Comparability between insecticide resistance bioassays for mosquito vectors: time to review current methodology?

Henry Frempong Owusu[1,2], David Malone[3], Hilary Ranson[4] and Pie Müller[1,2]

[1]Swiss Tropical and Public Health Institute, Basel, Switzerland. [2]University of Basel, Basel, Switzerland. [3]Innovative Vector Control Consortium, Liverpool, UK. [4]Liverpool School of Tropical Medicine, Liverpool, UK

Swiss TPH
Swiss Tropical and Public Health Institute
Schweizerisches Tropen- und Public Health-Institut
Institut Tropical et de Santé Publique Suisse

Associated Institute of the University of Basel

Introduction

The Centers for Disease Control and Prevention (CDC) bottle assay and World Health Organization (WHO) susceptibility test are the most frequently used methods in insecticide resistance monitoring. However, the two bioassays differ in terms of insecticide delivery and how insecticide susceptibility is measured. In the WHO bioassay, insecticide susceptibility is assessed by the proportion of mosquitoes killed after a 1 h exposure to a diagnostic insecticide concentration and a 24 h holding period. In contrast, the endpoint of the CDC bottle assay is the time to knockdown (TKD). To evaluate how equivalent data from the two assays are, we compared the two methods side-by-side.

Materials and methods

CDC vs. WHO assay

A literature search was conducted to identify publications that performed both the CDC bottle and WHO tube assays on the same mosquito population. Then the results were compared for agreement between the two tests using the kappa statistic. In addition to the literature search both assays were performed side-by-side by testing laboratory colonies of *Ae. aegypti* (ROCK), *An. stephensi* (STI) and *An. gambiae* (KISUMU, VK7 and NDJA) against permethrin, λ-cyhalothrin, DDT, bendiocarb and malathion following their standard protocols[1,2]. We also held mosquitoes from the CDC assay for 24 hours. Using kappa statistic, we compared results based on the WHO definition of resistance. We compared the original results and also after holding mosquitoes in the CDC assay for 24 hours. At least 100 mosquitoes were used in all assays except for VK7 (49 against λ-cyhalothrin) and NDJA (18 against permethrin).

Mortality as a function of time-to-knockdown

Individual mosquitoes of *Ae. aegypti* (ROCK), *Cx. quinqefasciatus* (JHB), *An. stephensi* (STI), *An. gambiae* s.s. (KISUMU, VK7 and ZAN/U) and *An. arabiensis* (NDJA) laboratory strains were exposed to 0.5 l glass bottles treated with recommended concentrations of permethrin, λ-cyhalothrin, DDT, bendiocarb and malathion until knockdown was observed. The mosquito was immediately transferred into a holding cup. The time to knockdown was recorded, 10% sugar solution was provided and the mosquito was kept for 24h and scored as either dead or alive. We used a generalised linear mixed model with a logit link function to test this association. Insect strain and insecticide were modeled as fixed effects, while we introduced a random effect term for the day of testing.

Results and discussion

CDC vs. WHO assay

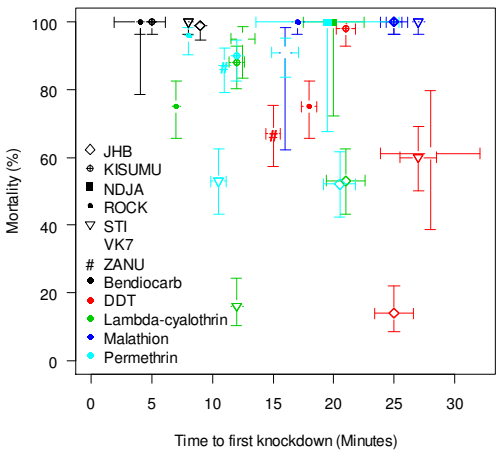
Nine publications reported results of the two assays on the same population[3-11].

Publication	Number of pairs compared	kappa	Agreement
Fonseca-González et al [3]	24	0.52	Moderate
Fonseca-González et al [4]	96	0.82	Almost perfect
Fonseca-González et al [5]	24	0.70	Substantial
Hargreaves et al [6]	21	-0.02	Poor
Matowo et al [7]	2	1.00	Perfect
Ocampo et al [8]	46	0.55	Moderate
Ochomo et al [9]	3	0.00	Poor
Perea et al [10]	2	1.00	Perfect
Aïzoun et al [11]	12	1.00	Perfect

In the present laboratory experiments, agreement was rather weak and only improved after also introducing a 24 hours holding period in the CDC assay

End point in CDC assay	Cut-off mortality rate (%)	kappa	Agreement
TKD at diagnostic dose and time	90	0.06	Slight agreement
	98	0.0086	poor
24 hours mortality	90	0.5148	Moderate
	98	0.5848	moderate

Mortality as a function of time-to-knockdown



Though statistically significant, the association between mortality and time-to-knockdown was weak (OR [95% CI] = 0.97 [0.95-0.99], *p*-value < 0.001). The mosquito colony and the type of insecticide had a much stronger effect on mortality.

References

1. WHO. 2013. Test Procedures for Insecticide Resistance Monitoring in Malaria Vector Mosquitoes.
2. Malaria Research and Reference Reagent Resource Center. 2010. Methods in Anopheles Research.
3. Fonseca-González et al. 2009. Parasitology Research 105 (5) (October): 1399–1409.
4. Fonseca-González et al. 2011. Pest Management Science 67 (4) (April): 430–437.
5. Fonseca-González et al. 2009. Memórias Do Instituto Oswaldo Cruz 104 (1) 18–26.
6. Hargreaveset al. 2000. Medical and Veterinary Entomology 14 (2) (June): 181–189.
7. Matowo et al. 2010. Malaria Journal 9: 193.
8. Ocampo et al. 2011. Acta Tropica 118 (1) (April): 37–44.
9. Ochomo et al. 2013. Medical and Veterinary Entomology 27, 156–164
10. Perea et al, 2009. Malar J 200, 8:208
11. Aïzoun et al, 2013. Parasit Vectors, 6:147

Conclusions and recommendations

Even though the two assays can detect insecticide resistance, they may not be used interchangeably. While the diagnostic dose in the WHO susceptibility test does not allow for detecting shifts at low or extreme resistance levels, time-to-knockdown measured in the CDC bottle assay is a poor predictor of 24 hours mortality. Therefore, we suggest dose-response assays would provide the most flexibility. New standardised bioassays are needed that produce consistent dose-response measurements with a minimal number of mosquitoes..

Acknowledgements
Paul Howell, Marlaria Research and Reference Resource Center; **Helen Williams**, Liverpool Insect Testing Establishment; **Mark Hoppé**, Syngenta Crop Protection AG; **Danica Jančáryová** and **Mohamad Sater**, Swiss Tropical and Public Health Institute

Swiss TPH, Socinstrasse 57, P.O. Box, 4002 Basel, Switzerland
T +41 61 284 81 11, F +41 61 284 81 01, www.swisstph.ch