

SOP for Cone Bioassay

Purpose: This document is to provide instructions on the conduct of cone bioassays in experimental huts for the correct measurement of bio efficacy with adult mosquitoes using knock down and mortality

Appendix: FORM AVECNET EH 004.01

Definitions

KD	Knock down
PI	Principal Investigator
PM	Project manager
SOP	Standard operating procedure

Scope

This document is applicable to all staff of the project that perform cone bioassays in experimental huts.

Responsibilities

1. It is the responsibility of the individuals performing the assay to follow this SOP AVECNET EH 004.01 and use the associated data sheet FORM AVECNET EH 004.01
2. It is the responsibility of the PI to ensure that the correct version of this SOP is in place at the study site and correct training has been given to all personnel that use this SOP.
3. It is the responsibility of all scientific staff to ensure that this SOP is correctly followed.

Instructions

1. Safety and precautions

- 1.1. Inspect the sprayer tank for damage and make sure sprayer hoses, regulator, lance and nozzle are clean and functioning properly.
- 1.2. Never point the spray lance in the direction of people or animals.

2. Equipment

- 2.1. Laboratory coat
- 2.2. Disposable Medical Examination Gloves or similar
- 2.3. Mouth aspirator (new or cleaned and decontaminated)
- 2.4. Stop watch
- 2.5. WHO bioassay cones from an approved batch (new, unused or used, clean)
- 2.6. Cups (new, unused internally waxed 200-500ml), rubber bands, netting (unused, new fine mesh)
- 2.7. Temperature/Humidity Recording Device

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- 2.8. Cotton wool
- 2.9. Masking tape
- 2.10. Data sheet and black biro pen
- 2.11. Permanent marker
- 2.12. Plastic Bin with lid (for transporting used Bioassay Cones)
- 2.13. Sugar solution (as described in SOP AVECNET EH 003.01 Transportation of Mosquitoes)
- 2.14. Data record sheets (FORM AVECNET EH 004.01)

3. Procedure

- 3.1. Prepare mosquitoes as per standard laboratory rearing practices
- 3.2. Transport mosquitoes as per SOP AVECNET EH 002.01 Transportation of Mosquitoes
- 3.3. Record temperature and humidity inside the hut and confirm they are within ranges deemed acceptable as per the study protocol
- 3.4. Complete study procedure log
- 3.5. Put on gloves (bioassay technician)
- 3.6. Unless otherwise stated in the study protocol, there will be two cones/bioassays on each sprayed surface
- 3.7. Determine the position of each cone prior to fixing the cones to the sprayed surface.
- 3.8. Determine the position of each cone according to the study protocol
- 3.9. Each cone is attached to the sprayed surface using masking tape in such a way that the cone is securely attached to the sprayed surface and that mosquitoes will not be able to escape under the lip of the bioassay cone
- 3.10. Once the cones have been attached, gently aspirate 10 mosquitoes into the first cone and plug cone with cotton wool
- 3.11. Start stop watch
- 3.12. Move to the next successive cone and aspirate in next batch of 10 mosquitoes
- 3.13. Continue to each cone in turn at 2 minute intervals
- 3.14. Stop adding mosquitoes when the stop watch shows 30 minutes
- 3.15. Return to first cone
- 3.16. Gently aspirate the mosquitoes from the first cone into the correctly labelled holding cup
- 3.17. Move to the next successive cone and aspirate out the next batch of mosquitoes
- 3.18. Continue to each cone in turn until all cones have had the mosquitoes removed

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- 3.19. To reduce the risk of contamination, use a new pair of gloves when moving from one experimental hut to another
- 3.20. To reduce the risk of contamination, use a new/different aspirator when conducting cone bioassays in treated huts and untreated huts
- 3.21. Place mosquitoes in labelled cups into a cool box following SOP AVECNET EH 002.01 Transportation of Mosquitoes
- 3.22. Take mosquitoes to the laboratory/field laboratory
- 3.23. Provide sugar source to the mosquitoes following SOP AVECNET EH 003.01 Sugar Soaked Cotton Wool
- 3.24. Score mosquitoes for knockdown 60 minutes after the mosquitoes have been exposed to the treated surface (i.e. 90 minutes after the start of exposure) using data sheet FORM AVECNET EH 004.01
- 3.25. If not already at the laboratory/field laboratory, transport mosquitoes back to the laboratory as per per SOP AVECNET EH 002.01 Transportation of Mosquitoes
- 3.26. Once all bioassays have been completed, ensure that all bioassay cones are removed from the huts and put into a plastic bin with a sealed lid for transport back to the laboratory for cleaning
- 3.27. Hold mosquitoes at 27 ± 2 °C and $70\pm 10\%$ relative humidity
- 3.28. At 24 hours after the mosquitoes were exposed, score mortality using data sheet FORM AVECNET EH 004.01
- 3.29. Take the study procedure log and data sheets to study director for verification.

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4. Outcome measures

4.1. Knockdown

4.2. Mortality

5. Glossary

- Knock-down and Mortality: For the purpose of insecticide bioassays, the definition of knock-down and mortality involves not only the state of the insect but also the time at which the observation is made.
- A mosquito is classified as dead or knocked down if it is immobile or unable to stand or take off (see below).
- The distinction between knocked down and dead is defined only by the time of observation. The assessment of knock-down is made within 60 min post exposure. Mortality is determined at least 24 h post exposure.
- The holding container may be tapped a few times before a final determination is made.

Classification of adult mosquitoes as alive, knocked down or dead in bioassays		
Alive	Knocked down = recorded 60 minutes after exposure Dead = recorded 24 hours after exposure	
	Moribund	Dead
Can both stand on and fly in a coordinated manner	<ul style="list-style-type: none"> • Any mosquito that cannot stand (e.g. has 1 or 2 legs) • Any mosquito that cannot fly in a coordinated manner • A mosquito that lies on its back, moving legs and wings but unable to take off • A mosquito that can stand and take off briefly but falls down immediately 	No sign of life: immobile; cannot stand

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