

SOP for Scoring Mosquito Mortality

Purpose: Once mosquitoes have been transported to the laboratory, there is a need for scoring mortality and their physiological status during the holding period.

Definitions

KD Knock Down (see below for definitions of KD and mortality)

SOP Standard operating procedure

Scope

This SOP is to be followed when adult mosquitoes collected from experimental hut studies are scored for knock down, mortality and physiological status.

Responsibilities

1. It is the responsibility of the Study Director and individuals scoring the mosquitoes to follow this SOP AVECNET EH 006.01 and record results using the corresponding record sheet.

Instructions

1. Procedure

- 1.1. Transport the mosquitoes to the laboratory following the SOP for Transportation of Mosquitoes (SOP AVECNET EH 002.01).
- 1.2. Once in the laboratory make counts of mortality/physiological status at post exposure intervals according to the study protocol (e.g. typically 30 minutes and 24 hours post exposure).
- 1.3. Unless otherwise stated in the study protocol, hold the mosquitoes in the same cups/cages that they were transferred into at the experimental hut site (preferably less than 10 mosquitoes per cup/cage).
- 1.4. Mosquitoes will have been provided with sugar solution prior to transportation. Maintain a fresh **supply of sugar soaked cotton wool** during the holding period (SOP AVECNET EH 003.01 Sugar soaked cotton balls).
- 1.5. Maintain mosquitoes at 27 ± 2 °C and $70\pm 10\%$ relative humidity 80%.
- 1.6. **Scoring 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
- 1.7. A mosquito is classified as dead or knocked down if it is immobile or unable to stand or take off (see below).

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1.8. 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.

1.9. 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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- 1.1. **Scoring Physiological Status:** Physiological status is recorded for each mosquito (alive/dead) collected from the experimental huts.
- 1.2. Blood-fed mosquitoes are those that contain blood meal in their abdomen at the time of scoring.
- 1.3. Non-blood-fed mosquitoes are those that have no trace of blood in their abdomen at the time of scoring.
- 1.4. Physiological status can be determined by carefully aspirating each mosquito from the holding cup/cage and examining the abdomen for signs of a blood meal (using binocular microscope/magnifier).
- 1.5. Once the physiological status has been recorded, return the mosquito to the holding cup/cage.
- 1.6. **Results Interpretation:** The entomological impact of each treatment is expressed relative to the control in terms of the following:

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- 1.7. **Deterrence:** percentage reduction in the number of mosquitoes caught in treated hut relative to the number caught in the control hut;
- 1.8. **Repellency:** (Induced exophily) percentage of the mosquitoes collected from the veranda trap of treated hut relative to percentage caught in veranda trap of control hut;
- 1.9. **Inhibition of blood-feeding:** reduction in blood feeding rate relative to the control. This will be calculated using the following model:

$$100(BFu - Bft)/BFu.$$

Where Bu = is the proportion of blood-fed mosquitoes in the untreated control huts and

Bt is the proportion blood-fed mosquitoes in the huts with insecticide treatment.

- 1.10. **Induced mortality:** percentage of dead mosquitoes in treated hut at the time of collection and after a 24 hour holding period relative to control hut (control corrected mortality using Abbot's correction)

$$\% \text{ Corrected Mortality} = \frac{\% \text{ Killed in treated} - \% \text{ killed in control} \times 100}{100 - \% \text{ Killed in control}}$$

- 1.11. The personal protective effect of the treatments which is described by a reduction in the number of mosquitoes that succeeded in blood feeding in the intervention relative to the control hut:

$$\% \text{ Personal Protection} = 100(Bu - Bt)/Bu \text{ Where}$$

Bu = is the number of blood-fed mosquitoes in the untreated control huts and

Bt is the number blood-fed mosquitoes in the huts with insecticide treatment.

- 1.12. If mortality in control is $\geq 20\%$, the result is discarded.

- 1.13. A sample is efficient if it performs equal or better than the positive control.

- 1.14. **Statistical analysis:** Differences in proportional outcome variables (mortality, blood-feeding inhibition and repellency) between treatment and control are analysed using logistic regression after adjusting for the effect of sleeper and hut position.

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1.15. The number of mosquitoes entering the huts, the numbers succeeding in feeding, and the numbers killed are analysed using negative binomial regression with adjustments for sleeper and hut position. These analyses shall be done using STATA version 11 or equivalent validated statistical package.

1.16. A mass killing effect is desirable to reduce transmission. The overall insecticidal effect of a treatment relative to the number of mosquitoes that would ordinarily enter an untreated hut can be estimated by using the following formula and expressed as a percentage:

Overall insecticidal effect (%) = $100 (K_t - K_u) / (T_u - K_u)$ where

K_t is the number killed in the treated hut,

K_u is the number dying in the untreated control hut, and

T_u is the total number collected from the control hut.

References

- 1.1. Guidelines for laboratory and field testing of Long Lasting Insecticidal mosquito Nets (LLIN). WHO/CDS/WHOPES/GCDPP/2005.11
- 1.2. Guidelines for testing mosquitoes adulticides for indoor residual spraying and treatment of mosquito nets. WHO/CDS/NTD/WHOPES/GCDPP/2006.3

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