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REDI-NET FV-1 FLYING VECTOR FIELD SAMPLING

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Remote Emerging Disea...

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Abstract

This protocol outline steps for properly collecting adult Nematoceran flies from the families Culicidae (mosquitoes), Ceratopogonidae (biting midges), and Psychodidae (sand flies) from the environment to evaluate the risk of zoonotic disease transmission by the detection of pathogens from arthropod nucleic acid.

Image Attribution

Fig. 1. CDC traps baited with dry ice coolers. In these images, the trap device is hung directly from the bottom of the cooler using a hook or D-ring that has been installed onto the cooler for this purpose.

Fig. 2. If the trap cannot be hung directly from the cooler, the cooler and trap may both be hung from the hook or post. In this instance, ensure that the bottom of the cooler (where the holes for CO2 release are located) is positioned just above the top of the trap.

Fig. 3. An assembled CDC gravid trap. The trap apparatus is mounted onto bars, which allows the trap to balance on top of the trap base.

Fig. 4. An assembled BG Sentinel trap showing the collection funnel in the open position.

Fig. 5. The BG Lure and its correct installation in the lid of the BG Sentinel trap. Additional attractants, including dry ice (CO2) can be placed inside the trap. Odors are pushed out through the perforated lid by the fan.

Guidelines

OBJECTIVE

To outline steps for properly collecting adult Nematoceran flies from the families Culicidae (mosquitoes), Ceratopogonidae (biting midges), and Psychodidae (sand flies) from the environment to evaluate the risk of zoonotic disease transmission by the detection of pathogens from arthropod nucleic acid.

SUMMARY/SCOPE

The overarching aim of the REDI-NET is to develop a collaborative laboratory network between domestic and international partnering institutions to address disease surveillance needs in order to effectively detect, predict and contain potentially emergent zoonosis. This SOP provides guidance on the protocol for choosing the appropriate sampling technique for mosquitoes, biting midges, or sand flies at CONUS/OCONUS sites to evaluate and predict the risk of zoonotic disease transmission.

RESPONSIBLE PERSON

Principal Investigator, Study Coordinator, Entomology Component Lead, Managers

Note

All study procedures must be conducted in compliance with national and local policies for prevention and control of COVID-19 infection.

MAINTENANCE OF EQUIPMENT

BEFORE EACH COLLECTION

1. Check that all batteries are charged.
2. Ensure all traps with movable parts are in working condition.
3. Ensure there are no holes or tears in collection bags.
4. Check weather and consider using rain guards to protect samples from rain. Note that these may impede the CO_2 flow near the fan intake. Adjust if able.

4.1. Rain guards are available for CDC and BGS traps, but may interfere with CO_2 dispersal if dry ice is used.

5. Make sure that dry ice (if applicable) is procured and that gas canisters (if applicable) have enough gas for the collection.

AFTER EACH COLLECTION

1. Charge all batteries.

1.1 Recommend labeling batteries with the date they were last charged.

2. Check all traps for damage and make repairs if able.
3. Procure replacement supplies if unable to repair.
4. Clean equipment as prescribed by the manufacturer.
5. If temperatures are very high, check that glued portions of the traps have not been delaminated.

QUALITY CONTROL

This SOP is reviewed by the applicable supervisor annually or as required in order to maintain its relevance.

Materials

EQUIPMENT AND MATERIALS

Note

NOTE: If product number is listed, please ensure use of this or equivalent product.

	A	B	C
	Equipment / Material	Description	Mfg / Product #
	CDC Miniature Light Trap	This version has an incandescent light. Other styles and variations can be used as available and appropriate.	John Hock Company/512.MIL/ 2740-01-106-0091
	Collection Bag, Single-ring, fine mesh	Collection bags for CDC traps	John Hock Company/1.42
	New Collection Cup, Standard Mesh	Collection cups for mosquitoes	John Hock Company/1.44
	New Collection Cup, Fine Mesh	Collection cups for biting midges/sand flies	John Hock Company/1.49/374 0-01-527-5618
	CDC Gravid Trap		John Hock Company/1712/654 5-01-457-5511
	Hay infusion	Attractant for CDC Gravid Traps	Sourced locally
	BG-Sentinel 2		Biogents (Clarke)
	BG Lure Mosquito Attractant	Attractant for BG Sentinel Traps	Biogents (Clarke)/BG10503
	Biogents Catch Bag	Collection bag for BG Sentinel Traps	Biogents (Clarke)/BG10060
	6-Volt Battery	For use with CDC Miniature Light	John Hock Company/2.30/614

	A	B	C
		Traps and CDC Gravid Traps	0-00-432-0490
	6-Volt Battery Charger		John Hock Company/2.92-6-90-240
	12-Volt Battery	For use with BG Sentinel 2 Traps	John Hock Company/2.35
	12-Volt Battery Charger		John Hock Company/2.70
	Insulated Dry-Ice Container (Igloo)	Can also be retrofitted from drink coolers or other insulated containers by drilling small holes in the bottom of the container and adding a D-ring or hook for hanging if desired	John Hock Company/1.10
	Cooler	Used for transport of collections from the field to the laboratory	Sourced locally
	Dry ice	Alternative CO2 sources can be used, including compressed gas and yeast	Sourced locally
	Shepherd's hooks	Used for hanging CDC Miniature Light Traps. Alternative hooks/poles/structures can be used for hanging traps in available	Sourced locally
	Permanent markers	For labeling collection containers in the field	Sourced locally
	Label tape	For labeling collection containers in the field	Sourced locally
	Flagging tape	For marking the location of traps in the field if the	Sourced locally

	A	B	C
		same trapping sites will be used for multiple collection events	
	Measuring tape	For measuring distances between traps	Sourced locally
	Mallet	For driving shepherd's hooks or other poles for trap hanging into the ground	Sourced locally
	Nylon String	Useful to have on hand for hanging or repairing traps in the field	Sourced locally

Safety warnings

NOTE: The CDC trap is a highly versatile design that can be used in a number of different configurations with different attractants to collect nearly all blood-feeding Nematocerans. UV lights have been shown to be more effective in catching certain types of mosquitoes. We suggest a combination of UV-light and CO2 to collect the greatest diversity of blood-feeding insects.

NOTE: CO2 may not be available at certain sites and/or too expensive, and can be considered optional.

NOTE: Collection cups may have large mesh (for mosquitos), fine mesh (for midges and sand flies), or a solid bottom (for a preservative liquid for midges). Ensure the style matches the intended target organism.

- Liquid collection media can be used for biting midge collections and can help preserve these very small insects compared to dry collections.
- If using liquid media, tap water with a small amount of detergent can be made up in bulk prior to trapping. Insects should be immediately transferred into 90% ethanol after collection if using this method. The use of ethanol for collection is not recommended due to evaporation and cost.

Note that mosquitos will lose identifying features when stored in a liquid medium and this should be avoided.

NOTE: The BGS trap was designed using visual cues that preferentially attract diurnal Aedes species mosquitoes (e.g. *Aedes aegypti*). When paired with additional attractants (bait lure purchased separately), the BGS can collect other mosquito species and other Nematocerans.

NOTE: CO2 may not be available at certain sites and/or too expensive, and can be considered optional.

Before start

Activities include reading instructions, taking notes, preparing supplies, deploying and retrieving traps, and handling specimens. There may be hazards in the field and having at least two individuals is advisable for safety.

SAMPLING TEAM

1 Field teams should consist of at least two people.

2 Activities include reading instructions, taking notes, preparing supplies, deploying and retrieving traps, and handling specimens.

3 There may be hazards in the field and having at least two individuals is advisable for safety.

TRAP SITE AND TYPE SELECTION

4 Refer to Appendix 1 to determine which trap type to use for the target organism(s) of interest.

5 Refer to Appendix 2 to determine when traps should be placed for the target organism(s) of interest.

6 Refer to Appendices 3-5 to determine ideal trapping locations for the target organism(s) of interest.

6.1 Traps should be separated by at least 20 meters so they don't interfere with one another. Multiple light traps may interfere with one-another, and considerations should be made when deciding where to place these.

6.2 It is suggested that at least two traps per trapping location are used.

CDC MINITAURE LIGHT TRAPS

7

Note

NOTE: The CDC trap is a highly versatile design that can be used in a number of different configurations with different attractants to collect nearly all blood-feeding Nematocerans. UV lights have been shown to be more effective in catching certain types of mosquitoes. We suggest a combination of UV-light and CO2 to collect the greatest diversity of blood-feeding insects.

Note

NOTE: CO2 may not be available at certain sites and/or too expensive, and can be considered optional.

8 Label the collection cup with a sample code using tape.

9 Drive a shepherd's hook into the ground in the location where the trap will be set.

9.1 Alternatively, any other type of post with a hook or structure can be used to hang traps.

9.2 Keep in mind the expected weight of the trap (particularly if dry ice will be used) when selecting the hanging device.

10 CDC-style traps should be set in an area with 1-2 meters of vertical space and hung at least 1.5m above the ground.

11 Prepare the trap assembly: attach the collection bag to the cup (see note below), attach the collection bag to the fan.

12

Note

NOTE: Collection cups may have large mesh (for mosquitos), fine mesh (for midges and sand flies), or a solid bottom (for a preservative liquid for midges). Ensure the style matches the intended target organism.

- Liquid collection media can be used for biting midge collections and can help preserve these very small insects compared to dry collections.
- If using liquid media, tap water with a small amount of detergent can be made up in bulk prior to trapping. Insects should be immediately transferred into 90% ethanol after collection if using this method. The use of ethanol for collection is not recommended due to evaporation and cost.

13 Bait the trap with CO2 (Appendix 6) (if using). If dry ice is used as the CO2 source, place ~2.5kg dry ice into an insulated drink cooler or other insulated container modified for CO2 dispersal. Pack the top of the cooler with newspaper to insulate the dry ice. The

cooler should be hung above the CDC trap. Some coolers have a D-ring on the bottom of the container (or one can be retrofitted), from which the CDC trap can be hung (Fig. 1).

Fig. 1. CDC traps baited with dry ice coolers. In these images, the trap device is hung directly from the bottom of the cooler using a hook or D-ring that has been installed onto the cooler for this purpose.

14 Otherwise, the cooler should be suspended from a height above the opening to the trap (Fig. 2).

Fig. 2. If the trap cannot be hung directly from the cooler, the cooler and trap may both be hung from the hook or post. In this instance, ensure that the bottom of the cooler (where the holes for CO2 release are located) is positioned just above the top of the trap.

15 Attach the power supply (6V battery). Ensure that the power cables are hooked up to the battery correctly (i.e. red/+ to red/+). Otherwise the fan will run backwards and you will not collect any insects.

15.1 The CDC trap is a down-draft trap- insects that fly over the top of the trap will be drawn down into the collection bag by the fan.

16 Ensure all components are functioning and note the start time.

17 The time of day to deploy traps will vary by target species of interest. If using lights, ensure other light sources are minimal and that the trap's lights will not interfere with operational activities.

18 When retrieving traps, remove the collection bag and quickly secure it by pulling the drawstring closed while the fan is running to ensure insects don't escape. Label the collection bag with a unique identifier and note the collection time.

19 Samples should be transported live prior to processing and can be placed into a cooler on wet ice (or ice packs) to reduce their activity and prevent escapes during transport.

20 Knock samples down using CO2 or a freezer before opening the bag.

21 Samples can be stored in bulk at -80 ºC (preferred) in a properly labeled tube (ex. 50 mL conical tube). If -80 ºC is unavailable, aim to store samples at -20 ºC or in a nucleic acid stabilizing solution (ex. DNA Shield).

Note

Note that mosquitos will lose identifying features when stored in a liquid medium and this should be avoided.

CDC GRAVID TRAPS (optional)

22

Note

NOTE: CDC gravid traps are designed to specifically target some *Culex* species mosquitoes (e.g. *Culex pipiens/quinquefasciatus*), and may not be appropriate for other Nematocerans. These traps also target females that have previously blood-fed and are looking for a place to lay eggs. As a result, pathogen infection rates in these collections may be higher than when using other traps, and host-seeking females will not be collected.

23 Label the collection cage with a sample code using tape.

24 Gravid traps are placed directly on the ground.

25 Gravid traps are baited with hay-infused water. Hay infusion should be prepared at least 5 days ahead of time. Prepare hay infusion by adding 0.5 kg of hay to 114 L of tap water.

25.1 Add at least 1 gal/3.8 L to the gravid trap base (use new hay infusion for each collection event).

26 Attach the fan apparatus to the metal support arms using the provided bolt. Attach the collection cage over the fan apparatus. Place the assembled trap over the top of the trap base (Fig. 3).

Fig. 3. An assembled CDC gravid trap. The trap apparatus is mounted onto bars, which allows the trap to balance on top of the trap base.

27 Attach the power supply (6V battery). Ensure that the power cables are hooked up to the battery correctly (i.e. red/+ to red/+). Otherwise the fan will run backwards and you will not collect any insects.

27.1 The gravid trap is an up-draft trap- insects that are attracted to the oviposition media (hay infusion) will be drawn up into the collection bag by the fan.

28 Ensure all components are functioning and note the start time.

29 When retrieving traps, remove collection bags and quickly secure them while the fan is running to ensure samples don't escape. Label the collection bag with a unique identifier and note the collection time.

30 Samples should be transported live prior to processing and can be placed into a cooler on wet ice (or ice packs) to reduce their activity and prevent escapes during transport.

31 Knock samples down using CO2 or a freezer before opening the bag.

32 Samples can be stored in bulk at -80 ºC (preferred) in a properly labeled tube (ex. 50 mL conical tube). If -80 ºC is unavailable, aim to store samples at -20 ºC or in a nucleic acid stabilizing solution (ex. DNA Shield).

Note

Note that mosquitos will lose identifying features when stored in a liquid medium and this should be avoided.

BG SENTINEL (BGS) TRAPS

33

Note

NOTE: The BGS trap was designed using visual cues that preferentially attract diurnal *Aedes* species mosquitoes (e.g. *Aedes aegypti*). When paired with additional attractants (bait lure purchased separately), the BGS can collect other mosquito species and other Nematocerans.

Note

NOTE: CO2 may not be available at certain sites and/or too expensive, and can be considered optional.

Label the trap funnel with a sample code using tape.

34 BGS traps are placed directly on the ground (Fig. 4).

Fig. 4. An assembled BG Sentinel trap showing the collection funnel in the open position.

35 BGS traps are baited with a commercially produced lure (BG-Lure), which mimics human-odors. When baited with the BG-Lure, the BGS preferentially attracts anthropophilic *Aedes* spp. (i.e. *Aedes aegypti* and *Aedes albopictus*). The lure is placed into the small hole in the trap lid and can be reused for up to five months.

35.1 BGS traps can be baited with other types of odor lures (e.g. CO2) to increase the diversity of species collected.

35.2 If other types of lures are used, they should be placed inside of the trap, as this trap functions by creating an odor plume from drawing air into the trap body and pushing it out of the holes in the perforated trap lid (Fig. 5).

Fig. 5. The BG Lure and its correct installation in the lid of the BG Sentinel trap. Additional attractants, including dry ice (CO2) can be placed inside the trap. Odors are pushed out through the perforated lid by the fan.

36 Attach the catch bag to the funnel with the shutter in the “unlatched” position. Place the funnel/catch bag into the center hole of the trap lid. The weight on the funnel lid should keep it closed until the trap is turned on.

36.1 The catch bag provided with the BG trap is sufficient to collect both mosquitoes and the smaller Nematocera, like biting midges and sand flies.

37 Attach the power supply (12V battery). Ensure that the power cables are hooked up to the battery correctly (i.e. red/+ to red/+). Otherwise the fan will run backwards and you will not collect any insects. When the trap turns on, the fan should cause the funnel lid to open.

37.1 This mechanism ensures that even if the battery dies during operation, the funnel lid will close automatically, preventing collected insects from escaping.

37.2 The BGS is a down-draft trap- insects that fly over the top of the trap will be drawn down into the collection bag by the fan.

38 Ensure all components are functioning and note the start time.

39 When retrieving traps, unplug the power source and close the funnel lid. There is a latch on the funnel lid that can be used to secure it. Tape can also be used to provide additional security for the lid. Label the collection bag with a unique identifier and note the collection time.

40 Samples should be transported live prior to processing and can be placed into a cooler on wet ice (or ice packs) to reduce their activity and prevent escapes during transport.

41 Knock samples down using CO2 or a freezer before opening the bag.

42 Samples can be stored in bulk at -80 ºC (preferred) in a properly labeled tube (ex. 50 mL conical tube). If -80 ºC is unavailable, aim to store samples at -20 ºC or in a nucleic acid stabilizing solution (ex. DNA Shield).

Note

Note that mosquitos will lose identifying features when stored in a liquid medium and this should be avoided.

APPENDICES

43 **APPENDIX 1. TRAP TYPE SELECTION CONSIDERATIONS**
Adapted from CDC Document CS 345089-A.

	A	B	C
	Trap Type	Target Organism(s)	Notes
	BG Sentinel Trap	Aedes spp., Culex spp., Biting Midges, Sand Flies	Can be used with CO2 and/or other chemical lures
	CDC Light Trap	Mosquitos, Midges, and Sand Flies	This is the broadest collection method, but lights will attract non-target organisms as well. Different models have different light sources. Incandescent, UV and LED are all available, but will have different efficacy and range of attraction.
	CDC CO2 Trap	Mosquitos, Midges, and Sand Flies	CO2 alone will reduce non-target organisms and attract host-seeking females
	Gravid Trap	Culex spp.	Hay water must be prepared ahead of time. Gravid females have already

	A	B	C
			taken blood and are an ideal stage to collect for pathogen surveillance.
	Resting Trap	Anopheles spp., Culex spp., and Culiseta spp.	Can be passive or paired with a fan.
	Aspiration	any	Non-selective collection from the environment may result in blood-fed females.

44 **APPENDIX 2. ARTHROPOD SAMPLING TIME SELECTION CONSIDERATIONS**

Many blood-feeding Nematocera have crepuscular or nocturnal host-seeking behavior. For most target species, traps can be set 2 hours prior to sunset and collected 2 hours after sunrise. In areas where Aedes aegypti and Aedes albopictus are target species, traps should be set during the mid-afternoon to accommodate their diurnal activity patterns.
Adapted from AFPMB-TG48.

	Genus/Group	Biting Period2	Flight Range	Associated Pathogens3
	Aedes	C, D	< 0.5 – 5 mi	CK, DG, YF, ZV
	Anopheles	C	0.5 – 2 mi	M, EEE, SLE, VEE, WEE, WNV
	Coquillettidia	C	1 – 2 mi	EEE, VEE, WNV
	Culex	C, N	0.5 – 2 mi	RRV, SLE, WEE, EEE, WNV, VEE
	Culiseta	C, N	0.5 – 2 mi	EEE, WEE, WNV, CE
	Mansonia	C, N	1 – 5 mi	VEE
	Psorophora	C, N	1 – 5 mi	VEE, WNV
	Sand Flies	C	< 0.5 – 2 mi	LM, SFF, BA
	Biting Midges	C, N	< 0.5 – 2 mi	ORV

2D = Day; N = Night; C = Crepuscular (dusk and dawn)

3Italics indicate secondary or suspected vectors; primary if otherwise. BA = Bartonellosis; CE = California group encephalitis; CK = Chikungunya; DG = dengue; EEE = Eastern equine encephalitis; LM = Leishmaniasis; M = malaria; ORV = Oropouche Virus; RRV = Ross River Virus; SFF = Sand Fly Fevers; SLE = St. Louis encephalitis; VEE = Venezuelan equine encephalitis; WEE = Western equine encephalitis; WNV= West Nile Virus ;YF = Yellow fever; ZV= Zika Virus

Note: Many species will shift their activity periods earlier in the day during the winter.

45 APPENDIX 3. SAND FLY SAMPLING HABITATS

Information is adapted from AFPMB-TG49.

Domestic Sites: abandoned buildings, basements/cellars, cracks in mud floors/walls, soil in dwellings

Peridomestic Sites: animal burrows/housing/shelters, caves, latrines, dikes/embankments, manure, debris, wells

Sylvatic Sites: ant/termite mounds, small animal burrows, drains, hollow trees, leaf litter, nests, large roots, under rocks, caves, soils under trees, soil under overhanging rocks

Work with your command and locals to determine areas with sand fly burdens that would be useful to trap at

46 APPENDIX 4. MOSQUITO SAMPLING HABITATS

Information is adapted from AFPMB-TG47.

Traps can be placed in a variety of areas but these should be free from direct sunlight and wind. Consider trapping in field locations where people will be working, or where people will be living.

47 APPENDIX 5. BITING MIDGE SAMPLING HABITATS

Information is adapted from McDermott and Lysyk 2020, Feitoza et al. 2023

Culicoides are primarily collected in close proximity to livestock. Traps can be placed near housed or managed animals on farms.

Pestiferous species or potential vectors of human pathogens can be collected near human domiciles or near wooded areas. For example, *C. paraensis* (vector of Oropouche virus) can be collected in banana plantations and around trees in villages.

48 APPENDIX 6. OPTIONS FOR CO2 LURES

Dry ice (solid carbon dioxide) is a preferred material for CO2 emission but may not be available or feasible for sampling budgets. If using, exercise caution when handling by using cold-proof gloves and implements. Place dry ice into an insulated container (NEVER seal a container containing dry ice) with an outlet that will release gaseous carbon dioxide around the fan's suction.

Compressed CO2 with an outlet tube reaching the fan's suction opening is a good option. Always take precautions when transporting compressed gas canisters and operating regulators (prior safety training is highly encouraged). Release rates may vary, but may range from 300 ml/min to 1,000 ml/min.
Brewer's/baker's yeast (*Saccharomyces cerevisiae*) can be used to produce CO2 when combined with water and sugar. This may be a low-cost method of CO2 production when other methods are inaccessible. Combine these in a container (such as a 2-liter soda bottle) and direct the outlet towards the intake of the fan trap (using tubing for example).
5 g yeast + 280 g/L sugar water in 300 mL liquid. (Dhanique et al. 2017)

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