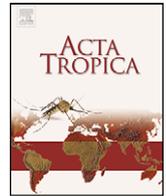




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Short communication

## Evaluation of the wash resistance of three types of manufactured insecticidal nets in comparison to conventionally treated nets

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### ABSTRACT

The present study evaluated the efficacy and wash resistance of several commercial deltamethrin-treated nets (PermaNet<sup>TM</sup>, from factory (PN-F) and market (PN-M), Yorkkool (Y) and AZ net) that were claimed by the manufacturers to be Long-Lasting Insecticide Treated Nets (LLITNs), compared to ITNs conventionally treated with deltamethrin (23–27 mg/m<sup>2</sup>, using one K-O Tab<sup>®</sup> tablet (KO) per net). Montpellier washing technique was used for washing the pieces of the nets. Insecticidal activity was assessed on dried pieces of nets after 0, 2, 5, 8, 11, 15, 18 and 21 washes, using two types of bioassay (mean median knock down times and mortality 24 h after a 3-min exposure) and reared female *Anopheles stephensi*. To evaluate the effect of heat on diffusion of insecticide from inside of the nets to the surface of them, some PermaNet nets were heated. For all the types of nets tested the median knock down time (MKDT) increased approximately linearly with number of washes. The slopes of the lines (increase of MKDT per wash) were low with the PN-F and PN-M, intermediate with Y and equally high with KO and AZ. No significant differences can be claimed with the 3-min exposure tests. The slopes of the regression lines did not differ significantly between the heated and unheated samples. It is concluded that diffusion at ambient temperature is fast enough to rapidly compensate for the loss of insecticide on the surface with no need to artificially stimulate diffusion by heating.

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### 1. Introduction

Much emphasis is now placed on insecticidal bednets for control of malaria transmission (Alonso et al., 1991; Curtis et al., 1992; Curtis, 1994; Curtis and Mnzava, 2000; D'Alessandro et al., 1995; Habluetzel et al., 1997; Nevill et al., 1996; Phillips-Howard et al., 2003a,b,c; Sexton, 1994). Conventionally treated nets lose insecticidal power if repeatedly washed and they have to be re-treated. The treatment and re-treatment rates of nets especially in most projects in Africa have been very low. There are several reasons for this. Firstly, there are weaknesses in the logistics re-treatment systems, secondly many African families are too poor to buy insecticide kits for re-treatment and thirdly, many of them may not want to re-treat their nets or do not think it is necessary. However, Maxwell et al. (2002) reported that more than 90% re-treatment could readily be achieved in 2–3 days per village by one supervisor bringing free insecticide to the village and working with two–three village

health workers. Clarke et al. (2001) and Schellenberg et al. (2001) reported that in practice, most ITN users do not re-impregnate their nets unless a strongly coordinated action is put into place at the primary health care level. There are now three main approaches for the treatment and re-treatment of nets: (1) impregnation of untreated nets by householders with insecticide treatment kits, (2) free re-treatment services and (3) use of Long-Lasting Insecticidal Treated Nets (LLITNs) (WHO, 2003).

Nets typically last 3–5 years. Routine methods of treatment are generally considered to be effective against malaria mosquitoes for 6–12 months if the net is only washed 3–5 times in above period; frequent washing may reduce the insecticidal power but the effective life of insecticide will be extended if special techniques are used for re-treatment (Curtis, 1996; Elissa and Curtis, 1995; Lines, 1996). Lines (1996) stated that regular re-impregnation of nets is necessary and a system must provide that. He also stated that most programmes for distributing subsidized nets have been more successful than systems for re-impregnation of nets. He emphasised that the health system in most areas of Africa is rudimentary and has problems in delivering even the basic drugs.

Long-Lasting nets (LLITNs) are ready-to-use pre-treated mosquito nets that have been given a special insecticide treatment that is more durable and resistant to washing than the

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conventional dipping method. Ideally, and in order to fit the (WHO, 2002) definition of an LLITN, the insecticidal activity should last as long as the nets expected life span (3–5 years).

There are now several brands of manufactured nets (Long-Lasting Insecticidal Nets or LLIN) which are claimed to have more wash-resistant insecticidal deposit than conventionally dipped nets (Adams et al., 2002; Graham et al., 2005; Jawara et al., 1998; Kayedi, 2004; Lindsay et al., 1991; Lines, 1996; Miller et al., 1991, 1995, 1999; Muller et al., 2002; Ordonez Gonzalez et al., 2002; Pleass et al., 1993; Vythilingam et al., 1999). So the persistence of insecticidal activity of various types of ITNs which are claimed by manufacturers to be LLINs (PermaNet™, Yorkool™ and AZ nets) and conventionally treated nets in routine domestic use was assessed (Graham et al., 2005; Kayedi et al., 2007a,b, 2008; Muller et al., 2002).

Different laboratories have reported different results of bioassay tests after washing of permanets (PNs). This may be due to variations of quality of different batches of PNs (Graham et al., 2005; Kayedi et al., 2007a,b, 2008; Ordonez Gonzalez et al., 2002). We compared by bioassay PNs that were received direct from the factory, with PNs that were bought from a market in Uganda using the Montpellier shaker method.

Several research showed that the number of washing is the main factor which may decrease the insecticidal activity of LLINs, while other factors such as sun exposure and dust may have lesser impacts (Graham et al., 2005; Kayedi et al., 2007a,b, 2008; Ordonez Gonzalez et al., 2002). It is believed that washing removes insecticide from the surface of netting fibres, that this is gradually replaced by insecticide diffusing from inside the resin layer which is applied to the PermaNet (PN). Duchon et al. (2002) reported that heating a PN accelerates the diffusion of insecticide to the surface. We re-tested this process of heat treatment to accelerate recovery of insecticidal power.

This study arose from interest in the use of wash-resistant on “Long-Lasting” insecticide treated nets, and from inconsistencies in the results from different laboratories from tests of these nets. The overall goal is to compare different methods of net treatment and factors that affect the duration of insecticidal activity of treated nets, particularly recently developed methods of producing “Long-Lasting” insecticide treatments, in order to guide malaria control planning.

Based on the above explanation, in our study we compared the residual insecticidal power of three types of ITNs which are claimed by manufacturers to be LLINs versus conventionally treated nets using standard Montpellier shaker method to assess if there is any difference between the nets available in markets and those we received directly from the factory. In addition we checked if heating does any effect on the recovery of insecticidal power.

## 2. Materials and methods

There are different methods of washing of the nets (hand washing, machine washing, etc.). One of the methods that Montpellier WHO reference laboratory has recently developed is a standard washing method which involves putting pieces of netting in soap solution in laboratory flasks, and agitating them not rubbing them as with hand washing (Duchon et al., 2002).

### 2.1. Descriptions of nets

We tested four types of nets which were PermaNet™ (white colour, 100 denier), Yorkool™ (white colour, 75 denier), AZ (green colour, 100 denier) and nets treated with K-O Tab® (white colour, 100 denier). The fibres of these nets were made from 100% polyester. PermaNet, Yorkool and AZ nets had been pre-treated with deltamethrin in the factory (PermaNet 55 mg/m<sup>2</sup>, Yorkool 40 mg/m<sup>2</sup>

and AZ 40 mg/m<sup>2</sup>). The “KO” treated nets contains 23–27 mg/m<sup>2</sup> deltamethrin.

Eight PN<sup>1</sup> from a factory, four PN<sup>2</sup> from a market, four Yorkool<sup>3</sup>, four AZ<sup>4</sup> nets and four nets treated with one K-O tab<sup>5</sup> were used in the experiments. Three pieces of netting (30 cm × 35 cm) were cut out from each net (one from the top, one from one of the long sides and one from one of the short sides). The pieces of nets were washed with a water bath shaker up to 21 times using the method of the WHO reference laboratory (Montpellier, France) using a Julabo SW22 water bath shaker (made in Germany).

Each netting sample was placed in a 1 l glass bottle and 500 ml of deionised water with 2 g/l of soap “savon de Marseille” (pH 10.2) was added to each bottle. The bottles were placed on the shaker with the water bath at 30 °C on a tray agitated with 155 strokes/min for 10 min. After washing with soap, nets were rinsed twice with deionised water at 30 °C for 10 min each, at the same agitation speed. The nets were dried on a line in a room at 27 °C and 80% RH and with artificial light (night/day 12 h/12 h). The intervals between washes were 2 days.

Eight PNs obtained direct from the factory of Vestergaard-Frandsen were sub-divided into two groups of four, PN-F and PN-H. The four PN-H nets were heated at 80 °C for 10 min, 24 h after the 5th, 8th, 15th, 18th and 21th wash. The other factory PN nets (labelled PN-F) were not given heat treatment and were stored between washes at 27 °C, as were all the other nets (PNs bought from a market in Uganda, “PN-M”, Yorkool nets, “Y”, A to Z nets, “AZ”, and nets conventionally treated with a KO-Tab, “KO”).

### 2.2. Bioassay tests

The bioassay methods were described by Curtis et al. (1998) and WHO (1998). One bioassay with continuous exposure and one with 3 min exposure were carried out on each piece of net after 0, 2, 5, 8, 11, 15, 18 and 21 washes. Nets were wrapped around a spherical frame and laboratory reared *A. stephensi*, Beech strain (3–5 days old), were placed inside so that the only place that they could stand was on the netting.

Median knock down time (MKDT) in continuous exposure bioassay and mortality 24 h after 3 min exposure were recorded. In total 576 continuous exposure tests and 576 3 min exposure tests were carried out using 12,672 mosquitoes. Bioassays on the PN-H groups of nets were carried out before heating [PN-H (before)] and after heating [PN-H (after)].

### 2.3. Statistical analysis

The SPSS version 15 package was used for analysis of data. We had two dependent variables, MKDT and the percent of mortality. We checked if the distribution of these two variables were comparable with normal distribution using Kolmogorov–Smirnov test. Since the percent of mortality was not distributed normally, we put its logarithm as the dependent variable in linear regression model.

The number of washing was our main independent variable in our regression models and we estimated the slope of the regression

<sup>1</sup> PermaNet™ received from the manufacturer in Vietnam (10-10 Js Textile Co 253 Mtnh Khat ST, Hanoi-Vietnam).

<sup>2</sup> PermaNet™ bought in a market in Kampala, Uganda.

<sup>3</sup> Yorkool™ received from the manufacturer in China. Yorkool (Tianjin) International Trading, Development Co., Ltd. Rm. 705, North China Finance Tower, #5, Youyi Road, Tianjin 300074, China.

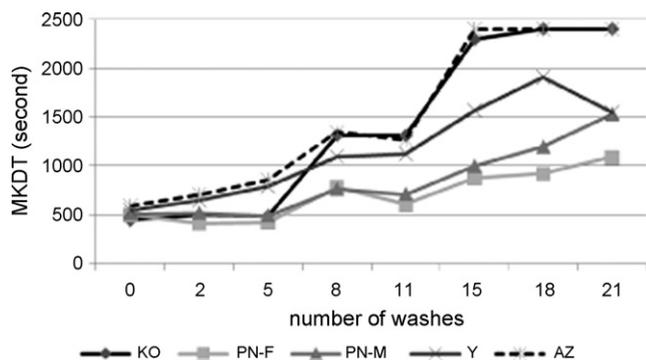
<sup>4</sup> AZ net received from the manufacturer in Tanzania.

<sup>5</sup> K-O Tab® treated net (Manufacturer: Agr Evo (Aventis), Address: Aventis Crop-science, South Africa, 268 West Avenue, Centurion PO Box 10441, Centurion, 0046 South Africa).

**Table 1**

The slope of linear regression lines between the numbers of washes and mean median knock down time (MKDT); and the number of washes and the percent of mortality 24 h after 3-min exposure (%mortality) classified by type of bed nets.

Type of bed nets	MKDT slope (95% C.I.)	%Mortality slope (95% C.I.)
KO	110.6 (98.5–122.8)	−4.79 (−5.7, −3.87)
AZ	99.9 (86.1–113.7)	−3.22 (−4.34, −2.11)
Y	60.4 (49.7–71.2)	−3.43 (−4.38, −2.48)
PN-F	30.7 (24.1–37.2)	−3.63 (−4.43, −2.84)
PN-M	46.6 (42.9–50.3)	−3.6 (−4.05, −3.15)



**Fig. 1.** Mean median knock down time (MKDT) of *A. stephensi* with continuous exposure to PN-F, PN-M, KO, Y and AZ nets.

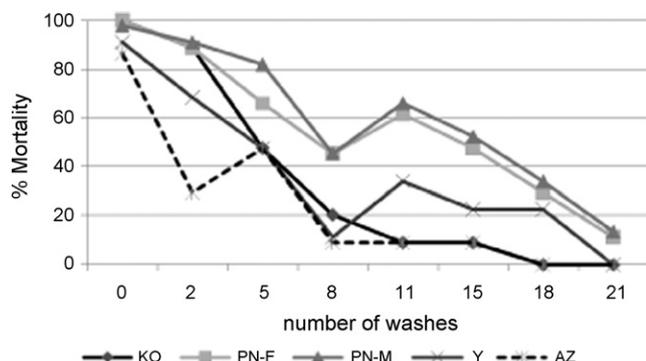
lines for each type of bednets to compare if washing had similar effects on the insecticidal effect of nets.

### 3. Results

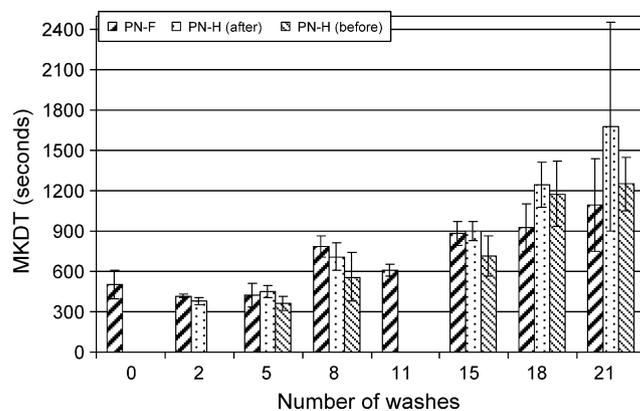
#### 3.1. Median knock down time (MKDT) and mortality 24 h after 3 min exposure

The median knock down time (MKDT) were around 500 s in all types of bednets before washing. However, by increasing the number of washing, the MKDT of different types of bednets were varied. The slopes of the lines (increase of MKDT per wash) were low with the PN-F and PN-M, intermediate with Y and equally high with KO and AZ (Table 1 and Fig. 1).

Similarly, the percentage of mortality after a 3 min exposure were more or less similar in different types of nets before washing and were close to 100%; but they were different after two washes (Fig. 2). Although the slope of KO nets were less than the other types which means KO nets were less wash resistance, their confidence intervals were overlapped and we did not find statistically differences (Table 1). Fig. 2 shows that the lines for AZ and KO nets had



**Fig. 2.** Mortality of *A. stephensi* 24 h after 3-min exposure to PN-F, PN-M, KO, Y and AZ nets.



**Fig. 3.** Mean median knock down time with 95% C.I. of *A. stephensi* with continuous exposure to PN-F, PN-H (after) and PN-H (before).

steep decline initially and less decline later as mortality approached zero.

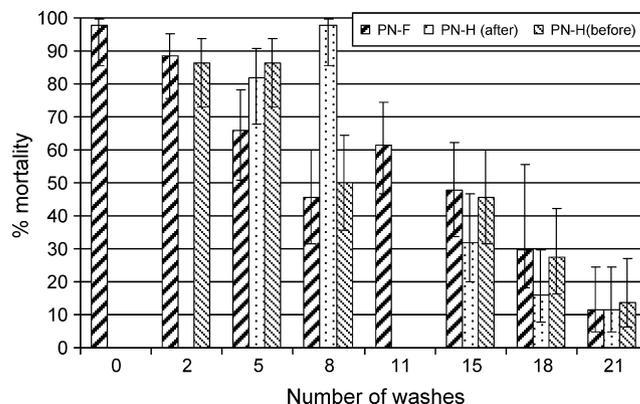
#### 3.2. Diffusion of insecticide to the surface of fibres

The effect of heat in stimulating insecticide to diffuse from inside the resin to the surface of PN-F nets was investigated as well. The results of bioassays measuring (MKDT and %mortality) of PN-F nets which had or had not been heated after washing are shown in Figs. 3 and 4. These figures show that the results of bioassay tests were more or less comparable and their confidence intervals were well overlapped. Moreover, the slopes of the regression lines did not have significant difference in the heated and unheated samples. Hence, it is concluded that diffusion at ambient temperature is fast enough to rapidly compensate for the loss of insecticide on the surface with no need to artificially stimulate diffusion by heating.

### 4. Discussion

The washing method used in the present study showed differences between LLITNs and non-LLITNs and between LLITNs and conventionally treated nets very clearly. In this washing method, there is no intense rubbing of nets, as in hand washing. Nevertheless, this method removes insecticide from the fibres after each wash. The hand washing method might more closely resemble the washing method used in practice in the field, which suggests that maybe this method of testing is detecting differences that might not be seen in the field.

PN showed the best insecticidal efficacy among all the types of net tested. However even with this net the insecticidal efficacy



**Fig. 4.** Mortality of *A. stephensi* 24 h after 3-min exposure to PN-F, PN-H (after) and PN-H (before) with 95% C.I.

declined with increasing number of washes. Gimnig et al. (2005) reported similar results with the PN nets. They used standard laboratory washing protocol approximately the same method that we used in our study. Their study showed that PN nets retains significant efficacy against *Anopheles gambiae* (Kisumu strain) after up to 20 washes.

In the present study the MKDT of PN-F after 21 washes had doubled. Similarly the mortality at 24 h after 3-min exposure of the PN net declined from 100% before washing to 11.4% after 21 washes with this washing method. This is not consistent with the claims of the manufacturer that there is 90% mortality after 21 washes with the same washing method (WHO, 2000).

Our MKDT results showed that Permanets obtained direct from the factory or from the market were, as claimed, more wash resistant than conventionally dipped nets. With the Yorkol there is some degree of enhanced wash resistance but AZ nets did not show wash resistance, so the Yorkol and AZ nets cannot be correctly described as “LLINs” and do not recommend to use for control of malaria without regular re-treatment. It has been claimed in some literature reviews and WHO (2000) that Permanets are fully wash resistant with no loss of power after 20 washes (Graham et al., 2005; Kayedi, 2004; Kayedi et al., 2007a,b, 2008; Ordonez Gonzalez et al., 2002). Our data do not confirm this, there is certainly some loss of insecticidal power. Maxwell et al. (1999) reported that Tanzanian villagers typically washed their nets 3–5 times/year. Thus, then after 4 years Fig. 1 suggests that the MKDT would have increased by about 130%.

From the data on loss of insecticidal power in laboratory bioassays it is difficult to assess what this would mean in terms of loss of power to protect a sleeper under the nets from biting and loss of killing power against free flying mosquitoes, as exposed to mosquitoes confirmed in a bioassay testing frame. These questions are best answered by tests in experimental huts in which people sleep under nets while wild mosquitoes can enter the huts (Curtis, 1996; Graham et al., 2005; Kayedi, 2004) or by research on routine domestic use of ITNs (Graham et al., 2005; Kayedi, 2004; Kayedi et al., 2007a,b, 2008; Ordonez Gonzalez et al., 2002). It is important that more work done on washed LLINs under such conditions and more investigations are necessary to evaluate the effect of heating on diffusion of insecticide from inside of the resin to the surface of it. Moreover our data do not confirm Duchon et al. (2002) results on diffusion of insecticide, but Gimnig et al. (2005) results demonstrated the same results of our study on regeneration of insecticide by heating.

Results showed that there was no significant difference between Permanets that were bought from market in Uganda with Permanets that were received from factory, so we conclude that the quality of impregnation with insecticide is the same with both Permanets that were evaluated.

We should mention that there was limitations to the present study such as physical durability which cannot be measured in laboratory wash-resistance studies. We assessed only the impact of washing while in further field studies, the impact of other factors such as physical durability, sun exposure and dust should be assessed.

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