of the 60 cm long half-inch diameter PVC pipe (step #10 on construction diagram).

NOTE: This aids in cutting through the dry-ice filled PETE soft-drink bottle during trap assembly.

- d. Drill two sets of perpendicular 10 mm through-and-through holes in the PVC pipe 25 cm from the notched end of the PVC pipe (or at length suitable for your PETE soft-drink bottles so that these holes remain inside the bottle when the PVC pipe is inserted into the dry-ice packed PETE soft-drink bottle (step #11 on construction diagram).
- e. Drill an 8mm hole through a single wall of the half-inch PVC pipe at a distance of 11 cm from the un-notched (upper) end of the PVC pipe. This hole should accommodate the 0.6 ml microcentrifuge tube securely (step #12 on construction diagram).

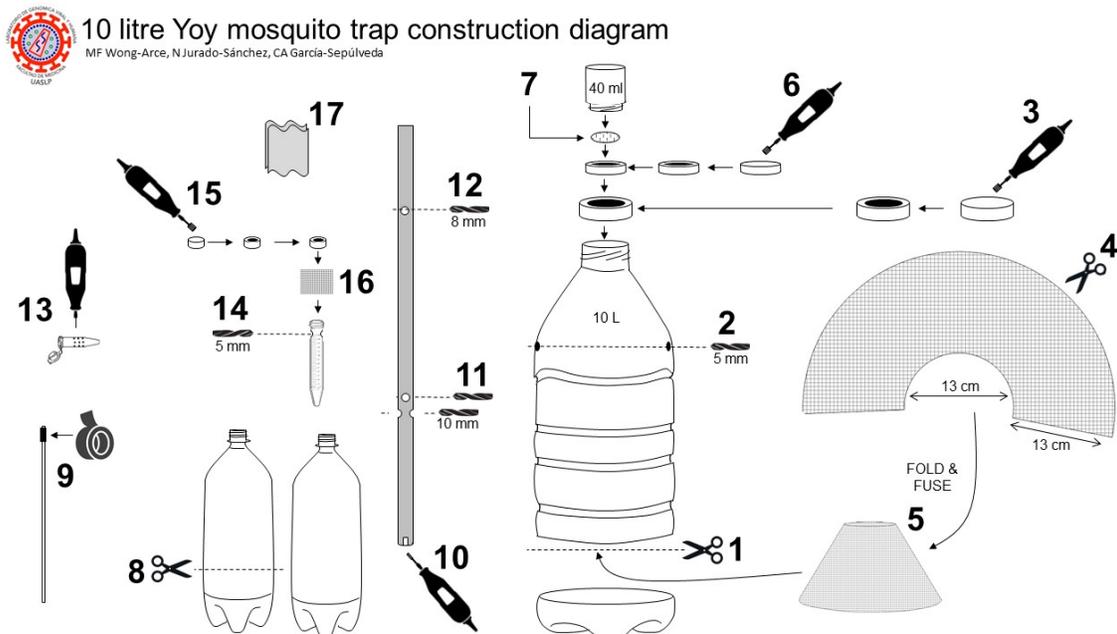


- f. Using the Dremel® rotary tool drill multiple 2 mm holes into 0.6 ml microcentrifuge tube (step #13 on construction diagram below).

NOTE: Octenol wafer will be placed inside this microcentrifuge tube once the microcentrifuge tube is inserted into half-inch PVC pipe. Holes allow for odour to dissipate into the main collection vessel when forced by the CO<sub>2</sub> flow within the half-inch PVC pipe.

- g. Drill a 5 mm hole through the upper part of the 15 ml conical centrifuge tube but below the screw-top cap (step #14 on construction diagram).
- h. Cut the bottom conical portion of the 15 ml conical centrifuge tube, approximately 1 cm from tip.
- i. Use the Dremel rotary tool and carving bit to make a 1.3 cm hole in the centre of the 15 ml conical centrifuge tube cap (step #15 on the construction diagram).
- j. Place the 3 x 3 cm square of plastic mosquito mesh on top of the 15 ml conical centrifuge tube and cap with the perforated top (step #16 on the construction diagram).
- k. Prepare a two-ply roll of 15 x 15 cm gauze (step #17 on the construction diagram).

## Construction diagram



A high-resolution version of this diagram is available for download at our website ([http://www.genomica.uaslp.mx/Protocolos/ARBO\\_Yoy\\_Construction\\_Diagram.pdf](http://www.genomica.uaslp.mx/Protocolos/ARBO_Yoy_Construction_Diagram.pdf)).

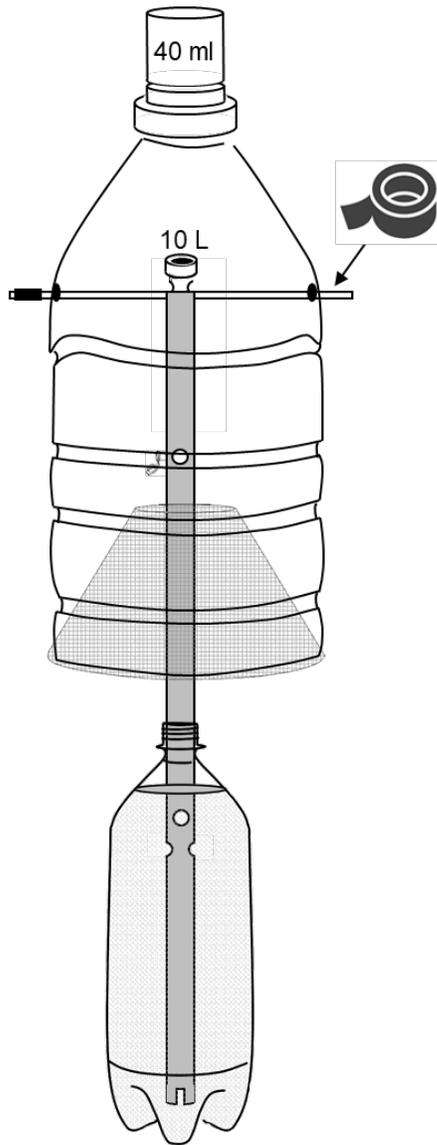


Diagram and photograph of assembled Yoy mosquito trap.



PVC pipe lower notch



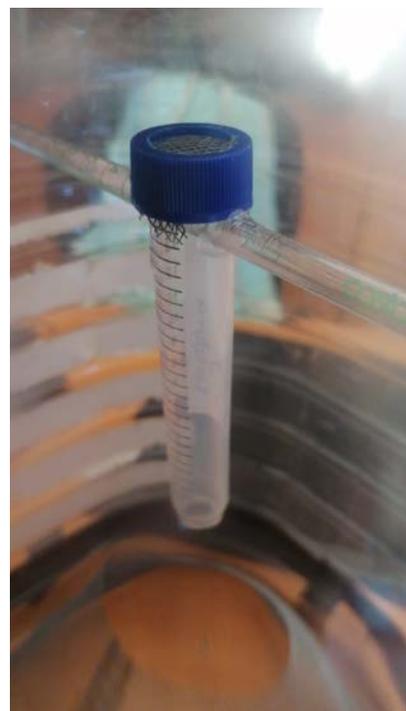
CO2 flow through holes



0.6 ml Octenol delivery tube



PVC pipe insertion



15 mL conical tube



2 ml pipette



Funnel entrance



PVC pipe through funnel



FTA card holder



FTA card holder



FTA card holder



## Notes

1. All dimensions, materials and tools can be easily customized to local resources.

## References

1. Wipf NC, Guidi V, Tonolla M, Ruinelli M, Müller P, Engler O. Evaluation of honey-baited FTA cards in combination with different mosquito traps in an area of low arbovirus prevalence. *Parasites and Vectors*. 2019 Nov 21;12(1).
2. Flies EJ, Toi C, Weinstein P, Doggett SL, Williams CR. Converting Mosquito Surveillance to Arbovirus Surveillance with Honey-Baited Nucleic Acid Preservation Cards. *Vector-Borne Zoonotic Dis*. 2015 Jul 1;15(7):397–403.
3. Melanson VR, Jochim R, Yarnell M, Ferlez KB, Shashikumar S, Richardson JH. Improving vector-borne pathogen surveillance: A laboratory-based study exploring the potential to detect dengue virus and malaria parasites in mosquito saliva. *J Vector Borne Dis*. 2017 Dec 1;54(4):301–10.
4. F van B, J R, A F, MH D. Mosquitoes Use Vision to Associate Odor Plumes with Thermal Targets. *Curr Biol [Internet]*. 2015 Aug 17 [cited 2021 Aug 26];25(16):2123–9. Available from: <https://pubmed.ncbi.nlm.nih.gov/26190071/>

## Revision history

- 1.0 Original document.

