# Yoy mosquito trap assembly.

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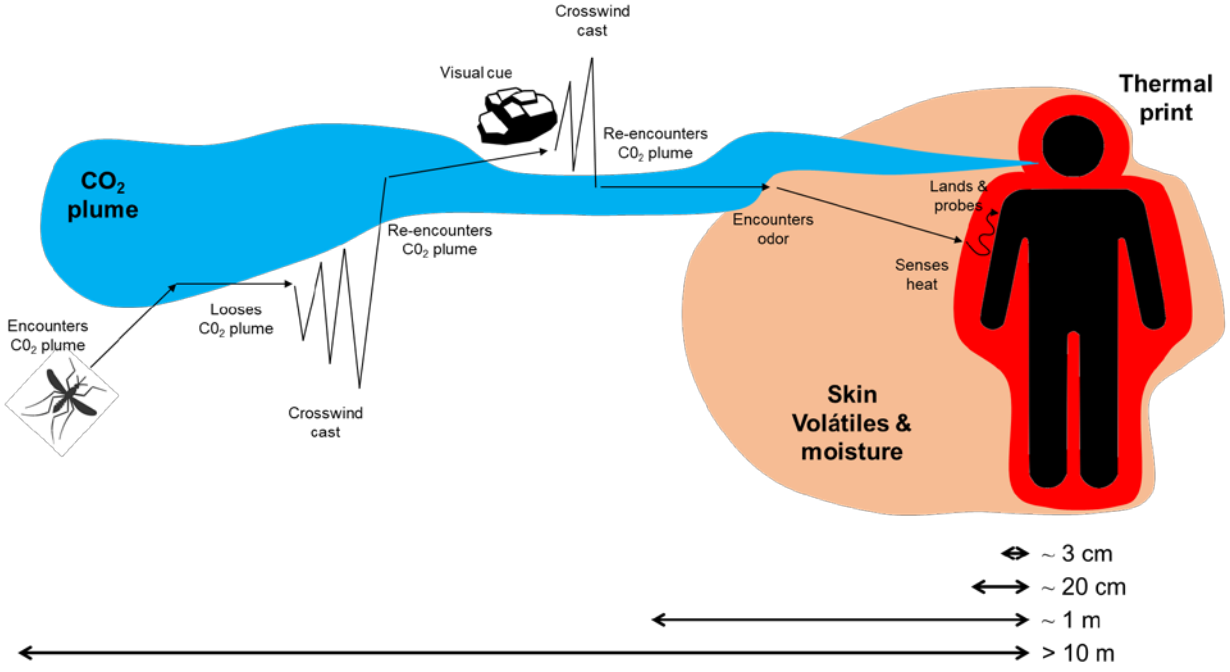
This protocol describes detailed instructions into the construction of the Yoy (Tenek Amerindian word for mosquito) trap developed by the Viral & Human Genomics Laboratory for use in vector and arbovirus surveillance. The trap incorporates different strategies to attract, confine and maintain live mosquitoes 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 $CO_2$ 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 $CO_2$ 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 $CO_2$ cue. Upon re-engaging the $CO_2$ 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 $CO_2$ 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.

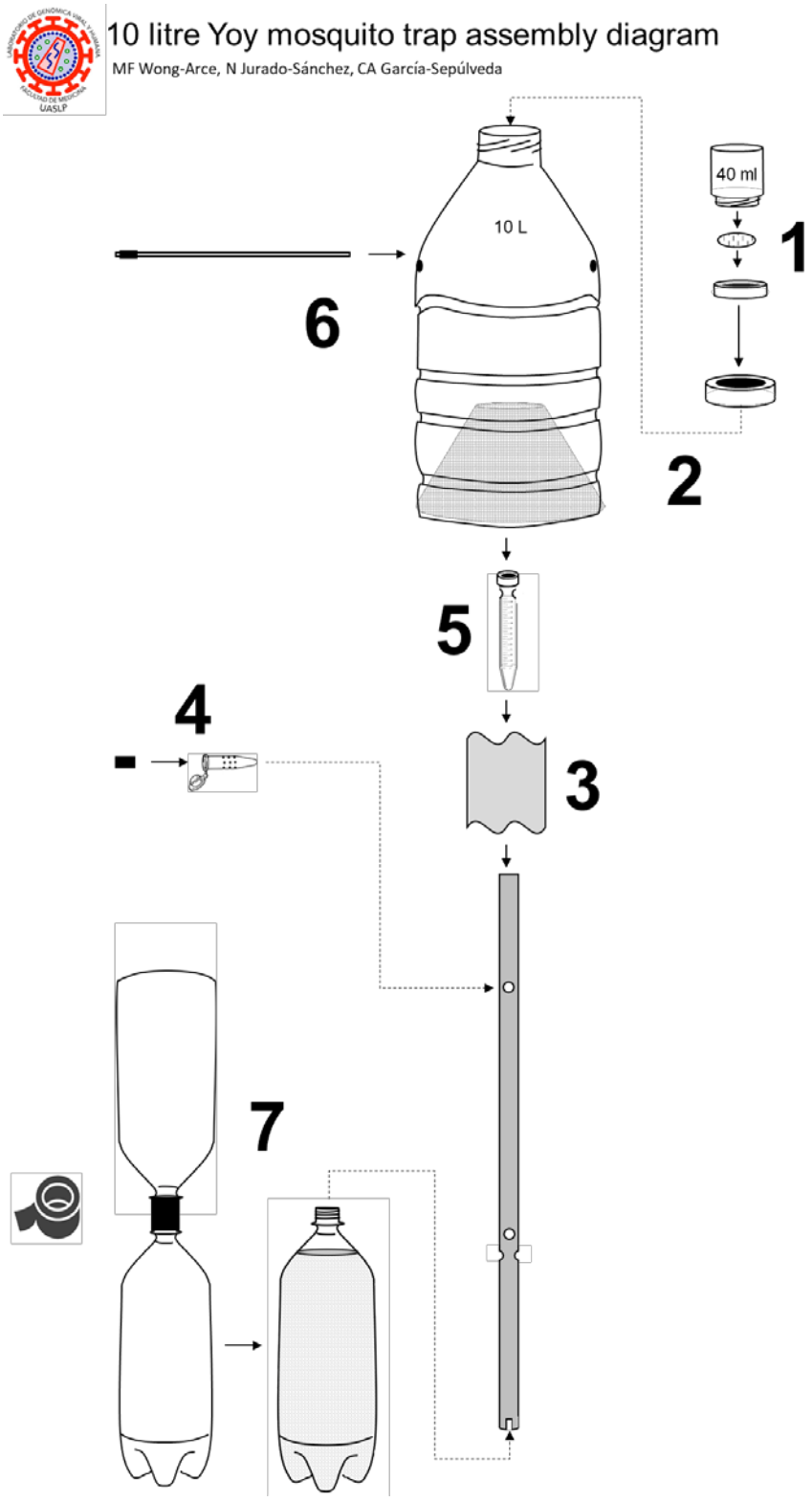
See the Yoy mosquito trap construction protocol for instructions on how to assemble the following parts needed (http://www.genomica.uaslp.mx/Protocolos/ARBO_Yoy_Construction_ENG.pdf).

**Parts needed per trap**

- 40 ml plastic bottle with pre-drilled top (FTA card holding vessel).
- 10 litre main mosquito collection vessel with plastic mesh funnel.
- One 2-litre PETE soft-drink bottle (per trap)
- One 2-litre PETE soft-drink bottle funnel (suitable for several traps)
- Pre-perforated 15 mL conical centrifuge tube ($CO_2$ delivery system).
- Pre-perforated 0.6 ml microcentrifuge tube
- Honey impregnated FTA card
- Two-ply roll of 15 x 15 cm gauze
- 50 mg Octenol wafer
- 20 ml tap water
- Pre-perforated 60 cm PVC pipe
- 2 kgs dry ice (solid carbo dioxide)

**Equipment needed**

- Scissors.
- Roll of PVC electrician’s tape




A high-resolution version of this diagram is available for download at our website (http://www.genomica.uaslp.mx/Protocolos/ARBO_Yoy_Assembly_Diagram.pdf).

## Assembly

NOTE: Follow the "*10 litre Yoy mosquito trap assembly diagram*" given in the previous page for a graphical depiction of the assembly process.

1. Fill the 40 mL plastic bottle acting as the FTA holding vessel with a synthetic sponge previously impregnated with a green food colouring dyed 50% honey solution (v/v in water).

2. Place a 1.25 inch in diameter 100% honey impregnated green-food colouring dyed FTA card inside the 40 mL plastic bottle top and screw onto 40 ml FTA holding vessel (step #1 on the assembly diagram).

3. Snap fit the 40 ml FTA holding vessel unto the 10-litre main mosquito collection vessel (step #2 on the assembly diagram).

4. Wet the two-ply roll of 15 x 15 cm gauze in tap water and place inside the top of the PVC pipe and below the 0.6 ml microcentrifuge port (step #3 on the assembly diagram).

5. Place the 50 mg of octenol wafer inside the pre-perforated 0.6 ml microcentrifuge tube and insert tube into its corresponding port on the PVC pipe (step #4 on the assembly diagram).

   NOTE: The gauze roll should be inserted below this microcentrifuge tube to avoid its expulsion by sublimated $CO_2$ back-pressure.

6. Place the 15 ml conical centrifuge tube on the upper end of the 60 cm PVC pipe (step #5 on the assembly diagram).

7. Insert the top end of the PVC pipe holding the 15 ml centrifuge tube into the bottom of the main mosquito collection vessel and through the centre of the plastic mech funnel. Traverse both the main mosquito collection vessel and the 15 ml conical centrifuge tube with a 2 ml serological pipette. Place two or three turns of electricians tap on both ends of the 2 ml serological pipette to hold it in place (step #6 on the assembly diagram).

8. Using the 2-litre soft-drink PETE bottle funnel, load as much dry-ice as possible into the other 2-litre bottle by either taping both bottle ends with PVC electrician's tape or by holding them together while using thermal mittens. A 2-litre bottle normally holds between 1.8 and 2.1 kg of flaked or pelleted dry-ice.

   NOTE: We have incorporated the use of a 3-inch-long tube cut from a 50 ml conical polypropylene centrifuge tube as an adaptor for this purpose.

9. Insert the bottom (notched end) of the 60 cm PVC pipe into the dry-ice packed 2-litre bottle.

10. Make sure that the two sets of perpendicular 10 mm through-and-through holes made in the PVC pipe 25 cm from the notched end are fully within the dry-ice packed PETE soft-drink bottle (step #7 on assembly diagram).

11. The fully assembled mosquito collection trap is shown in the following photograph.

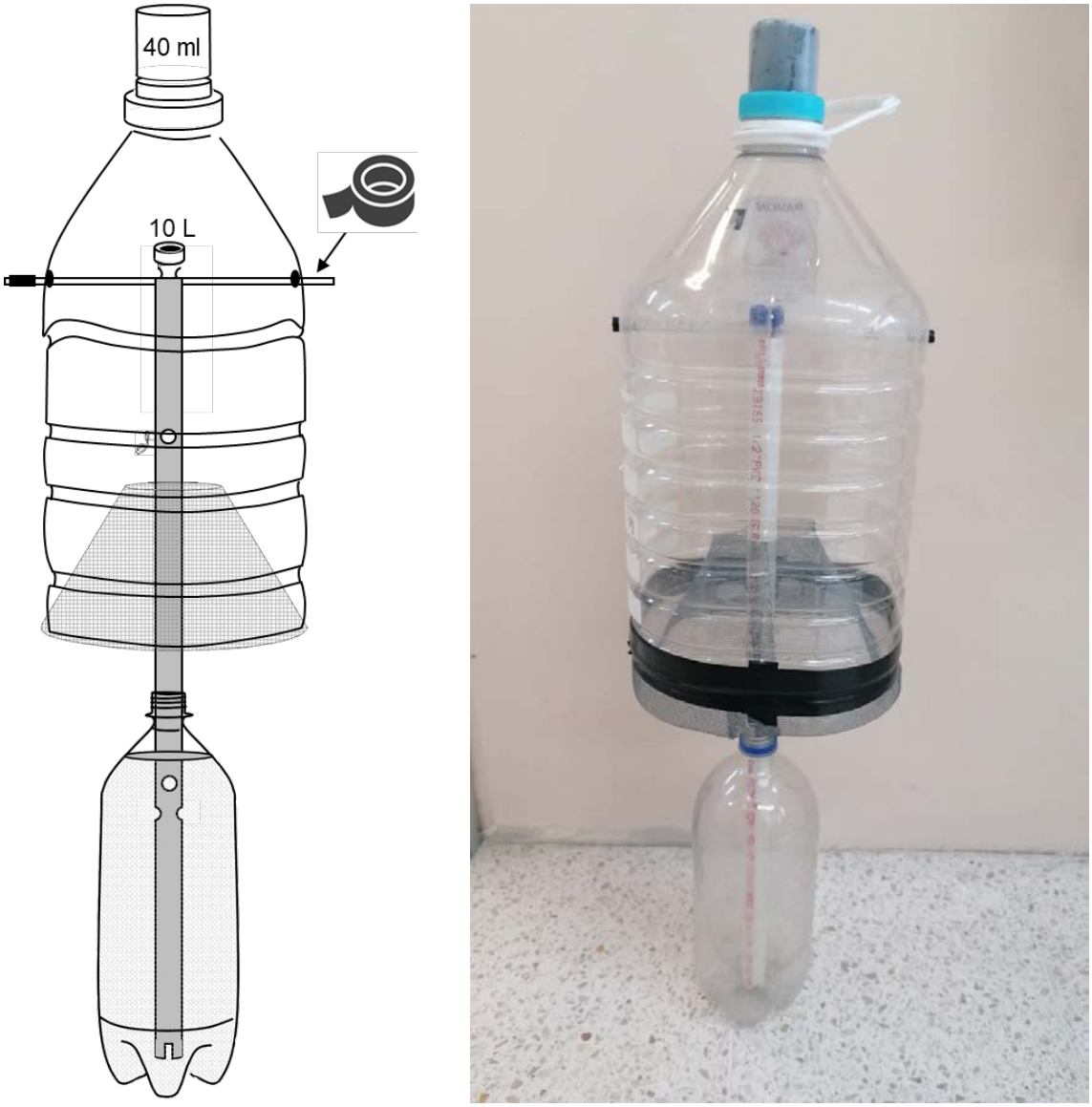



Diagram and photograph of assembled Yoy mosquito trap.

## Notes

1. The trap is normally transported as a main mosquito collection vessel (including a pre-assembled FTA card holding vessel and 15 ml conical centrifuge tube traversed with a serologic pipette) and a separate CO2 delivery system (including dry-ice packed 2-litre bottle and PVC pipe) to prevent damage during deployment.

## 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.