The Gnatwork

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Title of resource
GN_19: Protocol for use of CDC light traps with FTA cards
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DOI
Description
Protocol for use of CDC light traps with FTA cards. Describes the use of modified CDC light traps in combination with sugar-soaked FTA cards to collect sand flies and determine their infectiousness and hence leishmaniasis transmission in an area. Protocol from the Gnatwork project "The use of FTA cards to monitor *Leishmania* infection and infectiousness in sand flies and midges" created for field work in Ethiopia and Ghana (summer 2019).
Intended use
Scientific research use and training purposes.
Restrictions on use
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Resource history
N/A

GN_19: Protocol for use of CDC light traps with FTA cards

When using this protocol, the following should be referenced:
Mojca Kristan, Tom Walker, Matt Rogers (London School of Hygiene and Tropical Medicine)

A. Introduction

CDC light traps are commonly used for collection of malaria vectors in order to estimate sporozoite rates and human biting rates [1] and are frequently used to collect phlebotomine sand flies [2]. Recent findings demonstrate that infectious mosquitoes expectorate detectable malaria sporozoites onto FTA cards during sugar feeding [3]. FTA cards have previously been used for direct sampling from patients' leishmaniasis lesions [4] and also for blood meal identification and parasite detection in sand flies [5].

This protocol was prepared to determine the potential of using sugar-soaked FTA cards in combination with a CDC light trap to survey sand fly infectiousness and hence leishmaniasis transmission and has been used in the field in Ethiopia and Ghana. The use of food dye mixed with sucrose solution means the sand flies or midges will ingest it, allowing for confirmation of whether they have fed on the card [6].

B. Materials

Equipment

- FTA classic cards (Whatman® FTA® card technology, FTA classic card with 4 sample areas per card, Sigma-Aldrich WHAWB120205)
- Absorbent cotton wool
- Sealable plastic bags
- Bijou tubes

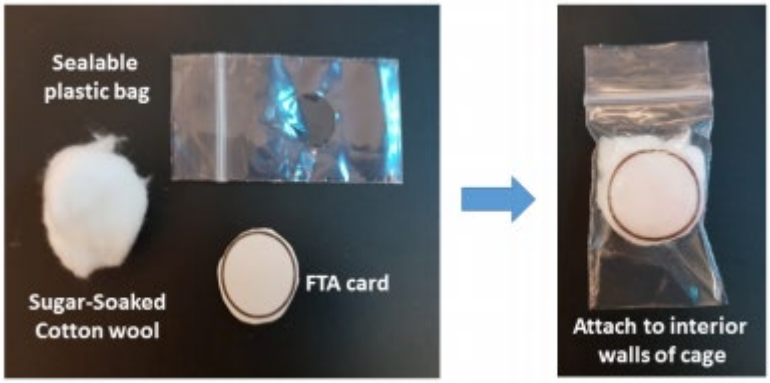
Reagents

- Sucrose powder (Sucrose for microbiology, ACS reagent, >99.0%, Sigma-Aldrich 84100)
- Patent Blue V sodium salt (Sigma-Aldrich 21605)
- Silica gel orange, with moisture indicator free of heavy metals (Honeywell 13767)

C. Method

C.1 Prepare a 50% (v/v) sucrose solution and add a tiny amount of blue dye to turn the solution blue.

C.2 Cut out a small circular opening from one side of the sealable bag (approximate size of an FTA card). With clean forceps or gloves, place one FTA card circle into the bag to cover this opening with its active side (i.e. where the black circle outline is) and place a large piece/pad of cotton wool at its back (it can fill the rest of the bag).



C.3 Use a plastic pipette and completely soak the cotton wool (after you put it into the bag), making sure the FTA card is wet with the sugar solution and is exposed through the opening. Seal the bag.

C.4 Attach two sealed bags containing sugar-soaked FTA cards to the plastic sides of the cage using tape.

Two panels of the cage (here front, where the sleeve is attached, and bottom) are made from plastic. Attach the sealable bags with FTA cards inside the cage onto the white plastic panel (which will be a side once the cage is hanging from the light trap).

Sealed bag with FTA card soaked with sucrose + food dye, attached inside the light trap collection cage.

C.5 Assemble the light trap using a small cage instead of a collection pot, hang in a good position and run it.

C.6 Carefully close the collection cage by using a string and remove it. Do not fold the sleeve into the cage so the insects have enough space.

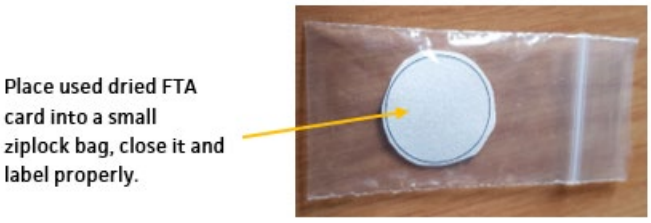
CDC light trap with a small cage instead of collection pot. A sealed bag with FTA card soaked with sucrose + food dye should be attached **inside** the light trap collection cage.

C.7 If any sand flies or midges were caught keep them in the same collection cage for another few days or until they die. The aim is to trap sand flies or midges and keep them alive inside the cage where they will take sugar meals from the FTA cards and (potentially) deposit parasites.

C.8 Kill any remaining live sand flies/midges by freezing or by using ethyl acetate/acetone.

C.9 Prepare a Bijou tube: place some silica gel in the bottom and cover by cotton wool. Collect all the mosquitoes caught and place them into one Bijou tube (unless there are too many – they should not be crushed) and cover with a bit of cotton wool, then close the lid. Label with the date of collection, collector initials and location.

C.10 Remove the FTA card that was in the cage with clean forceps and allow to completely dry at room temperature on a clean surface. Clean forceps between FTA cards by wiping with 70% (v/v) ethanol. Store each one individually in a small sealable plastic bag labelled with the date of collection, collector initials and location details (i.e. the same details as on the tube containing sand flies/midges).



C.11 Redeploy the CDC light trap with a fresh FTA card.

C.12 Repeat in the same location then if possible, find a new location. If not possible, keep using the same location.

Next steps

C.13 Extract gDNA or RNA from collected FTA cards.

C.14 If extracting RNA, generate cDNA.

C.15 Use qRT-PCR for detection of *Leishmania* parasites.

D. References

1. Wong, J. *et al*. (2013). Standardizing operational vector sampling techniques for measuring malaria transmission intensity: evaluation of six mosquito collection methods in western Kenya. *Malar J*, 12: 143.

2. Gaglio, G. *et al*. (2017), Field evaluation of a new light trap for phlebotomine sand flies. *Acta Trop*, 174: 114-117.

3. Brugman, V.A. *et al*. (2018). Detection of malaria sporozoites expelled during mosquito sugar feeding. *Sci Rep*, 8(1): 7545.

4. Kato, H. *et al*. (2019), Further insight into the geographic distribution of Leishmania species in Peru by cytochrome b and mannose phosphate isomerase gene analyses. *PLoS Negl Trop Dis*, 13(6): e0007496.

5. Sant'Anna, M.R. *et al*. (2008), Blood meal identification and parasite detection in laboratory-fed and field-captured Lutzomyia longipalpis by PCR using FTA databasing paper. *Acta Trop*, 107(3): 230-7.

6. Hall-Mendelin, S. *et al*., Exploiting mosquito sugar feeding to detect mosquito-borne pathogens. *Proc Natl Acad Sci USA*, 2010. 107(25): p. 11255-9.