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REDI-NET FF-1 FILTH FLY FIELD SAMPLING V.1

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Remote Emerging Disea...

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We use this protocol and it's working

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Abstract

To outline steps for properly collecting filth flies from cattle and environmental locations to evaluate the risk of zoonotic disease transmission by the detection of pathogens from vertebrate DNA (vDNA).

Guidelines

OBJECTIVE

To document the field processes for collecting filth flies from cattle and environmental locations.

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 the correct filth fly sampling at CONUS/OCONUS sites to evaluate and predict the risk of zoonotic disease transmission.

RESPONSIBLE PERSON

Principal Investigator, Study Coordinator, Entomology Component Lead, Managers

Materials

	A	B	C
	Equipment / Material	Description	Mfg / Product #
	Fly Cartons	See instructions in Appendix 1	Locally sourced
	Tulle fabric	For fly carton	Locally sourced
	Scissors	For fly carton construction	Locally sourced
	X-acto knife	For fly carton construction	Locally sourced
	Hot glue gun	For fly carton construction	Locally sourced
	Hot glue sticks	For fly carton constructions	Locally sourced
	Men's crew sock (size L)	For fly carton	Locally sourced
	Rubber bands	For fly carton	Locally sourced
	Sweep nets		Locally sourced
	GPS Unit	WGS 84 and precision of 5 decimal degrees	Locally sourced
	Tablet	For data entry and picture of sampling site	Locally sourced
	Writing utensils	Sharpies, pens, pencils	Locally sourced
	Cotton balls	Water-soaked	Locally sourced
	50 mL Falcon tubes	For storing collected flies	Locally sourced
	Forceps	For collecting flies from nets	Locally sourced
	Condiment cup	For the water-soaked cotton balls	Locally sourced
	Sugar	For house/face flies kept alive	Locally sourced
	Dehydrated milk	For house/face flies kept alive	Locally sourced
	Defibrinated cow blood	For horn/stable flies kept alive	Locally sourced
	Sanitary pads	For horn/stable flies kept alive	Locally sourced

Troubleshooting

Safety warnings

CATTLE COLLECTIONS

- Cattle are restrained in a squeeze chute for all procedures.
- **Never** stand in front of the chute until the animal has been completely restrained.
- **Always** be aware of the location of the gate hinge when standing next to the chute as it is opening and closing.
- **Always** make sure that all drop-down bars and fold-out panels are closed before the chute is opened.
- **Always** be aware of the fact that the animal can still move somewhat inside the chute. Be aware of where your hands and arms are, and be able to remove them from the chute quickly.
- **Always** communicate verbally with the team operating the squeeze chute when you are finished checking the animal so that they know when it is safe to open the chute.
- **If you ever feel uncomfortable checking an animal** (animal is too worked up or aggressive in the chute), or you are unable to safely access part of the animal in the chute, **skip it**.

ENVIRONMENTAL COLLECTIONS

- **Always** use caution when entering pastures with cattle. Use the buddy system whenever possible and avoid turning your back on the animals. If bulls are present in the pasture, always be aware of the location of the bulls and know how to exit the pasture quickly if necessary.
- If entering pastures for collections, don't forget to close and lock any gates that you open.

Before start

NOTE: Obey all safety procedures in of this SOP.

- **Never** stand in front of the chute until the animal has been completely restrained.
- **Always** be aware of the location of the gate hinge when standing next to the chute as it is opening and closing.
- **Always** make sure that all drop-down bars and fold-out panels are closed before the chute is opened.
- **Always** communicate verbally with the team operating the squeeze chute when you are finished checking the animal so that they know when it is safe to open the chute.
- **Always** be aware of the fact that the animal can still move somewhat inside the chute. Be aware of where your hands and arms are, and be able to remove them from the chute quickly.
- **If you ever feel uncomfortable checking an animal** (animal is too worked up or aggressive in the chute), or you are unable to safely access part of the animal in the chute, **skip it**.

Note

Prepare fly cartons prior to field day; see directions in Carton Construction Appendix 1.

SAMPLING TEAM

1 This SOP assumes that the animals' owners/handlers/veterinarians are on site to corral cattle, move them through the chute, and operate the chute. If collecting from cattle, animals should be restrained in a chute or squeeze chute or in a head gate. If animals are only restrained in a head gate, **be aware that cattle can kick to the side and keep an appropriate distance from the animal.**

2 Sampling of vDNA samples involves two people. One person serves as the 'sampler' and the other person serves as a 'helper'. The helper can look up details in these instructions when needed, keep track of samples, handle objects that are contamination risks, serve as a second set of eyes for potential contamination, and ensure safety of the sampler in potentially hazardous field conditions.

CATTLE COLLECTION

3 Label a previously prepared fly carton with the collection date, collection site, mark as "cattle".

4 Once the animal(s) is/are restrained, make three, quick forward and reverse sweeps with the net over the back/sides of the animal

5 At the end of the third sweep, quickly grab the net to close the opening with your hand.

5.1 **For cattle in the chute:** Stand on step or step stool to sweep over the back of animals.

5.2 **For cattle restrained in the squeeze chute:** Lower the drop-down bars and fold-out panels of the chute to sweep the sides and legs of the animal.

Note

Remember to close the panels before the animal is released from the chute and communicate clearly with the chute operator when you are finished working and the animal is safe to be released.

6 Untie the sleeve of the fly carton (while still holding the net closed) and carefully transfer the flies from the net to the carton by pushing the opening of the net into the sleeve and turning the net inside out.

7 Re-tie the carton sleeve.

8 Record the ear tag or identification number of the animal sampled.

9 Repeat this process until five animals have been sampled. After five animals have been sampled, start a new carton.

10 Continue sampling until at least 20% of the animals have been sampled. Ideally, all animals will be sampled, but occasionally this will not be possible due to time or personnel restraints.

11 Provide each carton with two water-soaked cotton balls in a condiment cup to help sustain flies until they can be returned to the lab.

ENVIRONMENTAL COLLECTION

12

Note

If entering pastures for collections, don't forget to close and lock any gates that you open.

Label a previously prepared fly carton with the collection date, collection site, mark as "environment".

13 Identify appropriate collection locations at the site. Common filth fly development and aggregation locations include manure piles (house/stable flies), intact dung pats (horn/face flies), silage (house/stable flies), litter (house/little house flies), barn/stable walls (house/little house flies) .

14 For each collection location-type sampled (e.g. silage, dung pats), use a new fly carton.

15 Label the fly carton with the collection location type.

16 Make three, quick forward and reverse sweeps with the net over the collection location.

17 At the end of the third sweep, quickly grab the net to close the opening with your hand.

18 Untie the sleeve of the fly carton (while still holding the net closed) and carefully transfer the flies from the net to the carton by pushing the opening of the net into the sleeve and turning the net inside out.

19 Re-tie the carton sleeve.

20 Repeat this process until the carton is full, or all flies have been collected.

21 Provide each carton with two water-soaked cotton balls in a condiment cup to help sustain flies until they can be returned to the lab.

FLY STORAGE

22 If flies **do not** need to be kept alive for research purposes:

22.1 Place fly cartons into a -20 °C freezer for at least two hours, and up to 24 hours to kill flies.

22.2 After flies are dead, label a 50mL Falcon tube with the same information that was written on the fly carton.

22.3 Remove the mesh covering on the fly carton and transfer the flies to the Falcon tube.

22.4 If samples can not be processed on the same day of collection, all samples should be stored at -80 °C until they are identified to species and processed for TNA extraction.

22.5 If -80 °C is not available, filth fly samples should be stored at -20 °C and processed as soon as possible.

23 If flies **are** to kept alive for research purposes:

23.1 Maintain flies in a separate room from the main laboratory if possible to limit escapes. Ideally, this should be an insectary space held at ~ 23 °C, 75% RH, and 16:8 L:D cycle. However, these conditions are not necessary if flies are only being held temporarily as long as the room temperature is appropriate.

23.2 For collections from cattle, or for environmental collections that are primarily horn or stable flies, pour 20 mL of cow blood onto a sanitary pad. Place that pad blood side-down on top of the mesh covering of the fly carton. Replace blood pad daily as necessary.

23.3 For environmental collections that are primarily house, little house, or face flies, mix 12 g dehydrated milk powder and 12 g table sugar together in a condiment cup and place it inside the fly carton. Also replace the water-soaked cotton balls as necessary.

APPENDIX 1. CARTON CONSTRUCTION

24 For mosquitoes, use the X-acto knife to cut a 3.5×2″ access hole in the side of the carton. For flies, cut a 5.5" diameter circle.

25 For mosquitoes, cut the toe off of the sock and cut a 0.5″ slit in the cuff. For flies, cut a 30″x24″ rectangle of organza or tulle and hot glue the long sides together, leaving the short ends open.

26 Apply a line of hot glue around the inside of the access hole and adhere the cuff end of the sock (or short side of organza) to the inside of the carton so that it forms a sleeve (Fig. 1).

27 Apply a small amount of glue at a time (1-2″) and adhere the sock so that the glue does not dry and you can stretch the sock enough to cover the opening.

Figure 1. Access hole with sock stretched over it to create a sleeve.

28 Apply additional glue as needed to secure the sock or organza.

29 Cut a 12×12″ square (or large enough to cover the top of the carton) of tulle screen. Apply a line of hot glue around the outside rim of the carton and adhere the screen over the top to cover it.

30 Apply a small amount of glue at a time (1-2″) and adhere the screen so that the glue does not dry and you can stretch the fabric enough to cover the carton.

31 Stretch an elastic band over the top of the screen-covered carton to further secure the screen.

32 Tie the sleeve before adding insects to the carton (Fig 2.)

Figure. 2. Completed mosquito carton with sock sleeve knotted to prevent escape.

Protocol references

REDI-NET Overview Summary