10-liter Yoy passive mosquito trap assembly.

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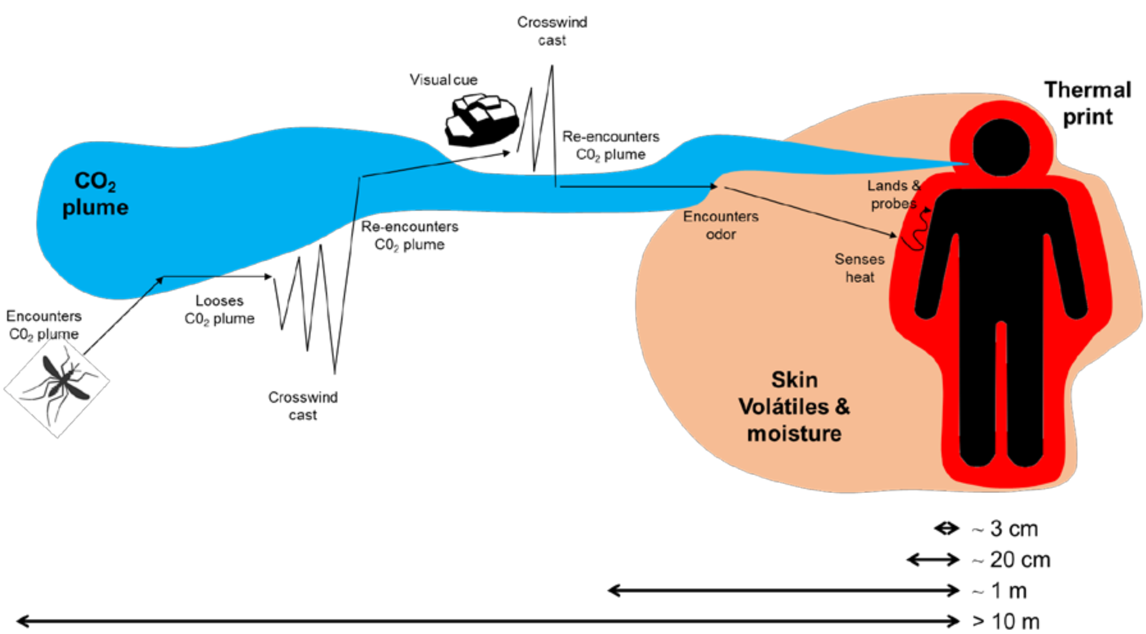
This protocol provides a detailed methodology for the construction of the Yoy trap, named after the Tenek Amerindian term for "mosquito," developed by the Viral & Human Genomics Laboratory for vector and arbovirus surveillance. The trap integrates multiple mechanisms to attract, capture, and sustain live mosquitoes for up to 24 hours in field conditions and up to 72 hours in laboratory settings. Designed for cost efficiency and adaptability in resource-limited environments, the trap is constructed from recycled materials to enhance accessibility and scalability. Mosquito attraction is facilitated by carbon dioxide (CO_2) released from dry ice, which serves as a long-range attractant, drawing mosquitoes from distances exceeding 10 meters. Additionally, the trap incorporates odor volatiles and humidity cues to enhance attraction within a one-meter range. Mosquitoes are funneled into the collection chamber through a conical entry structure, which restricts escape. The transparent plastic collection vessel further reduces exit attempts by exploiting ambient light orientation behaviors of mosquitoes. Once captured, mosquitoes have free access to a honey-impregnated FTA bait card, which provides an alternative food source, sustaining them for up to 72 hours. During feeding, mosquitoes regurgitate salivary secretions, depositing viral nucleic acids onto the FTA card, where they can remain preserved for up to seven days. Laboratory trials indicate that over 50% of captured mosquitoes probe and feed on the FTA card daily during the first three days, demonstrating the trap's efficacy in maintaining viable specimens for downstream molecular analysis.

Operating principle

When mosquitoes encounter a CO2 plume they will attempt to follow-it up-wind in the search of odor, humidity, and thermal cues indicative of a suitable host animal.



If the CO_2 plume is lost during their approach, mosquitoes will exhibit casting behavior, characterized by an upwind, perpendicular zig-zag flight pattern, to re-engage the olfactory cue. Upon re-establishing contact with the CO_2 plume, mosquitoes may utilize visual stimuli to locate a potential host. However, if these visual cues lead to objects lacking secondary attractants—such as skin volatiles, moisture, or thermal signatures—mosquitoes will resume casting behavior in an attempt to relocate the CO_2 source.

If the CO_2 plume is successfully tracked, mosquitoes will detect skin-emitted volatiles and continue orienting toward the host, guided by humidity and thermal gradients. Once these cues are confirmed, mosquitoes will land on the host, initiate probing behavior, and ultimately obtain a blood meal.

Parts required for trap assembly

- 40 ml plastic bottle with pre-drilled top (FTA card holding vessel).
- Honey/food coloring impregnated sponge for 40 ml FTA card holding vessel.
- Honey/food coloring impregnated FTA card.
- Pre-drilled 10-liter trap vessel cap (should securely hold 40 ml FTA card holding vessel cap).
- 10-liter trap vessel with plastic mesh funnel.
- Pre-cut 2-liter PET bottle funnel
- 2-liter PET bottle dry ice reservoir
- Pre-cut 50 ml centrifuge tube (used to adapt the 2 L funnel to 2 L reservoir).
- One 2 ml (5 mm outer diameter, OD) serological pipette (hanger)
- Pre-perforated 15 mL conical centrifuge tube (hanger adaptor) with perforated cap and mesh.
- Two-ply wad pf 15 x 15 cm still water humidified gauze.
- 60 cm PVC Charlotte pipe
- Pre-drilled 0.6 ml microcentrifuge tube with cap (Octenol diffuser)
- One 3x7 mm (50 mg) Octenol lozenge (see note 1)
- 2 kg of pelleted dry ice
- 1 roll of vinyl electrical insulating tape
- Scissors or field knife

For part manufacture consult the construction protocol available on the following link (www.genomica.uaslp.mx/Protocolos/ARBO_Yoy_Construction_ENG.pdf).

Procedure

1. FTA cards impregnated with honey and food coloring should be prepared at least one week prior to field deployment in accordance with the established protocol (refer to www.genomica.uaslp.mx/Protocols/ARBO_FTA_Card_Preparation_ENG.pdf). It is recommended to prepare a sufficient quantity to cover the entire sampling period for the year to ensure consistency and availability during field operations.

2. Approximately 24 hours prior to trap deployment, contact a local dry ice supplier to confirm availability. Each 10-liter Yoy trap requires an estimated 2 kg of dry ice for optimal operation.

However, additional dry ice should be procured to account for sublimation losses during transportation and handling.

3. Deploy traps approximately one hour before dusk to coincide with peak mosquito activity and retrieve traps one to two hours after dawn, ensuring collected mosquitoes remain viable for processing.

    a. In central Mexico (22°N, 100°W), this corresponds to a deployment window between 17:30 and 18:00 hours and a retrieval window between 07:00 and 08:00 hours. Refer to the table below for approximate sunrise and sunset times specific to San Luis Potosí City to ensure precise scheduling.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Sunset	18:20	18:40	18:50	19:00	19:10	19:20	19:20	19:10	18:50	18:30	18:10	18:10
Sunrise	07:20	07:10	06:50	06:30	06:10	06:00	06:10	06:20	06:30	06:40	06:50	07:10

4. Impregnate a sponge with a 50% (v/v) honey/food coloring solution and insert it into the FTA card holding vessel (40 mL bottle).

5. Place a honey/food coloring-impregnated FTA card onto the cap of the 40 mL vessel.

6. Secure the FTA card holding bottle onto the cap, ensuring the FTA card remains intact without creasing or tearing.

7. Fit 40 ml FTA card holder cap into 10-liter bottle cap.

8. Moisten two-ply gauze sheets using clean still water before inserting them into the upper section of the PVC pipe.

9. Retrieve 50 mg (3×7 mm lozenge) of refrigerated octenol and place it inside a perforated 0.6 mL microcentrifuge tube.

10. Insert the perforated 0.6 ml microcentrifuge tube into the designated port of the PVC pipe, ensuring that the gauze is positioned below the microcentrifuge tube inside the pipe.

11. Fill a 2-liter reservoir bottle with 2 kg of dry ice using a 2-liter funnel.

12. Couple the funnel to the reservoir using either a short section of a 50 mL centrifuge tube or vinyl electrical insulating tape.

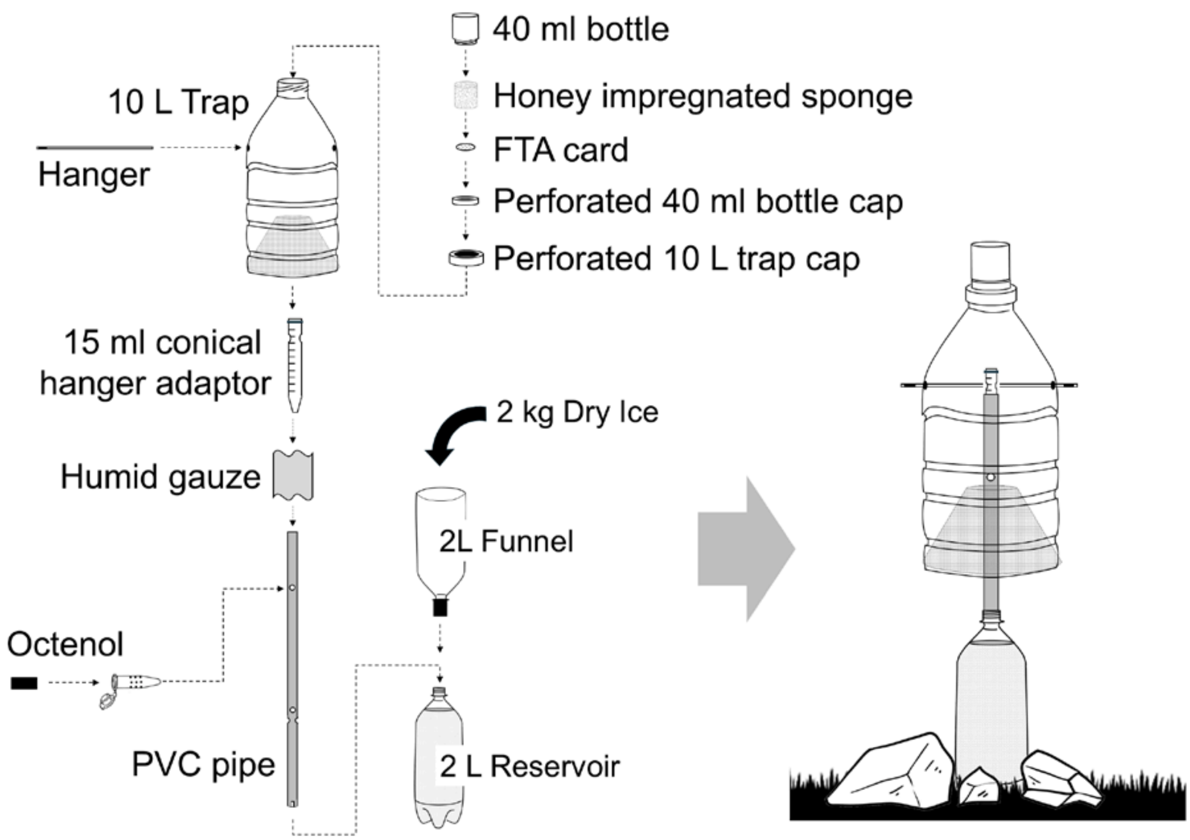
13. Use a serological pipette to dislodge any clogged dry ice in the funnel.

14. Insert the notched end of the PVC pipe into the 2-liter dry-ice reservoir using a single sharp blow to ensure a secure fit.

15. Seal one end of a 2 mL serological pipette with vinyl electrical insulating tape and insert it into the

hanger port on one side of the 10-liter trap.

16. Thread the hanger through the 15 mL centrifuge tube hanger adaptor inside the trap.
17. Pass the hanger through the hanger port on the opposite side of the trap and secure it with vinyl electrical insulating tape to prevent slippage.
18. The hanger assembly may remain permanently attached to the 10-liter trap for future use.
19. Transport 10-liter traps (with installed hangers and FTA card holders) separately from the 2-liter dry-ice reservoirs (coupled to the PVC pipe, octenol diffuser and gauze) to prevent damage during transit.
20. Position the trap in a vertical orientation at the sampling site.
21. Stabilize the trap using stones or by leaning it against surrounding vegetation to prevent displacement.



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Selection of sampling site and spatial arrangement of traps

1. Choose locations with high mosquito activity based on previous surveillance data, environmental conditions, public reports and/or host preference.

2. Avoid areas with strong winds, heavy rain exposure, or direct sunlight that could interfere with trap efficiency or mosquito survival within traps.

3. If studying specific vector species, consider their habitat preferences (e.g., shaded areas for *Aedes spp*., humid zones for *Anopheles spp*.).

4. Position traps in semi-shaded areas to protect them from direct sunlight, which could cause excessive heat buildup and dry ice sublimation.

5. Orient the trap downwind from known mosquito breeding or resting sites to maximize attraction.

6. Ensure the trap is upright and stable, using rocks, stakes, or natural support to prevent tipping over.

7. Place traps away from high-traffic areas, human dwellings, or artificial light sources that may interfere with mosquito behavior.

8. Minimize human scent contamination by handling traps with gloves and avoiding excessive movement around the trap after setup.

9. Keep traps away from insecticide-treated areas to prevent interference with capture rates.

10. Maintain a minimum distance of 10 meters between traps to prevent competition and ensure independent sampling results.

11. When using multiple traps in a study area, arrange them in a grid, transect, or cluster pattern, depending on research objectives.

12. If conducting comparative studies, ensure traps are placed in similar microhabitats to control for environmental variability.

13. During rainy conditions, place traps under natural cover (e.g., tree canopies) or construct simple rain shields to prevent water accumulation.

14. Adhere to local environmental regulations and ethical guidelines regarding trapping and handling of mosquito vectors.

15. Obtain necessary permits if trapping is conducted in protected areas or on private land.

16. Maintain a field logbook with trap location, deployment/retrieval time, weather conditions, and any

field observations.

17. Record temperature, humidity, wind speed, and precipitation at the time of trap deployment and retrieval, as these factors can influence mosquito capture rates.

18. Record exact GPS coordinates for each trap placement to facilitate repeat sampling and spatial analysis.

19. Verify that all trap components (e.g., dry ice reservoir, bait, collection vessel) are properly assembled before leaving the site.

Notes

1. Any commercially available octenol-based mosquito attractant in solid lozenge form may be used, including but not limited to:
    - ALLRoad®
    - AMVOG®
    - Carsum®
    - Mosquito Magnet®
    - Black Flag®
    - Flowtron®
    - ATRAKTA Mosquito Lure®

2. The selected attractant should be stored and handled according to the manufacturer's instructions to preserve potency and efficacy.

3. Personnel must not apply insect repellent sprays, lotions, creams, wristbands, or similar products when handling trap components, assembling, or deploying mosquito traps.

4. The presence of repellent residues on hands or clothing may interfere with mosquito attraction, leading to reduced trap efficiency.

5. If repellents must be used for personal protection they should only be applied after all trap-related tasks have been completed and personnel have exited the sampling site.

6. To minimize the risk of mosquito bites and vector-borne disease exposure, all personnel involved in trap deployment and retrieval must wear appropriate protective clothing, including:

    a. Long-sleeved, heavy-fabric shirts (to reduce mosquito penetration).
    b. Full-length, heavy-fabric pants (preferably with elastic cuffs to prevent mosquito entry).
    c. Long socks (worn over or under pant cuffs to create a barrier).
    d. Headgear (such as wide-brimmed hats or caps for additional protection).

e. Face and neck coverings, such as Shemaghs or Balaclavas, to protect exposed skin from bites.
f. Gloves (strongly recommended, particularly in high-density mosquito environments).
g. Light-colored clothing is preferable, as dark clothing is known to attract some mosquitoes.

7. Personnel should conduct self-inspections before and after fieldwork to check for mosquito bites or attached vectors (e.g., ticks).

8. In our experience, 10-liter Yoy traps assembled and deployed as described collect between 700 and 1600 mosquitoes per night.

References

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Revision history

1. Original document.
2. Detailed description of assembly incorporated, changed to new protocol format.