World Health Organization

GUIDELINES

FOR LABORATORY AND FIELD-TESTING OF LONG-LASTING INSECTICIDAL NETS

Guidelines for laboratory and field-testing of long-lasting insecticidal nets

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The Department would greatly appreciate receiving feedback and suggestions from national programmes, research institutions and industry on the guidelines in order to improve future editions.

1. Introduction

Guidelines for testing long-lasting insecticidal nets (LNs) were first published by WHO in 2005.[1] The original guidelines were designed for pyrethroid-treated nets and were based on the state of knowledge and LN technology at the time. Considerable experience in testing LNs has since been gained, and WHO recently published additional guidelines for monitoring the durability of LNs under operational conditions.[2] The current document represents a synthesis of those two documents and includes revisions based on lessons learnt in evaluating LNs. Furthermore, as pyrethroid resistance increases and threatens to undermine the efficacy of the current generation of pyrethroid-treated LNs, it is expected that LNs containing new insecticides and/or synergists will become available for use in the near future. This document also includes recommendations from the Fifteenth WHO Pesticide Evaluation Scheme (WHOPES) working group meeting held in Geneva, 18–22 June 2012, to evaluate LNs containing new insecticides or mixtures of insecticides.[3]

The revised guidelines were reviewed by a WHOPES informal consultation on innovative public health pesticide products, held at WHO headquarters on 22–26 October 2012. Industry was invited to attend the first 2 days of the meeting to exchange information and provide their views, after which their comments were further reviewed by a group of WHO-appointed experts, who finalized the guidelines by consensus.

The purpose of this document is to provide specific, standardized procedures and guidelines for testing LNs for personal protection and malaria vector control. It is intended to harmonize testing procedures in order to generate data for registration and labelling of such products by national

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[1] *Guidelines for laboratory and field testing of long-lasting insecticidal mosquito nets*. Geneva, World Health Organization, 2005 (WHO/CDS/WHOPES/GCDPP/2005.11).

[2] *Guidelines for monitoring the durability of long-lasting insecticidal mosquito nets under operational conditions*. Geneva, World Health Organization, 2011 (WHO/HTM/NTD/WHOPES/2011.5).

[3] *Report of the Fifteenth WHOPES working group meeting: WHO/HQ, Geneva, 18–22 June 2012: review of Olyset plus, Interceptor LN, Malathion 440 EW, Vectobac GR*. Geneva, World Health Organization, 2012.

authorities and provide a framework for industry in developing novel LN products. This document replaces the previous guidelines, published by WHOPES in 2005.[4]

An LN is a factory-treated mosquito net that is expected to retain its biological activity for a minimum number of standard washes and a minimum period of use under field conditions. Currently, an LN would be expected to retain its biological activity for at least 20 standard washes under laboratory conditions and 3 years of recommended use under field conditions, as defined in these guidelines.

The document describes laboratory and small- and large-scale field studies undertaken to determine the efficacy and operational acceptability of LNs. It is largely based on requirements for testing LNs containing WHO-recommended pyrethroids for treatment of mosquito nets.[5] Although some observations on the safety of such nets can be made in the field, a preliminary safety assessment must be undertaken with the generic risk assessment model devised by WHO for this purpose,[6] before any field study can be done.

Products submitted for laboratory studies and/or field trials should be accompanied by a material safety data sheet and the manufacturer's certification that the product meets their specifications or WHO specifications, when available. Independent physical and chemical analysis of the products for compliance with specifications in an accredited, qualified laboratory may be required before efficacy studies are initiated.

Biological tests are subject to variation. Studies should therefore be conducted under the close supervision of personnel familiar with the biological testing of LNs and with

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[4] *Guidelines for laboratory and field testing of long-lasting insecticidal mosquito nets*. Geneva, World Health Organization, 2005 (WHO/CDS/WHOPES/GCDPP/2005.11).

[5] For testing LNs containing novel insecticides, synergists and insecticide mixtures, see section 5.

[6] *A generic risk assessment model for insecticide-treated nets – revised edition.* Geneva, World Health Organization, 2012 (WHO/HTM/NTD/WHOPES/2012.3; http://whqlibdoc.who.int/publications/2012/9789241503419_eng.pdf).

sound scientific and experimental procedures. Standardized procedures and good quality assurance systems are essential for generating high-quality, reproducible data. The advice of an experienced statistician on design and analysis should be considered.

Studies on LNs should be undertaken in accordance with national ethical regulations. WHO guidelines for preparing an informed consent form are given in Annex 1 for experimental hut studies and Annex 2 for phase III field studies. The main parameters assessed in phase I–III studies are summarized in Table 1.

Table 1. Main parameters assessed in phase I, II and III studies of long-lasting insecticidal mosquito nets

Phase	Type of study	Parameters measured
I	Laboratory	Regeneration of insecticidal activity
		Efficacy and wash-resistance
II	Small-scale field trial	Wash-resistance
		Efficacy as measured by vector mortality and blood-feeding inhibition
III	Large-scale field trial	Long-lasting insecticidal efficacy
		Rate of loss or attrition of nets
		Physical durability of netting material
		Community acceptance
		Safety

Epidemiological end-points are not included in WHOPES evaluations of LNs treated with WHO-recommended pyrethroids. Proof of principle may, however, be required before WHOPES reviews LN products with a mode of action different from that of pyrethroid insecticides.

2. Laboratory studies (phase I)

The objectives of laboratory testing are to determine the efficacy and wash-resistance of an LN and to study the dynamics of the insecticide on the netting fibre, including regeneration time (the time required to restore the biological efficacy of a net when the surface insecticide has been

depleted by washing). The aim of these experiments is not to simulate washing under field conditions but rather to provide a standardized protocol to allow consistent comparisons among laboratories and among different LN products.

The test includes determination of:

- the time required for insecticidal regeneration of the LN after washing and
- the efficacy and wash-resistance of the LN against a fully susceptible *Anopheles* vector species.

2.1 Regeneration time and wash-resistance

2.1.1 Preparation of nets for testing

Four candidate LNs are required for phase I studies, from at least two different production batches. From each net, 14 pieces (25 cm x 25 cm) are sampled, as shown in Figure 1.

The tests conducted on the 56 pieces are as follows:

- Eight pieces (four unwashed and four washed) are used to estimate regeneration time (section 2.1.2).
- 28 net pieces are used to evaluate wash-resistance (section 2.1.3). Four pieces are tested after 1, 3, 5, 10, 15, 20 and 25 washes (4 x 7 = 28 bioassays), although only 20 washes are considered standard procedure for determining wash-resistance. If the manufacturer's claim cites more than 20 washes, additional net pieces may be cut and used for further washing and bioassays. After bioassays, the net pieces are tested in chemical assays to determine the wash-resistance index (section 2.1.3).
- 20 pieces (five pieces from four nets) are wrapped in aluminium foil and held at 4 ^{o}C for chemical analysis in order to determine the between- and within net variability.

Net pieces should be handled with care to avoid contamination or excessive abrasion. Nets should be stored wrapped in aluminium foil at 30 ^{o}C between washes.

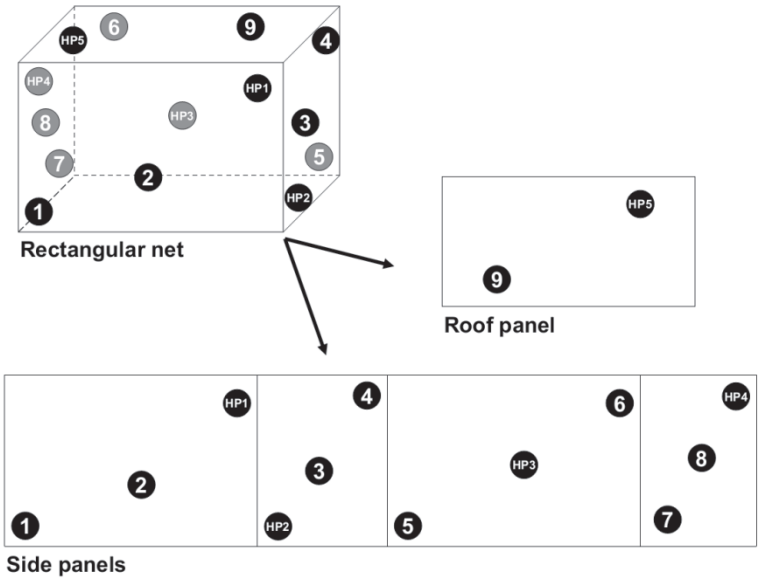

Figure 1. Sampling scheme for 14 pieces of netting from each net, including positions HP1–HP5 for chemical assay. A different sampling scheme is required for combination nets (section 5.4).

2.1.2 Regeneration time

Washing removes insecticide from the surface of the LN, but it is replenished over time by migration from within the fibres. The 'regeneration time' is that required to restore an effective insecticide level; it must be estimated in order to determine the washing frequency in wash-resistance testing. As there are no chemical methods to measure the level of surface insecticide on an LN reliably, regeneration is assessed indirectly in bioassays.

To determine the time necessary to regenerate insecticide in an LN after a standard wash and holding at 30 °C, bioassays are first conducted on unwashed net samples; then, the net samples are washed and dried three times consecutively in a single day to deplete the insecticide on the net surface and held at 30 °C. The nets are then subjected to WHO cone bioassays on days 1, 2, 3, 5, 7 and longer if necessary after the final wash. 'Efficacy curves' (of 24 h mortality and 60 min

knock-down) are established by exposing susceptible *Anopheles* mosquitoes for 3 min in cone bioassays. The time required (in days) to reach a plateau is considered the regeneration time. If the knock-down and mortality curves are different, the longer time will be adopted as the washing interval for phase I and II studies. Details of the standard washing procedure are given in section 2.1.4, and cone bioassays are described in section 2.2.1.

2.1.3 Wash-resistance

The resistance of an LN to washing is determined in standard bioassays with nets washed at intervals corresponding to the regeneration time (as determined above), by the standard wash procedure. Bioassays should be conducted after 0, 1, 3, 5, 10, 15, 20 and 25 washes or more according to the number of washes stated in the manufacturer's claims (see Figure 2). One piece of net is selected randomly from each of four nets. The efficacy of unwashed nets is tested, but these pieces are not used for chemical assay (see section 2.1.1 for a sampling plan for chemical assay of unwashed pieces). Each of the remaining pieces is washed once, three, five, 10, 15, 20 or 25 times, tested for efficacy and stored at 4 °C for chemical analysis.

Each bioassay should be performed just before the next wash. For practical reasons, LNs are not washed during a weekend but are stored at 30 °C until the next wash (5 days of washes plus 2 days of storage). Knock-down and mortality of mosquitoes should be plotted against the number of washes, and the number of washes after which mortality or knock-down is above the cut-off point (≥ 80% mortality after 24 h or ≥ 95% knock-down 60 min after exposure) is reported. If the efficacy of an LN falls below the cut-off point, the study should be continued until 20 washes are reached, and then a tunnel test (see section 2.2.2) should be conducted.

Net pieces[a]	Number of standard washes																									
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
4	Efficacy of unwashed nets tested[b]	Washed once, efficacy tested and net pieces stored at 4 ^{0}C for chemical analysis																								
4	Washed 3 times, efficacy tested and pieces stored at 4 ^{0}C for chemical analysis																									
4	Washed 5 times, efficacy tested and pieces stored at 4 ^{0}C for chemical analysis																									
4	Washed 10 times, efficacy tested and pieces stored at 4 ^{0}C for chemical analysis																									
4	Washed 15 times, efficacy tested and pieces stored at 4 ^{0}C for chemical analysis																									
4	Washed 20 times, efficacy tested and pieces stored at 4 ^{0}C for chemical analysis																									
4	Washed 25 times, efficacy tested and pieces stored at 4 ^{0}C for chemical analysis																									

[a] One piece is selected randomly from each of the four nets

[b] These unwashed pieces are not used for chemical assay; see section 1.1.1 for sampling plan for chemical assay of unwashed pieces

Figure 2. **Scheme for washing of nets to determine wash resistance and insecticide retention rates**

2.1.4 Washing procedure[7]

Net samples (25 cm x 25 cm) are introduced individually into 1-l beakers containing 0.5 l deionized water, with 2 g/l soap[8] (pH 10–11) added and fully dissolved just before washing. The beakers are introduced into a water-bath at 30 °C and shaken for 10 min at 155 movements per minute. The samples are then removed, rinsed twice for 10 min in clean, deionized water under the same shaking conditions as above, dried at room temperature and stored at 30 °C in the dark between washes.

2.2 Efficacy

The efficacy of nets is determined in standard WHO cone bioassays and, if necessary, tunnel tests. All bioassays should be conducted with a strain of *Anopheles* mosquitoes that is fully susceptible to the insecticide used in the candidate LN. The susceptibility of the mosquito colony should be confirmed every 6 months, by the procedures described in the WHO guidelines.[9]

2.2.1 WHO cone bioassays

Five susceptible, non-blood-fed, 2–5-day-old female *Anopheles* (species to be stated in the test report) mosquitoes are exposed to each piece of netting (25 cm x 25 cm) for 3 min under standard WHO cones (Figure 3), after which they are held for 24 h with access to sugar solution. Knock-down is recorded 60 min after exposure and mortality after 24 h. One

[7] A standard washing method has been devised by the Collaborative International Pesticides Analytical Council (CIPAC) in collaboration with WHO for development of WHO specifications and for determining the insecticide retention index for use in quality control of LNs. Further validation of the method for use in determining biological efficacy in laboratory studies is required. The CIPAC method is described in Annex 3.

[8] Currently, savon de Marseille is recommended as the standard soap for efficacy studies in phase I.

[9] *Guidelines for testing mosquito adulticides for indoor residual spraying and for treatment of mosquito nets*. Geneva, World Health Organization, 2006 (WHO/CDS/NTD/WHOPES/GCDPP/2006.3; http://whqlibdoc.who.int/hq/2006/WHO_CDS_NTD_WHOPES_GCDPP_2006.3_eng.pdf, accessed 7 February 2013).

piece each from four different nets should be tested. Up to four cones at a time may be attached to a piece of netting, and five mosquitoes at one time should be exposed in a cone. This procedure should be repeated until a total of 50 mosquitoes have been exposed to each piece. Results should be reported for each net tested and for the four nets (4 pieces x 10 cone tests x 5 mosquitoes = 200 mosquitoes). Mosquitoes exposed to untreated net pieces are used as controls; they should be tested each day, just before and just after testing treated netting material. If the mortality in controls on any day is < 10%, the results for that day should be adjusted by Abbott's formula.[10] If the mortality in controls is > 10%[11] on a given day, the results for that day are considered invalid and should be discarded. Bioassays should be carried out at 27 ± 2 °C and 75% ± 10% relative humidity.

Figure 3. Cone bioassay of long-lasting insecticidal mosquito nets. Courtesy of Dr Vincent Corbel, Institut de Recherche pour le Développement, Montpellier, France. The holding board is held slanted at 45°.

[10] Abbott's formula: Adjusted mortality (%) = 100 x (*X*–*Y*) / (100–*Y*), where *X* is the percentage mortality with the candidate LN, and *Y* is the percentage mortality with the untreated control sample.

[11] In the previous WHO guidelines, a study was considered to be invalid when mortality among controls was > 20%. The present guideline recommends a maximum of 10% mortality in control as the criterion for correction.

The definitions of mortality and knock-down are those recommended by WHOPES.[12] Mosquitoes are considered to be alive if they can both stand upright and fly in a coordinated manner. Mosquitoes that are moribund or dead are classified and recorded as knocked down at 60 min and as dead at 24 h. A mosquito is moribund if it cannot stand (e.g. has one or two legs), cannot fly in a coordinated manner or takes off briefly but falls immediately. A mosquito is dead if it is immobile, cannot stand or shows no sign of life.

A sample data collection sheet for cone bioassays is provided in Annex 4.

Nets washed at least 20 times that do not meet the criteria in the WHO cone test in phase I (section 2.3) should undergo tunnel tests, as described in section 2.2.2.

The bioavailability curves of coloured LNs should be compared with those of white nets of the same brand. If they are found to be significantly different, they should be considered a separate product, requiring full testing and evaluation.

2.2.2 Tunnel tests

The efficacy of treated nets may be underestimated if judged based on the outcome of standard cone bioassays. This is true particularly for insecticides that have a high excito-repellent effect, such as permethrin and etofenprox. In such cases, the efficacy (mortality and blood-feeding inhibition) of LNs washed 20 times or more that no longer meet the criteria in standard cone bioassays should be studied in a tunnel in the laboratory. The netting piece that results in mortality closest to the mean mortality in the cone bioassay is used in the tunnel test.

The tunnel test is used to measure the mortality and blood-feeding success of host-seeking mosquitoes in an experimental chamber (Figures 4 and 5). The assay is carried out in a laboratory by releasing non-blood-fed female anopheline

[12] *Report of the Fifteenth WHOPES working group meeting, 18–22 June 2012*. Geneva, World Health Organization, 2012 (WHO/HTM/NTD/WHOPES/2012.5; (http://apps.who.int/iris/bitstream/10665/75304/1/9789241504089_eng.pdf).

mosquitoes aged 5–8 days into a 60-cm tunnel (25 cm x 25 cm square section) made of glass.[13] At each end of the tunnel, a 25-cm square cage covered with polyester netting is fitted (extension). The LN netting sample, held in a disposable cardboard frame, is placed at one third the length of the glass tunnel. The surface of netting available to the mosquitoes is 400 cm^2 (20 cm x 20 cm), with nine holes 1 cm in diameter; one hole is located at the centre of the square, and the other eight are equidistant and located 5 cm from the border. In the shorter section of the tunnel (Figure 5, area C2), a suitable bait (e.g. guinea-pig or rabbit) is placed, which is unable to move and is available for mosquito biting. One hundred female mosquitoes are introduced into the cage at the end of the longer section of the tunnel (Figure 5, area C1). They are free to fly in the tunnel but have to make contact with the piece of netting and locate the holes in it before passing through to reach the bait. After taking a blood meal, the mosquitoes may fly back to the cage at the end of this compartment and rest. A tunnel with untreated netting is always used as a negative control.

Figure 4. Tunnel made of glass for studying the efficacy of long-lasting insecticidal nets. Courtesy of Dr Vincent Corbel, Institut de Recherche pour le Développement, Montpellier, France

[13] The tunnel should be made of glass and not made of absorbent material.

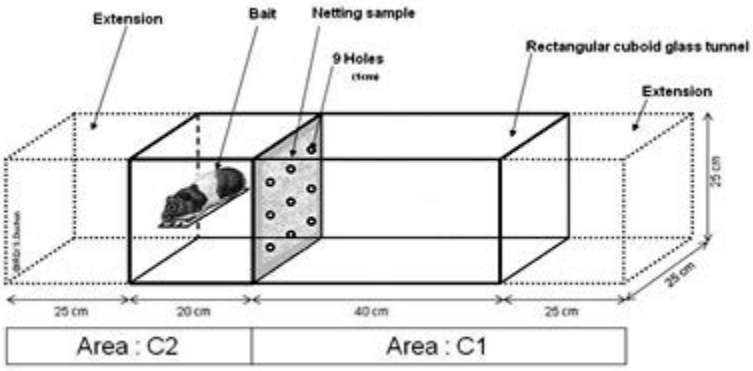

Figure 5. Tunnel used for studying the efficacy of long-lasting insecticidal nets, with extensions on either side to fix mosquito cages. Courtesy of Dr Stéphane Duchon, Institut de Recherche pour le Développement, Montpellier, France

During the tests, the tunnels and cages are held at 27 ± 2 °C and 75% ± 10% relative humidity at night in full darkness. After an exposure of 12–15 h, the mosquitoes are removed from each section of the tunnel with a glass suction tube and counted separately; mortality and blood-feeding rates are recorded. Blood-feeding inhibition is assessed by comparing the proportion of blood-fed females (alive or dead) in treated and control tunnels. Overall mortality is measured by pooling the mortality rates of mosquitoes from the two sections of the tunnel.

Mortality on the LNs should be corrected for mortality in the controls with Abbott's formula. If mortality in the controls is > 10%, the test should be considered invalid.

As blood-feeding by controls has a considerable effect on mortality in the presence of treated samples (i.e. the host-seeking behaviour increases the chance of contact with treated fabric), a 50% minimum cut-off value of the blood-feeding rate in controls should be established for tunnel tests.

A sample data collection sheet for tunnel tests is provided in Annex 5.

2.2.3 Chemical analysis

After cutting or testing, all netting samples should be properly labelled, wrapped individually in aluminium foil and stored at 4 °C until they can be analysed for their insecticide content to determine their wash-resistance index.[14] The insecticide content of each net sample should be analysed to estimate between- and within-net variation, and the density of netting (i.e. mass of net per unit area) should be measured. The samples should be analysed by the methods published by the Collaborative International Pesticides Analytical Council (CIPAC)[15] or, if those are unavailable, with tests devised by the manufacturer and validated. The results should be expressed in grams of active ingredient per kilogram as well as in milligrams of active ingredient per square metre of netting material. The decrease in insecticide content after successive washes can be used to estimate the wash-resistance index of the LN.[16]

2.3 Efficacy criteria for phase I studies

Nets washed at least 20 times that meet the criteria of WHO cone bioassays (≥ 80% mortality or ≥ 95% knock-down) or of the tunnel test (≥ 80% mortality or ≥ 90% blood-feeding inhibition) meet the criteria for undergoing phase II testing.

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[14] *Report of the Eleventh WHOPES working group meeting, 10–13 December 2007*. Geneva, World Health Organization, 2007 (WHO/HTM/NTD/WHOPES/2008.1).

[15] *http://www.cipac.org/cipacpub.htm.*

[16] The wash resistance index (w) is expressed as a percentage by the following formula:

$$w = 100 \times \sqrt[n]{(t_n/t_0)}$$

Where, n = number of washes, tn = total active ingredient content (in g/kg) after n washing cycles; t0 = total active ingredient content (in g/kg) before washing of nets (no washing).

3. Small-scale field trials (phase II)

Candidate LNs that meet the requirements of phase I testing should subsequently be tested in phase II studies in experimental huts, where the efficacy of LNs against free-flying, wild mosquitoes in terms of inhibiting blood-feeding, deterring mosquitoes and inducing exophily and mortality are assessed. While it is recommended that phase II studies be conducted in areas where mosquitoes are susceptible to pyrethroids, it is recognized that resistance to pyrethroids is expanding rapidly and that there may be no areas with fully susceptible vector populations in the future. Studies conducted in areas with pyrethroid-resistant mosquitoes can provide equally valuable information, as the results can be compared with positive control LNs.

In the sites where phase II studies are to be carried out, the susceptibility of the wild vector population to the active ingredient in the candidate LN product must be evaluated at the beginning of the study by the procedures detailed in WHO guidelines.[17]

Once resistance has been detected at a study site, the intensity or strength of resistance should be measured by exposing field samples to a range of doses, in order to establish whether individuals in the local population can survive very high doses, which would compromise the effectiveness of the candidate LN.

It is also helpful, as background information, to measure the frequency of *kdr* alleles in the local population. It is not easy to measure the population frequency of metabolic resistance mechanisms, but testing with synergists can help to establish the presence of metabolic resistance.

[17] *Guidelines for testing mosquito adulticides for indoor residual spraying and for treatment of mosquito nets*. Geneva, World Health Organization, 2006 (WHO/CDS/NTD/WHOPES/GCDPP/2006.3; http://whqlibdoc.who.int/hq/2006/WHO_CDS_NTD_WHOPES_GCDPP_2006.3_eng.pdf, accessed 7 February 2013).

3.1 Design of experimental huts

Experimental huts allow evaluation of LNs under controlled conditions that resemble those in which mosquitoes enter a human habitation and contact an LN in normal use. Experimental huts have structural features that enable collection of live, exiting or dead or dying mosquitoes without loss. Reductions in mosquito biting and blood-feeding attributed to the LN and the mortality among host-seeking mosquitoes that enter the huts represent the most accurate estimates of personal protection or mosquito mortality.

Experimental huts are designed to resemble local housing but have design features in common:

- The huts are identical, each having eave gaps or entry slits that allow host-seeking mosquitoes to enter and forage, and are arranged or positioned in an array in proximity to mosquito breeding sites to allow a uniform rate of entry into each hut.
- A water-filled channel surrounds each hut to prevent entry of ants that would scavenge killed mosquitoes, which would result in underestimates of mosquito mortality.
- Each hut has traps at exits (window or veranda) to capture exiting mosquitoes, allowing accurate estimates of the population, blood-feeding rates and repellency rates.

Several LN treatments can thus be tested simultaneously, with positive and negative controls, allowing a full assessment of efficacy after a range of washes.

Experimental huts have several physical designs. Each of those described below has been tested in a suitable geographical region and shown to be effective for sampling local mosquito populations. The hut design may be adapted, or novel designs may be used in different settings. Some huts may require a period of acclimatization before use in trials, as mosquitoes may be reluctant to enter newly constructed huts.

Before an experimental hut study is started, it is important to ensure that adequate numbers of mosquitoes will be attracted and that all the huts are equally attractive, with little or no bias due to position. In phase II studies, baseline information should be obtained on the attractiveness of the huts and the recapture rates of live and dead mosquitoes released in the huts. Contact bioassays should be conducted on the walls to rule out contamination that could affect the outcome of the study. If the huts were used recently to evaluate a residual insecticide, replastering of the walls is recommended. All mosquitoes collected during the study should be preserved in a desiccant or other medium (e.g. silica gel, ethanol) and labelled with the location of collection in the hut, the intervention, and the status of the mosquitoes at the time of collection (dead or alive, blood-fed or unfed) for quality control and future studies of genetic markers of insecticide resistance.

3.1.1 West African-style huts

Huts of the typical West African style are made from concrete bricks with a corrugated iron roof, a ceiling of polythene sheeting and a concrete base surrounded by a water-filled moat to prevent the entry of ants (Figure 6). Mosquitoes can enter the huts through four window slits constructed from pieces of metal fixed at an angle to create a funnel with a 1-cm gap. The design of the window slits allows easy entry but greatly limits the egress of mosquitoes once they have entered the hut.

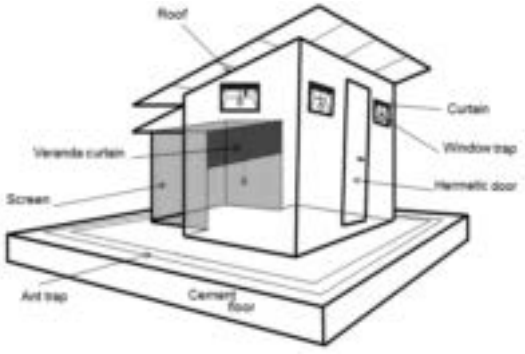

Figure 6. Design of the experimental huts commonly used in West Africa. Courtesy of Dr J.M. Hougard, Institut de Recherche pour le Développement, Benin

A veranda trap made of polythene sheeting and screening mesh (2 m long, 1.5 m wide and 1.5 m high) is fitted at the back of each hut. Mosquitoes are allowed to move unimpeded to and from the veranda trap during the night.

3.1.2 East African-style huts

East African style huts have veranda traps on all four sides, but two sides remain open each night to allow entry of mosquitoes[18] (Figure 7). The huts have brick walls plastered with mud on the inside, a wooden ceiling lined with hessian sackcloth or plastic sheeting, an iron roof, open eaves and window and veranda traps on each side.

The huts are built on concrete plinths and surrounded by a water-filled moat to deter entry of scavenging ants. Two opposite sides of the huts have closed verandas, screened to capture mosquitoes that leave via the eaves; the other two verandas are left open so that mosquitoes can enter through the eaves. The total number of mosquitoes collected in the hut is estimated by adding the number of mosquitoes collected in the main hut, window trap and twice those collected in the two veranda traps. The number of mosquitoes in a trap is multiplied by 2 to adjust for unrecorded escapes through the two verandas that were left unscreened to allow entry of wild mosquitoes through the 2-cm gaps under the eaves.

Figure 7. Design of the experimental huts commonly used in East Africa (United Republic of Tanzania). Courtesy of Professor C.F. Curtis, London School of Hygiene and Tropical Medicine, London, England

[18] Curtis CR, Myamba J, Wilkes TJ. Various pyrethroids on bednets and curtains. *Memorias do Instituto Oswaldo Cruz*, 1992, 87 (Suppl. III), 363–370.

At the end of each Latin square rotation, the north and south verandas are closed and the east and west sides opened, or vice versa, to compensate for possible selective exit in one compass direction. Modifications that have been tested recently include unidirectional eave baffles that funnel host-seeking mosquitoes into the hut but prevent them from exiting the other way, so that exiting mosquitoes are channelled into closed verandas or window traps.

3.1.3 Asian-style huts

Each hut measures 3 m x 3 m (Figure 8) and is built of wood on a concrete floor. The roof is covered with cement tiles with wooden ceiling. The front of the hut has four entry slits (0.75 m), two on each side of the door, and one long slit over the entire width of the front above the door (3 m). The back also has three entry slits (0.75 cm each), with two on the wall and one in the eaves between the wall and roof. A screened veranda is connected to the hut and can be closed by a door.

The hut is surrounded by a water-filled moat to avoid the entry of scavengers.

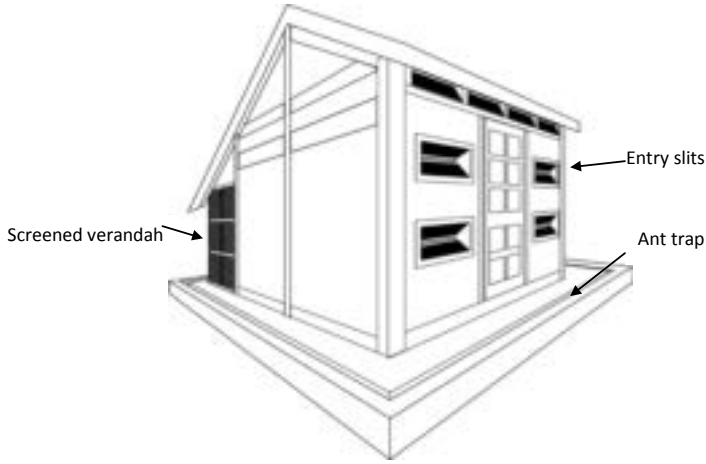

Figure 8. Design of the experimental huts commonly used in Asia. Courtesy of Dr Marc Coosemans, Institute of Tropical Medicine, Antwerp, Belgium

3.2 Study arms

Candidate LNs are compared with a negative control (an untreated net of the same or similar netting material, denier and mesh size) and a positive control (a WHOPES-recommended LN[19] with the same or similar specifications in terms of insecticide, treatment technique, netting material, denier and mesh size). One candidate LN and one positive control should be unwashed and one of each that has been washed 20 times. The study arms are therefore:

- untreated net of the same or similar material (if not available, a polyester net may be used);
- reference LN, unwashed (positive control);
- reference LN, washed 20 times (positive control);
- candidate LN, unwashed; and
- candidate LN, washed 20 times.

Additional arms consisting of candidate LNs washed more than 20 times, depending on the manufacturer's claims, may be included.

3.3 Preparation of nets

Six replicate nets should be prepared for each treatment arm. The nets should be from three different production batches. One net from each treatment arm should be retained for laboratory bioassays and chemical analysis. After five netting pieces have been removed for baseline bioassays and chemical assays, the net is washed 20 times (or more, depending on the manufacturer's claim) as described below; then bioassays and chemical assays are performed on pieces cut from adjacent positions. The other five nets are then washed 20 times (or more as appropriate) and used in the hut trial. At the end of the trial, one of the five nets from each treatment arm is selected at random for bioassays and chemical assays. Figure 9 shows net preparation schematically.

[19] The reference LN may have either a full or an interim recommendation. The specification number of the reference LN should be reported, but the brand name should not.

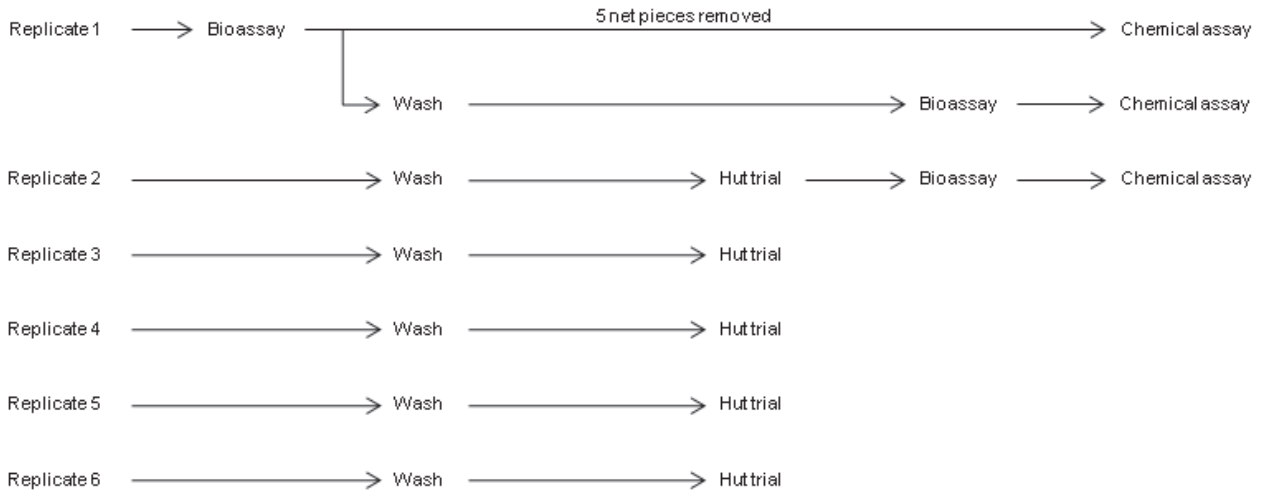

Figure 9. Washing, bioassay and chemical assay of each of six net replicates for candidate and reference long-lasting insecticidal nets in an experimental hut trial

The bioassay cones should be placed at the five positions indicated in Figure 10. Ten mosquitoes should be tested at each position, for a total of 50 mosquitoes tested against each net. The pieces that are used for bioassays should be stored individually in aluminium foil at 4 °C until chemical assays are performed.

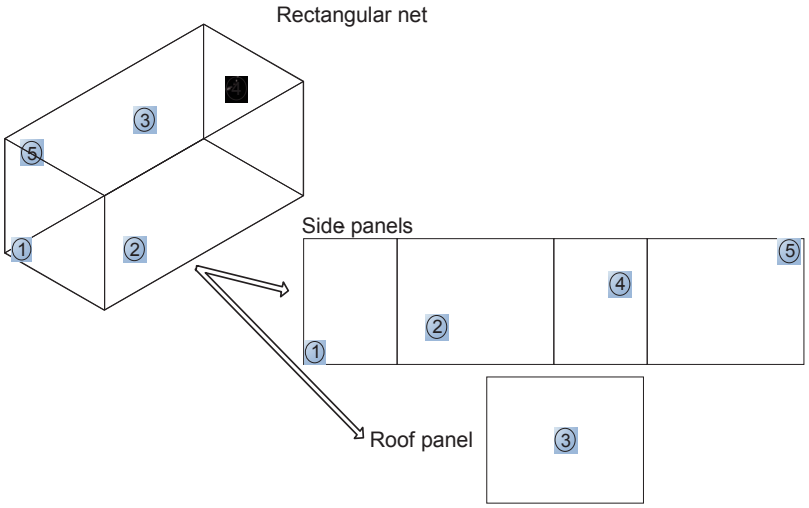

Figure 10. Recommended positions from which netting pieces should be taken

For washing, the nets should be placed in non-plastic bowls (e.g. aluminium) with 10 l of water and 2 g/l of soap such as savon de Marseille. The water should have a maximum hardness of 5 dh. The nets should be washed for a total of 10 min. It is suggested that the nets be agitated for 3 min, allowed to soak for 4 min and agitated again for 3 min. The nets should be agitated by stirring them with a pole at 20 rotations per minute. They should then be rinsed with clean water by a similar procedure, dried horizontally in the shade and stored at ambient temperature between washes.

The time between two consecutive washes should correspond to the regeneration time determined in phase I studies (see section 2.1.2).

Preparation of nets may take several weeks or even months for LN nets that have a long regeneration time and must be washed 20 times. To maintain equivalence between the various treatments in the trial, washing in each treatment arm should be completed at the same time, which means starting washing of long-regeneration nets weeks or months earlier than those with a shorter regeneration time or those that are washed fewer times.

Once washing has been completed, bioassays should be conducted again (as described above) on all nets. One net is removed from each treatment arm and retained for chemical analysis (Figure 9), and the remaining five nets are used in the experimental hut study. Six holes (4 cm x 4 cm) should be cut in each net, with two holes on each of the long side panels and one on each of the short side panels. The holes in the short side panels are located in the centre of the net, while those in the long side panels should be spaced evenly along the length of each panel; i.e. the first hole is made at one third of the distance and the second at two thirds of the distance from the edge of the side panel. The holes should be centred vertically on all sides of the net.

Results should be presented for nets before washing, after washing but just before the hut trial and after the end of the hut trial (Annex 6).

3.4 Latin square rotation of treatments, nets and sleepers

The number of huts and the number of sleepers required depends on the number of treatments being compared. In order to compare five treatment arms, the treatments are rotated among the huts weekly (with five treatment arms, five nights of study and two nights of break), and the sleepers are rotated through the huts each night. Also, each night, a different replicate net is used in each treatment arm to ensure that all the volunteers sleep under a net from each treatment arm

during each rotation. It also ensures that five different nets are tested in each arm during each rotation.

For a comparison of five treatment arms, 25 nights are required to complete a full Latin square rotation. For a comparison of six treatment arms, 36 nights are required (with six nights of study per week and one night of break). If there are few mosquitoes, two full rotations may be needed to achieve an adequate sample size.

At the end of each weekly rotation of nets, all the huts should be thoroughly cleaned and ventilated to avoid contamination among the treatment arms.

Rotation with a balanced Latin square design avoids potential contamination between treatments. A balanced six-treatment design with a rotation schedule in which each treatment follows every other treatment exactly once a week is as follows:

Period	Hut 1	Hut 2	Hut 3	Hut 4	Hut 5	Hut 6
1	1	2	3	4	5	6
2	2	3	4	5	6	1
3	6	1	2	3	4	5
4	3	4	5	6	1	2
5	5	6	1	2	3	4
6	4	5	6	1	2	3

A balanced Latin square cannot be constructed for odd-order squares, such as 3 × 3, 5 × 5. To achieve balance, two Latin squares are required, as illustrated below.

All treatments are preceded and followed by all other treatments twice. Therefore, addition of a sixth treatment may actually reduce the duration of an experimental hut trial, if adequate numbers of mosquitoes are captured in one rotation.

Period	Hut 1	Hut 2	Hut 3	Hut 4	Hut 5
1	1	2	3	4	5
2	2	3	4	5	1
3	5	1	2	3	4
4	3	4	5	1	2
5	4	5	1	2	3
6	4	5	1	2	3
7	3	4	5	1	2
8	5	1	2	3	4
9	2	3	4	5	1
10	1	2	3	4	5

3.5 Experimental procedures

Sleepers are recruited into a study preferably from the local area, and an information sheet (Annex 1) is given or read to them to apprise them of the procedures involved.

A bed or mattress should be placed in each hut and, at a specified time each night, sleepers should enter each hut. At least one sleeper is needed for each hut. To attract more mosquitoes, it may be desirable to have more than one sleeper inside each hut, although the number should be standardized for all huts. The sleepers should ensure that the nets are tied to walls with strings and tucked under the mattress and should then remain inside the huts until a specified time in the morning. Provisions such as water, food and a chamber pot may be provided to minimize the risk of sleepers leaving the huts during the night. Smokers should be excluded to avoid potential bias in the results.

At a specified time in the morning, mosquitoes should be collected from inside the hut. Dead and live mosquitoes are first collected from inside the nets. The verandas are then closed to prevent movement of mosquitoes between the different compartments. Then, dead and live mosquitoes are collected from inside the hut. Lastly, dead and live mosquitoes are

collected from inside the exit and veranda traps. Mosquitoes should be scored by location as dead or alive and as blood-fed or unfed. Live mosquitoes should be placed in small cups, given sugar solution and held at 25 ± 2 °C and 75 ± 10% relative humidity for 24 h to assess delayed mortality.

3.6 Outcome measures

The primary outcomes measured in experimental huts are:

- deterrence: the reduction in hut entry relative to control huts (untreated nets);
- exophily: the proportion of mosquitoes found in the exit and veranda traps;
- blood-feeding inhibition: the reduction in blood-feeding in comparison with the control huts (untreated nets); and
- immediate and delayed mortality: the proportions of mosquitoes entering the hut that are found dead in the morning (immediate mortality) or after being caught alive and held for 24 h with access to a sugar solution (delayed mortality).

Deterrence and blood feeding inhibition are indicators of personal protection. The personal protection effect of a treated net can be estimated from:

$$\text{Personal protection (\%)} = 100 \times (B_u - B_t) / B_u,$$

where B_u is the total number blood-fed mosquitoes in the huts with untreated nets and B_t is the total number of blood-fed mosquitoes in the huts with treated nets.

Mortality (immediate and delayed) is an indicator of the potential mass killing effect of the LNs, i.e. a reduction in the density and/or longevity of mosquitoes in an area with high net coverage, resulting in community-wide protection that also benefits those who are not using nets. The potential killing effect of a treated net can be estimated from:

$$\text{Killing effect (\%)} = 100 \times (K_t - K_u) / T_u,$$

where K_t is the number of mosquitoes killed in the huts with treated nets, K_u is the number of mosquitoes killed in the huts

with untreated nets, and T_u is the total number of mosquitoes collected from the huts with untreated nets.

A sample table for recording the primary outcomes measured in phase II trials is provided in Annex 7.

3.7 Statistical analysis

The primary analysis should be a test of the non-inferiority of the candidate LN washed 20 times relative to the standard LN washed 20 times (positive control). The non-inferiority margin shall be set subject to expert statistical advice.

The number of mosquitoes entering each hut is compared among the different treatment arms by Poisson or negative binomial regression or another non-parametric test, such as the Kruskal-Wallis test. The proportion of mosquitoes leaving early (induced exophily), the proportion that were blood-fed (blood-feeding rate) and the proportion that were killed (mortality rate) can be compared in logistic regression or generalized linear mixed models, which provide a framework for regression modelling of non-normal outcome data (such as mosquito mortality) while naturally adjusting for clustering effects. The models should be adjusted for the effects of sleepers and huts. Variance estimates should be adjusted for clustering by each hut-night of collection.

3.8 Perceived adverse effects

A risk assessment of LNs[20] is performed before phase II studies. Nevertheless, the sleepers in the huts should be questioned during the study about any perceived adverse or beneficial effects of each treatment. Volunteers should be asked to report any adverse events associated with use of treated nets and given medical care if necessary. Such observations can neither be associated with, nor are they intended to be associated with, participation in any particular

[20] *A generic risk assessment model for insecticide-treated nets – revised edition.* Geneva, World Health Organization, 2012 http://www.who.int/whopes/guidelines/en/; accessed 22 April 2012).

treatment arm. Phase II studies are not designed to evaluate the safety of LN products in the field. The small number and the rotation of sleepers preclude any association between a given LN product and adverse events.

3.9 Chemical analysis

Chemical analysis of nets should be done according to the sampling scheme presented in Figures 9 and 10. The chemical content and density (mass of net per unit area) should be analysed in each of the nets to estimate between-net variation. If it is necessary to analyse these parameters in each of the five net samples from a net, such as to correlate chemical content with bio-efficacy, density and within- and between-net variation should be estimated. The chemical analysis should be conducted with the methods published by CIPAC for each LN or, if unavailable, tests developed by the manufacturer and validated. Results should be expressed in both grams of active ingredient per kilogram of netting and milligrams of active ingredient per square metre of netting.

Results should be presented for nets before washing, after washing and after completion of the experimental hut study.

3.10 Ethical considerations

The experimental hut studies involve some risk, as volunteers are asked to sleep under nets in which holes have deliberately been cut. Furthermore, they are asked to sleep one night per week under nets that are untreated. Although the sleepers will probably be at lower risk than if they had not used a net, the investigators should minimize the risk. Only adults (excluding pregnant women) should be allowed to participate, and, depending on the setting, it may be advisable to allow only adult males to participate. Participants should be given chemoprophylaxis or prompt diagnosis and treatment of malaria with an effective antimalarial as appropriate. Routine blood smears and checking volunteers for malaria are also recommended. National ethical guidelines should be followed for the study.

An information sheet should be given or read to all sleepers participating in phase II studies, apprising them of the procedures involved. Written informed consent should be obtained. A generic consent form for sleepers is shown in in Annex 1. It is the responsibility of the principal investigators to obtain necessary clearances or waivers before starting experimental hut study.

3.11 Efficacy criteria for phase II studies

A candidate LN is considered to meet the phase II efficacy criteria if, after 20 washes, it performs as well as or better than the reference LN when washed 20 times in terms of blood-feeding inhibition and mortality. Such candidate LNs are given an interim recommendation.

4. Large-scale field trials (phase III)

Candidate LNs that pass phase I and phase II testing may receive an interim recommendation for use. For a full recommendation, however, a demonstration of durability (i.e. bio-efficacy, net survivorship or attrition and fabric integrity) for 3 years under field conditions is required. The objectives of phase III field studies are to demonstrate the longevity of the insecticidal activity of candidate LNs, to document their physical durability (i.e. fabric integrity) and survivorship and to assess their user acceptability.

4.1 General principles and site selection

Phase III studies are prospective 3-year studies to compare a candidate LN with a reference LN (positive control) according to general guidelines for monitoring durability. An LN is expected to retain a minimum three years of biological efficacy. Phase III studies may however be extended beyond 3 years to verify a manufacturer's product claims. A reference LN is included as a positive control to determine site-specific differences in net handling (e.g. use, washing frequency and severity) that may affect their durability. Comparison with a

reference LN will not be used to determine whether the candidate LN meets WHOPES efficacy requirements in phase III. In view of eco-epidemiological and socio-cultural differences among settings, which may affect LN efficacy over time, it is recommended that trials be conducted at a minimum of three sites.

4.2 Community sensitization and informed consent

When a community is selected and visited, the assistance of opinion leaders should be sought in order to obtain permission to use the community as a study site and to inform community members of the study's objectives and methods.

Written informed consent is required from all households at the time of the baseline census (section 4.3). An information sheet and consent form are suggested in Annex 2, which should be adapted and translated into the local language. For householders who cannot read the informed consent form, it should be read out and explained by a member of the investigating team in the local language in the presence of a literate community witness. Once they have consented, these people will be asked to mark a thumb impression on the form, and the witness will be asked to sign it.

It is important to advise potential participants that they can refuse to participate in the follow-up interview and may keep their LN. They should also be informed that even if they refuse to participate in the study, they may accept the nets. Participants should also be advised to seek medical care at the nearest health facility if they observe any sign or symptom of malaria or other vector-borne disease and any adverse effects of using the nets.

4.3 Baseline census

A census of all the households in the selected study sites should be carried out before LNs are distributed, to form the basis for random allocation of LNs and for sampling LNs at follow-up (Annex 8). At a minimum, the household census should record the name of the village, the name of the head of the household, the household identification number, the

number of adults and children living in the house, the number of nets already in the household and the number of sleeping spaces in the household. For ethical reasons, the names of households will not appear in the reports of the study. It is also recommended that the global positioning system (GPS) coordinates be recorded to assist in identifying houses during follow-up. For quality assurance, the name of the interviewer should also be recorded. The information should be entered into a computer database to serve as the master list (Annex 9) and sampling frame for subsequent LN monitoring. The master list should be updated after each monitoring round, with a record of households that are no longer present in the study or those that have been excluded from the study.

4.4 Study design

The objectives of phase III studies are to determine the duration of insecticidal activity, net survivorship or attrition, the fabric integrity of candidate LNs and user acceptability over 3 years. Attrition is defined as the rate of loss of LNs from study households due to wear and tear or other causes.

Assessment of insecticidal activity requires destructive sampling of nets for biological and chemical assays. For assessment of fabric integrity, however, LNs can be inspected in households. Longitudinal monitoring of individual nets throughout the study is preferable to their withdrawal, as the former allows monitoring of accumulated holes and net integrity for individual nets every 6–12 months until a point is reached when the net may be discarded by the user.

Households should be randomized into two groups. The nets of the first group will be assessed for insecticidal activity, and those of the second group will be assessed for elements of durability, such as changes in fabric integrity and rate of attrition. If this is not done, the same household may have some nets destined for destructive sampling and replacement for purposes of insecticide testing and also nets to be monitored for durability, which is likely to lead to errors in sampling. In practice, households in the two groups are sited close together, some being visited for monitoring of attrition and fabric integrity and others for sampling of nets for bioassays and chemical assays of insecticidal activity.

The estimate of the number of LNs for the study should be based on the sample size needed for longitudinal monitoring of attrition and fabric integrity and the sample size needed for 6-monthly assessment of insecticidal activity. A sample size calculation should be made for each study, taking into account assumptions about effect size, length of follow-up and degree of clustering specific to that study. This should be done in consultation with a statistician.

4.4.1 Monitoring attrition and fabric integrity

The sample size necessary to detect differences in attrition rate between LNs should be based on a conservative attrition rate to ensure that there are enough nets left to monitor at the end of 3 years. The sample size should also be adjusted for the effect of a cluster design (within-cluster correlation) of net assessment and for the expected attrition. When possible, the number of study LNs distributed to households should be in excess of that needed on the basis of the initial sample size calculation, to allow for any unexpected losses, such as family migration.

For example, a total of 250 LNs per product will allow detection of a 10% point difference in LN attrition rate if the best-performing product has an attrition rate of 10% per year. This sample size will also allow detection of a 12% point difference in LN attrition rate if the best-performing product has an attrition rate of 20%.

Assessment of integrity requires close inspection of each net, whereas attrition can be assessed relatively quickly by recording the presence or absence of nets from houses. The number of nets available for monitoring fabric integrity will decrease over time. With an attrition rate of 50% over 3 years, a sample of 150 LNs per product drawn from the master list will provide approximately 75 nets for fabric integrity measurement after 3 years, which is considered sufficient to detect major differences between products in a given setting. Measuring attrition in all 250 LNs would improve precision and is highly recommended.

The estimate of the total number of households for the insecticide-testing arm must make allowance for attrition rates

and for nets that are destructively sampled for testing during the study. To ensure that enough nets are available to estimate attrition rates and fabric integrity after 3 years of use, it is recommended that enough nets be distributed so that at least 250 nets in each arm could be included in the study cohort for measuring attrition rate and fabric integrity. Adjustment should be made for natural loss of households from the study area over the 3 years and for loss of nets for reasons other than physical attrition (e.g. given away to extended family members). Stable communities are to be preferred.

4.4.2 Assessment of insecticidal activity

From the households allocated for assessment of insecticidal activity, a subsample of nets should be randomly selected and withdrawn (after replacement with the same brand of LN) for testing. In previous trials, 30 nets per LN product at each time was found sufficient for bio-efficacy testing, but a larger sample (e.g. 50 nets per survey) will provide greater precision. Ideally, nets for bio-efficacy testing should be selected randomly from the net master list (Annex 9). The numbers of nets that should be sampled at baseline and at each follow-up during the study for measuring the attrition rate, fabric integrity, bio-efficacy and chemical content are shown in Table 2.

Table 2. Sample size required for each arm of the study at each follow-up for measuring attrition and fabric integrity and for testing bio-efficacy and chemical content

Months after distribution	Cohort of nets inspected		Number of nets withdrawn for bio-efficacy and or chemical assays
	Number of nets for attrition[a]	Number of nets for fabric integrity survey[b]	
0	–	–	30
6	250	150	30[c]
12	250	150	30
18	–	–	30[c]
24	250	150	30
30	–	–	30[c]
36	250	150	50
Total number of nets distributed	250	250	460[d]

Note that the table provides sample sizes for two different groups of nets under study. The first group will be followed-up for net attrition and field estimates of net integrity. These nets will not be withdrawn. The second group will be followed-up for bio-efficacy and chemical analysis and will require withdrawal of nets at each follow up.

[a] For net attrition in a 3-year study, a minimum of 250 nets are distributed. The same nets are followed-up in each survey as long as they are available with the households included in the study cohort. When nets are no longer present, the reason for their loss should be recorded.

[b] Field measurement of fabric integrity is done on a sub-sample of the nets distributed for assessment of attrition. Note that the sample size for fabric integrity represents a minimum target. Where possible, it would be ideal to measure fabric integrity on all nets that are followed for attrition. If sampling is done at household level, the number of households sampled should be adjusted to reflect the estimated number of nets per household.

[c] These nets are sampled for bioassay alone.

[d] A minimum of 230 nets are sampled over a 3-year period for bio-efficacy and chemical assays. Assuming a 50% attrition rate through 3 years, a total of 460 nets are required to be distributed in this group. It may be desirable to assume an even larger loss to follow-up to ensure adequate numbers are available after 3 years.

4.5 Allocation of nets

The candidate and reference (positive control) LNs should be allocated at household level rather than to individuals or sleeping places in order to avoid any bias in usage, such as a preference for one brand of LN over another. The number of LNs that each household receives should be based on the number of sleeping places available. Equal numbers of households in a given community should be assigned to receive the candidate and reference LN products.

A household census should be conducted well in advance to allow for random allocation of the candidate and reference LN products from the net master list. Pre-printed lists of household identifiers and the LN product to be given to each household should be provided to field teams at the time of net distribution. Each trial LN should be marked with a unique identification number, which should be recorded on the master list with the name of the householder and the location (preferably GPS coordinates) to help in tracking the net during follow-up. To reduce the risk of loss of labels over time, use of permanent ink, car paint or coloured tear-proof thread knotted into the netting is recommended. After the nets have been labelled, they are packed individually in plastic bags until they are distributed to the households.[21]

The specifications for some LN products allow for a range of denier (fabric weights). For phase III studies, unless the durability of each type is studied separately, manufacturers should provide nets of the *lowest* fabric weight listed in their specifications so that the results of the phase III studies represent estimates of the minimum durability of the candidate LN. Low-denier nets are likely to be less durable.

The following steps may be taken to encourage use of trial nets by study participants:

[21] Ideally, any existing nets should be removed from the household and substituted with the nets distributed as part of the study. If removal is not possible, the owners should be asked to store any existing nets for use when the study LNs are worn out, although it may be difficult to ensure that householders will use the trial nets rather than existing nets.

- Nets should not be distributed in their original sealed package.
- Before distribution, nets should be taken out of the original package, labelled with a unique code with a permanent marker to indicate that they are part of the study and put back in the plastic bag or original packaging, which should then be removed before net distribution.
- Materials for hanging the nets (hooks, ropes or poles, as appropriate) should be provided with the nets.
- Householders should be asked to begin using their nets immediately.
- Nets should be observed over sleeping places within 1 month of distribution, for example during the survey of adverse effects.
- It is advisable to conduct a 'hang-up' campaign within a month of distribution to ensure that recipients are using their new nets.
- Revisit all households 1 month after distribution to check that they are using the nets. Households that are not using their nets should be revisited; if they are still not using them, they should be excluded from the study and censored in the net master list, although they have the option of keeping the trial nets.

4.6 Follow-up surveys

4.6.1 Monitoring attrition and fabric integrity

Follow-up surveys should be conducted:

- within 1 month of distribution to record perceived adverse effects (Annex 10) and to ensure that recipients are using their new nets; and
- 6, 12, 24 and 36 months after distribution to measure the survivorship (or attrition rate) and physical integrity of the nets. Additional follow-ups may be conducted at 18 and 30 months or beyond 36 months if desired.

Households may be located for follow-up by suitable identifiers e.g. by the name of the head of the household, by GPS coordinates or the net code on the label. Families that have moved (and taken their nets), refused to participate or refused to allow inspection of their LNs should be censored on the master list of households. Households should be visited up to three times before they are recoded as unresponsive.

During surveys of LN attrition and fabric integrity, a standard questionnaire should be used to collect data on the status of each LN and on their use and handling (see example questionnaire in Annex 11).

The use of mobile technology (e.g. 'personal digital assistants' or 'smartphones' equipped with GPS) for recording responses to the questionnaire are recommended for automated data checking. GPS readings, scanning bar codes on nets if provided by the manufacturer and photographic records of nets in the field are also valuable.

Fabric integrity (hole index) and condition should be observed for a minimum of 150 nets per arm. It is assumed that if there are initially 250 LNs in each arm, at least 150 LNs per arm will remain available for inspection at the end of the 3-year study. A list of randomly selected nets with their unique code numbers and information on the household to which they were distributed (e.g. household identification, name of head of household, GPS coordinates) should be given to the staff who are sampling the nets in the field.

The nets or households should be sampled by simple random sampling, so that the probability that each net or household on the list will be selected is equal. The simplest sampling strategy will be to randomly select individual nets from the master list. However, this approach may be difficult in some settings, in which case sampling may be done at household level. If sampling is done at household level and all the nets in the household are sampled, the sample size should be adjusted for a clustering effect and the questionnaire adjusted accordingly. Nets sampled for physical integrity are inspected in the household and returned to the family. The survey team should inspect the study nets outdoors in the light to determine the hole index, ideally using a portable frame over which the net can be draped during inspection.

4.6.2 Sampling of nets for insecticidal activity

Nets should be sampled for insecticidal activity at 0, 6, 12, 18, 24, 30 and 36 months after distribution to determine their bio-efficacy. Adjacent netting pieces are cut for chemical assays from the nets sampled for bioassays at 0, 12, 24 and 36 months.

A subsample of nets should be randomly selected from the net master list of households that remain for bio-efficacy testing, after selection of those for attrition and fabric integrity assessment, and should be replaced by new LNs of same brand. These are used for measuring insecticidal activity and chemical content. In previous studies, 30 nets per LN product were found to be sufficient for bio-efficacy testing, although a larger sample, especially for bioassays at 36 months, will provide more precision. As nets may be lost to follow-up, it is best to anticipate the number of losses and randomly select more than 30 LN codes from the master list, so that at least 30 nets can be identified at the time of sampling. It has also been found useful to update the net master list after each follow-up survey to eliminate the codes of lost nets and re-randomize for sampling just before the next follow-up survey.

4.7 Outcome measures

4.7.1 Net attrition

To measure survivorship or attrition, the physical presence of the LN in the household should be recorded during each follow-up survey. The investigator should record whether the net is being used for its intended purpose. Nets that have never been used or are used for other purposes should be recorded as present but should be excluded from the analysis. If the net is no longer in the house, the investigator should ask the owner why it was missing (Annex 11).

Attrition should be determined for all nets recorded during the exercise at each interval but stratified by LN product. The number of nets in the sample, the proportion of the indicator and the 95% confidence interval should be reported (taking account of the sampling design, i.e. cluster sampling, if applicable). The following indicators should be used and

disaggregated by survey time (e.g. 6, 12, 24 or 36 months, or more if necessary):

Survivorship:

The numerator is the total number of each LN product present in surveyed households (and available for sleeping under) x 100. The denominator is the total number of each LN product distributed to surveyed households in the study cohort at the beginning of the study.

Attrition is calculated as 1 minus survivorship. Attrition can be due to discarding of nets because of excessive loss of fabric integrity (true attrition); movement of nets by selling them, giving them away or using them in another location (migration); or use for other than the intended purpose, although still owned by the household (misuse). Nets that are worn out but stored in the house and no longer used for their original purpose should be considered to have undergone true attrition. Attrition due to migration or misuse is likely to occur with any type of net, whereas true attrition is usually associated with the physical characteristics of the net. The cause of true attrition can be further disaggregated according to the type of damage, such as wear and tear from regular use or damage due to animals or fire.

For each product, the non-response rate or the proportion of nets that cannot be traced should also be reported, as high non-response rates may indicate a bias.

4.7.2 Fabric integrity

Fabric integrity is assessed from the questionnaire by counting the number of holes (including tears and split seams) by their location on the net and their size. Holes can be classified into:

size 1: smaller than a thumb (0.5–2 cm),

size 2: larger than a thumb but smaller than a fist (2–10 cm),

size 3: larger than a fist but smaller than a head (10–25 cm) and

size 4: larger than a head (> 25 cm).

Holes smaller than 0.5 cm should be ignored. Evidence of repairs to the net fabric and the type of repair should also be recorded on the form.

In follow-up field surveys, holes in nets are usually counted in the field. Nets should be examined outside, either held by at least two people and inspected by a third or draped over a portable frame. In some cases, the cause of the holes may be deduced from their physical characteristics or by questioning the user (Annex 11, question 4.2).

The three indicators of interest are the proportion of LNs with holes, the hole area and the hole index.

For the *proportion of LNs with any holes* (with 95% confidence interval), the numerator is the total number of each LN product with at least one hole of size 1–4, while the denominator is the total number of each LN product found and assessed in surveyed households.

This indicator may also be calculated for each category of hole size.

The *hole area* is calculated by assuming that the holes in each size category are circular, with a diameter that is equal to the mid-point of the category (except for the largest category, for which an arbitrary diameter, say 30, is selected, as there is no upper limit). For the four sizes listed above, the diameters would be 1.25 cm, 6 cm, 17.5 cm and 30 cm, respectively. The area (A) of each hole can then be estimated from the equation:

$$A = \pi r^2,$$

where $\pi = 3.142$ and r = the diameter divided by 2 and summed over each net (Table 3). For the hole categories listed above, the estimated hole areas are 1.2 cm^2, 28.3 cm^2, 240.6 cm^2 and 706.9 cm^2.

Table 3. Calculation of hole index

Hole size (cm)	Hole diameter (*d*; cm)	Hole radius (*r* = *d*/2)	r^2	Area of hole ($\pi * r^2$)	Hole Index[a]
0.5–2.0	1.25	0.625	0.390625	1.23	1
2–10	6	3	9	28.28	23
10–25	17.5	8.75	76.5625	240.56	196
> 25	30[b]	15	225	706.95	576

A, area of the hole; π = 3.142
[a] Area divided by 1.23
[b] Assumed diameter

The *hole index* is calculated by weighting each hole by size and summing for each net. If the weight of hole sizes 1, 2, 3 and 4 is A, B, C and D, respectively, the hole index is calculated as:

> hole index = (A x no. of size-1 holes) + (B x no. of size-2 holes) + (C x no. of size-3 holes) + (D x no. size-4 holes).

The holes should be weighted according to the average area of each hole category. For the hole size categories described above, the weights would be 1, 23, 196 and 576, which correspond to the areas estimated on the assumption that the hole sizes in each category are equal to the mid-points.

For each product type, the mean (and standard deviation) as well as the median (and interquartile range) hole index should be determined. The hole index for different products can be compared by analysis of variance for normally distributed data or the Kruskal-Wallis test or Poisson regression for non-parametric data.

A sample table for the presentation of data on fabric integrity is provided in Annex 12.

4.7.3 Insecticidal activity

The insecticidal activity (biological efficacy) of the nets should be determined in WHO cone tests and, when necessary, in tunnel tests (sections 2.2.1 and 2.2.2). Baseline bioassays should be conducted on five pieces of netting measuring 25 x 25 cm taken as shown in Figure 10, with 20 mosquitoes exposed on each piece of netting (total = 100 mosquitoes per net). In subsequent follow-ups, the piece removed from the bottom of the net (position 1) should be excluded, as it may be exposed to excessive abrasion if tucked under the bed (total = 80 mosquitoes per net). As in phase I studies, when knock-down is < 95% and mortality is < 80% on a given LN, it should be subjected to a tunnel test. For each net that fails to meet the criteria of the WHO cone test, the tunnel test should be conducted on the piece of netting that results in the mean mortality closest to that in the WHO cone assay.

A sample table for presenting data on insecticidal activity is shown in Annex 13.

4.7.4 Chemical analysis

Chemical analysis should be done at baseline and every year thereafter until the end of the study. At baseline, five pieces of netting measuring 30 cm x 30 cm should be cut from adjacent positions, following the sampling scheme presented in Figure 10. In subsequent sampling, the piece from position 1 is excluded, as it is considered to be tucked under the bed and exposed to excessive abrasion. Net samples should be measured to estimate their density (mass of net per unit area), and then samples from the same net should be combined for chemical analysis. The analysis should be conducted with the CIPAC methods cited in WHO specifications for each LN or, if unavailable, tests that have been developed by the manufacturer and validated. The results should be expressed both in grams of active ingredient per kilogram and milligrams of active ingredient per square metre of netting material. A sample table for presenting the results of chemical analysis is shown in Annex 14.

Nets should be analysed at baseline to confirm that they meet WHO specifications, before the study is initiated. Nets that do not meet the specifications should not be distributed.

4.8 Ethical considerations

Although these studies present a minimal risk to participants, approval should be obtained from appropriate ethical committees before they are conducted. Written informed consent should be obtained from the head of each household enrolled in the study (Annex 2).

4.9 Efficacy criteria for phase III studies

A candidate LN is considered to meet the criteria for efficacy for testing in phase III studies if, after 3 years, at least 80% of sampled nets are effective in WHO cone tests (≥ 95% knock-down or ≥ 80% mortality) or tunnel tests (≥ 80% mortality or ≥ 90% inhibition of blood-feeding). No widely accepted criteria for fabric integrity are available but are needed.

5. Adaptations for long-lasting nets with novel insecticides, synergists and insecticide mixtures

The massive scale on which malaria vector control is applied and the consequent problems of resistance to insecticides are increasing the demand for new public health pesticides, including LNs with new active ingredients or mixed formulations.

The mechanisms of action and performance criteria of some novel LNs are well understood and familiar, and these can be assessed with established WHOPES methods and criteria. LNs that have new intended functions or purposes (e.g. formulations with a synergist), however, will require additional test procedures and criteria. In cases where the mechanism of action is entirely different and the conditions for effectiveness are not yet known (e.g. LNs with slow-acting insecticides),

epidemiological evidence of effect on malaria or other vector-borne diseases (proof of principle) may be required.

The recently launched *Global plan for insecticide resistance management in malaria vectors*[22] calls on governments of malaria-endemic countries and other stakeholders to use strategies to combat the growing threat of insecticide resistance and to facilitate the development of innovative vector control tools and strategies. The present document provides guidance for evaluating anticipated LN products with new insecticides or combinations of insecticides that may become part of a resistance management strategy.

5.1 Characterization of insecticide resistance

New, non-pyrethroid insecticides brought to the public health pesticides market must be tested against a range of resistant mosquito strains in phase I studies. Therefore, new mosquito strains with novel resistance mechanisms should be established and characterized. Ideally, resistant strains should be characterized by target site modification (e.g. *kdr*) and the presence of different metabolic resistance mechanisms.

Exchange of such colonies among laboratories is to be encouraged. Nevertheless, the establishment of such colonies must be based on stringent biosafety precautions, to prevent the risk that genes for insecticide resistance will accidentally be introduced from a resistant colony into the wild mosquito population.

In phase II and phase III studies, insecticide resistance should be monitored regularly in the wild population of mosquitoes by bioassays and, when possible, biochemical or molecular tests. In phase II studies, phenotypic resistance should be measured just before the trial or within the 6 months before the trial. In phase III studies, phenotypic resistance should be measured before distribution of nets, at the mid-point of the study and at the end of the study.

[22] *Global plan for insecticide resistance management in malaria vectors.* Geneva, World Health Organization, 2012 (http://www.who.int/malaria/vector_control/ivm/gpirm/en/index.html).

5.2 Efficacy testing of nets with insecticides other than pyrethroids

LNs may contain compounds with entirely new modes of action on mosquitoes. If the new insecticide acts primarily through contact toxicity, like pyrethroids, causing rapid knock-down and mortality, the general framework for evaluating LNs will be applicable, although modifications may be required in each phase of testing. LN products that act by causing mortality alone, repellency alone or by an alternative mode of action on mosquitoes, may require, as proof of principle, epidemiological studies to demonstrate their efficacy in reducing malaria transmission or in controlling the disease.

A number of modifications are recommended to phases I, II and III studies for LNs containing novel insecticides.

Phase I studies: This type of testing is designed to assess the efficacy, wash-resistance and regeneration time of the insecticide on the netting. The current guidelines recommend that LNs be tested against susceptible strains of mosquitoes. When new insecticides are used in the manufacture of LNs, cross-resistance to other insecticides should be assessed.

It may be necessary to modify certain test procedures, depending on the mode of action of the new insecticide. For example, for LNs with slow-acting insecticides, mortality may be recorded 24, 48 and 72 h after exposure. For LNs that contain growth regulators, it may be necessary to measure the fertility and fecundity of females exposed to the netting.

Phase II and III studies: In Phase II studies, the efficacy of LNs should be determined against wild, free-flying mosquitoes susceptible both to pyrerthroids (where possible, given the spread of pyrethroid resistance) and to the insecticide on the candidate LN. For phase III studies, the recommendations for phase II studies of pyrethroid-treated LNs should be followed, although some modifications may be required, depending on the mode of action of the insecticide on the novel LN.

The reference LN should be a WHOPES-recommended net with the same or similar specifications in terms of netting material, denier and mesh size. Currently, the reference LN will necessarily be a pyrethroid-treated LN. As LNs containing

novel insecticides with new modes of action become available, further modification of these guidelines and evaluation methods may be necessary.

A net will be considered to have met the requirements for WHO interim recommendation if the mortality and blood-feeding inhibition of the candidate LN washed 20 times is equal to or better than that of the positive control washed 20 times.[23] If the candidate LN meets these criteria when tested against a vector population that is susceptible to both pyrethroids and the novel compound, further tests should be conducted in areas where the vector population is resistant to pyrethroids but susceptible to the novel compound.

Where pyrethroid-susceptible populations are not available for phase II testing, a reference LN should still be included in the comparison as best practice; however, the decision to recommend the novel product as an LN will be made on the basis of its own performance.

Phase III studies should include a positive control LN arm as recommended above, and WHO cone bioassays and tunnel tests should be done with pyrethroid-susceptible and pyrethroid-resistant strains. The susceptible strain serves for quality control, while the resistant strain is used to estimate the durability of the candidate LN under field conditions.

Laboratory evaluations of novel LNs may have to be modified. As noted above, candidate LNs treated with insecticides with effects on mosquitoes that differ from those of pyrethroids may require proof of principle and new assays.

5.3 Efficacy testing of nets with a mixture of insecticides

In some circumstances, mixtures offer benefits for managing insecticide resistance. Therefore, the use of mixtures is

[23] The benefits of novel non-pyrethroid insecticidal nets that withstand fewer than 20 standard washes but meet the grave threat of pyrethroid resistance may have to be considered.

identified as a desirable strategy in the *Global plan for insecticide resistance management in malaria vectors.*[24]

It is anticipated that novel LN products will contain mixtures of at least two unrelated insecticides. 'Mixtures' are products in which at least two insecticides are co-formulated, such that an insect on contact is exposed to both insecticides at the same time. A combination of an insecticide with a synergist is not considered a mixture in this context; however, the modifications for testing LNs described below may also be applied to nets with mixtures of insecticide plus a synergist.

For the purpose of resistance management, the insecticides used in mixtures should have different modes of action and should not show cross-resistance. Mosquitoes that are not killed by one insecticide because they are resistant to it will probably be killed by the other insecticide(s). Mixtures can also be used to capitalize on the different modes of action of different insecticides, such as personal protection and direct toxicity.

Unless more than one of the elements in a mixture require additional testing because of their different modes of action, the basic requirements for phase I studies should be fulfilled. In all cases, the efficacy, wash-resistance and regeneration of the candidate LN should be determined for both the product as a mixture and for the individual components of the product. This is necessary in order to understand and demonstrate the benefit of combining them.

The following modifications to phases I, II and III studies are recommended for LN products with mixtures of insecticides:

Phase I testing should be conducted against both a susceptible and one or more pyrethroid-resistant mosquito strains. The regeneration time and washing interval should be those of the final product. The following treatment arms are recommended for LNs in which two compounds in the mixture are active

[24] *Global plan for insecticide resistance management in malaria vectors.* Geneva, World Health Organization, 2012 (http://www.who.int/malaria/vector_control/ivm/gpirm/en/index.html).

against mosquitoes, in order to determine the efficacy of the individual insecticides and the added benefit of the mixture:[25]

- candidate LN with compounds A and B
- candidate LN with compound A only
- candidate LN with compound B only.[26]

Phase II trials should initially be conducted in an area with mosquitoes susceptible to both pyrethroids and the compounds in the mixture in the candidate LN. If the product is as effective as the reference LN, it should also be tested in an area with pyrethroid-resistant mosquito populations that show reduced mortality and blood-feeding inhibition when conventional LNs with pyrethroid are used. The following treatment arms should be tested in phase II:

- an untreated net, preferably of the same material as the candidate LN; if not available, a polyester net.
- candidate mixture LN, unwashed
- candidate mixture LN, washed 20 times
- reference LN, unwashed
- reference LN, washed 20 times

The ultimate decision is based on a comparison of the candidate LN washed 20 times and the positive control washed 20 times. The efficacy of the candidate LN in terms of mortality and blood-feeding inhibition should be equal to or better than that of the positive control.[27]

Bioassays of nets before washing, after washing and after the hut trial should be done with colony mosquitoes as well as with wild-caught pyrethroid-resistant mosquitoes. As noted above, mosquitoes collected in experimental hut studies should be

[25] Additional treatment arms may be required if more than two compounds are present in the candidate LN.

[26] If removal of one compound from the candidate LN significantly alters the migration or release of the other compound, conventionally treated nets might have to be included to test each compound individually (arms 2 and 3).

[27] The benefits of novel net products with mixtures of insecticides that withstand fewer than 20 standard washes but meet the grave threat of pyrethroid resistance will be considered.

preserved for quality control or future studies of genetic markers of insecticide resistance and their relation to efficacy in the experimental huts.

Phase III studies should include at least two LN products: the candidate LN and a reference LN. It is not necessary to test the component parts of the candidate LN separately. It is recommended that both pyrethroid-susceptible and pyrethroid-resistant mosquitoes be tested in WHO bioassays and tunnel tests.

5.4 Efficacy testing of combination nets

Combination LNs contain two or more different nettings, each of which has a different specification for fibres and/or active ingredient(s), with or without synergists.

In phase I, each netting component must be assessed separately. In phase II, the full product should be studied. When the netting contains a mixture of insecticides or of insecticide plus a synergist, the principles for evaluating LNs with mixtures as described above generally apply. In phase III, the full product should be compared with a reference LN, but bioassays should be conducted separately on each netting component of the LN, as in phase I. Depending on the specifications of the net, the sampling scheme for bioassays and chemical assays may require modification of phase II and III studies.

Annex 1. Informed consent form for sleepers in experimental hut studies (template)

Note: This is a proposed template, which can be modified and adapted according to national ethical guidelines. It should be printed in the local language.

Name of project: Evaluation of <*product name*> in experimental huts

Name of principal investigator: _____

Name of organization: _____

Household identification No. _____

Part 1. Information sheet

Introduction

My name is <*name of investigator*>, and I work for <*name of institution*>. I invite you to carefully read this document <*or understand its contents as read by a literate witness*> before accepting to participate in this study. The aim of this study is to determine how effective factory-treated insecticidal mosquito nets are in killing malarial mosquitoes when people sleep under them. To test these nets, we invite you to participate as users of the nets in experimental huts.

This study has been cleared by the ethical committee of <*name of institution or government*>.

Purpose and background of the study

Malaria is a major disease in <*name of study area or country*> and is transmitted from one person to another through the bites of certain mosquitoes. These mosquitoes usually bite after dark. Sleeping under a mosquito net protects against mosquitoes that bite in the night. If the net has been treated

with a chemical that kills insects (insecticides), it gives better protection against mosquito bites. Some kinds of nets are given a special chemical treatment in the factory and do not require re-treatment until the end of their useful life; these are called long-lasting insecticidal nets (LNs or LLINs).

We want to test and compare two LN brands in specially constructed huts. One LN is called <*product name*>. It has been tested and recommended by the World Health Organization (WHO) Pesticide Evaluation Scheme for the prevention and control of malaria. The other LN is called < *product name* >. It has been tested in a laboratory and found to be effective. A risk assessment has shown them to be safe for human use. However, we want to know if it works against wild mosquitoes in your village. We will test new LNs and those that have been washed 20 times with normal soap. We will compare the numbers of mosquitoes that enter the huts, feed on the sleepers and die each night of the study. The results will be compared with those with a mosquito net that has not been treated. These results will help us determine if the new LN can be recommended for malaria prevention and control.

Procedure and type of intervention

We will check how effectively the treated nets kill mosquitoes. Your participation is voluntary. As a volunteer, you will be asked to sleep under a bed net inside an experimental hut. There will be five huts.[28] Four will have nets treated with <*insecticide*>, while the fifth will have an untreated net for comparison. All the nets will have holes to simulate the conditions of a torn net and to ensure that we are testing whether the insecticidal treatment rather than the net prevents biting of sleepers. We will recruit at least five adult volunteers > 18 years of age from among the inhabitants of your area so that one sleeper can occupy each hut every night.[29] The selection will ensure that an equal opportunity is given to

[28] The number of huts should be adjusted to the study design.
[29] In some settings, two volunteers may sleep in each hut.

everybody; however, women are excluded.[30] You will be enrolled as a 'sleeper'. You should enter the hut at a standard, fixed time in the evening after supper or dinner and stay until a fixed time in the morning. During this period, you are not allowed to smoke or drink alcohol. You will be free to use the toilet facility. You should bring drinking-water. You will be given a commonly used reed mat bought on the local market, but you can opt to bring your own bedding. The mat should be laid on the floor, and the net should be tied to the walls of the hut with strings.

The sleepers will rotate among the five huts each night so that every sleeper sleeps under a different net each night. The study will last up to 10 weeks; in each week, you will sleep in the huts for five nights and will have a break on the sixth and seventh nights.

Adverse effects

WHO has recommended that the insecticide <*name*> can be used to treat nets for malaria control. Nets treated at the recommended dose do not cause considerable risk or discomfort to the users. Previous experience shows, however, that use of treated nets can cause certain adverse effects in some people during the first few days. These may include: itching of the skin, facial burning or tingling, sneezing, liquid discharge from the nose, feeling of headache, nausea, eye irritation and tears, experience of bad smell and body rashes. These events are usually transient and go away shortly on washing or bathing.

Risks and discomforts

Even though nets are used, there is still a low risk of contracting malaria during the study, if mosquitoes manage to penetrate the holed nets or bite through the untreated net. For this reason, we will give you medicines to protect you from

[30] Depending on the cultural setting and the safety and privacy around the huts at night, women may be included in the study. In some settings, couples have been sleepers in experimental huts.

malaria for the duration of the study and for 4 weeks afterwards. These tablets must be taken <*daily or weekly*> to be effective. Taking the protective medicine is voluntary.

Benefits

If you participate in the research, you may not get any personal benefit, but your participation is likely to help us to find the answer to the research question, that is, whether the new type of LN gives better personal protection from mosquitoes that spread malaria. However, malaria treatment will be available free of charge when necessary.

Incentives and cost

We will compensate you for the time spent in participating in the study and your travel costs according to local rates. The set rate is <*local rate*>.

Confidentiality

All information about your participation will be kept confidential and will not be revealed to anyone, except if required by law, such as in a legal request for the list of beneficiaries. Your identity will not be revealed in any reports or publications resulting from the study. The results of the interview will be put into a computer, with the code number of the household but without the names of the people interviewed.

The data collected will be kept for analysis. It will be stored for some time on paper and in a computer but may eventually be destroyed.

Right to refuse or withdraw consent

You do not have to take part in this research if you do not wish to do so, and refusing to participate will not affect your right to obtain nets from us or to obtain the routine medical care available to you. You may stop participating in the research at any time that you wish without losing any of your rights.

Who to contact

If you have any questions, please ask them, either now or later. If you wish to ask questions later, you may contact <*name, address and telephone number of the principal investigator*>.

Any important new information concerning the results of our study will be made known to you. This proposal has been reviewed and approved by <*name of the ethics committee*>, whose task it is to make sure that study participants are protected from harm. If you wish to find out more about this committee, please contact <*name, address and telephone number*>.

We are leaving a copy of this informed consent form with you for your information and future reference.

Part 2. Certificate of consent

(This is an integral part of the information sheet and not a stand-alone document.)

I have read this information in <*local language*>, or it has been read to me in my native language. I have had the opportunity to ask questions about it, and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate in this study, and I now know that I have the right to withdraw from the study at any time without in any way affecting my rights. I am told that the principal investigator of the study can exclude me from the study without my consent. I have been given a copy of this consent form.

Print name of participant	Date and signature of participant
_____	__/___/___ (*dd/mm/yy*)

For illiterate people

I have witnessed the interviewer reading the consent form to the potential participant. The reading was careful and accurate,

and the individual had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of independent literate witness:

_____

(If possible, this person should be selected by the participant and should have no connection to the research team.)

Signature of witness and date _____
___/___/___ (dd/mm/yy)

I have read or witnessed the reading of the consent form to the potential participant. The reading was careful and accurate, and the individual had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of researcher _____

Signature of researcher and date _____
___/___/___ (dd/mm/yy)

Annex 2. Informed consent form for net users in phase III studies (template)

Note: This is a proposed template, which can be modified and adapted according to national ethical guidelines. It should be printed in the local language.

Name of project: Evaluation of <*product name*> under field conditions

Name of principal investigator: _____

Name of organization: _____

Household identification No. _____

Part 1. Information sheet

Introduction

My name is <*name of investigator*>, and I work for <*name of institution*>. I invite you to carefully read this document <*or understand its contents as read by a literate witness*> before accepting to participate in this study. The aim of this study is to check or compare the action of various factory-treated insecticidal mosquito nets that are expected to retain their power to kill malaria mosquitoes after several washes and 3 or more years of use.

This study has been cleared by the ethical committee of <*name of institution or government*>.

Purpose and background of the study

First, some background information. As you probably know, malaria is a major disease in <*name of study area or country*> and is transmitted from one person to another through the bites of certain mosquitoes. These mosquitoes usually bite after dark. Sleeping under a mosquito net protects against

mosquitoes that bite in the night. If the net has been treated with a chemical that kills insects (insecticides), it gives better protection against mosquito bites. Some kinds of nets are given a special chemical treatment in the factory and do not require re-treatment until the end of their useful life; these are called long-lasting insecticidal nets (LNs or LLINs).

National malaria programmes are now distributing LNs for malaria prevention and control in areas targeted for this intervention. The community you live in has been targeted to receive LNs. We want to measure how long the LNs being tested by us actually last in routine use in the households in your community.

Your area <*name of village or area*> has been selected for this study. As you may be aware, we first asked your community leaders <*names of community leaders*> to give permission for this study. Now, we have come to you to obtain your consent before we start the study. We would like to invite you to participate in this study. With your consent, we will undertake a population census (enumeration of people by household). Then, various LNs will be distributed to cover all members of your household. Your household will receive one brand of the LNs being tested. To find out their mosquito killing and malaria prevention properties and to know how durable these nets are, my team has now come to your village, in consultation with your community leader or village headperson, to investigate these issues.

Information on study nets

The study nets to be given to your household are factory-treated LNs. In this study, the insecticide(s) used to treat the nets is/are: <*name of each LN product, its manufacturer and the insecticide used*>. These products are not new: they are all well established. They have been tested by the World Health Organization and are recommended as safe and effective.

Type of study

In this study, we are following the nets over time, to see how quickly they get holes and wear out. Nets of the different

brands have been given to many or all the families in the area. A smaller number of these households, chosen at random, will be re-visited every few months, to see whether the nets are still in use and still in good condition.

Voluntary participation: right to refuse or withdraw consent

Your participation in the study and interviews is entirely voluntary. You are not under any obligation to participate, and you have the right to refuse this invitation.

By participating in this study and allowing inclusion of your house, you are expected to sleep under the net regularly, maintain the nets, allow the study team to take back the nets for further checks, participate in surveys of the usefulness of the nets and report any adverse effects, if any. You can refuse to participate but will still have the same option of getting nets as households that are participating in the study.

If at any time during the study or interviews you decide not to participate further, you are free to withdraw immediately, with no further discussion. This will have no adverse consequences for you. The study nets that have been given to your household belong to you and are yours to keep. In a few cases, we may ask you to give an old net back to us in exchange for a new one, but you may refuse this request if you wish.

Procedures

I would therefore like to have your consent to be interviewed; this will last about <*approximate number*> minutes. During the interview, I will ask you some questions about your household and about any nets owned or being used by you. If you consent to participate, we will give you nets that are marked with a water-soluble ink to assess the washing of the nets. You will be told how to use and maintain them properly. The team will visit the village twice during the first month, and your house may be selected randomly to assess your experience of use of the nets. Thereafter, the team will visit the village every 6 months until 3 years. If your household is selected for sampling, they will check condition of the net and interview you about your

household, the status of the nets given to you or your family members, how you use and handle your net and whether you and your family members experienced any problem in using them. The interviews may last about 30 minutes. The team may or may not take your nets back. If they withdraw the net, they will give you a new net of the same type.

At the completion of the study, all villagers will be told the main outcomes in a community meeting in the village.

Risks, adverse effects and benefits

There is a remote possibility that you may get malaria even while using the nets. This might be possible due to biting by mosquitoes outdoors or if you or a family member failed to sleep under the net. Thus, if you suffer from fever, you should immediately approach the health staff in your village or the nearest centre <*name of centre*> for treatment, if adequate facilities exist for treatment of malaria. You may even seek advice or assistance from our institute <*name of institute*>; the contact details are given below.

The insecticide used to treat the nets has been tested before and has not been found to have any undue adverse effects in most people at the dose used in the nets. Transitory tingling or runny nose has been recorded when the nets are used for the first time after being taken from the package. There is no cause for alarm, as these effects pass within a day or two. In certain people, use of treated nets may cause other adverse effects during the first few days, such as headache, numbness (paraesthesia), itching, sneezing, discharge from eyes, nausea and an unpleasant smell. Should you perceive any adverse effects of using the nets, please consult a doctor at the local health facility mentioned above or report this to one of our staff immediately at the contact details given below, and we will give you all the necessary medical care.

By participating in this research, you are not likely to experience any long-lasting discomfort. For the purposes of our research, we may interview you within 1 month we distribute the nets to ask you about any adverse effects or symptoms from using the nets.

If you participate in this research, you are not likely to get any personal benefit or incentives.

Confidentiality

All information related to your participation will be kept confidential and will not be revealed to anyone, except if required by law, such as in a legal request for the list of beneficiaries. Your identity will not be revealed in any reports or publications resulting from the study. The results of the interview will be put into a computer with the code number of the household but without the names of the people interviewed.

The data collected will be kept for analysis. They will be stored for some time on paper and in a computer but may eventually be destroyed.

Who to contact

If you have any questions, please ask them, either now or later. If you wish to ask questions later, you may contact: <*name, address and telephone number of the principal investigator*>.

Any important new information about the results of our study will be made known to you. This proposal has been reviewed and approved by <*name of the ethics committee*>, whose task it is to make sure that study participants are protected from harm. If you wish to find out more about this committee, please contact <*name, address and telephone number*>.

We are leaving a copy of this informed consent form with you for your information and future reference.

Part 2. Certificate of consent

(This is an integral part of the information sheet and not a stand-alone document.)

I have read this information in <*local language*>, or it has been read to me in my native language. I have had the opportunity to ask questions about it, and any questions that I have asked

have been answered to my satisfaction. I consent voluntarily to participate in this study, and I now know that I have the right to withdraw from the study at any time without in any way affecting my rights. I am told that the principal investigator of the study can exclude my household from the study without my consent. I have been given a copy of this consent form.

Print name of participant _____

Signature of participant and date

_____ __/__/____(*dd/mm/yy*)

For illiterate people

I have witnessed the interviewer reading the consent form to the potential participant. The reading was careful and accurate, and the individual had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of independent literate witness: _____

(If possible, this person should be selected by the participant and should have no connection to the research team.)

Signature of witness and date

_____ ___/___/___ (dd/mm/yy)

I have read or witnessed the reading of the consent form to the potential participant. The reading was careful and accurate, and the individual had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of researcher _____

Signature of researcher and date

_____ ___/___/___ (dd/mm/yy)

Annex 3. Washing procedure devised by the Collaborative International Pesticides Analytical Council (CIPAC)

The CIPAC method 4827/m was provisionally accepted in 2012 for determination of wash resistance index of LNs.[31] In this method, a stock solution of the CIPAC washing agent is prepared as follows. Heat one bottle of polyoxyethylene glycol (25) monostearate (CAS number 9004-99-3 or 37231-60-0) to approximately 50 °C to melt and reduce its viscosity. Turn the bottle 180° a few times to ensure homogeneity. In a suitable glass flask, add 80 ml of water, 12 g of sodium oleate (CAS number 143-19-1) and 8 g of polyoxyethylene glycol (25) monostearate. Heat the mixture to approximately 50 °C, turning 180° frequently or stirring with a magnetic stir bar until the mixture becomes clear and homogeneous. The CIPAC washing agent can be used for up to 4 weeks if kept sealed in the dark at 4 °C.

For each wash, 2.5 ml of the stock CIPAC washing agent solution is added to 500 ml of de-ionized water at 30 °C ± 2 °C in a 1-l glass bottle. A piece of netting (25 cm x 25 cm) is inserted and the bottle capped and inverted 10 times. The bottle is turned 180° by hand and brought back to its upright position with both these steps being completed in approximately 2 s. The bottle is then placed in a water bath or in an oven with thermostat at 30 °C ± 2 °C in an upright position free from vibration for 10 min, after which the piece of netting is removed with tweezers, and excess fluid is removed by gentle shaking.

After washing, the piece of netting is rinsed twice by placing it in a 1-l glass bottle containing 500 ml of de-ionized water at 30 °C ± 2 °C. The bottle is capped, inverted 10 times and placed in a water bath or in an oven with thermostat in an

[31] The method will be published in a CIPAC handbook and made available on the CIPAC web site (http://www.cipac.org/cipacpub.htm).

upright position free from vibration for 10 min. The sample is then removed with tweezers.

After the second rinse, excess water is removed by gently shaking the net sample, which is then allowed to dry on a line for 30 min at room temperature out of direct sunlight. Once dry, the net samples are folded once or twice in each direction, placed in a bottle which is then closed and stored at 40 ^{o}C ± 2 °C for 22 hours ± 2 hours before starting the next washing cycle.

The washing–rinsing–heating process is repeated 3 more times. After the 4 wash cycles, the net samples are analysed using the appropriate CIPAC method for determination of total active ingredient content, and the wash resistance index is calculated using the following equation:

$$w = 100 \times \sqrt[4]{(t_4/t_0)}$$

where: w = wash resistance index, expressed as a percentage; t_4 = total active ingredient content (in g/kg) after 4 washing cycles; and t_0 = total active ingredient content (in g/kg) before washing (no washing).

Annex 4. Cone bioassays of nets collected in households

Name of person performing bioassays:

Date of test (dd/mm/yyyy): |__|__|/|__|__|/|__|__|__|__|

LN code: |__|__|__|__|__|__|__|__|__|__|

Temperature: |__|__| °C Relative humidity: |__|__| %

Test mosquito species:

Age of mosquitoes: |___| days

Net position	Replicates [a]	Test start & end time (h/min)	No. of mosquitoes exposed [b]	No. knocked down after 1 h	No. dead after 24 h	No. alive after 24 h	% Knocked down	% Mortality
1[c]	1							
	2							
	3							
	4							
2	1							
	2							
	3							
	4							
3	1							
	2							
	3							
	4							
4	1							
	2							
	3							
	4							
5	1							
	2							
	3							
	4							
Control	1							
	2							
	3							
	4							

[a] Four cones on each net sample (replicates 1–4).

[b] Usually, five mosquitoes per cone; exposure time, 3 min.

[c] Net position 1 should be tested only in baseline bioassay.

Annex 5. Tunnel bioassay of nets collected in households

Name of person performing bioassays: ……...

Date of test (dd/mm/yyyy): |__|__|/|__|__|/|__|__|__|__|

LN code: |__|__|__|__|__|__|__|__|

Temperature: |__|__| °C Relative humidity: |__|__| %

Test mosquito species and strain: …….

Age of mosquitoes: |___| days

Test start time* (h/min): End time (h/min):

* Females are introduced at 18:00 h and collected at 09:00 h.

		Blood-fed females		Unfed females		Total	
		Alive	Dead	Alive	Dead	Alive	Dead
Control	Compartment 1						
	Compartment 2						
	Total						
Treatment (LN)[a]	Compartment 1						
	Compartment 2						
	Total						

Compartment 1, long section of tunnel into which mosquitoes are released (area C1, Figure 5); compartment 2, section between test netting and animal bait

[a] Add additional treatment rows when more than one subsample of the same net or samples of other nets are tested in parallel.

Annex 6. Sample table for bioassays in phase II experimental hut trials

Treatment	Before washing			After washing, before hut trial			After hut trial		
	N	% KD	% Mortality	N	% KD	% Mortality	N	% KD	% Mortality
Untreated net									
Candidate LN, unwashed									
Candidate LN, washed 20 times									
Reference LN, unwashed									
Reference LN, washed 20 times									

N, number of mosquitoes; KD, knock-down

Annex 7. Sample table for experimental hut data

	Research arms				
	Untreated net	Reference LN, unwashed	Candidate LN, unwashed	Reference LN, washed 20 times	Candidate LN, washed 20 times
Total number of females caught					
Females caught/night					
Deterrence (%)[a]					
Females in veranda					
Exophily (%) and 95% confidence limits					
Blood-fed females					
% blood-fed and 95% confidence limits					
Blood-feeding inhibition (%)					
Dead females					
Overall mortality and 95% confidence limits					
Mortality corrected for control (%)					
Killing effect (%)					

[a] Deterrence is calculated relative to untreated net.

Annex 8. Household census and demographic data form

Village:	District:
Date:	Enumerator:

Household identification	Name of head of household	GPS coordinates	No. of children < 5 years	No. of children 5–15 years	No. of adults (> 15 years)	No. of existing LNs	No. of existing other nets	No. of sleeping places	No. of LNs to be allocated

Annex 9. Net master list

Village code	Name of head of household	Household identification code	Study arm (type of LN)	Net identification (net code)	Date of distribution	Date of withdrawal of net

Annex 10. Assessment of adverse effects among net users

Name of project: Evaluation of <*product name*> under field conditions

Name of principal investigator: _____

Name of organization: _____

Household identification No. _____

Village code: |__|__| Household code: |__|__|__|__|

Household number: |__|__|__|

Date of survey (dd/mm/yyyy): |__|__|/|__|__|/|__|__|__|__|

Date of receipt of LN (dd/mm/yyyy): |__|__|/|__|__|/|__|__|__|__|

Number of nets provided by the project: |__|__|

Number of project nets used: |__|__|

Confirmation of number of nets by interviewer: |__|__|

Code number of selected net: |__|__|__|__|__|__|__|__|__|

Number of people sleeping under **this** net |__||__|

Proper use of nets provided by the project (record past and present experience): (0, no answer; 1, Yes; 2, No)

Individuals: P1, P2, P3

a. Do you sleep under the net every night? |__| |__| |__|

b. Any itching of your skin or paraesthesia? |__| |__| |__|

c. Any facial burning? |__| |__| |__|

d. Any sneezing? |__| |__| |__|

e. Any liquid discharge from your nose? |__| |__| |__|

f. Feeling headache? |__| |__| |__|

g. Any nausea? |__| |__| |__|

h. Eye irritation? |__| |__| |__|

i. Tears coming from your eyes? |__| |__| |__|

j. Experienced bad smell using nets? |__| |__| |__|

k. Any other symptoms? Please specify ……

l. Will you use your net regularly? |__| |__| |__|

If the respondent answers positively to any of the above questions, ask whether he or she has reported to a physician:

Yes |__| No |__|

Annex 11. Sample questionnaire for monitoring durability of nets in phase III studies

Questions should be adapted to local settings.

To be filled in before the interview

Identification number I___I___I___I (to be filled in by supervisor)

1. Code of interviewer I___I___I
2. Date (day/month/year) I__I__I / I__I__I / I__I__I__I__
3. Name of village I_____I
4. GPS coordinates of household I_____I
5. Household identification number I___I___I___I___I
6. Long-lasting insecticidal net number I___I___I___I___I

To be filled in by the supervisor at the end of the day

7. Code of supervisor I____I____I

Comments_____
_____

I confirm that the questionnaire is complete.

Date : I___I___I / I___I___I / I___I___I___I___I

Name I_____I

Signature I_____I

To be filled in by data entry clerks during data entry

Data entry clerk 1	Data entry clerk 2
Date I__I__I/I__I__I/I__I__I__I__I	Date I__I__I/I__I__I/I__I__I__I__I
Signature_____	Signature_____

Section 1: I would like to ask you (*head of household or adult > 18 years*) some questions about your household.			
Q #	Questions and filters	Coding category	Answer (enter coding categories)
1.1	Who is responding to the questions?	1. Yes 0. No	Head of household I____I User of net I____I Parent or guardian of user(s) of net I___I Other adult in household I____I
1.2	What is the highest level of education of the head of the household?	1. None 2. Religious school 3. Primary school 4. Secondary school 5. Higher education 6. Other, specify	I____I Other _____
1.3	Does your household have electricity?	1. Yes 0. No	I____I

1.4	What is the principal type of toilet facility used by members of the household?	1. Own flush toilet 2. Shared flush toilet 3. Own pit latrine 4. Shared pit latrine 5. Bush or field 6. Other	I____I Other _____
1.5	What is the principal household source of drinking-water?	1. Piped water into home 2. Protected well in home 3. Unprotected well in yard 4. Open well in yard 5. Protected well in yard 6. Unprotected public well 7. Protected public well 8. Tap in yard 9. Tanker truck 10. Bottled water 11. Public tap	I____I Other _____

		12. Rainwater 13. Surface water 14. Spring 15. Other	
1.6	How many people slept in your household last night?		Adults > 15 years I____I____I 5–15 years I____I____I < 5 years I____I____I
1.7	How many sleeping places were used last night in your household? (including sleeping places outside and temporary spaces)		I____I____I
1.8	How many mosquito nets that can be used for sleeping does your household have? *(Probe for any nets currently not in use: stored, saved, still in packaging)*		I____I____I

1.9	Of the total number of mosquito nets, how many are LNs? *(Observe)*		|____|____|
Section 2: LN status (for selected net identification |____|____|____|____|)			
2.1	Is this net still in the household, and can it be used for sleeping under? *(Look for the net in the household, including those still in packaging or being used for another purpose)*	1. Yes → Skip to section 3 0. No	|____|
2.2	If No, why is this net no longer available for sleeping under in the household?	1. Net was damaged and thrown away 2. Net was given away to others 3. Net was stolen 4. Net was sold 5. Net is being used in another location	|____| Other _____

		6. Net is being used for another purpose 7. Other, specify	
2.3	How many months ago did this net become unavailable for sleeping under in the household?	1. 0–6 months → End questionnaire 2. > 6 months → End questionnaire 9. Don't know→ End questionnaire	I____I
Section 3: LN use and handling (for selected net identification I__I__I__I__I)			
3.1	Has this net ever been used for sleeping under?	Yes No → End questionnaire	I____I
3.2	Was this net used last night to sleep under?	1. Yes → Skip to 3.4 0. No	I____I

3.3	If No, why was this net not used last night?	1. Yes 0. No	Too hot I____I Don't like the smell I____I Feel "closed in" I____I No malaria now I____I No mosquitoes I____I The net is too torn or old I____I Net not available I____I Used another net I____I User did not sleep here I____I Other I____I _____ Don't know I____I
3.4	In the past week, how often was the net used?	1. Every night (7 nights) 2. Most nights (5–6 nights) 3. Some nights (1–4 nights) 4. Not used at all (0 nights) 9. Don't know	I____I

3.5	How many adults (> 15 years) slept under this net last night?		I____I
3.6	How many children 5–15 years slept under this net last night?		I____I
3.7	How many children < 5 years slept under this net last night?		I____I
3.8	During which periods of the year is this net used to sleep under?	1. All year 2. Only the rainy season 3. Only the dry season 9. Don't know	I____I
3.9	Is this net ever used for sleeping under away from the main house? If yes, where?	1. Taken to the fields 2. Taken to the beach 3. Taken to the forest 4. Taken to the farm hut 5. Other, specify 6. Not used away →Skip to Q. 3.11 9. Don't know →Skip to Q. 3.11	I____I Other _____

3.10	During which periods of the year is this net used to sleep under away from the main house?	1. All year 2. Only the rainy season 3. Only the dry season 9. Don't know	I____I
3.11	Has this net ever been used over the following types of sleeping places?	1. Yes 0. No	Reed mat I____I Cut bamboo I____I Grass I____I Foam mattress I____I Wooden bed frame (finished) I____I Wooden bed frame (sticks) I____I Metal bed frame I____I Bare floor or ground I____I Other, specify I____I_____
3.12	Do you tuck the net in at night?	1. Yes 0. No 9. Don't know	I____I

3.13	Has the net ever been washed?	1. Yes 0. No →Skip to Q. 4.1 9. Don't know →Skip to Q. 4.1	I____I
3.14	When was the last time you washed the net?	1. 1 week ago 2. 1 week to 1 month ago 3. 1–3 months ago 4. 3–6 months ago 5. > 6 months ago 9. Don't know	I____I
3.15	What type of soap was used?	1. None 2. Local bar soap 3. Detergent powder 4. Mix (bar and detergent) 5. Bleach 9. Don't know	I____I

3.16	How long did the net soak for?	1. Did not soak the net 2. < 1 h 3. > 1 h 9. Don't know	I____I
3.17	Was the net scrubbed hard or beaten on a hard surface (e.g. rocks, with sticks)?	1. Yes 0. No 9. Don't know	I____I
3.18	Where was the net dried?	1. Outside in the sun 2. Outside in the shade 3. Inside 9. Don't know	I____I
Section 4: LN condition (for selected net identification I____I____I____I____I)			
4.1	In the past month, have any new holes appeared in the net that you are aware of?	1. Yes 0. No 9. Don't know	I____I

4.2	What caused these new holes?	1. Yes 0. No	Tore or split when caught on an object I____I Was burnt I____I Was caused by animals I____I Was caused by children I____I In another way I_____I, specify _____ Don't know I____I
4.3	How is the net found? (*Observe*)	1. Hanging loose over sleeping place 2. Hanging tied in knot 3. Hanging folded 4. Visible but not hung up 5. Stored away	I____I
4.4	What type of sleeping place is the net hanging over? (*Observe*)	1. Reed mat 2. Cut bamboo 3. Grass 4. Foam mattress	I____I Other _____

		5. Wooden bed frame (finished) 6. Wooden bed frame (sticks) 7. Metal bed frame 8. Nothing 9. Other, specify	
4.5	Where is it found? (*Observe*)	1. Inside 2. Outside → Skip to Q. 4.9	I____I
4.6	What is the principal type of flooring in the room where the net is found? (*Observe*)	1. Soil or sand 2. Wood, palm, bamboo 3. Cement (including vinyl) 4. Cement 5. Carpet 6. Other	I____I Other _____
4.7	What are the walls of the room in which the net is found made of? (*Observe*)	1. Mud brick 2. Mud with wood frame 2. Concrete	I____I____I Other _____

		3. Twigs 4. Wood 5. Straw 6. Bamboo 7. Corrugated iron 8. Lime-plastered 9. No walls (used outside) 10. Other, specify	
4.8	What is the roof or ceiling of the room in which the net is found made of? (*Observe*)	1. Grass thatch 2. Corrugated iron 3. Concrete 4. Reed mats 5. Wood 6. Tiles 7. Other, specify	I____I Other _____

4.9	Do you use an open flame for cooking, heating or lighting where the net is found?	1. Yes 0. No	Wood fire I____I Charcoal fire I____I Wax candle I____I Oil lamp with a glass I____I Oil lamp without glass I____I Other, specify I____I _____
4.10	What types of holes are observed?	1. Yes 0. No	Horizontal tears at bottom I____I Holes at hanging points I____I Open seams I____I Burn holes I____I Holes from rodents I____I Whole section missing I____I
4.11	Number of holes of size 1	Less than size of thumb (0.5–2 cm)	Roof I____I____I Upper I____I____I Lower I____I____I Seams I____I____I

4.12	Number of holes of size 2	Larger than thumb, smaller than fist (2–10 cm)	Roof I____I____I Upper I____I____I Lower I____I____I Seams I____I____I
4.13	Number of holes of size 3	Larger than fist, smaller than head (10–25 cm)	Roof I____I____I Upper I____I____I Lower I____I____I Seams I____I____I
4.14	Number of holes of size 4	Larger than head (> 25 cm)	Roof I____I____I Upper I____I____I Lower I____I____I Seams I____I____I
4.15	Number of holes repaired		Stitched I____I____I Knotted I____I____I Patched I____I____I

Annex 12. Sample table for fabric integrity measured in phase III studies

Survey (month)	Candidate LN					Reference LN				
	% nets with at least 1 hole	Hole index		Hole area		% nets with at least 1 hole	Hole index		Hole area	
		Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)		Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)
0										
6										
12										
24										
36										

SD, standard deviation; IQR, interquartile range

Annex 13. Sample table for biological efficacy in phase III studies

For each cell, the proportion of nets meeting the criteria (number passing/number tested) should be presented as in the example shown for survey month 0.

Survey (month)	Candidate LN			Reference LN		
	Cone bioassays [a]	Tunnel tests [b]	Cone & tunnel tests combined (a+b)	Cone bioassays [a]	Tunnel tests [b]	Cone & tunnel tests combined (a+b)
0	96.7 (29/30)	100 (1/1)	100 (30/30)	93.3 (28/30)	50 (1/2)	96.7 (29/30)
6						
12						
18						
24						
30						
36						

WHO criteria:

[a] cone test: ≥ 80% mortality or ≥ 95% knock-down

[b] tunnel test: ≥ 80% mortality or ≥ 90% blood-feeding inhibition; control tunnel test should preferably show 50% blood-feeding.

Tunnel tests are carried out on pieces of nets that did not satisfy the cone test criteria.

Annex 14. Sample table for data on chemical content of nets in phase III studies

Survey (month)	Candidate LN		Reference LN	
	No. of nets	Mean concentration of AI g/kg (95% CI)	No. nets	Mean concentration of AI g/kg (95% CI)
0				
12				
24				
36				

AI, active ingredient; CI, confidence interval

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