

A simple field test for detecting pyrethroids on impregnated nets

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Summary

We tested a modified Beilstein method for detecting pyrethroids on bednets under laboratory conditions using an emulsifiable concentrate of permethrin (50 EC) for viability as a simple standardized field test and to judge its reliability for detecting different insecticide doses. At the recommended doses of permethrin (0.5 g/m²), sensitivity was near 100%, even when small pieces of fabric were tested and time of extraction was limited. In unwashed nets sensitivity stayed high (80–95%) down to 0.1 g active ingredient/m². In untreated nets false positives were rare (0–2%). The test could become a valuable tool in vector control programmes: it is cheap, easy to learn and to perform. The Lot Quality Assurance Sampling method, using an upper and lower performance threshold, could be applied for monitoring the impregnation campaigns.

keywords insecticide-treated bednets, monitoring, permethrin

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Introduction

During the last decade, the use of insecticide-treated mosquito nets has emerged as an essential tool for malaria control (Rozendaal 1989; Sexton 1994). For the first time, a method for vector control is available which is so simple and safe that it can be organized and used locally by nonspecialists. Increasingly, the WHO, health ministries and donor agencies are considering impregnation of nets with insecticides as an integral component of the national malaria control strategies. However, measuring the deposits of insecticide on an impregnated net, essential for quality control of routine operations, appears not to be done (Carnevale & Coosemans 1995; Lines 1996). Currently such information can only be provided by the costly and sophisticated gas chromatography technique (Hossain *et al.* 1989; Lindsay *et al.* 1991), which is not readily available and cannot be used in the field. Bio-assays, in which susceptible mosquitoes are exposed to impregnated material, reveal residual activity of the insecticide deposit (Lindsay *et al.* 1991; Miller 1994). Indirectly they can also be used to estimate net treatment quality. But they are technically demanding and often not feasible. In routine practice, monitoring of bednet impregnation is limited to reports of health staff and questioning net users whether and when the nets were impregnated. Therefore the development of a field

test to measure the dose of insecticides on bednets is a research priority (Lines 1996).

Recently a modified Beilstein test (Falbe & Regitz 1989) has been used in The Gambia to detect the presence or absence of insecticide (Müller *et al.* 1994). Preliminary results suggested that this simple and inexpensive qualitative test could be a useful tool for monitoring the impregnation status of bednets. The technique was described for use in laboratories only, but a simple field test would be of special interest. In our study a series of variables involved in the test were examined in order to develop a standardized, user-friendly field test. The test was then performed for different doses of permethrin and on different netting material. The washing status of the nets was also evaluated.

Materials and methods

The study was conducted in the Department of Entomology at the Institute for Malariology, Entomology and Parasitology in Hanoi, Vietnam. Light cotton and polyester netting material were used, both obtained from the factory in Hanoi which produces mosquito nets used by the National Malaria Control Programme. The netting material was impregnated with permethrin 50 EC (Imperator®, Zeneca, ref F6283).

The test used in the Gambia was based on the Beilstein method (Falbe & Regitz 1989). A piece of net was pushed

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into a universal glass bottle containing 20 ml of acetone. The container was agitated to extract permethrin from the net, which then was removed. After evaporation of the acetone (2–3 days), a piece of copper wire was scraped around the inside of the bottle and held in the outer part of the flame of a gas Bunsen burner (Müller *et al.* 1994). A green colouration indicated the presence of halogens of the pyrethroid insecticide.

In our study, the colouration was tested in the flame of an alcohol burner because gas Bunsen burners are rare in Vietnam. The tests were performed by a person unaware of the impregnation status of netting material. Each sample was processed three times. A result was considered positive when a green flame was observed at least once during the repeated observations of the same sample. All tests were performed within two weeks after impregnation.

During preliminary tests, the technique was first tried out using different types of glass containers (beakers, Petri dishes and test tubes) and several kinds of copper wires (e.g. ordinary electrical cable, thick electrical wires, full copper wires) purchased on the local market. After selection of a glass container and copper wire, the size of material and extraction time were studied at an impregnation dose of 0.5 g active ingredient (a.i.) per m². Ten pieces of 1 cm², 25 cm², 100 cm² and 750 cm² of both cotton and polyester netting material were immersed for 5 s, 30 s and 1 min in 20 ml of acetone. Two days later, after the acetone had evaporated, the residue was scraped from the glass and held into the flame.

In the next phase, cotton and polyester netting material were treated with permethrin diluted in water to give final doses of 0.01, 0.05, 0.1, 0.2 and 0.5 g of a.i./m². After treatment, one third of the netting material was hand-washed with cold water and one third was washed with cold soapy water (Lyx® powder). For each dose, each washing status and for the controls, 30 pieces of 100 cm² netting material (both cotton and polyester) were put into each of 2 ml, 5 ml and 10 ml of acetone. For all these tests an extraction time in acetone of 15–30 s was used. Half of all tests were performed with acetone manufactured in China (US\$ 4/l) and half with acetone manufactured in Vietnam (US\$ 2.5/l). Additional tests were performed on non-impregnated coloured nets verifying if dye may induce false positive results. Only blue polyester bed nets were available on the market for this purpose.

Results

Selection of basic material to perform the Beilstein test

Petri dishes (6 cm diameter) proved to be more suitable than test tubes or beakers because evaporation was rapid and scraping easy. No positive reaction was obtained from any of

30 glass containers washed with soap, after being used for testing netting material impregnated at a dose of 0.5 g of a.i./m².

The best green colourations in the flame were obtained with an ordinary electrical cable, stripped of its insulation, with the thin copper wires spread out in the form of a little brush. The same piece of wire could be used over and over again. To avoid green colouration by the previous sample, the wire had to be held in the flame prior to the scraping until any green colouration had disappeared. When a piece of polyvinyl chloride (PVC) mounted on a copper wire was put in the flame, it also produced a green colouration. Therefore the PVC of the electrical wires has to be cut away. Single-strand copper wires gave less satisfactory results.

Selection of fabric size and extraction time

Three hundred tests were performed on netting material impregnated with permethrin at a dose of 0.5 g a.i./m² using different sizes of material and different extraction times (Table 1). Based on these results (see below), a standard size of 100 cm² (100% sensitivity at a dose of 0.5 g permethrin/m²) and an extraction time in acetone of 15–30 s were chosen.

Acetone

Time of evaporation was very variable, depending on a combination of factors. Two ml of acetone evaporated in 10 min to two hours, while evaporation of 20 ml of acetone required more than 24 h on a cool day. Netting material was never damaged by the acetone in any of about 4000 tests. No significant differences in results could be detected with different quantities of acetone (2, 5 and 10 ml) and with acetone of different origin. Therefore these results were pooled for further analysis.

Table 1 Numbers of netting samples found positive with a modified Beilstein test after impregnation with permethrin 50 EC at a dose of 0.5 g a.i./m², using different materials and sizes of netting material and for 3 different extraction times (*n* = 10 for each situation, *N* = 300)

| Bed net material | Light cotton | | | Polyester | | |
|---------------------|--------------|------|-------|-----------|------|-------|
| | 5 s | 30 s | 1 min | 5 s | 30 s | 1 min |
| Control* | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 cm ² | 3 | 5 | 4 | 5 | 4 | 4 |
| 25 cm ² | 9 | 10 | 10 | 10 | 10 | 10 |
| 100 cm ² | 10 | 10 | 10 | 10 | 10 | 10 |
| 750 cm ² | 10 | 10 | 10 | 10 | 10 | 10 |

*100 cm² of netting material that was never impregnated.

Table 2 Sensitivity of a modified Beilstein test after impregnation with permethrin 50 EC at a dose of 0.5 ga.i./m², using different materials and sizes of netting material and for 3 different extraction times ($n = 10$ for each situation, $N = 300$)

| Treatment | Light cotton | | | Polyester | | |
|----------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| | U | W | WS | U | W | WS |
| Untreated | 1% (0–3.3) | 0% | 1% (0–3.3) | 2% (0–5.3) | 1% (0–3.3) | 1% (0–3.3) |
| 0.01g/m ² | 13% (6.3–20.4) | 3% (0–7) | 1% (0–3.3) | 19% (10.8–27) | 11% (4.6–17.6) | 10% (3.8–16.2) |
| 0.05g/m ² | 61% (51–71.2) | 34% (24.6–44.3) | 27% (17.5–35.8) | 77% (67.9–85.4) | 57% (46.4–66.9) | 39% (28.8–49) |
| 0.1g/m ² | 87% (79.6–93.7) | 79% (70.5–87.3) | 41% (30.9–51.3) | 88% (81–94.5) | 73% (64.2–82.5) | 58% (47.6–68) |
| 0.2g/m ² | 94% (89.7–99.2) | 94% (89.7–99.2) | 67% (56.9–76.4) | 98% (94.7–100) | 94% (89.7–99.2) | 49% (38.2–59.2) |
| 0.5g/m ² | 100% | 97% (93–100) | 93% (88.2–98.5) | 100% | 88% (81–94.5) | 78% (69.2–86.4) |

Percentage of positive bed net samples (95% Confidence Intervals) unwashed (U), washed with water (W) and washed with soap (WS) using 2 ml to 10ml acetone (combined data).

Sensitivity of the test

The sensitivity of the test for detecting different amounts of permethrin is presented in Table 2 (3240 tests). In general slightly more positive reactions were obtained with unwashed polyester netting material than with unwashed cotton material. These differences were not statistically significant and the tendency was not so clear among washed samples.

For the same netting material impregnated at the same dose, more positive test results were obtained from unwashed samples than from samples washed with water, which in turn were more often positive than the samples washed with soap (χ^2 for trend >4 , $P < 0.05$ for all doses except 0.01 g a.i./m² in polyester nets). Only two false positive test results were found in 300 samples of non-impregnated light cotton, 4 in 300 non-impregnated white polyester samples and none in 30 samples of non-impregnated blue polyester material.

Discussion

The results did not vary with the quantity of acetone used. If only small amounts of acetone are required, this is an asset for the use of the test in the field. Depending on the origin and the amount of acetone used, the cost per test (calculating that on average one glass container can be used for 50 tests) ranged from US\$ 0.01 to US\$ 0.08, mainly determined by the amount and the origin of the acetone. However, in the field the test should be carried out without cutting a sample out of the bednet. During preliminary testing in the field, 2 ml of acetone were sometimes completely absorbed by the net and could not

be recovered in the Petri dish. The best choice under field conditions appeared to be 5 ml of acetone per test. With similar results obtained with acetone of different origin, the cheaper one can be used and the cost in Vietnam can be estimated at US\$ 0.03 per test in the future. The proposed procedure for executing this modified Beilstein test in the field is summarized in Table 3.

Our study confirmed high sensitivity (close to 100%) of the test at recommended doses of impregnation as observed by Müller *et al.* (1994). Even when small amounts of netting material were tested and time of extraction was limited, sensitivity was high (Table 1). In unwashed nets (Table 2) sensitivity stayed high (87%) down to 0.1 g of a.i./m².

In countries such as Vietnam, where impregnation of bednets is applied on a large scale (MOH 1997), a simple and cheap field test to evaluate the correct implementation of the instructions of impregnation would be very useful. Our data indicate that the Beilstein test has a potential to respond to this need in the days after the impregnation. However, in routine

Table 3 Optimal measurement method for executing a modified Beilstein test in the field

| | |
|--|---|
| Required material | – alcohol burner – Petri-dishes (6 cm diameter) – electrical cable with thin copper wires |
| Standard size of tested netting material | 100 cm ² |
| Extraction solvent | 5 ml acetone/per sample |
| Extraction time | ± 30 seconds |

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operations, wide variations of insecticide deposits can be expected within nets and between nets (Alonso *et al.* 1993; Lines 1996). With different netting materials and different sizes of bednets available, it is difficult to define the exact amount of water that will be absorbed. Therefore it seems justified to consider results of the Beilstein test of the lower dose than the one aimed for as the lowest acceptable limit. One could test the net at different places but conclusions will always be limited. The Beilstein technique is dichotome and does not show whether the net was washed or not, or the dose applied to an individual net.

The aim of monitoring impregnation campaigns with the Beilstein test is to determine if the nets in the communities have been treated with sufficient amounts of insecticide. Lot Quality Assurance Sampling (Lanata & Black 1991) can be used for selecting nets to be tested in the villages covered by the programme. According to the directives of the National Malaria Control Programme in Vietnam, nets should be impregnated at a dose of 0.2 g a.i./m² (MOH 1997). On the basis of the data for unwashed nets (Table 2), 6% and 20% of negative tests would then form suitable upper and lower programme performance thresholds to determine whether treatment of the nets reached acceptable levels or not. Sample sizes of 38 nets per village suffice to classify the effectiveness of impregnation in a village. In villages where up to 3 nets test negative in the Beilstein test, net impregnation can be considered acceptable with an α error of 5% (probability of falsely classifying programme performance as acceptable). Net impregnation with more than 3 negative results of 38 nets tested in one village counts as unacceptable. The probability of falsely classifying a village as unacceptable (β error) is 20%. This type of error has less serious implications from the point of view of monitoring programme performance.

In the long term, resistance development of *Anopheles* mosquitoes against pyrethroids should be regarded as unavoidable, particularly when insecticides are used on a large scale. For resistance monitoring, WHO kits can be used, exposing mosquitoes to known insecticide doses for specified periods to assess the extent to which a population of a given mosquito species is susceptible to a particular insecticide (Magesa *et al.* 1994). So far this technique seems not to have been incorporated into routine activities. A first indicator of resistance might be people reporting loss of insecticide effect against mosquitoes or other nuisance insects. However, this could also indicate that the net was not properly treated. The latter possibility could be excluded by the Beilstein method.

The technique was mastered quickly, it is cheap, and results might immediately lead to action. This study, however, only provided data concerning the test during the days after impregnation. In the field false positives might be more frequent because of contamination by halogens of other origin (e.g. other insecticides used in the house, plastics). The test

should now be studied under field conditions, repeated over time after impregnation and re-impregnation, and compared with results of gas chromatography and bio-assays.

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