

APPENDIX I

SOP FOR PRESERVATION OF MOSQUITO SPECIMENS IN RNALATER

RNAlater (Ambion Inc) is a non-toxic tissue storage reagent that rapidly permeates tissue to stabilise and protect RNA in unfrozen specimens. RNAlater treatment eliminates the need to immediately process or snap freeze specimens. Tissue submerged in RNAlater can be stored without evidence of RNA degradation for 1 day at 37 °C, 1 week at 25 °C, 1 month at 4 °C.

1. Equipment and reagents

- 1.1. 100% ethanol and RNAlater® Solution
- 1.2. 1.5 mL microcentrifuge tubes (sterile, DNase & RNase-free)
- 1.3. Paper towel, Mouth aspirator (new or cleaned and decontaminated), Pipet and tips (200µL - 1 mL)
- 1.4. Clean forceps (sterilise with ethanol before usage)
- 1.5. Fridge (4 °C, for overnight incubation and/or storage for up to 1 month) and Freezer (-20 °C or -80 °C, for long-term storage)
- 1.6. Optional: Cotton wool (to make sure specimen stay fully submerged in RNAlater)

2. Procedure

- 2.1. Blow aspirated mosquitoes directly into a Petri dish with 100% ethanol to kill them (*and makes the tissue more permeable to the RNAlater*)
- 2.2. Retrieve mosquitoes with forceps and **remove the ethanol by gently** putting the mosquitoes on a paper towel for 10 seconds.
- 2.3. Immediately transfer mosquitoes into a 1.5 ml microcentrifuge tube with RNAlater. Using forceps push the mosquitoes down into the RNAlater. For 50 mosquitoes use 1 ml of RNAlater.
- 2.4. Leave samples at 4°C overnight to allow the solution to thoroughly penetrate the tissue. **Ensure mosquitoes are fully submerged in RNAlater** for this overnight step.
Samples can be kept at 4 °C for 1 month. To prevent mosquitoes from floating on the surface of the RNAlater, a small cotton wool ball can be placed on top or the microcentrifuge tube can be filled to the top with RNAlater (for example for shipment of specimen at ambient temperature).
- 2.5. The next day ideally retrieve mosquitoes from RNAlater using sterile forceps and **remove the excess RNAlater by gently putting the mosquitoes on a paper towel** for 10 seconds.
- 2.6. Transfer mosquitoes into a clean 1.5 ml tube and move samples to -20°C or below for long term storage.