RESEARCH NOTE

BIOEFFICACY OF A LONG-LASTING INSECTICIDE IMPREGNATED NET AGAINST *ANOPHELES MACULATUS* THEOBALD AND *CULEX QUINQUEFASCIATUS* SAY

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Abstract. This study was conducted to evaluate the bioefficacy and effects of unwashed and washed PermaNet® 2.0 nets treated with extrinsic heat during washing against mosquitoes under laboratory condition. The effectiveness of the washed nets was bioassayed soon after washing using the WHO cone test method. The results indicated that the treated nets exhibited high durability to washing (five washes) and persistent bioefficacy against *An. maculatus* on both unwashed and washed nets. The unwashed net exhibited mortality of 98.33±2.06%, with KT50 and KT90 ranging from 17.57-to-66.38 minutes and 34.65-to-311.30 minutes, respectively (n=9) for >44 months. Nets washed at 30ºC for three successive washes exhibited mortality of 98.00±2.65% (n=3) for 7 months.Twenty-one months later, two washes at 80ºC on the same nets caused a mortality of 87.50±3.54% (n=2). The overall cone mortality of five washes over a total wash period of 33 months was 94.75±6.85% with KT50 and KT90 ranging from 23.98 to 58.25 minutes and 51.63 to 230.21 minutes, respectively (n=5). However, the treated net exhibited lower bioefficacy against *Culex quinquefasciat*us on the unwashed nets with mortality of 9%. KT50 and KT90 ranged from 129.17 to 136.78 minutes and 315.35 to 716.05 minutes, respectively, over an unwashed period of 3 months (n=1), and washed nets had a mortality of 30%; KT50 of 64.04 minutes and KT90 of 347.56 minutes for only one wash at 30ºC (n=1). Application of extrinsic heat treatment during washing at 30ºC followed by increased heat at 80ºC on the same net did not enhance any significant increase in mortality of *An. maculatus* and *Culex quinquefasciatus* (p>0.05). There was a difference in the delayed knock down time soon after washing at 80ºC. Based on these promising results, the PermaNet® 2.0 should be field-tested.

Keywords: PermaNet®, bioefficacy, knock down time, laboratory evaluation, WHO cone test, Malaysia

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INTRODUCTION

Insecticide-treated nets (ITN), especially long-lasting nets (LLN) are widely used in malaria control. Treated nets are useful and provide protection to the people; however, the retreatment of nets

has always posed serious problems in the implementation of ITN. Retreatment rates of nets are very low due to several factors. Many countries do not have the resources for retreatment; these are the cost of the insecticides as well as a lack of understanding of its importance as a form of personal protection proven to reduce severe disease in endemic regions (CDC, 2014). People also do not like to have their nets treated in bulk together with others (Vythilingam *et al,* 2003). Therefore, to avoid the need for periodic re-treatment, it would be advantageous for nets to be able to retain insecticidal efficacy for years and withstand repeated washing (N'Guessan *et al*, 2001).

PermaNet® 2.0(VestergaardFrandsen, Lausanne, Switzerland) is a type of net pretreated in the factory with deltamethrin, a pyrethroid, which is a neurological insecticide rapidly absorbed by the legs of the mosquito while in contact with the insecticide treated nets. Mosquitoes often dispose off the affected legs when the poison is detected. Therefore, with only three or fewer legs, they can no longer fly, land, and bite, thereby reducing man/vector contact and overall vector density. The World Health Organization (WHO) recommended permethrin as one of the pyrethroids to be widely used in vector control programs (WHO, 1985).

The aim of this study was to evaluate the bioefficacy of the treated net against mosquitoes under laboratory conditions and the efficacy of washed nets with extrinsic heat at 30ºC followed by 80ºC on the same nets during washing.

MATERIALS AND METHODS

Study site

The study was conducted at the main laboratory of the Medical Entomology Unit/WHO Collaborating Centre for Vectors, Infectious Disease Research Centre, Institute for Medical Research, Kuala Lumpur. The temperature and humidity was maintained at 27±2ºC and 80±2%, respectively with a photoperiod of 12:12 hours (L:D).

Mosquito net tested

PermaNet® 2.0(VestergaardFrandsen, Lausanne, Switzerland) was made of 75 denier polyester with a mesh size of 25 holes/cm² and treated in the factory with 55 mg/m² deltamethrin. The size of the net was 160 cm x 150 cm x 180 cm (width x height x length). A similar but untreated net was also tested as a control net**.** Four samples of the net (25 x 25 cm) were cut from each of the nets and used in the test.

Mosquitoes tested

The mosquitoes used in the study were laboratory-bred *Anopheles maculatus* Theobald and *Culex quinquefasciatus* Say,which were susceptible to insecticides including pyrethroid. The mosquitoes were fed with 10% sucrose solution and maintained in the insectarium at 27±2ºC and 80±2% RH with 12:12 hours (L:D) photoperiod regime.

Laboratory washing procedure

The net samples (25 cm x 25 cm) were individually introduced into 1 liter beakers containing 0.5 liter distilled water, with 2 g/l soap (Savon de Marseille, pH10-11) fully dissolved. The beakers were then placed into a shaking water bath at 30ºC and 80ºC**,** and shaken for 10 minutes at 155 rotations per minute. The net samples were then removed and rinsed twice for 10 minutes in clean distilled water in the same shaking condition as stated above. After washing and drying at room temperature, the net samples were tested to determine effectiveness soon after washing. In this trial, the same nets were

washed three times at 30ºC followed by two final washes at 80ºC and shaken for 10 minutes at 155 rotations per minute. The purpose of washing the net in hot water was to determine if heat could regenerate the activity of the net after washing.

Bioassay of mosquitoes

Four pieces of net were cut from each net. The standard WHO plastic cones were placed on each net and labeled 1-4, accordingly. Five sugar-fed, 2-to-5 days old female mosquitoes were introduced into each cone. The cones were then plugged with cotton wool and covered with a black cloth to ensure total contact of the mosquitoes with the treated net. The exposure time was 3 minutes, after which the mosquitoes were transferred into clean paper cups accordingly, and mortality rate was recorded at 10-minute intervals until 60 minutes.

The mosquitoes were held in these paper cups for 24 hours at 27±2ºC and 80±2% RH with 12:12 hours (L:D) photoperiod, and 10% sucrose solution was provided. Each sample was tested using a total of 25 mosquitoes, and four such replications were carried out. Tests were also conducted on the two pieces of control (untreated) nets in parallel to the test and delayed mortality observed after 24 hours. Test net materials used for residual were kept indoor in the laboratory at ambient temperature.

Data analysis

The number of dead mosquitoes was recorded. If the control mortality was between 5%-20%, the mortalities will be corrected by Abbott's formula (Abbott, 1925):

$$\frac{\%\text{treated mortality} - \%\text{control mortality}}{100\% - \%\text{control mortality}} \times 100\%$$

When results obtained in test where control mortalities exceed 20%, the test would be discarded and the experiment repeated. All data were expressed as the mean ± SEM. A value of $p<0.05$ was considered significant. Data obtained from the test were subjected to statistical analysis using SPSS® (version 16.0, SPSS: Chicago, IL), while knockdown data were subjected to probit analysis using similar software to obtain the KT50 and KT90 values.

RESULTS

An. maculatus

With unwashed treated nets, *An. maculatus* exhibited KT50 and KT90 ranging from 17.57-to-66.38 minutes and 34.65-to-311.30 minutes, respectively for >44 months (Table 1). Bioefficacy of knock down time of washed treated net against *An. maculatus* exhibited KT50 and KT90 ranging from 23.98-to-58.25 minutes and 51.63-to-230.21 minutes, respectively for five washes over a test period of 33 months with a difference in the delayed knock down time of 34.27 minutes soon after washing at 80ºC (Table 2).

The residual activity of treated net against *An. maculatus* on unwashed nets was >44 months with a mean mortality of 98.33±2.06% (n=9), while the nets washed at 30ºC for three successive washes exhibited mortality of 98.00±2.65% (n=3) for 7 months and 21 months later (from 28th-to 33rd month). Two washes at 80ºC on the same nets exhibited mortality of 87.50±3.54% (n=2). However, the overall mortality of five washes (30ºC followed by 80ºC) was 94.75±6.85% (n=5) over a total wash period of 33 months (Table 4). There was no significant difference between unwashed and washed treated nets ($p>0.05$).

Culex quinquefasciatus

*Culex quinquefasciat*us exhibited lower

Table 1
Knockdown rate of *An. maculatus* on unwashed nets.

Unwashed (Months)	KT50 (Min) (95% Confidence limit)	KT90 (Min) (95% Confidence limit)	Regression line
0	17.57 (14.37-20.55)	34.65 (29.21-44.39)	y=4.35 x-5.41
3	21.01 (19.21-22.75)	45.61 (41.44-51.24)	y=3.81 x-5.04
8	25.39 (15.37-34.80)	71.95 (48.33-233.45)	y=2.83 x-3.98
16	14.40 (12.35-16.28)	41.13 (36.38-47.97)	y=2.81 x-3.26
29	46.38 (34.56-84.87)	163.50 (87.78-1,683.85)	y=2.34 x-3.90
34	52.83 (43.80-71.11)	433.93 (231.61-1,392.23)	y=1.40 x-2.41
39	41.69 (35.62-50.97)	280.85 (172.41-650.04	y=1.55 x-2.51
41	54.08 (41.08-114.39)	150.61 (84.76-1,769.17)	y=2.88 x-4.99
>44	66.38 (44.99-336.56)	311.30 (121.83-56,467.30)	y=1.91 x-3.48

Table 2
Knockdown rate of *An. maculatus* on washed nets.

Washed (Months)	KT50 (Min) (95% Confidence limit)	KT90 (Min) (95% Confidence limit)	Regression line
1st 30ºC	23.98 (22.07-25.84)	51.63 (46.82-58.24)	y=3.85 x 5.31
2nd 30ºC	23.61 (14.22-31.73)	59.35 (41.98-151.26)	y=3.20 x-4.40
3rd 30ºC	25.42 (23.25-27.56)	61.13 (54.38-70.96)	y=3.36 x-4.73
4th 80ºC	59.15 (50.71-73.78)	250.51 (167.31-483.01)	y=2.04 x-3.62
5th 80ºC	58.25 (45.04-100.12)	230.21 (122.63-1,272.99)	y=2.15 x-3.79

Table 3
Knockdown rate of *Cx. quinquefasciatus* on unwashed and washed nets.

Unwashed (Months)	KT50 (Min) (95% Confidence limit)	KT90 (Min) (95% Confidence limit)	Regression line
0	136.78 (94.79-285.37)	716.05 (327.34-3,635.30)	y=1.78 x-3.81
3	129.17 (91.35-338.61)	315.35 (168.05-1,895.00)	y=3.31 x-6.98
1 wash, 30ºC	64.04 (53.08-85.47)	347.56 (207.95-844.21)	y=1.75 x-3.15

bioefficacy of knock down times of KT50 and KT90 ranging from 129.17-to-136.78 minutes and 315.35-to-716.05 minutes over an unwashed period of 3 months. Bioefficacy of washed treated nets exhibited knock down times of KT50 (64.04 minutes) and KT90 (347.56 minutes) for only one wash at 30ºC with a difference in the knock down time of 65.13 minutes soon after washing (Table 3).

Table 4
Mortality of *An. maculatus* and *Cx. quinquefasciatus* on unwashed and washed nets.

Treatment	Months	Species	Replicate (*n*)	Mortality (%)	*p*-value
Unwashed	>44	*An. maculatus*	9	98.33 ± 2.06	0.00
3 washes, 30ºC	7	*An. maculatus*	3	98.00 ± 2.65	0.00
2 washes, 80ºC	28th - 33rd	*An. maculatus*	2	87.50 ± 3.54	0.0018
Overall washed 30ºC and 80ºC	33	*An. maculatus*	5	94.75 ± 6.85	0.00
Unwashed/washed		*An. maculatus*	5	3.00 ± 3.94	0.164
Unwashed	1	*Cx. quinquefasciatus*	1	9.00	nil
1 wash, 30ºC	1	*Cx. quinquefasciatus*	1	30.00	nil

The mortality of *Cx. quinquefasciatus* on unwashed nets was 9% (*n*=1), while the nets washed at 30ºC exhibited mortality of 30% (*n*=1) (Table 4). Because the mortality was so low, no further test was carried out at 80ºC.

DISCUSSION

The bioefficacy of insecticide treated bednets in preventing morbidity and mortality from malaria endemic countries had been studied and reported by several researchers (Alonso *et al*, 1993; Dolan *et al*, 1993; Jaenson*et al*, 1994; Stich *et al*, 1994; D'Alessandro *et al*, 1995a, 1995b; Binka *et al*, 1996; Nevill*et al*, 1996; Rowland *et al*, 1996; Goodman *et al*, 1999; Armstrong-Schellenberg *et al*, 2001; Guyatt and Snow, 2002; Lengeler, 2004; Rafinejad *et al*, 2008).

Our cone bioassay indicated that PermaNet exhibited high durability to washing (five washes) and persistent bioefficacy against *An. maculatus* on both unwashed,with mortality of 98.33±2.06% (*n*=9) for >44 months, and nets washed at 30ºC for three successive washes with mortality of 98.00±2.65% (*n*=3) for 7 months and 21 months later (from 28th-to-33rd-months), followed by two washes at 80ºC on the same nets with a mortality of 87.50±3.54% (*n*=2) over a washed period of 33 months.

However, the overall cone mortality of five washes over a total washed period of 33 months was 94.75±6.85% (*n*=5). Our study concurred with previous research done in Colombia and Bolivia using a cone test for the evaluation of PermaNet®, lambda-cyhalothrin, deltamethrin, and alpha cypermethrin impregnated polyester nets against *Anopheles* sp where 100% mortality was reported after three washes (Gonzalez *et al*, 2002).

However, PermaNet® 2.0 exhibited lower bioefficacy against *Cx. quinquefasciatus* on unwashed and washed nets. Our study concurred with Hougard *et al* (2003) that *Cx. quinquefasciatus* exhibited lower susceptibility to most chemical insecticides.

In comparison to the tunnel test, higher mortality was observed in cone bioassay that has forced tarsal contact for mosquitoes to rest on the insecticide treated nets. Our previous study (Tan *et al*, 2014) on tunnel mortality using the same nets concurred with the results of Bhatt *et al* (2005) and Rafinerad *et al* (2008) where higher mortality of their cone and

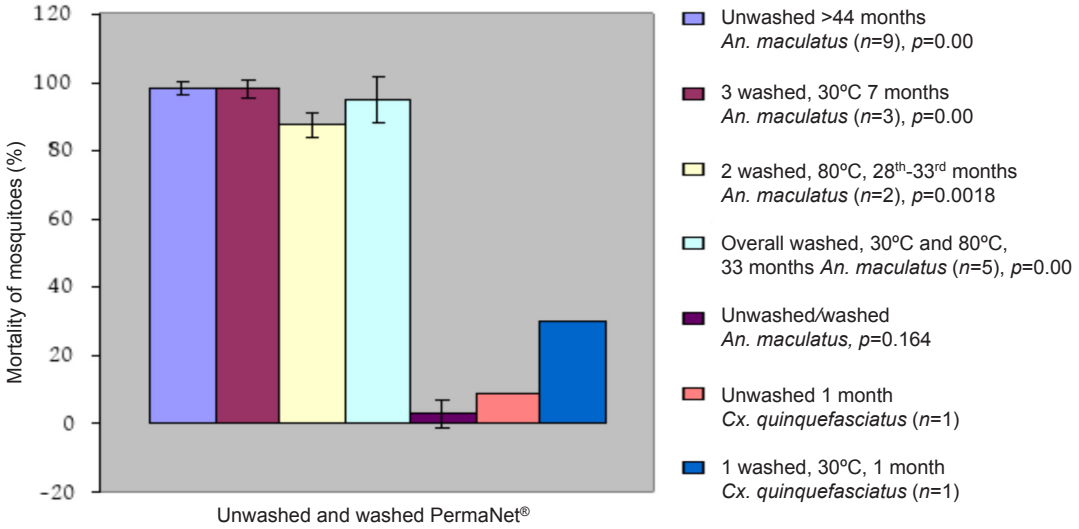

Fig 1–WHO cone test on bioefficacy of unwashed and washed PermaNet® 2.0 against *An. maculatus* and *Cx. quinquefasciatus*.

tube test on six times washed nets were observed and the percentage of 1 hour knockdown on unwashed and six times washed nets was 100% in both cone and tubes. In tunnel tests, 44% and 74% mortality rates were recorded on unwashed and six times washed nets, respectively. Our tunnel mortality on the same nets exhibited 74.36±25.77% (n=14) mortality on unwashed and 75.00±18.46% (n=10) after five washes. Cone mortality exhibited higher mortality of 98.33±2.06% (n=9) (unwashed) and 94.75±6.85% (n=5) (five washes). The variation in the magnitude of decline in tunnel mortality may be due to the large space of tunnel; therefore, mosquitoes are not obliged to come into contact with the nets impregnated with insecticides (Rafinejad *et al*, 2008).

This study also suggested that there was no significant difference in knockdown time and mortality of *An. maculatus* and *Cx. quinquefasciatus* between unwashed and washed nets (p>0.05).Therefore, application of extrinsic heat treatment of 30ºC followed by 80ºC on the same nets during washing did not enhance any significant decrease in the knock down time and increase in mortality of both *An. maculatus* and *Cx. quinquefasciatus.*

Since washing at 30ºC was done only once, and no further washing was conducted at 80ºC, future study on larger sample size of *Cx. quinquefasciatus* should be conducted to obtain better and reliable results.

Lindblade *et al* (2005) concluded that PermaNet® 2.0 performed significantly better than other conventional nets. The wash resistance offered by PermaNet® 2.0 was much better and long lasting (Rafinejad *et at*, 2008). Graham *et al* (2005) found 97% mortality against *Anopheles* in Pakistan, Iran, and Tanzania trials using PermaNet® 2.0 after 21 washes, whereas our study found that after 5 washes, PermaNet® 2.0 still remained effective in killing susceptible lab-strain *An. maculatus*

of up to 94.75±6.85% (n=5). Based on data obtained, we suggest PermaNet® 2.0 be field-tested to assess its impact on malaria.

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