EVALUATION OF PERMANET® 2.0 MOSQUITO BEDNETS AGAINST MOSQUITOES, INCLUDING *ANOPHELES MINIMUS* S.L., IN INDIA

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Abstract. Wash resistance and field bioefficacy of PermaNet® 2.0 nets, long lasting insecticidal nets, against mosquitoes were evaluated in Assam, northeastern India. After repeated hand washings at 12-day intervals a decline in the mosquito killing ability of PermaNet nets was noted (trend χ^2 38.9, p <0.0001), yet these nets retained good insecticidal efficacy for up to 15 wash cycles, producing a 72.5% mean mortality of *An. minimus* 24 hours after a 3-minute exposure in World Health Organization cones after 15 washings. Significantly fewer (p<0.001) mosquitoes were captured in self-baited landing collections in houses equipped with PermaNet nets than in houses using untreated nets in the case of culicines (p<0.001) but not with anophelines, including *An. minimus*. The use of PermaNet nets resulted in noticeably fewer bites from *Culex pseudovishnui* (68.5%) and *Cx. quinquefasciatus* (70%). Blood-feeding inhibition of mosquitoes in deliberately torn PermaNet nets was seen, indicating a protective effect for those sleeping under these nets. No serious adverse effects of the PermaNet nets were reported by users.

INTRODUCTION

Insecticide-treated bed nets (ITNs) have emerged as a potent tool globally in preventing morbidity and mortality due to mosquito-borne diseases, especially malaria (Lengeler, 2005). ITNs not only provide personal protection against biting mosquitoes for the users but also are considered to produce a public health effect by killing large numbers of mosquitoes and substantially reducing inoculation rates, provided large scale coverage of the community with ITNs is achieved (Hawley *et al*, 2003). Frequent washing of ITNs by users for hygienic, cultural and religious reasons along with the need for periodic re-treatment at regular intervals in order to maintain insecticidal efficacy (Lines, 1996) are obstacles in exploiting the full potential of ITNs. Although ITNs have been included in the national malaria control programs of many countries as a vector control option, low re-treatment rates of ITNs in most countries (Cham *et al*, 1997; Winch *et al*, 1997; Dabire *et al*, 2006) are seriously affecting ITN programs. To meet this challenge long lasting insecticidal nets (LLINs), have been developed incorporating synthetic pyrethroid in the net yarn at the manufacturing stage to ensure its bio-availability on the surface of the net even after

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multiple washings (Guillet *et al*, 2001). Presently two brands of WHO recommended LLINs are available in the Indian market: the Olyset® net (M/S Sumitomo Chemicals, Japan), made of polythethylene netting material with 2% permethrin (corresponding to 1 g/m^2 concentration) incorporated into the polymer before monofilament yarn extrusion, and the PermaNet® net (M/S Vestergaard Frandsen, Denmark), made of polyester netting material with deltamethrin at 55 mg/m^2 incorporated into a resin coating the fibers. Both these nets have shown remarkable field bio-efficacy in several countries, such as Kenya (Lindblade *et al*, 2005), Tanzania (Maxwell *et al*, 2006; Kulkarni *et al*, 2007), Columbia (Kroeger *et al*, 2004), Burkina Faso (Dabire *et al*, 2006), Ivory Coast (N' Guessan *et al*, 2001) and India (Ansari *et al*, 2006). Good wash resistance has also been seen with laboratory studies (Gimnig *et al*, 2005; Graham *et al*, 2005; Kayedi *et al*, 2007a). In Delhi, India, 3 studies have involved field, and laboratory evaluation of these nets against mosquito vectors *An. stephensi* and *An. culicifacies* (Olyset® nets-Ansari *et al*, 2006; Sreehari *et al*, 2007b and PermaNet® 2.0 nets-Sreehari *et al*, 2007a). There is a lack of information regarding the performance of LLINs in the high malaria endemic northeastern region of India, where conventionally treated deltamethrin nets have been found effective in controlling malaria transmitted by *An. minimus* (Jana-Kara *et al*, 1995) and *An. dirus* (Prakash *et al*, 2003) complex mosquitoes. In view of this, we carried out a village level study in the Assam state of northeastern India evaluating PermaNet® 2.0 nets for wash resistance against the local malaria vector, *An. minimus* s.l. and their impact on biting densities of other mosquitoes.

MATERIALS AND METHODS

White colored PermaNet® 2.0 nets (henceforth called PermaNet), measuring 100 x 150 x 180 cm comprised of 100 denier polyester yarn having a mesh of 156/inch^2 (25/cm^2) containing 55 mg/m^2 deltamethrin and untreated polyester nets of similar specifications, both manufactured by M/S Vestergaard Frandsen, India were used in the study.

Laboratory wash-resistance of PermaNet

The impact of repeated washings on the insecticidal activity of PermaNet, up to 22 washes, was tested through a WHO contact cone study using a 3-minute fixed time exposure (WHO, 1998) between August 2005 and April, 2006. Two nets (representing 2 replicates) each of PermaNet and untreated nets were used in the wash-resistance study.

Washing protocol. Nets were hand-washed every 12 days for 22 washes in the laboratory. For each net, a 20 g sachet of Surf Excel Quickwash® detergent powder (M/S Hindustan Lever, Mumbai) was dissolved in a plastic trough containing 10 liters of cold tap water (pH of the detergent solution ~9.5) and the net was soaked for 10 minutes. The soaked net was rubbed thoroughly between the palms for 3 minutes and thereafter rinsed in plain water 3 times to remove the detergent. After washing the nets were shade dried for 6-8 hours in a horizontal position. The ambient temperature during the drying period ranged between 20-34^{o}C depending on the month. Between washings and evaluations, the nets were stored individually in polythene bags at room temperature (temperature range 11-30^{o}C depending on the month).

Cone bio-assays. Contact bio-assays (WHO, 1998) were carried out 6-7 days after each wash by exposing wild caught female *An. minimus* s.l. mosquitoes (for 22 washes) and *An. philippinensis /nivipes* mosquitoes (for 5 washes), collected the previous night and maintained on a sugar solution. Each test

was carried out for 3 minutes using WHO cones fixed to the PermaNet nets (test) or untreated nets (control) in a horizontal position in the camp laboratory. Depending on availability, 10-42 mosquitoes were exposed in 2-4 replicates to each category of net and then transferred into plastic cups with a net cover and provided with a 10% sugar solution during the 24 hour post-exposure period after which mortality was scored. The ambient temperature during the post-exposure period ranged between 25-30ºC. The data were pooled and a corrected mortality rate was calculated using Abbott's formula (Abbott, 1925).

Village level bio-efficacy of the PermaNet

Study site. Paninara village in the Titabor Primary Health Center of Jorhat District, Assam, India was selected. The village had a population of about 700 living in 112 houses. The village was divided into 3 sections. The study was carried out in the Tanti Basti section with a high incidence of malaria. It contained 14 thatched, split-bamboo, mud-plastered houses (population 81) located at the base of the Naga Hills. PermaNet nets were distributed to odd numbered houses and untreated nets were distributed to even numbered houses, 7 houses each during the last week of July 2005 based on the number of beds in each house ensuring complete coverage. A total 21 PermaNet nets and 20 untreated nets were distributed to the 14 houses in the study section. House owners were asked to use the provided nets only and not to wash these nets during the study period.

Impact on biting densities of mosquitoes. Dusk-to-dawn, self-baited mosquito-landing catches were carried out in 2 fixed houses (1 house each with PermaNet nets and untreated nets) simultaneously for 1 night each month. The two houses were situated about 100 meters apart, had similar in characteristics and were fairly equidistant from mosquito breeding sites. Two experienced collectors, working in 6-hour shifts in each house, collected mosquitoes landing on their bare legs below the knee while sitting on a stool in the bedroom at a distance of 4-5 feet from the beds where occupants slept under the nets. Mosquitoes collected each hour were kept separately in plastic containers and identified next morning under the microscope in the camp laboratory using standard keys. The collectors were provided weekly chemoprophylaxis with chloroquine during the study period. Biting densities were expressed as mosquitoes landing per person per night. The index houses were not changed during the study period.

Impact on blood feeding inhibition of mosquitoes. Two in-use nets (one PermaNet and one untreated net) in 2 randomly selected houses were deliberately torn (six holes of 4 x 4 cms), simulating a torn net. Each month different houses were selected for this study. One family member slept under the torn net as usual for one night. The next morning all dead/alive mosquitoes found inside the nets were collected using a suction tube and flash light, identified and their abdominal status noted. Torn nets were replaced by new nets of similar type the following day of the study.

Acceptability of PermaNet nets. Users of PermaNet nets were interviewed using a semi-structured questionnaire after 4 weeks of net distribution to elicit their responses regarding the net and to know if any adverse effects were experienced by them.

Statistical analysis

Z-test was employed to evaluate significant differences in mosquito mortality between two sets of washes. Significant differences in mean densities of landing mosquitoes between PermaNet net houses and untreated net houses were calculated using *t*-test.

Ethical approval

The study protocol was reviewed and approved by the Institutional Ethics Committee of the Regional Medical Research Center, Dibrugarh. Written informed consent was obtained from the residents of the Tanti Basti section of the Paninara Village before initiating the field study.

RESULTS

Wash resistance of PermaNet nets

Average 24-hour post-exposure mortalities of *An. minimus* s.l. and *An. philippinensis/nivipes* exposed to PermaNet nets after repeated washings are presented in Table 1. PermaNet caused 100% mortality in both the species of anopheles at base line (0 wash). Repeated washings resulted in a gradual decline in insecticidal activity (Fig 1) which declined relatively slowly until 10 washes (82.7% mean mortality of *An. minimus* after 6-10 washes). The decline in insecticidal activity progressed more rapidly after 11-15 washes, but still the mortality rate averaged over 72%, which was not significantly different than the mean mortality rate recorded after 1-5 washes (p=0.059) and 6-10 washes (p=0.110). The insecticide activity declined significantly (p<0.0001) after 16-22 washes when mean mortality rate declined to 47.6%. The overall trend of declining insecticide activity of the PermaNet nets with repeated washings was highly significant (trend χ^2 38.9, p<0.0001). Evaluation of activity against *An. philippinensis/nivipes* could not be carried out beyond 5 washes (mean mortality 87.6%) due to the non-availability of sufficient numbers of this species of mosquito.

Field bio-efficacy of PermaNet

In mosquito-landing catches 12 species of culicines and 11 species of anophelines were captured. Of the known malaria vectors in Assam, *An. minimus* s.l. was the predominant species whereas, *An. dirus* s.l. was

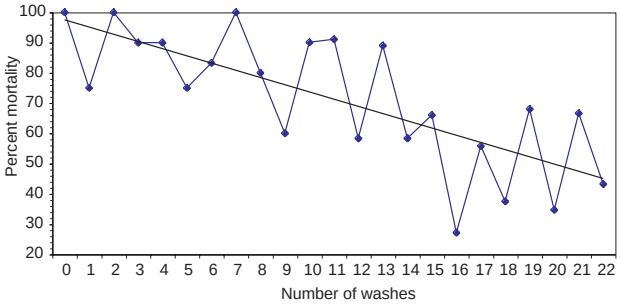

Fig 1–24-hour post-exposure mortality trend against *Anopheles minimus* s.l. after repeated washes of PermaNet® 2.0 nets.

collected in fewer numbers. The protective effect of PermaNet nets on mosquito biting was observed with significantly fewer (p<0.001) numbers of landing mosquitoes collected from PermaNet house compared to untreated net house. However, this protective effect was restricted to culicine mosquitoes only. The mean biting densities of culicines (36.8 per person per night) in the PermaNet house, during the study period, were 62% lower and significantly less (p<0.001) than that of corresponding biting densities in the untreated net house (97.6 per person per night) with a highly pronounced reduction in biting densities of *Culex pseudovishnui* (68.5%) and *Cx. quinquefasciatus* (70%) mosquitoes. Surprisingly, no protective effect of PermaNet use was noted with *Anopheles* biting. The overall mean number of landing anophelines, including *An. minimus* s.l., was significantly higher (p=0.016) in the PermaNet house (Table 2).

In addition to reducing biting densities of culicine mosquitoes, the PermaNet also prevented blood feeding by mosquitoes on the users sleeping inside the nets. In the torn mosquito-net tests relatively fewer mosquitoes entered the PermaNet net and more importantly none succeeded in feeding on the sleeper before dying (Table 3). In com-

Table 1
WHO cone bio-assay (3 minutes exposure) test results using PermaNet® 2.0 nets after repeated washings[a].

Mosquito species[b]	24-hour post exposure mean % corrected mortality (Range) on PermaNet® 2.0 nets after no. of washings[c]				
	0	1-5	6-10	11-15	16-22
An. minimus s.l.	100	86(75-100)	82.7(60-100)	72.5(58.3-91.1)	47.6(27.2-68.0)
An. philippinensis /nivipes	100	87.6(80-100)	ND	ND	ND

ND = Not done
[a]Two nets were hand-washed in the laboratory at 12-day intervals using 2 g of Surf Excel® Quick wash detergent per liter of cold water for each net. WHO cone bio-assays were performed 6-7 days after each wash.
[b]Wild caught and sugar fed females.
[c]Mean % corrected mortality for a set of 5 number of washes.

Table 2
Mean number of landing mosquitoes caught per bait per night in houses equipped with PermaNet® 2.0 nets or untreated nets[a].

Mosquitoes	Mean densities (± SD) of landing mosquitoes in		Significance level[b]
	PermaNet® 2.0 net house	Untreated net house	
All mosquitoes	49.6 ± 49.2	105.1 ± 107.1	$p < 0.001$
All culicines[c]	36.8 ± 47.8	97.6 ± 104.4	$p < 0.001$
All anophelines[d]	12.8 ± 14.3	7.5 ± 3.0	$p = 0.016$
An. minimus s.l.	10.5 ± 11.5	3.8 ± 2.5	$p = 0.007$

[a]6 night collections (1 night per month) in each category of house between August 2005 and March 2006. No collections made during December 2005 and January 2006 due to operational reasons.
[b]*t*-test
[c]12 species (*Culex bitaeniorhynchus, Cx. fuscocephala, Cx. gelidus, Cx. pseudovishnui, Cx. quinquefasciatus, Cx. tritaeniorhynchus, Cx. vishnui, Cx. whitmorei, Mansonia annulifera, Ma. uniformis, Ma. dives, Aedes* sp)
[d]11 species (*Anopheles aconitus, An. culicifacies, An. dirus* s.l., *An. hyrcanus* gp, *An. jeyporiensis, An. maculatus* gp, *An. minimus* s.l., *An philippinensis /nivipes, An. splendidus, An. vagus, An. varuna*)

parison, 73% mosquitoes that entered the torn untreated nets fed successfully on the sleeper and all remained alive till morning.

User response to the PermaNet net

Altogether 27 adult users of PermaNet nets were interviewed for their perceptions regarding the nets. More than half the users (56%) complained of mild tingling/burning sensation of the face (7/15), hands (4/15) or upper body (4/15) after sleeping under the nets in the begining. The burning sensation was self limited and in the majority (93%) disappeared by 2-4 days. No severe adverse

Table 3
Mosquito blood-feeding inhibition by PermaNet® 2.0 net[a].

Type of net	Abdominal condition of mosquitoes	Mosquitoes collected in the morning inside the net			
		Live		Dead	
		Culicines	Anophelines	Culicines	Anophelines
Untreated net[b]	Full fed	7	1	0	0
	Unfed	2	1	0	0
PermaNet® net[b]	Full fed	0	0	0	0
	Unfed	0	0	3	2

Culicines includesd *Cx. pseudovishnui, Cx. vishnui, Cx. quinquefasciatus*
Anophelines included *An. aconitus, An. minimus* s.l., *An. vagus*
[a]Cumulative data for August, September, October and November, 2005.
[b]Nets having six holes (4 x 4 cm), simulating torn nets, with one sleeper inside.

effects were reported by users. Nearly all respondents (93%) were appreciative of the nets for the collateral benefits of killing head lice, cockroaches, ants, house flies, crickets and spiders after using the nets in their houses. The good quality yarn was also noted by all users.

DISCUSSION

Favorable climatic conditions for malaria transmission during most of the year, efficient multiple vector species, geographical features, such as an abundance of hills, foot-hills, forested inaccessible areas and poor surface communication, make control of malaria logistically difficult in northeastern India. Although ITNs have shown promise in controlling malaria in this part of the country (Jana-Kara *et al*, 1995; Prakash *et al*, 2003) retreatment, especially in remote areas, poses a serious operational challenge. LLINs, requiring no re-treatments due to wash resistance have the potential to meet this challenge, making this study important.

We found good wash-resistance with PermaNet, maintaining a good level of efficacy against *An. minimus* s.l. and *An. philippinensis/nivipes*. Nearly 82% mortality was noted against *An. minimus* s.l. after 10 hand washes of PermaNet nets. This declined to 72% after 15 washes, then 47% after 22 washes. These results confirm the long lasting insecticidal action of these nets, yet indicate relatively more rapid loss of insecticidal activity after repeated washing in the climatic conditions of Assam, compared to results obtained elsewhere. Graham *et al* (2005) reported an approximately 82% knock down and mortality rate after 20 washings of the PermaNet net in 3-minute exposure studies in Pakistan against *An. stephensi*. This dropped to 43.2% after 30 washes. Similarly, Sreehari *et al* (2007a) recorded >80% mortality of *An. culicifacies* and *An. stephensi* after 20 washes of the PermaNet in India near Delhi. Kroeger *et al* (2004) found 80% mortality of *An. nunentovari* and *An. rangeli* after 23 washes and 3 years of field use of PermaNet in Columbia. In western Kenya, 82.2% mortality of *An. gambiae* was recorded after 2 years of field use of the PermaNet

(Lindblade *et al*, 2005). There are 3 possible reasons for the more rapid loss of insecticide action with the PermaNet in the present study: 1) different vector species and different susceptibilities to deltamethrin, 2) use of higher amounts of detergent per wash. We used 2 g of detergent per liter with each wash. In other studies (Graham *et al*, 2005; Sreehari *et al*, 2007a) the quantity of detergent ranged from 0.6 to 1.0 g/liter. Kayedi *et al* (2007a) speculated various washing conditions and the use of different detergents and soaps in the wash resistance tests with LLINs in various laboratories and field conditions contributed to different results with the same product causing difficulty in comparing results; 3) relatively longer intervals between successive washes: 12 days in the present study compared to 2-6 days in other studies (Graham *et al*, 2005; Sreehari *et al*, 2007a) allowing higher diffusion of deltamethrin from inside to the outer surfaces of the fibers thereby making it more susceptible to removal with the next wash (Kayedi *et al*, 2007b).

We recorded significantly reduced biting densities of culicines when PermaNet nets were used in houses but surprisingly this effect was not evident with anophelines or *An. minimus* s.l., It may be that culicines in general, and *Cx. pseudovishnui* and *Cx. quinquefasciatus* in particular, have lower excito-repellent thresholds than the anophelines against the deltamethrin insecticide impregnated in PermaNet nets. This finding needs to be further investigated. In other field studies, a significant reduction in indoor resting *An. culicifacies* and *An. stephensi* (Sreehari *et al*, 2007a), *An. gambiae*, *An. funestus* and *Cx. quinquefasciatus* (Dabire *et al*, 2006) mosquitoes with using PermaNet nets was found in comparison to untreated or no net houses.

In spite of higher biting densities of *Anopheles* in houses using PermaNet® 2.0, the nets protected the persons sleeping inside them by killing the mosquitoes which came in contact with the nets. Although this study was not designed to evaluate this aspect, PermaNet® 2.0 nets appear to prevent blood feeding by mosquitoes, including anophelines and kills those which entered through holes before feeding, thus, protecting the sleepers from getting bitten by hungry mosquitoes. This fact is corroborated by the observation that during the 8 months of the study period no malaria positive cases were recorded from the Tanti Basti section of the village (where the present study was carried out) by the case detection mechanism of the primary health center, whereas this section used to report a good number of malaria cases in the past, mostly due to *Plasmodium falciparum* (RMRC-unpublished data).

PermaNet® 2.0 nets had reasonably good wash resistance for up to 15 washes against *An. minimus* s.l, the major malaria vector in northeastern India under the environmental conditions of Assam. PermaNet® 2.0 nets reduced the biting densities of culicines in houses and inhibited blood feeding by mosquitoes. They were accepted by the community. Large scale studies are warranted to study the bio-efficacy of PermaNet® 2.0 nets after long term use in field conditions using local washing practices to confirm its insecticidal efficacy against different malaria vector species found in northeastern India and their impact on malaria transmission.

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