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Title of resource
GN_16: Protocol for staining small biting flies prior to micro-computed tomography scanning
Authored by
When using this protocol, the following should be referenced: Martin Hall, Daniel Martín-Vega and Brett Clark (Natural History Museum, London, UK)
DOI
N/A
Description
Protocol for preparing and staining sandflies and blackflies prior to micro-computed tomography scanning. This protocol was developed as part of Gnatwork Transformative Science project “Micro-CT visualisation of the parasite-host interface in small biting flies”.
Intended use
Scientific research use and training purposes.
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Resource history

GN_16: Protocol for staining small biting flies prior to micro-computed tomography scanning

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Martin Hall, Daniel Martín-Vega and Brett Clark (Natural History Museum, London, UK)

A. Introduction

Staining of insects prior to micro-CT examination is necessary to increase x-ray absorption of low density tissues and enhance overall contrast. The following protocol is based on our experience [1] and that of others [reviewed in 2] in staining small insects for micro-CT studies. Large insects, e.g. blow flies (Calliphoridae) and bot flies (Oestridae), stain rapidly with 1% iodine solutions and retain the stain in their tissues for periods sufficient for most micro-CT scans [3]. However, we found that iodine staining of small flies, such as sandflies (Phlebotominae, Psychodidae) and blackflies (Simuliidae), was problematic, especially for long scan runs, because the iodine leached rapidly out of the tissues into the ethanol preservative. This resulted in low quality scans, with poor contrast. Therefore, we used a phosphotungstic acid solution instead, which worked very well for our study, producing images of high contrast. In addition, the stain binds permanently to tissues so specimens can be re-scanned or stored for scanning without loss of stain. However, there is great scope for further work to develop staining techniques, for example testing different staining time frames and different presentations of the insects themselves (e.g. with/without limbs).

B. Materials

Equipment

- Pipettes (100-1000 µl)
- 0.3 - 1.5 ml microcentrifuge tube
- 1.5 ml microcentrifuge tube rack
- Laboratory shaker/swirler
- Green floral foam
- Forceps and micro-knife
- Pin
- Marker pen

Reagents

- 80% ethanol
- 1% phosphotungstic acid (PTA) in 80% ethanol

C. Method

C.1 Be aware of the health and safety aspects of the ethanol, phosphotungstic acid and floral foam used in this protocol.

C.2 Specimens can be freshly killed or preserved in 70-80% ethanol prior to staining.

C.3 Remove legs of specimens below level of the coxa to facilitate penetration of the staining solution (leaving specimens intact is likely to require a longer staining time than that detailed in section C.6).

C.4 Place individual specimens into a 1.0 - 1.5 ml microcentrifuge tube and fill the tube with a solution of 1% phosphotungstic acid (PTA) in 80% ethanol.

C.5 Place the tube in a rack on a laboratory shaker/swirler set to a gentle action.

C.6 Leave the specimens in stain for one week, changing the solution for fresh solution half-way through this period.

C.7 After one week, remove specimens from the staining solution and transfer to 80% ethanol within the container used for scanning (e.g. 0.3 ml microcentrifuge tube).

C.8 If specimens are allowed to move within the tube during scanning the resultant images will be blurred. To avoid this it is necessary to restrict specimen movement. This can be done simply using green floral foam to hold the specimen at the bottom of the tube. Push the tube about half way into a block of floral foam, then twist the tube and remove it which will leave a cylinder of foam in the tube. This foam cylinder can be carefully pushed down the tube to restrain the specimen at the bottom of the tube (Fig 1).

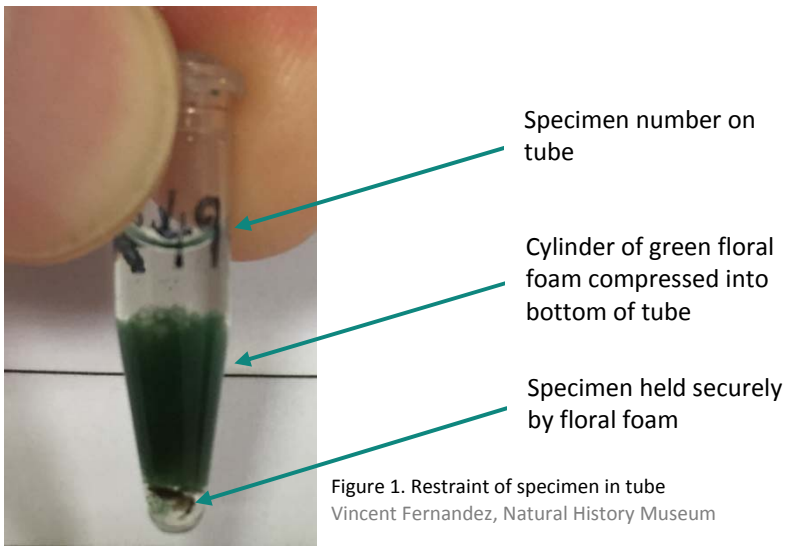

Figure 1. Restraint of specimen in tube
Vincent Fernandez, Natural History Museum

C.9 Take care not to force the foam so much that the specimen is distorted. Allow any trapped air bubbles to float to the top of the tube – this can be facilitated by using a pin or forceps to make an escape passage between the foam and the inside of the tube.

C.10 Use a marker pen to label the tube and then store prepared tubes in a rack prior to scanning.

C.11 Depending on the scanner, tubes can be mounted individually or within a stacked column of two to three tubes to enable several to be scanned sequentially.

D. References

1. Martín-Vega, D., Garbout, A., Ahmed, F., Wicklein, M., Goater, C.P., Colwell, D.D. and Hall, M.J.R. (2018). 3D virtual histology at the host/parasite interface: visualisation of the master manipulator, *Dicrocoelium dendriticum*, in the brain of its ant host. *Scientific Reports*, 8: 8587. (DOI: 10.1038/s41598-018-26977-2)

2. Hall, M.J.R. and Martín-Vega, D. (2019). Visualization of insect metamorphosis. *Philosophical Transactions of the Royal Society B*. 374: 20190071. (DOI: 10.1098/rstb.2019.0071)

3. Martín-Vega, D., Simonsen, T.J. and Hall, M.J.R. (2017). Looking into the puparium: Micro-CT visualisation of the internal morphological changes during metamorphosis of the blow fly, *Calliphora vicina*, with the first quantitative analysis of organ development in cyclorrhaphous Diptera. *Journal of Morphology*, 278: 629-651. (DOI: 10.1002/jmor.20660)