The Gnatwork

Intended use of resource / data

Open access resources and data provided by The Gnatwork should be used for the intended purpose only, as specified below.

Title of resource
GN_18: Protocol for use of FTA cards for *Leishmania* parasite detection in laboratory-infected sand flies and midges
Authored by
Mojca Kristan, Tom Walker, Matt Rogers (London School of Hygiene and Tropical Medicine)
DOI
Description
Protocol for the use of FTA cards for *Leishmania* parasite detection in laboratory-infected sand flies and *Culicoides* midges. How to use sugar-soaked FTA cards for detection of parasites in the expectorate of laboratory-infected vectors. Protocol from the Gnatwork project "The use of FTA cards to monitor *Leishmania* infection and infectiousness in sand flies and midges" created for laboratory work undertaken at LSHTM (Oct 2018 – Sep 2019).
Intended use
Scientific research use and training purposes.
Restrictions on use
Content is not to be redistributed in the public domain (e.g. presentation, lecture, online or in publications).
Resource history
N/A

GN_18: Protocol for use of FTA cards for *Leishmania* parasite detection in laboratory-infected sand flies and midges

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

A. Introduction

Recent findings demonstrate that infectious mosquitoes expectorate detectable malaria sporozoites onto FTA cards during sugar feeding [1]. FTA cards have previously been used for direct sampling from patients with cutaneous leishmaniasis [2] and also for blood meal identification and parasite detection in sand flies [3].

This protocol was prepared to determine if sugar-soaked FTA cards can be used for detection of *Leishmania* parasites in the expectorate of laboratory-infected sand flies and *Culicoides* midges. 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 [4].

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

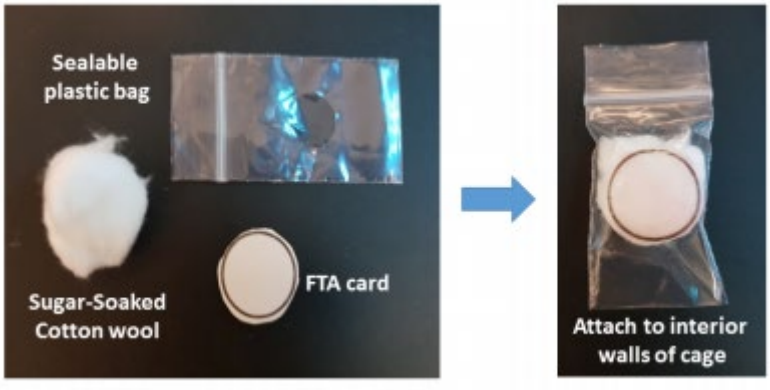
Reagents

- Sucrose powder (Sucrose for microbiology, ACS reagent, >99.0%, Sigma-Aldrich 84100)
- Patent Blue V sodium salt (Sigma-Aldrich 21605)

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).

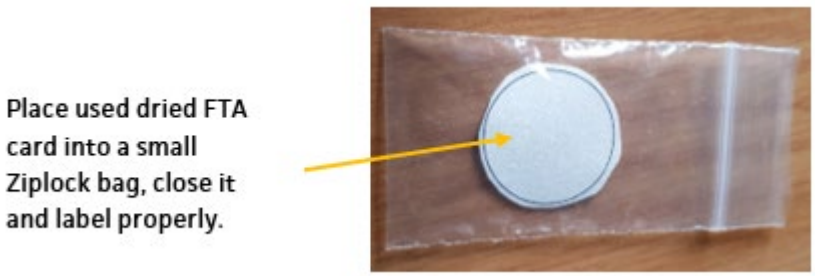


C.3 Use a plastic pipette and completely soak the cotton wool (after you put it into the bag), making sure the FTA card is wet with the sugar solution and is exposed through the opening. Seal the bag.

C.4 Either place sealed bags containing sugar-soaked FTA cards on top of the cage with infected sand flies or midges or place them inside on the bottom of the cage.

C.5 Change either daily or at regular intervals, making sure that dead insects do not get stuck on the FTA card. Remove FTA card from its plastic pouch with clean forceps. Clean forceps by wiping with 70% (v/v) ethanol.

C.6 Allow the FTA card to completely dry on a clean surface and store each one individually in a small sealable labelled plastic bag.



C.7 Store the FTA cards either dry at room temperature or in a freezer until further processing.

Next steps

C.8 Extract gDNA or RNA from collected FTA cards.

C.9 If extracting RNA, generate cDNA.

C.10 Use qRT-PCR for detection of Leishmania parasites.

D. References

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

2. Kato, H., *et al.*, 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.

3. 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.

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