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A biological test to quantify pyrethroid in impregnated nets

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Summary First instar larvae of *Aedes aegypti* (L.) (Diptera: Culicidae) are very susceptible to deltamethrin, which kills all larvae at a very low dose (0.1 μg/l). Thus the sensitivity of this insect to detect that pyrethroid is in parts per billion, as obtained with most common chromatographic methods of analysis. Here we describe a biological test (BT) to quantify deltamethrin in long-lasting insecticidal nets (LLINs) by exposing first instar larvae of *Ae. aegypti* to serial extracted insecticide solutions from net samples. The deltamethrin concentration in the net was calculated at the doses killing 50% of larva, from the LC_{50} of deltamethrin (6.5×10^{-5} mg/l) and the dilution factor (DF_{50}) of the extracted net solution. The pyrethroid quantification in LLINs after 0–25 washes with this BT was correlated with those obtained by direct chromatographic analysis ($r^2 = 0.84$). This BT did not require sophisticated equipment and could be extended to other molecules and materials. It appeared accurate, robust, cheap and well adapted to the national malaria programmes as the eggs of *Ae. aegypti* might be used for some months. This method was adapted to provide an easy to use kit test for the quality control of LLINs in the field.

keywords insecticide-treated net, long-lasting insecticidal nets, deltamethrin, biological analysis, *Aedes aegypti*

Introduction

Extensive experience has been gathered over the past 10 years on technical and managerial aspects of insecticide treated net interventions (ITNs) as well as their efficacy and, to a lower extent, their effectiveness (Miller *et al.* 1991; Hougard *et al.* 2002; Lengeler 2004). Emphasis is now on long-lasting insecticidal nets (LLINs) that are factory pre-treated and retain their biological efficacy for at least 20 standard washes under laboratory conditions and 3 years of use under field conditions (WHO 2005a). Demand for LLINs has considerably increased over the past 2 years, representing more than 90% of ITNs currently ordered by national programmes and institutional buyers (WHO 2005b). It has been estimated that 30–40 millions are required annually for the next 5 years to satisfy the demand (WHO 2004).

To achieve the expected protection, it is essential to ensure quality of ITNs that are purchased and used in the field, including in priority insecticide concentration. Concentrations lower than expected would result in financial losses as well as poor protection provided by the nets in the field (WHO 2003), whereas an excessive concentration of insecticide might have safety implications. All LLINs that have been recommended by WHO for use against malaria vectors have their own specifications developed for quality control. However, testing compliance to specifications (insecticide concentration and wash resistance) is a long and expensive process because it has to be done by analytical laboratories using sophisticated equipment. High-performance liquid chromatography and gas liquid chromatography are among the most accurate methods to identify and dose pyrethroid active ingredient and its metabolites (Mourot *et al.* 1979; Akhtar 1982; Pavan *et al.* 1999; Valverde *et al.* 2002). Moreover, only few countries in Africa have such laboratory available at national or regional levels. Checking insecticide concentration on field-used ITNs might also be important for programme managers to ensure that at any time, insecticide concentration is at least or above the minimum threshold level. Therefore, there is a need for a simple and robust method that could be used in the field or at least at country level to detect pyrethroid on nets and dose it with maximum accuracy.

Some methods have already been proposed based on enzyme-linked immunosorbent assay (Lee *et al.* 2002) or iodometric colouration (Enayati *et al.* 2001). However, these methods also require minimum laboratory equipment and well-trained technicians to use it. In addition, there might be interferences with dye and dirt when testing coloured nets already in use. The iodometric approach has

great potential if it can be simplified and be used with coloured or dirty nets (Carnevale 2003, unpublished report to WHO). Other simple methods have been proposed such as a modified Beilstein test based on the green colour that halogens from some pyrethroids have when burning (Muller *et al.* 1994; Verlé *et al.* 1998). However, these methods do not work for all pyrethroids; these tests can detect the presence of pyrethroids but cannot quantify them; also they are unsuitable for dirty or coloured nets (Drakeley *et al.* 1999).

Here we describe a biological assay based on mosquito larvae used to quantify deltamethrin concentration on LLINs. This test is based on the precise and stable correlation that exists between insecticide concentration, e.g. extracted from LLINs, and mortality of mosquito larvae of a reference susceptible strain. Biological titration when carefully carried out is known to be a reliable and robust way of dosing insecticides. For easy implementation, *Aedes aegypti* (Diptera: Culicidae) has been preferred to anopheline larvae. Insecticide has been extracted from LLINs unwashed then washed respectively 5, 10, 15, 20 and 25 times and results were compared with those obtained by chromatographic analysis. The objective of this study was to develop a simple test kit based on biological titration that can be used at country level by national malaria control programmes to check insecticide concentration on nets.

Materials and methods

Biological material

First to early second instar larvae of *Ae. aegypti* of a reference susceptible strain (Bora Bora) were used. This strain originating from French Polynesia has been maintained in laboratory colony for more than 25 years. Its susceptibility is regularly checked using both bioassays and molecular diagnostic for kdr based pyrethroid resistance. Eggs on filter paper are hatched in distilled water with yeast added, then transferred 24 h later to rearing pans with grounded pet food provided (room temperature 28–31 °C, photoperiod 12–12 h).

Treated netting

Three LLINs (PermaNet 2.0 nets provided by Vestergaard Frandsen) have been used in this study. They were made of blue 75 denier multifilament polyester complying with WHO specifications for netting material with a minimum deltamethrin concentration of 55 mg/m^2. A non-treated blue PermaNet (yarn coated with the same resin but without insecticide) has been used as a control.

Insecticide

Technical grade deltamethrin (kindly provided by Bayer Crop Science, France).

Biological analysis

A preliminary calibration test was conducted with a bioassay using *Ae. aegypti* larvae and a solution of technical grade deltamethrin (reference solution). Larvae were exposed to serial dilutions obtained from ethanolic solutions. Each egg-batch used during this study was assayed using solutions of technical grade deltamethrin. By simple comparison of LC50s obtained from the reference solution it has been possible to estimate the amount of insecticide extracted from nets. For each LLIN tested (unwashed or washed), about 100 circular samples (1 cm in diameter) were collected using a punch. Nets were folded as many times to punch the requested number of samples evenly distributed. For chemical analysis, 25 × 25 cm sample were cut from the nets following WHO procedure (WHO 2006). We used three unwashed and one washed net 5, 10, 15, 20 and 25 times. All collected samples were stored at 4 °C in sheets of aluminium before being used. One LLIN was washed once a day in cold water (about 25 °C) with Marseille soap (Le Chat®, Henkel, Boulogne Billancourt, France) following WHO specified procedure (WHO 2006). For bioassays, insecticide was extracted by soaking for 30 min, 10 pieces of netting (1 cm diameter) in a glass vial containing 10 ml of 90% ethanol.

Larvae were tested 18 h after immersion of eggs in distilled water at 27 °C. Batches of 20 larvae were tested in 9.9 ml of distilled water in 50 ml plastic cups to which 100 μl of insecticide ethanolic solution was added (final concentration 1%). Control cups received 100 μl of either extract from non-treated PermaNet (bioassay) or 90% ethanol (calibration test). Larvae were supplied with baker's yeast during testing. Three batches of 20 larvae were used for each concentration and 7–8 serial dilutions were tested for each sample. Water temperature was maintained at 27 °C during the 24 h exposure period after what mortality was recorded. Deltamethrin concentration on LLINs was calculated as follows:

$$D = LC_{50}/DF_{50} \times V/S$$

where D (mg active ingredient (a.i.)/m^2) is the concentration of the LLIN sample tested, LC_{50} in (mg a.i./l) is the lethal concentration obtained with technical grade deltamethrin (calibration), DF_{50} is the factor by which the LLIN sample extraction solution has to be diluted to get 50% mortality, V (l) is the extraction volume of the LLIN sample and S (m^2) the sample size.

Six samples collected from an unwashed PermaNet then washed 0, 5, 10, 15, 20 and 25 times, respectively were made using the analytical gaz-chromatography method. These samples were sent to Walloon Agricultural Research Centre, WHO Collaborating Centre, Gembloux, Belgium for chemical analysis. Deltamethrin has been extracted from the composite samples by heating under reflux for 60 min using 40 ml xylene. Extracts were left to reach room temperature then entirely transferred into a 100 ml volumetric flask. The flask was then filled with xylene then the solution was further diluted in xylene (25 times). The final extract was analysed by capillary gas chromatography with Ni electron capture detection using the external standard calibration.

Statistical analysis

Mortality rates were estimated from homogeneous batches of larvae. The deltamethrin concentration (LC_{50}) and the dilution factor of the extracts giving 50% mortality with their 95% confidence limits have been calculated using GOSA® software (www.bio-log.biz). The fit was performed on cumulated Gauss equations. Differences between respective LC_{50}s and DF_{50}s were considered significant when 95% confidence limits did not overlap.

Results

The baseline susceptibility for deltamethrin was determined using first instar larvae with a LC_{50} of 6.4×10^{-5} mg/l ($5.3–7.5 \times 10^{-5}$) and a slope of 3.8 (±1.4). Respective slopes and DF_{50}s for three different unwashed nets were not significantly different showing relative homogeneity of the tested LLINs (Table 1). The amount of deltamethrin extracted from nets using ethanol was on average three times lower than that one extracted with xylene and dosed by chromatographic analysis. One can consider that almost 100% of deltamethrin was recovered with the former method. Indeed, a second extraction operated on the same samples showed residue levels lower than the detection threshold (0.1 mg/m^2). Chromatographic dosage of unwashed LLINs revealed a concentration about twice the target concentration (108 *vs.* 55 mg/m^2).

There was no significant difference in DF_{50} between an unwashed and a five times washed net. Then, DF_{50} significantly increased after 10, 15 and 20 washes, respectively. However, there was no difference in bioassay results between 20 and 25 washes with an estimated concentration of about 8 mg/m^2. The trend in deltamethrin loss between wash 0 and wash 25 was exponential. The same pattern was observed in the chromatography results. The two results were correlated (Pearson $r^2 = 0.84$, $P = 0.036$).

Table 1 Deltamethrin content in three unwashed LLINs (A, B and C) and in LLIN (C) after 5–25 washes calculated from a biological test (BT) on first instar larvae of *Ae. aegypti* and from a chemical test by chromatographic analysis

LLIN identification	Number of washes	n*	Slope† (95% CI)	DF_{50}‡	95% CI	Deltamethrin content (mg/m^2)			
						BT§	95% CI	Chemical test¶	Correction coefficient**
A	0	615	4.22 ± 0.78	2.50×10^{-5}	2.39×10^{-5}–2.62×10^{-5}	32.6a	22.8–42.4	–	–
B	0	645	3.97 ± 0.68	2.83×10^{-5}	2.61×10^{-5}–3.05×10^{-5}	28.8a	23.6–34.0	–	–
C	0	877	3.93 ± 1.83	2.28×10^{-5}	1.81×10^{-5}–2.75×10^{-5}	35.8a	33.4–38.2	107.8	3.0
C	5	893	3.19 ± 0.72	2.00×10^{-5}	1.77×10^{-5}–2.23×10^{-5}	40.8a	35.9–45.7	65.8	1.6
C	10	581	5.01 ± 0.86	4.75×10^{-5}	4.45×10^{-5}–5.06×10^{-5}	17.2b	13.5–20.9	53.1	3.1
C	15	583	6.60 ± 1.06	6.93×10^{-5}	6.64×10^{-5}–7.22×10^{-5}	11.8b	15.6–15.6	39.4	3.3
C	20	581	4.20 ± 0.81	9.34×10^{-5}	8.59×10^{-5}–1.01×10^{-4}	8.7c	7.2–10.2	27.8	3.2
C	25	610	4.24 ± 0.66	9.57×10^{-5}	8.95×10^{-5}–1.02×10^{-4}	8.5c	6.7–10.3	24.1	2.8

*Number of larvae tested.
†Slope of the regression line dose/mortality of the extracted sample net.
‡DF_{50} is the dilution factor of the extracted sample net solution at the lethal concentration for 50% of larvae.
§ Calculated from LC_{50} deltamethrin and DF_{50} LLIN.
¶Mean of two chromatographic injections.
**Calculated by comparing values of biological analysis to those of chemical analysis.
Values with different letters differ significantly ($P < 0.05$).
CI, confidence interval; DF_{50}, dilution factor; LC_{50}, deltamethrin concentration; LLIN, long-lasting insecticidal nets.

We observed a linear relation between results of the two methods with an average threefold ratio between them (Table 1).

Discussion

As the first instar *Ae. aegypti* larvae are killed at very low deltamethrin concentration (0.1 μg/l), the biological titration was a very sensitive method. It can detect concentration from 0.1 to a few parts per billion as obtained with common chromatographic test methods (Pavan *et al.* 1999; Valverde *et al.* 2002). Thanks to this sensitivity, combined with the ability of *Ae. aegypti* eggs to survive for months in dry conditions, the proposed biological titration is relatively simple to carry out and reliable. With adequate calibration, it could be adapted for other pyrethroids and LLINs based on similar technologies (coating of fibres). The results obtained with three different nets showed both homogeneity of its treatment as well as the reliability of the biological titration. Results were closely correlated with chromatographic analysis results. However, considering the differences observed in results after five washes, the chromatographic method is more reliable. The absence of difference after 20 and 25 washes using biological titration results from a very small decrease in concentration (flat curve) usually observed after 20 washes.

The relatively low recovery rate obtained with biological titration is likely due to the lower extraction capacity of ethanol compared with xylene (threefold difference). The resin and insecticide mixture used to coat to the yarn is most likely less soluble in ethanol than in xylene. Ethanol is by far the best solvent but it is not toxic to young mosquito larvae and humans. It is also widely available in developing countries and cheap.

The initial concentration on the three LLINs tested was about twice the target concentration claimed by the manufacturer. Variable concentrations (27–142 mg/m^2) have already been obtained with this product in other studies (Graham *et al.* 2005). These results confirm the interest for national programmes purchasing LLINs to be able to carry out a simple and rapid check without necessarily going through the full quality control procedure that is long and expensive. An important factor that should be taken into consideration with this method is that it can be used for rapid wash resistance testing. Suitability of the extraction process should be tested with LLINs where insecticide is incorporated within the yarn and therefore much more difficult to extract. The method might be suitable but the calibration may be more complex to establish.

If unacceptably low initial concentrations are detected (e.g. below 50% for PermaNet that corresponds to the lower range of concentration minus the acceptable variation margin) from a newly imported consignment, it might be decided to carry out a full quality control test. The same approach is also valid for nets already in use to ensure that their average concentration is higher than a pre-determined threshold that corresponds to the minimum active concentration, preferably using a safety margin. Until duration of biological efficacy of a given LLIN product has been established under local conditions, programme managers might wish to monitor efficacy or residual insecticide concentration to determine more precisely when time has come to renew the nets.

The expected application of the proposed approach is not to have full bioassays carried out with mosquito larvae but the use of a simple kit providing a qualitative answer whether the concentration on the net is above a given threshold. We have developed such a kit for testing new PermaNet 2.0. It is based on the sampling and extraction procedures above described, 1 ml of a 1/100 dilution of extract solution is added to 99 ml of water poured in a plastic cup containing eggs of *Ae. aegypti* with some larval food. If the concentration of deltamethrin on a new PermaNet is over 50% of the minimum level stated in the specifications (55 mg/m^2), no mosquito larvae should survive in the cup 24 h after introduction of the solution. The kit will include a negative control with water and a positive control with the LD_{100} of deltamethrin for this strain. If some larvae do survive in the test bag, doubts would be raised about insecticide concentration and a quality control should be initiated based either on chromatographic methods or other suitable methods (Muller *et al.* 1994; Wengatz *et al.* 1998; Enayati *et al.* 2001). The dilution factor can be adjusted to various LLIN products, even if treated with another insecticide than deltamethrin, to test either newly purchased nets or nets that were already used in the field. In this case, the dilution factor should be based on the lowest concentration that, for this specific LLIN, provides the expected biological efficacy against adult malaria vectors.

Further development of this kit will imply calibration with different LLIN products and insecticides (WHO 2006). LLINs based on incorporation of insecticide into polymer before yarn extrusion will deserve special attention because ethanol will extract mostly insecticide that is bioavailable (at the surface of the yarn) that represents only a small fraction of the total insecticide content. Additionally, non- destructive methods will have to be tested that can be used in the field without damaging the sampled nets, as proposed by Enayati *et al.* (2001). For biosafety reason, a local susceptible colony of *Ae. aegypti* will be used, avoiding the introduction of new strains. Some National Programmes could eventually have access to *Ae. aegypti* eggs which will further simplify the procedure.

There is a need for a simple test such as the one as proposed, based on biological titration that can be used at country level by National Malaria Control Programmes. Such test will have to work with all kind of LLINs, white as well as coloured nets, new nets as well as old and dirty nets. The most practical approach will likely be to provide a yes/no answer to programme managers rather than an attempt to precisely quantify insecticide concentration on nets. What is important for them to know is: (i) if the total insecticide concentration on new nets is what it should be; and (ii) if the nets that are used in the field still have the minimum insecticide content required to provide the expected biological efficacy. The extraction process and the kit test will remain the same but the dilution factor will differ depending on whether new or in-use nets are to be tested. Ideally, the extraction process should remain the same for all nets and calibration will be established for all products, taking into account the proportion of insecticide that is extracted.

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References

Akhtar MH (1982) Gas chromatographic determination of deltamethrin in biological samples. *Journal of Chromatography* **246**, 81–87.

Carnevale P (2003) *Detection of Pyrethroids on Mosquito Nets with the Newly Developed Iodometric Method. Travel Report to Liverpool School of Tropical Medicine and Hygiene*. Document IRD-LIN Montpellier, France, pp. 15.

Drakeley CJ, Armstrong Schellenberg J, Abdulla S & Lengeler C (1999) Short report: lack of specificity of Beilstein test in detecting pyrethroid insecticide on coloured mosquito nets. *Tropical Medicine and International Health* **4**, 639–640.

Enayati AA, Vontas JG, Small GJ, McCarroll L & Hemingway J (2001) Quantification of pyrethroid insecticides from treated bednets using a mosquito recombinant glutathione-S-transferase. *Medical and Veterinary Entomology* **15**, 58–63.

Graham K, Kayedi MH, Maxwell C *et al.* (2005) Multi-country field trials comparing wash-resistance of PermaNet^{TM} and conventional insecticide-treated nets against anopheline and culicine mosquitoes. *Medical and Veterinary Entomology* **19**, 72–83.

Hougard JM, Fontenille D, Chandre F, Darriet F, Carnevale P & Guillet P (2002) Combating malaria vectors in Africa: current directions of research. *Trends in Parasitology* **18**, 283–286.

Lee HJ, Shan G, Watanabe T, Stoutamire DW, Gee SJ & Hammock BD (2002) Enzyme-linked immunosorbent assay for the pyrethroid deltamethrin. *Journal of Agricultural and Food Chemistry* **50**, 5526–5532.

Lengeler C (2004) *Insecticide-treated Bed Nets and Curtains for Preventing Malaria (Cochrane Review). The Cochrane Library, Issue 2*. John Wiley & sons Ltd, UK.

Miller JE, Lindsay SW & Armstrong JRM (1991) Experimental hut trials of bednets impregnated with synthetic pyrethroid or organophosphate insecticide for mosquito control in the Gambia. *Medical and Veterinary Entomology* **5**, 465–476.

Mourot D, Delepine B, Boisseau J & Gayot G (1979) High-performance liquid chromatography of deltamethrin. *Journal of Chromatography* **173**, 412–414.

Muller O, Martha Q, Kabbir C, Moses A & Greenwood B (1994) Detecting permethrin on treated bednets. *Lancet* **344**, 1699–1700.

Pavan FA, Dallago RM, Zanella R & Martins AF (1999) Determination of deltamethrin in cattle dipping baths by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry* **47**, 174–176.

Valverde A, Aguilera A, Rodriguez M & Boulaid M (2002) What are we determining using gas chromatographic multiresidue methods: tralomethrin or deltamethrin? *Journal of Chromatography* **943**, 101–111.

Verlé P, Ruyen NT, Huong NT *et al.* (1998) A simple field test for detecting pyrethroids on impregnated nets. *Tropical Medicine and International Health* **3**, 833–836.

Wengatz I, Stoutamire DW, Gee SJ & Hammock BD (1998) Development of an enzyme-linked immunosorbent assay for the detection of the pyrethroid insecticide fenpropathrin. *Journal of Agricultural food Chemistry* **46**, 2211–2221.

WHO (2003) *Report of the Seventh WHOPES Working Group Meeting. WHO/HQ, Geneva, 2–4 December 2003. Review of: Vectobac WG, Permanet, Gokilaht-S 5EC. (WHO/CDS/WHOPES/2004.8)* WHO, Geneva.

WHO (2004) News: drive to produce more long-lasting insecticidal mosquito nets for malaria. *Bulletin of the World Health Organization* **82**, 884–886.

WHO (2005a) *Guidelines for Laboratory and Field Testing of Long-lasting Insecticidal Mosquito Nets. (WHO/CDS/WHOPES/GCDPP/2005.11)* WHO, Geneva.

WHO (2005b) *Malaria Control Today. Current WHO Recommendations. Working Document, March 2005*. Roll Back Malaria Department, World Health Organization, Geneva, pp. 75.

WHO (2006) *Guidelines on the Use of Insecticide-treated Mosquito Nets for the Prevention and Control of Malaria in Africa. (WHO/CDS/WHOPES/ CTD/MAL/AFRO/97.4)* WHO, Geneva.

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Un test biologique pour la quantification des pyréthroïdes dans les moustiquaires imprégnées

Les larves de 1er stade de l'*Aedes aegypti* (L.) (Diptera: Culicidae) sont très sensibles à la deltaméthrine, qui tue toutes les larves à une très faible dose (0.1 μg/l). Dès lors la sensibilité de cet insecte à détecter ce pyréthroïde est en parties par milliard (ppb), comme les valeurs obtenues avec la plupart des méthodes d'analyse chromatographiques. Nous décrivons ici un test biologique pour quantifier la deltaméthrine dans les moustiquaires imprégnées d'insecticide en exposant les larves de 1er stade d'*Ae. aegypti* à des solutions d'insecticides extraites d'échantillons de moustiquaires. La concentration de deltaméthrine dans la moustiquaire a été calculée à partir de la dose léthale pour 50% des larves ($LC_{50} = 6.5 \times 10^{-5}$ mg/l) et du facteur de dilution (DF_{50}) de la solution extraite de moustiquaire. La quantification de pyréthroïde dans les moustiquaires imprégnées d'insecticide après 0 à 25 lavages en utilisant cet test biologique est bien corrélée à celle obtenue par analyse chromatographique directe ($r^2 = 0.84$). Ce test biologique n'a pas exigé d'équipement sophistiqué et peut être appliqué à d'autres molécules et matériaux. Le test s'est avéré précis, robuste, bon marché et bien adapté aux programmes nationaux de malaria comme les oeufs d'*Ae. aegypti* peuvent être utilisés pendant quelques mois. Cette méthode a été adaptée pour proposer un test en kit facile à utiliser pour le contrôle qualité des moustiquaires imprégnées sur le terrain.

mots clés Moustiquaires imprégnées d'insecticides, delthamétrine, analyse biologique, *Aedes aegypti*

Prueba biológica para cuantificar el piretroide en mosquiteras impregnadas

Las larvas de primer instar de *Aedes aegypti* (L.) (Diptera: Culicidae) son muy susceptibles a la deltametrina, la cual mata todas las larvas con una dosis muy baja (0.1 μg/l). Por lo tanto, la sensibilidad de este insecto para detectar piretroides está a un nivel de partes por billón (ppb), el mismo que se obtiene con los métodos de análisis cromatográficos más comunes. Describimos una prueba biológica para cuantificar deltametrina en mosquiteras impregnadas con insecticida de larga duración (LLINs), exponiendo las larvas de primer instar de *Ae. Aegypti* a una serie de soluciones de insecticida extraídas de muestras de redes mosquiteras. La concentraciones de deltametrina en las redes se calculó en la dosis que mataba un 50% de las larvas, a partir del LC_{50} de la deltametrina (6.5×10^{-5} mg/l) y del factor de dilución (DF_{50}) de la solución extraída de la red. La cuantificación de piretroides en LLINs después de 0 a 25 lavados estaba correlacionada con aquella obtenida por análisis cromatográfico directo ($r^2 = 0.84$). Esta prueba biológica no requirió de equipo sofisticado y podría extenderse a otras moléculas y materiales. Resultó precisa, robusta, barata y bien adaptada a los programas nacionales de malaria, puesto que los huevos de *Ae. Aegypti* pueden ser utilizados durante varios meses. Este método fue adaptado para proponer un kit 'fácil de usar' para el control de calidad de LLINs en el campo.

palabras clave Mosquitera impregnada, mosquitera impregnada con insecticida de larga duración, deltametrina, análisis biológico, *Aedes aegypti*