

**Streamlined durability monitoring of Insecticide-Treated Nets (ITNs) distributed during the [year] mass campaign in [Country]**

**Study Protocol**

[Month Year]

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# Background

## Evolution of ITN durability monitoring

Between 2010 and 2018 the proportion of households in sub-Saharan Africa with at least one insecticide-treated net[[1]](#footnote-2) (ITN) increased from 47% to 72%. The percentage of the population that could be protected by an ITN assuming each ITN in a household can be used by two people increased from 33% to 57% over the same period[[2]](#footnote-3). It is important to sustain coverage and monitor net durability and the average useful life of a net. WHO recommends that countries routinely monitor net durability following mass distribution campaigns[[3]](#footnote-4) and provided technical guidance on estimating physical survival and bio-efficacy in 2013[[4]](#footnote-5),[[5]](#footnote-6),[[6]](#footnote-7).

Since 2013, durability monitoring studies have been initiated and completed in 11 countries for 10 standard pyrethroid-only ITN brands (see Annex 1)[[7]](#footnote-8). Studies have measured the effect of normal daily use on: attrition (as measured by the loss of nets for any reason as well as due to wear and tear from households); physical durability (as measured by the number and size of holes in the net); and insecticidal efficacy (as measured by cone bioassay, tunnel test, and chemical content analysis, depending on type of net). Results from these studies suggest that the physical durability of similar products may vary significantly, between less than two years to four or more years, and that differences are largely driven by environmental and behavioral factors. Median all cause attrition for the same studies was estimated as 12.5% of ITNs in the first 6 months following distribution, most often driven by reallocation of nets to other households and family members. Between 6 and 36 months, median net attrition per month was 2.1%. Mean 24-hour mortality for deltamethrin ITNs ranged from 29-97%, while there was less variation among alpha-cypermethrin nets (64-85%).

While vector control has contributed substantially to the global reduction in malaria burden recorded since 2000, global progress towards malaria control and elimination has stalled in recent years and the long-term effectiveness of malaria vector control is threatened by the emergence and intensification of insecticide resistance in key mosquito populations. New types of ITNs that use more than one active ingredient and are effective against insecticide resistant mosquitoes have been developed, but large-scale uptake has been slow prior to 2020 and very limited durability monitoring data exists for these new products.

The United States President’s Malaria Initiative (PMI) has long supported ITN durability monitoring. To date, PMI has supported a full durability monitoring protocol to generate basic information on the physical integrity and bioefficacy of ITNs as well as information on ITN care and use. These studies generally compare two types of nets in one area or one type of net in two areas of a given country. More recently, PMI has developed a streamlined protocol for durability monitoring for countries that have already generated considerable ITN durability data and have more focused questions, particularly around durability of new types of nets.

This protocol outlines the approach for streamlined ITN durability monitoring in [Country] for ITNs distributed as part of the [Year] mass campaign.

## Overview of [year] mass distribution campaign

*Describe the recent ITN mass campaign in terms of dates, coverage, characteristics of ITN brands deployed (netting material, denier, active ingredient(s)) and quantities distributed (or planned if the campaign is yet to take place).*

Table 1 Characteristics of [Country] [year] ITN mass campaign

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Geographic area** | **ITN brand** | **ITN type** | **Active Ingredients** | **Quantity** | **Distribution date** |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |

## Overview of streamlined ITN durability monitoring in [Country]

### Monitoring sites

*Name and describe the monitoring sites.*

### Characteristics of ITN brands monitored

*Name and briefly describe the ITN brand(s) included in the monitoring activity.*

*e.g., PermaNet 3.0 brand ITNs, manufactured by Vestergaard, are rectangular, white, with polyester sides and a polyethylene roof. The side yarn is incorporated with deltamethrin at 2.8 g/kg, while the roof yarn has deltamethrin at 4.0 g/kg combined with synergist piperonyl butoxide at 25 g/kg.*

### Activity timing

*Complete the table below to describe the planned timing of survey timepoints.*

Table 2 Monitoring activity timing

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  |  |  | **Date of survey timepoints** |
| **Study site** | **ITN brand** | **ITN type** | **Campaign date** | **Pre-distribution** | **12-month** | **24-month** | **36-month** |
| *e.g. North* | *Brand A* | *PBO-synergist* | *March 2021* | *Jan 2021* | *March ‘22* | *March ‘23* | *March ‘24* |
| *e.g. West* | *Brand B* | *Dual AI* | *March 2021* | *Jan 2021* | *March ‘22* | *March ‘23* | *March ‘24* |

## Objectives

1. To assess the insecticidal efficacy of [brand or brands to be monitored] in [“one” or “multiple” as required] sites, as measured by cone bioassays, tunnel tests and chemical testing, over a three-year period of field use; and compare the insecticide effectiveness across these [“sites” or “brands” as appropriate] and identify major determinants of field performance (e.g. characteristics of net users, washing behaviors, etc.).
2. To monitor the physical integrity of the nets as measured by a net hole assessment and short questionnaire.
3. To estimate indirectly the level of attrition of the nets at each timepoint.

## Expected Benefits and Value

The results of the proposed study will:

* Provide the NMCP, PMI, and Roll Back Malaria (RBM) partners with valuable information regarding the new ITN brands distributed during the mass campaign, e.g. insecticidal efficacy, and whether and how this varies by [“site” or “brand”, as appropriate].

# Methods

## Study design

The principal design for streamlined ITN durability monitoring is a prospective study using repeat cross-sectional visits to sampled clusters with randomly sampled ITNs withdrawn from selected households at each timepoint.

Once ITNs have landed in country and before the ITN distribution campaign begins, 20 ITNs per site/brand will be sampled from the central stores to undergo bioassay and chemical residue testing. All 20 ITNs will undergo bioassays first, then run chemical testing on random selection of 10 out of the same 20 nets, following methods described later in this protocol. If results are as expected for the brand, then no further tests will be conducted. If results are not as expected – for example, they do not meet manufacturer specifications or results are variable – then chemical analysis will be conducted on the remaining 10 nets. Results from these tests will form the pre-distribution time point against which study results will be compared.

The first fieldwork activities will occur 12-months following the distribution. During this timepoint, a representative sample of approximately 258 campaign nets from each study site will be identified through a cluster household survey with all campaign nets from consenting households forming the sampling frame. These nets will be labelled with a unique identifier. A random sample of 30 campaign nets will be selected from the sampling frame and withdrawn for physical integrity assessment, bioassays, and chemical residue testing. At each subsequent survey time point (24- and 36-months) a random sample of 30 campaign nets will be selected from the sampling frame and withdrawn for bioassays, chemical residue testing and, physical integrity assessment. A household survey capturing household characteristics and net care behaviors will be administered at each household from which one or more campaign nets are withdrawn. Withdrawn nets will be replaced on a like-for-like basis with new ITNs, marked with the date so they are not sampled in future timepoints. Prior to undergoing destructive bioassay and chemical residue testing, the physical integrity of sampled ITNs will be measured at each timepoint using the standard hole assessment approach[[8]](#footnote-9). At each survey timepoint, an ordered list of ITNs will be randomly generated for sampling. Sampling will be done in the order of the list. In the 12-month survey round, attrition is estimated from the number of ITNs that were reportedly received by cluster households during the mass campaign and the number of ITNs remaining at the time of net listing. In the 24- and 36-month survey rounds, net attrition will be indirectly estimated based on the number of alternate ITNs in the sample list that must be sought before the required sample size of 30 ITNs is attained.

Figure 1 Overview of study design



## Study sites

*Edit the description below as appropriate depending on your study design (comparing different brands in similar sites or the same brand in different sites).*

The study will be carried out in [Site(s)] (Table 3).

Table 3 Study site characteristics

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Site 1** | **Site 2** |  |
| Study site |  |  |  |
| ITN brand |  |  | **Source** |
| **Environment** |
| Elevation (m) |  |  |  |
| Annual precipitation (mm) |  |  |  |
| Mean max temperature (°C) |  |  |  |
| Mean min temperature (°C) |  |  |  |
| Climate classification (Koppen-Geiger) |  |  |  |
| Population main economic activity |  |  |  |
| **Demographics and health** |
| Population (Province) |  |  |  |
| Total fertility rate |  |  |  |
| Percentage of children under 5 years with fever in the past two weeks |  |  |  |
| **Malaria epidemiology** |
| Description of malaria transmission season(s) |  |  |  |
| Prevalence of anemia in children under 5 years |  |  |  |
| Percentage of households with at least one ITN for every 2 people (%) |  |  |  |
| Population access to ITN (%) |  |  |  |
| Population use of ITN (%) |  |  |  |
| Ratio of ITN use:access |  |  |  |
| Malaria prevalence among children under five, by RDT (%) |  |  |  |
| Malaria prevalence among children under five, by microscopy (%) |  |  |  |
| *Population access*: Proportion of population that would be able to use an ITN if each ITN in a household was used by two people. *Use:Access ratio*: Ratio of population use to population access |

Figure 2: Streamlined ITN durability monitoring sites

*Map of Streamlined ITN durability monitoring sites*

## ITN brands

*Complete the table below for each ITN brand included in the monitoring activity. Add or remove columns as required for the number of brands being monitored.*

Table 4 Characteristics of ITN brands

|  |  |  |  |
| --- | --- | --- | --- |
| **Brand** |  |  |  |
| **Type*Pyrethroid-onlyPBO-synergistDual AI*** |  |  |  |
| **Chemical content(g/kg and mg/m2)** |  |  |  |
| **Fabric** |  |  |  |
| **Denier** |  |  |  |
| **Shape** |  |  |  |
| **Manufacturer** |  |  |  |

## Sample size

Sample size determination for this activity is informed by the standard WHO guidance for field testing of mosquito nets[[9]](#footnote-10). Thirty (30) ITNs per site will be collected at each assessment timepoint (12-, 24, 36-months). Logistically, these ITNs will be sampled as 2 ITNs from households in each of 15 clusters. Assuming, in the worst-case scenario, that the level of the main outcome measure of interest (which will vary based on ITN type) is 50% after three years, the sample will provide a precision of 12%-points in a one-sided analysis.

An analytic sample size of 30 ITNs at monitoring completion (the 36-month timepoint) must be translated into an operational sample to inform the size of the sampling frame required at the first study timepoint (12-months). Secondary analysis of attrition data from 12 PMI-funded durability monitoring studies completed or initiated since 2015 identified the monthly estimates of attrition shown in Table 5[[10]](#footnote-11). The median values have been used in this analysis.

Table 5 Estimates of ITN attrition

|  |  |  |
| --- | --- | --- |
| **Time period** | **Monthly median attrition** | **Monthly upper-quartile attrition** |
| Distribution to 12-months | 2.0% | 3.5% |
| 12-months to 36-months | 2.1% | 3.0% |

Assuming that at 36-months it is desirable to have at least 100 ITNs present in the sampling frame in each location (from which to select the final 30 nets), then a minimum of 258 ITNs must be listed in the sampling frame for one location at the 12-month timepoint. These 258 ITNs at 12-months are the surviving ITNs from 360 distributed ITNs assuming the ITNs are subject to median monthly attrition.

For consistency with the sampling approach used for standard durability monitoring, the study will work with 15 clusters in each location. The required number of households per cluster will depend on the mean household size, assuming the standard distribution strategy of 1 ITN for every 1.8 people was followed during distribution planning. To reduce intra-cluster correlation considering ITNs potentially sourced from the same household, while maintaining efficiency in study design, the number of ITNs included in the sampling frame from any one household is capped at 2. Table 6 provides a look-up for the number of households per cluster, based on the mean household size in a location. To use the table, household size in the study sites is rounded down to the nearest table entry.

Table 6 Required cluster sizes

|  |  |  |
| --- | --- | --- |
| **Mean household size in study sites is at least…** | **Campaign ITNs per household (assuming 1 per 1.8 people)** | **Required cluster size (households)** |
| 3.2 | 1.8 | 14 |
| 3.4 | 1.9 | 13 |
| 3.7 | 2.1 | 12 with net cap |
| 4 | 2.2 | 12 with net cap |
| 4.3 | 2.4 | 12 with net cap |
| 4.7 | 2.6 | 12 with net cap |
| 5.2 | 2.9 | 12 with net cap |
| 5.8 | 3.2 | 12 with net cap |
| 6.6 and above | 3.7 | 12 with net cap |

Mean household size in [Site(s)] is [mean household size] and so this study will sample 15 clusters with [number] households per cluster for each location for X households in total.

## Sampling procedures

### Stage one: selection of clusters

The campaign ITN distribution registers for the study sites will be sourced from the National Malaria Control Program. A cluster will be defined as the lowest level geographic area used for campaign planning. Cluster selection will be done with probability proportional to size sampling (PPS), with the number of ITNs distributed per cluster as the measure of size. If registers are not available, census enumeration areas will be sampled using PPS with population as the measure of size.

### Stage two: selection of households and study net sampling frame

Within each selected cluster, [number] households will be selected using the following methodology: if the cluster is less than 200 households the field team will list or map all inhabited houses and the team leader will randomly sample the required number of households with equal probability using a random number generator. In addition, half the number of required households (rounding up) will be selected as alternate households, which will be used if a sampled household reports never to have received any nets from the campaign. Following the household definition used in the ITN distribution campaign, the definition of a household will be “people eating from the same pot”. The *Household Listing and Sampling Form* will be used for this stage.

If the cluster exceeds 200 households, an equal size section-approach will be used. With the help of local leaders, the cluster will be divided in sections of approximately equal size (80-100 households). One of these sections will be randomly selected by the team leader using a random number generator and within this section all households will be listed and/or mapped. Household selection will then proceed as above. The number of sections will be recorded by the team leader so that weights can be correctly applied for analysis. To facilitate household selection, the use of satellite images and building footprints to map households before fieldwork begins will be explored.

Sampled households will be screened to determine whether they participated in the ITN distribution campaign. Screening will consist of a brief introduction by the study team and a set of questions to determine respondent and household eligibility. If a household is not eligible for the study because they did not participate in the mass campaign or they participated but have no campaign ITNs remaining in the household, they will be dropped and will not be included in the sampling frame. A record of lost campaign ITNs will be made during sampling to inform the estimate of attrition during the study. If a household confirms participation in the campaign and the presence of one or more campaign ITNs, information on the study will be given and oral consent sought using the consent script (Annex 2). The information sheet and consent form will be available in [“English”, “French” or “Portuguese”] and [Local written language(s)] and will be read to the respondent in their local language by a member of the field team fluent in that language. If the household does not give consent, it will be dropped, and one of the alternate households will be visited until the total of [number] households is reached.

For each consenting household, the GPS coordinates and the full name of the head of household will be recorded and entered in the *Net Listing Questionnaire* which will be used to identify households during annual follow-up visits.

Within each household, all remaining campaign ITNs will be identified by the field team based on the net label, net characteristics, and confirmation of net source from the household members. Each campaign net will be labeled with a unique identifying number that will be used to create a master list of nets which will serve as the sampling frame at each assessment timepoint. Campaign net IDs will be entered in the *Net Listing Questionnaire.* Compiled campaign net information from several households will be transferred to the *Net Sampling Form*at the 12-month timepoint to facilitate random sampling for ITNs in-situ during the first study timepoint. In each household, the number and status of nets lost between distribution and the 12-month timepoint will be recorded to inform attrition estimates.

### Stage three: selection of nets for analysis

Using the ITN sampling frame generated above, 2 ITNs per cluster will be randomly selected and withdrawn for analysis at each assessment timepoint. For the 24- and 36-month follow-up assessment timepoints, unselected ITNs will be listed in a random order and provided to study teams as alternate ITNs. Should they be required, alternate ITNs will be used in sequence in case any of the ITNs intended to be sampled are lost-to-follow-up or are otherwise not available during the visit (for example, if the identified ITN is not available on the day of visit). No alternate ITNs should be required at 12-months as ITN selection and interviews will occur immediately following the creation of the sampling frame and all households and ITNs should be available. Oral consent will be sought from the head of household or their representative prior to proceeding (Annex 3), and respondents will be administered a shortened version of the standard ITN durability monitoring questionnaire. Households will receive a new ITN to replace the one withdrawn; the new ITN will be the same brand as the one withdrawn or, if this is not possible, have the same active ingredients. Study ITNs will be relabeled and packaged in individual plastic bags for transport to the laboratory.

## Questionnaires

The monitoring activity will use two questionnaires. The first, *Net Listing Questionnaire*, will capture information on study clusters, selected household sites and tagged campaign ITNs at the time of screening. The second will capture information on the ITNs selected at each assessment timepoint for analysis.

The *Net Listing Questionnaire* will be used to identify eligible households and list eligible households and their campaign ITNs in the sampling frame (the *Net Sampling Form* at 12-months). It comprises sections for:

* Screening questions to determine household eligibility for listing (these will be administered as part of sharing study information with selected households; no information will be recorded for ineligible households or those that refuse to participate except for the outcome of the screening visit [ineligible/refusal]).
* Household identification, including GPS coordinates and head of household’s full name.
* ITN identification, recording the number of campaign ITNs present and assigning each a unique ID.

The main monitoring questionnaire will be used to capture information associated with ITNs selected to undergo laboratory analysis at each timepoint. It comprises sections for:

* Household characteristics (composition, assets and other factors potentially associated with insecticide deterioration)
* Selected campaign ITN handling and use patterns, and washing and drying habits
* Laboratory-based hole assessment

This study will field questionnaires using publicly available Open Data Kit (ODK) software to conduct electronic data collection (EDC). Additionally, in the 12-month timepoint, the *Net Sampling Form* will be generated on paper to permit immediate random selection of ITNs for the first round of analysis. Questionnaires will be adapted from ODK durability monitoring questionnaires and tested repeatedly to ensure fully functioning versions are available at the start of field work. Questionnaires will incorporate skip patterns and filters, and internal consistency checks, range checks and logical checks to strengthen data quality. Questionnaires will be fielded using electronic data collection and will be available in [“English”, “French”, or “Portuguese”] and [Local written language(s)].

## Field procedures

### Preparatory phase

During the preparatory phase ITN campaign distribution registers will be sourced and cluster sampling in each study location will be completed. After experiences in other countries where the ITN brand found during data collection was not as anticipated, steps will be taken to verify the target nets were distributed in the monitoring sites through discussion and confirmation with the NMCP and staff closest to the sites. A detailed set of master training materials will be modified to match the country context. A visual aid for ITN brand identification will also be prepared in advance. This will be a laminated sheet with photographs of the campaign ITN brands with one photograph of the label and one of the net. Visual aids and tally sheets for the hole assessment will also be prepared in advance.

Working with [name of in-country implementing partner], job descriptions for monitoring team positions will be developed and competent staff and a laboratory capable of conducting the required bioassays identified. Chemical residue testing will be conducted by [Name of laboratory]. The same laboratory will be used for analysis from all data collection timepoints during the life of the activity.

### Field work

#### Teams and training

Each monitoring location will have its own implementation team, with two technicians and one driver [customize as required for country set-up]. It is estimated that the time needed at 12-month timepoint to list/map households, generate the ITN sampling frame and interview households in each of the 15 clusters is 18 days per location, including travel to and from the study sites. This assumes that for each cluster, the household listing, sampling, and interviews can be completed in one day in addition to 3 days of travel. During follow-up timepoints (24- and 36-months), each location can typically be completed by one team in 8 days (assuming passable road conditions, as travel from one cluster to another will be the determining factor for fieldwork time during follow-up timepoints).

Technicians will be carefully selected so that they are culturally acceptable, have good knowledge of the local languages, have experience performing bioassays, and experience in entomological monitoring or household surveys. Prior to the first fieldwork timepoint there will be a three-day training that will include the following components:

* Understanding the study design and procedures to generate the sampling frame
* General approach to ethics of field work (consent and interview)
* Introduction to and practical use of the data entry software
* Detailed study of interview with role play
* Labeling and packaging of ITNs withdrawn for analysis
* Practice hole assessment at laboratory using frame
* Overview of bioassay standard operating procedures (SOPs)

Just prior to each survey follow-up timepoints, a two-day refresher training will be given, anticipating that the same technicians will be engaged during the life of the study. Exact training lengths will depend on the level of experience among technicians engaged in the activity. Training for the first fieldwork timepoint will be led in-country by a team of local, regional and/or international experts in durability monitoring. Refresher trainings may be conducted in-person and led by the same team as the first fieldwork timepoint, or remotely with the use of video conferencing software.

#### Logistics and administration

Partner laboratories or field team agencies will ensure sufficient administrative and logistics staff are budgeted to support fieldwork.

#### Sensitization

As soon as clusters are selected the local authorities and key influencers such as chiefs will be informed of the purpose and expected timing of the survey and their support sought. Communities within clusters will be sensitized to the study objectives and activities to obtain maximum cooperation for the surveys.

#### Household consent and interviews

During screening to create the ITN sampling frame, selected households will be visited and the head of household or their representative will be interviewed to determine whether the household qualifies for the study. Study information will be provided in [“English”, “French” or “Portuguese”] and [Local written language] and, if necessary, will be read to the respondent in their local language by a member of the field team fluent in that language. Written consent will be sought to include the household in the study sampling frame and capture GPS coordinates and the full name of the head of household. Each household will receive a unique identification number consisting of the cluster and the household’s number. If a selected household is not available for any reason (refusal or moved out of the cluster at follow-up timepoints), the household will be dropped and one of the sampled alternates used instead.

At each fieldwork time point (12-, 24- and 36-months), ITNs will be sampled for withdrawal from the sampling frame. Following ITN sampling, the respective households in a cluster will be visited. At follow-up timepoints, study information will be provided again and – for all timepoints – oral consent will be sought before the sampled ITN is withdrawn and the short questionnaire administered to the head of household or their representative. If a selected household is not available at the time of visit for any reason, the team will continue with selection of any remaining ITNs in the cluster before revisiting unavailable households. If all other ITNs have been sampled and selected households are still unavailable, these ITNs/households will be dropped and one or more alternate ITN(s) targeted for inclusion, from the predetermined list of alternate ITNs for the cluster.

Example information sheets and consent forms for each study element are included in Annexes 2, 3 and 4.

#### Identification and labeling of campaign net

In order to identify an ITN as coming from the mass campaign and create the ITN sampling frame, technicians will inspect each net in households selected for the sampling frame and compare the label and net characteristics with the visual aids previously prepared. If the label matches the campaign net brand, respondents will be asked about the source and time of obtaining the net. If this information confirms the net as a campaign net, it will be tagged with a unique ID in indelible ink. Nets that cannot be verified as campaign nets will not be included in the sampling frame.

Prior to each follow-up timepoint (24- and 36-months), the ITN sampling frame will be updated to remove nets withdrawn during the previous timepoint and a new random selection of 30 nets per location will be performed. For each selected net, the net ID, associated household ID and name of the head of household will be printed by cluster. The field team will use these details to locate households and ITNs for follow-up. All paper forms will be destroyed by team leaders in the field as the teams complete data collection.

#### Net removal and replacement for analysis

Following receipt of oral consent, technicians will administer the questionnaire, remove the net, and pack it in an individual plastic bag for transport to the laboratory for hole assessment, bioassays, and onward shipping of samples for chemical residue testing. A like-for-like new replacement net will then be given to the household and marked with the date so they are not sampled in future timepoints.

#### Data collection, management and safety

For data collection, electronic devices will be used that allow a detailed programming of skip patterns and internal controls to ensure that all necessary data is collected and consistent. Depending on local conditions, data from each interview will be uploaded to a secure, web-based database at the end of each day, or as soon as a data connection can be established thereafter.

From the data, an ITN master list (the ITN sampling frame) will be created and updated after each assessment timepoint (in addition to the cross-sectional data collected as part of the timepoint).

The ITN master list will include the campaign ITN ID, GPS coordinates for the household, full name of head of household, a record of whether the net has previously been sampled and withdrawn, and a record of whether ITNs or whole households are known to no longer be available for sampling. Between surveys, this file will be password encrypted and safely kept on a fixed and secure data storage device (server), also with password protection. Access will only be available to monitoring study investigators. The ITN master list file(s) containing names and GPS coordinates will be deleted immediately following the final data collection at 36-months.

It is important to record precise geolocations of durability monitoring. To allow sufficient accuracy for geographic analysis of results, but not permit the location of any study households, GPS coordinates will be modified by introducing a small random error during data cleaning. Full name data will be removed during cleaning so that analytical data files will contain no personal identifiers.

#### Supervision and field support

Daily reports, including unforeseen challenges, will be shared with the study PI for discussion and resolution. External quality control will be provided by [Implementing partner / role] by monitoring data collection remotely in real-time and providing feedback to local teams via WhatsApp, email, and phone calls.

## Laboratory activities

Sampled ITNs will be sent to the study bioassay laboratory partner [Name of laboratory] for hole assessment, bioassay testing, and sample preparation for shipping to [Name of laboratory] for chemical analysis.

### Net hole assessment

Prior to conducting bioassays and cutting samples for chemical residue testing each net will be assessed for physical integrity and signs of repair using a frame to hang the net in the laboratory. Separately inspecting each side and roof of the net, technicians will count and categorize present holes into four different sizes based on the WHO guidelines: 0.5 to <2 cm, 2 to <10cm, 10 to <25 cm and larger than 25 cm in diameter. Measuring rulers and hole templates will be used to ensure accurate measurement. The presence and number of repaired holes will be noted, but these will not be counted as holes. Data will be entered using electronic data collection in a copy of the main questionnaire for the selected ITN.

### Laboratory analyses

Insecticidal effectiveness testing of pyrethroid-only nets will be performed according to modified WHO guidelines. Testing for new types of nets (PBO-synergist and dual AI nets) will use standard operating procedures (SOPs) based on the Innovation-to-Impact (i2i) published SOPs and developed in collaboration with PMI. Adjacent net samples will be cut from each net panel for bioassays and chemical residue testing, as described in 2.8.3.1 (bioassays), 2.8.6 (chemical testing) and the SOPs. Table 7 provides an overview of the methods used for new types of ITN for which standard approaches exist; a brief description of laboratory methods then follows. Full SOPs are provided in Sections 10 (PBO), 11 (dual AI with the insect growth regulator, pyriproxyfen) and 12 (dual IA with chlorfenapyr).

Table 7 Overview of bioassay procedures

|  |  |  |  |
| --- | --- | --- | --- |
| **Type of ITN** | **Target ingredient** | **Cone assays** | **Tunnel tests** |
| Standard pyrethroid-only  | Alpha-cypermethrin, deltamethrin, permethrin | Susceptible strain on field samples and negative control |  |
| PBO-synergist | Pyrethroid | Susceptible strain on field samples and negative control |  |
| Piperonyl butoxide (PBO) | Pyrethroid-resistant strain on field samples, on positive PBO-synergist control, on pyrethroid-only positive control and on negative control |  |
| Dual AI with chlorfenapyr | Chlorfenapyr |  | Pyrethroid-susceptible and pyrethroid-resistant strains on two pieces per field sampled net and on one negative control\*. |
| Dual AI with pyriproxyfen | Pyrethroid | Susceptible strain on field samples and negative control |  |
| Pyriproxyfen\*\* | Pyrethroid-resistant strain on field samples, on positive pyrethroid + pyriproxyfen control and on negative control |  |
| \* A new dual AI positive control and positive control of pyrethroid-only precursor to dual AI net (where this exists) are used to characterize strain prior to testing\*\* Oviposition measured by chambering following the cone bioassays |

### Cone and/or tunnel bioassays

*Summarize the SOPs for cone bioassays and tunnel tests, depending on the ITN brands included in the monitoring activity. Text is included below as an example.*

#### Net preparation

*Pyrethroid-only ITNs*

For each sampled net, four 30cm × 30cm pieces will be cut from standard positions 2, 3, 4 and 5 (standard position 1 will be included for pre-distribution testing but excluded from post-distribution testing as it may be exposed to excessive abrasion in routine use through tucking and untucking under the bed or sleeping surface). Netting samples will be labeled with the net ID and the cutting position and will be stored in a cool, dry place at 4 degrees Celsius until the tests start.

*Dual AI ITNs containing chlorfenapyr*

For each sampled net, two 30cm x 30cm pieces will be cut: 1 piece from side panels and 1 piece from the roof panels, as indicated in the SOPs. Netting samples will be labeled with the net ID and the cutting position and will be stored in a cool, dry place at 4 degrees Celsius until the tests start.

*Dual AI ITNs containing pyriproxyfen and PBO-synergist ITNs*

For each sampled net, four 30cm x 30cm pieces will be cut: 2 pieces from side panels and 2 pieces cut from the panel, as indicated in the SOPs. Netting samples will be labeled with the net ID and the cutting position and will be stored in a cool, dry place at 4 degrees Celsius until the tests start.

#### Testing residual efficacy of pyrethroids for PBO nets and dual AI nets containing pyriproxyfen

To test the residual efficacy of pyrethroids, insectary-raised, 2-5-day old, unfed females of a pyrethroid-susceptible strain will be used. For each net piece tested (see 2.8.3.1 above), five mosquitoes at a time will be introduced into two WHO cones (10 mosquitos per piece in total). The total number of mosquitoes tested will depend on the type of ITN. The same cutting procedure will be followed for a sample of untreated netting as a negative control, and the same number of mosquitoes tested against the negative control. Control net results can be shared by all bioassays done the same day. If control mortality is over 10%, the test will be repeated. The knock-down effect will be measured 60 minutes after exposure (KD60) and mortality will be recorded after 24 hours.

#### Testing the overall impact of PBO nets on resistant mosquitoes using cone bioassays

To evaluate the overall impact of PBO nets on resistant mosquitoes, a pyrethroid-resistant strain will be used in cone bioassays. Strains will be characterized before bioassays begin following standard SOPs. Cone bioassays on field samples will follow the same procedures as described in 2.8.3.2 above. The same number of mosquitos will be tested against the same net pieces from the same sites for both susceptible and resistant strains. In addition, the following controls will be used when testing PBO ITNs: one untreated netting negative control, five new PBO-brand nets as positive controls and 1 new ITN with the same pyrethroid active ingredient (e.g., PermaNet 2.0 when testing PermaNet 3.0).

### Tunnel tests for dual AI nets containing chlorfenapyr

#### Testing residual efficacy of pyrethroids and chlorfenapyr using tunnel tests

Three tunnels will be used for each net to be evaluated using a pyrethroid-susceptible and pyrethroid-resistant strain. The first will be a tunnel with an untreated control net piece; the second and third will have two pieces from the IG2 field sample to be evaluated. One side piece and one roof piece from each field ITN will be used. If more than 3 tunnels per night can be run, only one set of positive and negative controls need to be run, and the results can be shared amongst all the IG2 field samples being tested. A minimum of 10 positive IG2 control ITNs will be used for comparison during the test, given the high variation in mortality recorded in tests on new IG2 nets recorded in Burkina Faso.

Fifty resistant nulliparous female mosquitoes, aged 5-8 days and sugar-starved for 6 hours, will be introduced into the end opposite the bait at 18:00. The lights of the room will be turned off, and only turned on when the tunnel test is finished the following morning at 7:00. The overall exposure period should be 12-15h. The environmental conditions in the room during the night should be 27 ± 2 °C and 75% ± 10% relative humidity.

At 7:00, a narrow insert will be slid down between the two compartments of the tunnel, to prevent mosquitoes from moving between the compartments. All mosquitoes will be carefully collected from the tunnel, noting the compartment in which the mosquitoes were collected (initial compartment/animal compartment), the blood-feeding status (fed/unfed), and mortality (living/dead).

Surviving mosquitoes from the tunnels will be put into cups covered with untreated netting, and cotton wool soaked in sugar solution will be placed on top of the cups, allowing mosquitoes to feed ad libitum. The mortality will be recorded at 18:00 (24 hours after the tunnel test started), and then again at 48 and 72 hours. Data on blood feeding will also be recorded to estimate the corrected blood-feeding inhibition due to chlorfenapyr.

### Measuring oviposition for dual AI nets containing pyriproxyfen

#### Testing residual efficacy of pyriproxyfen

The procedures described under 2.8.3.2 are implemented for pyrethroid-susceptible and pyrethroid-resistant mosquito strains using 3-to-5-day-old blood-fed female *Anopheles* mosquitoes. Mosquitoes should be blood-fed 6-12 hours prior to exposure. Mosquitoes should remain in their holding containers until day 4 (three days post-exposure). Mosquitoes alive on day 4 should be transferred individually into their own artificial egg laying chamber and provided with a sugar meal. On day 8 (four days post-chambering) the number of mosquitoes alive and dead is recorded, together with whether each mosquito has laid eggs. At the end of testing, discard mosquitoes safely. Only the chambering method will be used to measure sterility, as described within Section 4.7 of the SOPs for dual AI nets with the insect growth regulator, pyriproxyfen (SOPs are Section 7, below).

### 2.8.6 Chemical residue

For each sampled net, two 30cm × 30cm pieces will be cut from each position on either side of the piece cut for bioefficacy testing.

For the analysis of insecticidal content for dual AI nets containing chlorfenapyr, a total **four** 30cm x 30cm samples will be cut from each sampled net at locations adjacent to those cut for the bioassays.

For the analysis of insecticidal content for other dual AI, PBO, and standard pyrethroid nets, **eight** 30cm x 30cm samples will be cut from each sampled net at locations adjacent to those cut for the bioassays. Netting samples will be labeled with the net ID and the cutting position and will be stored in a cool, dry place at 4 degrees Celsius until ready for shipping to the testing laboratory.

Net pieces will be labeled with the net ID number, wrapped individually in aluminum foil, and shipped to the [Name of laboratory] for chemical residue testing using the ISO 17025 accredited analytical method RESMM002.

## Outcome measures

### Bioassay results

The primary outcome of insecticidal efficacy will be based on the bioassay results using the following criteria. Counts will be recorded for each net piece and replicate individually, and replicate data will be pooled for analysis.

For **pyrethroid-only ITNs** and **PBO-synergist ITNs**, the proportion of ITNs achieved optimal and minimal effectiveness will be estimated at each assessment timepoint.

Optimal Effectiveness is defined as:

* KD60 ≥ 95% or 24-hour mortality ≥ 80% by the cone assay

Minimal Effectiveness is defined as:

* KD60 ≥ 75% or 24-hour mortality ≥ 50% by the cone assay, or
* Mortality ≥ 80% or blood-feeding inhibition ≥ 90% by the tunnel test (where conducted)

For **PBO-synergist ITNs**, additional efficacy criteria will be quantified using the definition:

* The proportion of samples that are within 10% of the positive control values of KD60 or 24-hour mortality

For **dual-AI ITNs containing chlorfenapyr**, the following outcome measures will be recorded:

* The proportion of mosquitos dead at 72 hours (mortality at 24 hours and 48 hours will also be recorded), reported by compartment and blood-feeding status
* The proportion of blood-fed mosquitoes
* Blood-feeding inhibition

If control mortality is >10% or blood-feeding is <50% for the day the test results should be discarded, and the test repeated.

For **dual-AI ITNs containing pyriproxyfen**, mosquito counts on day 4 and day 8 will be used in addition to cone bioassay counts to calculate the following outcome measures:

* The proportion of mosquitoes knocked-down at one hour (KD60)
* The proportion of mosquitos dead t 24 hours (24-hour mortality)
* Oviposition, defined as the number of mosquitoes (living or dead) which laid eggs divided by the total number of surviving blood-fed mosquitoes placed into oviposition chambers.
* Oviposition inhibition, defined as the difference between the proportion of surviving blood-fed females from the control which laid eggs (Oc) and the proportion of surviving blood-fed females from the treatment which laid eggs (Ot), divided by Oc.

### Chemical residue

Two metrics will be used to report the chemical residue test results:

1. The mean insecticide content across the overall sample and for each location
2. The proportion of nets with a g/kg value for each active ingredient that falls within the approved level defined by the WHO Pre-Qualification specification, or manufacturer specification.

### Net integrity

Net integrity will be measured at each timepoint using the proportionate Hole Index (pHI) as recommended by WHO. Data from the net hole assessment will be transformed into the proportionate Hole Index (pHI) for each net in the following way:

*pHI= # size 1 holes + (# size 2 holes x 23) + (# size 3 holes x 196) + (# size 4 holes x 576)*

Based on the pHI each net is then categorized as “serviceable” or “torn”, with a subset of serviceable nets categorized as “good”, as follows [2-3]:

 Serviceable: total hole surface area ≤ 0.1 m² or pHI ≤ 642

Torn: total hole surface area > 0.1m² or pHI > 642

Good: total hole surface area < 0.01m² or pHI ≤ 64

### Net attrition rate

In the 12-month survey round, attrition is estimated from the number of ITNs that were reportedly received by households during the mass campaign and the number of ITNs remaining at the time of net listing. In the 24- and 36-month survey rounds, net attrition will be indirectly estimated based on the number of alternate ITNs in the sample list that must be sought before the required sample size of 30 ITNs is attained.

## Data analysis and reporting

Electronic data files will be available immediately following the completion of household data collection. Hole assessment results will be added to ITN-specific data files in the laboratory. Completed data files will be exported from the secure online database and imported to Stata. Standard Stata do files adapted from previous durability monitoring studies will be used to apply consistent cleaning, management, and analysis steps. Staff from [Name of implementing partner] or a suitably qualified consultant will review and clean the data, doing further consistency checks and preparing files for analysis. Personal identifiers will be dropped from final data sets that may be shared, on request, outside the study team. All processes will be documented using Stata do files so that any interested partner can repeat the steps on their own copy of the data set.

Bioassay testing data will be entered in standard Excel worksheets, capturing information on number of mosquitoes tested, number of mosquitoes knocked down: 3 min, number of mosquitoes knocked down: 60 min, number of mosquitoes dead: 24 hours, number of mosquitoes alive: 24 hours. Summary data from these worksheets will be produced in Excel and exported to Stata for standard data cleaning and analysis. Final analysis will follow the previously defined outcome measures (see above). Excel sheets will be used until durability monitoring data is added to VectorLink Collect (expected 2021).

Results will be shared and discussed among partners after each assessment time point and a summary report issued for each timepoint. Once the final report is completed, a dissemination meeting will be organized to present findings and recommendations to malaria vector control stakeholders and partners in the country. Following the final study timepoint, anonymized study data will be shared with PMI/USAID for archiving on the U.S. Government’s open data portal, [www.data.gov](http://www.data.gov). Final reports and anonymized study data will also be posted to the website, www.durabilitymonitoring.org.

# Ethical Considerations

The proposed study will be conducted according to the principles of the Declaration of Helsinki and the International Guidelines for Ethical Review of Epidemiological Studies.

This study has been determined to be “research with human subjects” and will be initiated only after receiving written approval from a recognized local ethics review board and [Name of implementing partner] Institutional Review Board (IRB). Those implementing this study will comply with all policies and procedures of all reviewing boards. Informed consent will be sought for all participants in this study.

This study has been designed to address the following ethical principles: respect for persons, beneficence, and justice. Efforts are made to protect individual autonomy, minimize harm, and maximize benefits and equitably distribute risks and benefits by using procedures which are consistent with sound research designs that take these issues into consideration. Since this is an exclusive interview survey without taking of samples of any kind no harm is expected to the participants.

## Informed Consent

Respondents in households selected to form the sampling frame will be informed about the purpose and nature of the study, what participation in the study requires and possible risks and benefits. Written consent will be obtained from the head of household or their representative. Respondents in households selected from which to withdraw an ITN for analysis will be informed as above and oral consent will be obtained before withdrawing and replacing the ITN and administering the study questionnaire.

Participants will be informed of all risks and protections through the study information sheet. Participants will also be informed of their right to withdraw from the study and to not answer any questions they do not feel comfortable answering. Respondents will be provided contact information for the PI and co-investigators who will be available to answer any questions about the study.

Information sheets and consent forms will be written in [“English”, “French” or “Portuguese”] and [Local written language] and, if necessary, will be read to the respondent in their local language by a member of the field team fluent in that language.

## Respect for persons and individual autonomy

*Potential Risks*

Potential risks to subjects are breach of confidentiality.

*Breach of Confidentiality*

The most significant risk is a breach of confidentiality. In this study, a breach of confidentiality could occur if private information from the surveys could be linked to an individual research respondent and this information was obtained by person(s) outside of the research project or something a participant says will be heard or found out by someone else.

*Strategies to Address Risks*

Steps will be taken to protect participants against potential risks posed by their participation in this research. Participants will be encouraged to contact the local co-investigators at any time to discuss any concerns they might have. All data and other information will be maintained confidentially and anonymous to the greatest extent possible. The following steps will be taken to protect against breaches of confidentiality.

*Identification of data sources*

None of the information registered is sensitive. Household GPS location and household members full names for the sampling frame will be recorded in raw data files. The master lists of ITNs will maintain a record of these identifiers so that follow-up visits can be made. This list will be an electronic data file only and will be kept in a secure location with password protection and access only by the PI and co-investigators. This database will be destroyed after the final assessment timepoint.

GPS coordinates will be modified by introducing a small random error during data cleaning. This will permit sufficient accuracy for geographic analysis of results but will not permit the location of any study households. Full name data will be removed during cleaning so that analytical data files will contain no personal identifiers. Anonymized files are the only ones which will be shared publicly, for example with the [www.data.gov](http://www.data.gov) repository.

Written consent documents will be stored in locked containers and destroyed at the conclusion of the final assessment timepoint.

*Data reporting*

All results of the study will be reported anonymously.

*Staff ethical training*

All research staff including interviewers and supervisors will be trained in human subject’s protection, especially the importance of protecting privacy and confidentiality.

## Beneficence (maximizing benefits and minimizing harm)

There is unlikely to be any direct benefit to participants themselvesat the time of the study or after the study. However, the proposed study may result in knowledge that can be applied to the design of future interventions to promote net use, and net care to increase net durability. Primary study results will support public health donors, governments and ITN manufacturers to better design, plan and implement ITN products and campaigns based on field results on insecticide effectiveness.

# Implementation timeline and study personnel

## Roles and Responsibilities

Study implementation will be conducted jointly by the National Malaria Control Program, [Name of implementing partner], [Name of any additional laboratories] and PMI. While all partners will give input into the study tools, analysis, and interpretation, [Name of lead implementing partner], with technical support from other partners, will be responsible for quality assurance of the design, implementation, and analysis of the study.

## Timeline

The proposed study timeline for the first 12 months of implementation is shown below (covering 2 data collection timepoints).

|  |  |  |
| --- | --- | --- |
|  | **Pre-distribution** | **Post-distribution (months)** |
| **Activity** | **4** | **3** | **2** | **1** | **0** | **1** | **2** | **//** | **11** | **12** | **13** | **14** | **15** | **16** | **17** |
|  | **Set-up** |
| Protocol drafted and shared with PMI and NMCP for review | X | X |   |   |  |   |   |  / |     |   |   |   |   |   |   |
| Protocol and tools for IRB submission finalized |    |  | X |  |  |   |   |  / |     |   |   |   |   |   |   |
| ITN campaign distribution |    |   |   |  | X |   |   |  / |     |   |   |   |   |   |   |
|  | **Pre-distribution activities (illustrative timing)** |
| Withdraw ITNs from central stores |  |  | X |  |  |  |  | / |  |  |  |  |  |  |  |
| Bioassays on 20 samples |  |  | X |  |  |  |  | / |  |  |  |  |  |  |  |
| Chemical tests on 10 samples |  |  |  | X |  |  |  |  |  |  |  |  |  |  |  |
| Additional tests if required |  |  |  | X |  |  |  | / |  |  |  |  |  |  |  |
| Pre-distribution reporting submitted to PMI |  |  |  |  | X |  |  | / |  |  |  |  |  |  |  |
|  | **12-month timepoint**  |
| Ethical review by [Name of implementing partner] |  | X | X |  |  |  |  | / |  |  |  |  |  |  |  |
| Ethical review by [country] IRB |  |  | X | X |  |  |  | / |  |  |  |  |  |  |  |
| 12-month training |  |  |  |  |  |  |  | / |  | X |  |  |  |  |  |
| 12-month fieldwork |  |  |  |  |  |  |  | / |  | X |  |  |  |  |  |
| 12-month hole assessment |  |  |  |  |  |  |  | / |  |  | X |  |  |  |  |
| 12-month bioassays |  |  |  |  |  |  |  | / |  |  | X | X |  |  |  |
| 12-month chemical residue testing |  |  |  |  |  |  |  | / |  |  | X | X | X |  |  |
| 12-month data cleaning and analysis |  |  |  |  |  |  |  | / |  |  | X |  | X | X |  |
| Draft ITN Durability Monitoring 12-month Report submitted to PMI |  |  |  |  |  |  |  | / |  |  |  |  |  |  | X |

The timeline for the follow-up timepoints (24- and 36-month timepoints) are shown below taking as an example the 24-month timepoint.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Activity** | **M****21** | **M****22** | **M****23** | **M****24** | **M****25** | **M****26** | **M****27** | **M****28** | **M****29** |
| **Follow-up timepoint (24-month)** |
| Draw 12-month sample and program tools | X |   |   |   |   |   |   |   |   |
| Revise training materials | X |   |   |   |   |   |   |   |   |
| Submit for continuing review from [country] IRB |  |  | X | X |  |  |  |  |  |
| 24-month training |  |  |  | X |  |  |  |  |  |
| 24-month fieldwork |   |   |   | X |   |   |   |   |   |
| Follow-up hole assessment |  |  |  |  | X |  |  |  |  |
| Follow-up bioassays |   |   |   |   | X | X |  |   |   |
| Follow-up chemical residue testing |   |   |   |   |  X | X | X |  |   |
| Follow-up data cleaning and analysis |   |   |   |   |   |   | X | X |  |
| Draft ITN Durability Monitoring Follow-up Report submitted to PMI |   |   |   |   |   |   |   |   | X |

## Study personnel

The following personnel are named investigators on this study.

Principal Investigator:

Co-investigator:

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# Annex 1: List of durability monitoring completed since 2011

Source: www.durabilitymonitoring.org

|  |  |  |
| --- | --- | --- |
| **Country** | **Product** | **Monitoring Areas** |
| Burma2016-2019 | DawaPlus 2.0PermaNet 2.0 | Tamu Township |
| DRC2016-2019 | DuraNet/MAGNetDawaPlus 2.0 | Sud-Ubangi / Mongala |
| Guinea2016-2019 | PermaNet 2.0 | Boffa / Dinguiraye |
| Tanzania (Zanzibar)2016-2019 | OlysetPermaNet 2.0 | Wete / North B |
| Malawi2016-2019 | YorkoolRoyal Sentry | Mangochi / Kasungu |
| Nigeria2015-2018 | DawaPlus 2.0 | Zamfara / Ebonyi / Oyo |
| Mozambique2015-2018 | MAGNetRoyal Sentry | Inhambane / Tete / Nampula |
| Madagascar2015-2017 | PermaNet 2.0 | Nosy / Varika / Maintirano / Tulear II / Ankazobe |
| Zimbabwe2015-2019 | DawaPlus 2.0DuraNet | 13 malaria endemic districts in Mashonaland Central and West Provinces |
| Ethiopia2015-2018 | PermaNet 2.0MAGNet | Oromia / Tigray / SNNP / Amhara |
| Benin2014-2017 | PADNETLifeNetPermaNet 3.0 | Oueme |
| Madagascar2013-2015 | NetProtectYorkoolRoyal Sentry | Ambanja / Morondava / Diego-Suarez / Mandoto / Sakaraha / Toamasina II, Randriamaherijaona |
| Nigeria2012-2014 | PermaNet 2.0DawaPlus 2.0 | Zamfara / Nasarawa / Cross River |
| Madagascar2011-2015 | YorkoolRoyal SentryNetProtect | Toamasinall/Morondava / Mandoto/Sakaraha / Ambanja/Diego |
| Liberia2018-2021 | DuraNet | Grand Gedeh / Lofa |

# Annex 2: Information sheet and consent form for net listing

**STREAMLINED DURABILITY MONITORING OF ITNS IN COUNTRY**

**NET LISTING AND SAMPLING (12-MONTHS)**

**INFORMATION SHEET**

Study Title: *Streamlined durability monitoring of insecticide-treated nets (ITNs) distributed during the [year] mass campaign in [Country]*

**SPEAK TO THE HOUSEHOLD HEAD OR THEIR REPRESENTATIVE**

**PURPOSE**

[Greeting]

My name is \_\_\_\_\_\_\_\_\_, from [Name of implementing partner].

We are conducting a study to monitor how long mosquito nets continue to kill and repel mosquitos after distribution during a mass distribution campaign, like the one that was conducted here in [Date of campaign]. We are also interested in understanding how long nets last until they are too torn to be used for sleeping under. Before the study begins, we need to create a list of mosquito nets that some households in your community possess. We will select nets from this list to be part of the study now and in future years. We are working in collaboration the [Country] National Malaria Control Program. The survey is funded by the United States government through the President’s Malaria Initiative. Your household has been selected at random to be approached to participate in this study.

I would like to talk to you about mosquito nets you have in your household. If your household is eligible and you agree to participate in the survey, I will ask to see the nets you received from the campaign in [Date of campaign] and write a code on the net labels. We will do this for [Number] households in your community and then select 2 nets at random for our study at this time. One of your nets may be selected, but the choice is random and a net from your household may not be selected at this time.

May we speak with you about this study?

***If Yes, continue***

***If No, thank the respondent for their time and visit the next household in the sampling list.***

Before we continue, can I check:

* Are you the household head or their representative?
* Are you at least 18 years old?
* Did your household receive any mosquito nets during the campaign in [Date of campaign]?
* Do you still have one or most mosquito nets from this campaign still in your possession?
* Are you free to talk with me today?

***If Eligible, continue***

***If Ineligible, thank the respondent for their time and visit the next household in the sampling list.***

**PROCEDURES**

I will ask you to show me all the nets that you received from the [Date of campaign] campaign. We will check that these nets came from the campaign by examining them. For each net you have from the campaign, we will write a unique code on the net label and record the code in our questionnaire. We will also ask for your first name and record the location of your household so we can visit you again in future if one of your nets is selected for the study.

This week, and again in [Date of 24-month round] and [Date of 36-month round], we will pick at random some of the nets we tag in households like yours. If your household is picked, we will visit you again to see if the net is still in your possession. If it is, we will ask if we can take the net for our study and we will replace it with a similar new net at no cost to you. We will ask a few questions about your household and the mosquito net that we select. During this visit it will take no more than 20 minutes to ask the questions and take down the net.

**Voluntary participation:** Participation in the study is completely voluntary. If you do not participate, there will be no disadvantage for you or your household. If you agree to participate, you can change your mind at any time. If your household is picked for an interview, you do not have to answer any question you do not want to, and you will be able to stop the interview at any time. You can leave the study at any time. We will let the community know about the results of the study when it ends.

**Risks/discomforts:** At this visit we are asking whether we can examine your nets from the last campaign, write a code on their label, and record your first name and the household location. If you say yes, this will recruit your nets into the study, and we may visit you again to request one of your nets for analysis. If we revisit your household, we will ask you questions about your household and the mosquito net that we select. There is a slight risk that someone might learn about your answers, but we will not share any information that identifies your household with anyone outside the study team. At the end of the study, the list containing your first name and the exact location of your household will be destroyed. We will not share this information with anyone outside the study team.

**Benefits:** We will not pay you to join the study, but we will give you a new mosquito net to replace the one we take if we pick one of your nets for the study. Your participation in this study will also inform the authorities which measures to take to make nets last longer.

**Protecting data confidentiality:** All research projects carry some risk that information about you may become known to people outside of a study. Your answers will not have your name attached to them. Your personal information will be destroyed at the end of the study as we will not need it to locate your house again once the study has ended.

**Protecting subject privacy during data collection:** We will do everything we can to prevent other people learning your answers to the study questions. Your answers will not be shown to anyone outside of the study team. After each round of data collection, the data sets we create will be de-identified. This means your first name and the location of your house will be removed from the data, so no one will know this information. The de-identified data sets may be used by others for future research without additional informed consent.

Do you have any questions?

**QUESTIONS**

If you have questions or concerns after the interview, you may contact the study investigators:

|  |  |
| --- | --- |
| [Name of local study investigator][Name of implementing partner][Telephone contact][Email contact] | [Name of HQ study investigator][Name of implementing partner][Telephone contact][Email contact] |

If you have any questions about your rights as a research participant, or if you think you have not been treated fairly, you may contact the [Name of Country IRB] at [Telephone contact].

**You may either read this form yourself or I will read it to you**

**STREAMLINED DURABILITY MONITORING OF ITNS IN COUNTRY**

**NET LISTING AND SAMPLING (12-MONTHS)**

**CONSENT FORM**

Study Title: *Streamlined durability monitoring of insecticide-treated nets (ITNs) distributed during the [year] mass campaign in [Country]*

**WRITTEN CONSENT TO BE INCLUDED IN THE STUDY SAMPLING FRAME**

**QUESTIONS**

If you have questions or concerns after the interview, you may contact the study investigators:

|  |  |
| --- | --- |
| [Name of local study investigator][Name of implementing partner][Telephone contact][Email contact] | [Name of HQ study investigator][Name of implementing partner][Telephone contact][Email contact] |

If you have any questions about your rights as a research participant, or if you think you have not been treated fairly, you may contact the [Name of Country IRB] at [Telephone contact].

**What does your signature or mark on this consent form mean?**

Your signature or mark on this form means:

* You have been informed about this study’s purpose, procedures, possible benefits and risks.
* You have been given the chance to ask questions before you sign.
* You have voluntarily agreed to list the mosquito nets that you have in your possession from the [Date of campaign] campaign for the study.
* You understand that registering the mosquito nets that you have in your possession from the [Date of campaign] campaign for the study does not guarantee that you will be visited again as part of the study; we will select mosquito nets for the study at random from all the households we visit.

|  |
| --- |
|  |
| *Signature or mark of study participant* |
|  |
| *Print name of person obtaining consent* |
|  |
| *Signature of person obtaining consent* |
|  |
| *Date* |

# Annex 3: Information sheet and consent form for 12-month round

**STREAMLINED DURABILITY MONITORING OF ITNS IN COUNTRY**

**12-MONTH INTERVIEW WITH SELECTED HOUSEHOLD**

**INFORMATION SHEET**

Study Title: *Streamlined durability monitoring of insecticide-treated nets (ITNs) distributed during the [year] mass campaign in [Country]*

**SPEAK TO THE HOUSEHOLD HEAD OR THEIR REPRESENTATIVE**

**PURPOSE**

[Greeting]

My name is \_\_\_\_\_\_\_\_\_, from [Name of implementing partner]. Staff from [Name of implementing partner] visited your household earlier today to identify some mosquito nets and record details about them and your household. We are working in collaboration the [Country] National Malaria Control Program. The survey is funded by the United States government through the President’s Malaria Initiative.

We have selected [Number of nets] of your nets at random to part of the study at this time. We would like to ask you to continue participating in the study about mosquito nets.

May we please speak with you about it?

***If Yes, continue***

***If No, thank the respondent for their time and visit the next household.***

Can I just check:

* Are you the household head or their representative?
* Are you at least 18 years old?
* Are you free to talk with me today?

***If Eligible, continue***

***If Ineligible, thank the respondent for their time and visit the next household in the sampling list.***

**PROCEDURES**

I will ask you to show me the net(s) that we have selected at random for the study. If the net(s) are no longer in your possession, then we will end the visit. If the net(s) are still in your possession I will ask if we can take the net(s) for our study and we will replace it with a similar new net at no cost to you. I will also ask a few questions about your household and the mosquito net(s). During this visit it will take no more than 20 minutes to ask questions and take down the net.

**Voluntary participation:** Participation in the study is completely voluntary. If you do not participate, there will be no disadvantage for you or your household. If you agree to participate, you can change your mind at any time. If we ask you a question you don't want to answer, let me know and I'll move on to the next question. You can stop the interview at any time. We will let the community know about the results of the study when it ends.

**Risks/discomforts:** You may be uncomfortable answering some of the questions. You do not have to answer all the questions and you may stop at any time.

**Benefits:** We will not pay you to join the study, but we will give you a new mosquito net to replace the one we take. If you have any problems in hanging your new net, we will be able to help you. Your participation in this study will also inform the authorities which measures to take to make nets last longer.

**Protecting data confidentiality:** All research projects carry some risk that information about you may become known to people outside of a study. Your answers will not have your name attached to them. Your personal information will be destroyed at the end of the study as we will not need it to locate your house again once the study has ended.

**Protecting subject privacy during data collection:** We will do everything we can to prevent other people learning your answers to the study questions. Your answers will not be shown to anyone outside of the study team. After each round of data collection, the data sets we create will be de-identified. This means your first name and the location of your house will be removed from the data, so no one will know this information. The de-identified data sets may be used by others for future research without additional informed consent.

Do you have any questions?

**QUESTIONS**

If you have questions or concerns after the interview, you may contact the study investigators:

|  |  |
| --- | --- |
| [Name of local study investigator][Name of implementing partner][Telephone contact][Email contact] | [Name of HQ study investigator][Name of implementing partner][Telephone contact][Email contact] |

If you have any questions about your rights as a research participant, or if you think you have not been treated fairly, you may contact the [Name of Country IRB] at [Telephone contact].

**You may either read this form yourself or I will read it to you**

**STREAMLINED DURABILITY MONITORING OF ITNS IN COUNTRY**

**12-MONTH INTERVIEW WITH SELECTED HOUSEHOLD**

**CONSENT FORM**

Study Title: *Streamlined durability monitoring of insecticide-treated nets (ITNs) distributed during the [year] mass campaign in [Country]*

**ORAL CONSENT TO PARTICIPATE**

**QUESTIONS**

If you have questions or concerns after the interview, you may contact the study investigators:

|  |  |
| --- | --- |
| [Name of local study investigator][Name of implementing partner][Telephone contact][Email contact] | [Name of HQ study investigator][Name of implementing partner][Telephone contact][Email contact] |

If you have any questions about your rights as a research participant, or if you think you have not been treated fairly, you may contact the [Name of Country IRB] at [Telephone contact].

Do you agree to participate in the study at this time?

Check if the respondent accepts to participate □

“I have read the consent form completely to the study participant and the study participant voluntarily agreed to participate in the study.”

|  |
| --- |
|  |
| *Print name of person obtaining consent* |
|  |
| *Signature of person obtaining consent* |
|  |
| *Date* |

# Annex 4: Information sheet and consent form for 24- and 36-month rounds

**STREAMLINED DURABILITY MONITORING OF ITNS IN COUNTRY**

**FOLLOW-UP INTERVIEW WITH SELECTED HOUSEHOLD (24-MONTHS AND 36-MONTHS)**

**INFORMATION SHEET**

Study Title: *Streamlined durability monitoring of insecticide-treated nets (ITNs) distributed during the [year] mass campaign in [Country]*

**SPEAK TO THE HOUSEHOLD HEAD OR THEIR REPRESENTATIVE**

**PURPOSE**

[Greeting]

My name is \_\_\_\_\_\_\_\_\_, from [Name of implementing partner]. Staff from [Name of implementing partner] visited your household in [Date of baseline round] to identify some mosquito nets and record details about them and your household. You may remember this visit. We are working in collaboration the [Country] National Malaria Control Program. The survey is funded by the United States government through the President’s Malaria Initiative.

We have selected [Number of nets] of your nets at random to part of the study at this time. We would like to ask you to continue participating in the study about mosquito nets.

May we please speak with you about it?

***If Yes, continue***

***If No, thank the respondent for their time and visit the next household.***

Can I just check:

* Are you the household head or their representative?
* Are you at least 18 years old?
* Are you free to talk with me today?

***If Eligible, continue***

***If Ineligible, thank the respondent for their time and visit the next household in the sampling list.***

**PROCEDURES**

I will ask you to show me the net(s) that we have selected at random for the study. If the net(s) are no longer in your possession, then we will end the visit. If the net(s) are still in your possession I will ask if we can take the net(s) for our study and we will replace it with a similar new net at no cost to you. I will also ask a few questions about your household and the mosquito net(s). During this visit it will take no more than 20 minutes to ask the questions and take down the net.

**Voluntary participation:** Participation in the study is completely voluntary. If you do not participate, there will be no disadvantage for you or your household. If you agree to participate, you can change your mind at any time. If we ask you a question you don't want to answer, let me know and I'll move on to the next question. You can stop the interview at any time. We will let the community know about the results of the study when it ends.

**Risks/discomforts:** You may be uncomfortable answering some of the questions. You do not have to answer all the questions and you may stop at any time.

**Benefits:** We will not pay you to join the study, but we will give you a new mosquito net to replace the one we take. If you have any problems in hanging your new net, we will be able to help you. Your participation in this study will also inform the authorities which measures to take to make nets last longer.

**Protecting data confidentiality:** All research projects carry some risk that information about you may become known to people outside of a study. Your answers will not have your name attached to them. Your personal information will be destroyed at the end of the study as we will not need it to locate your house again once the study has ended.

**Protecting subject privacy during data collection:** We will do everything we can to prevent other people learning your answers to the study questions. Your answers will not be shown to anyone outside of the study team. After each round of data collection, the data sets we create will be de-identified. This means your first name and the location of your house will be removed from the data, so no one will know this information. The de-identified data sets may be used by others for future research without additional informed consent.

Do you have any questions?

**QUESTIONS**

If you have questions or concerns after the interview, you may contact the study investigators:

|  |  |
| --- | --- |
| [Name of local study investigator][Name of implementing partner][Telephone contact][Email contact] | [Name of HQ study investigator][Name of implementing partner][Telephone contact][Email contact] |

If you have any questions about your rights as a research participant, or if you think you have not been treated fairly, you may contact the [Name of Country IRB] at [Telephone contact].

**You may either read this form yourself or I will read it to you**

**STREAMLINED DURABILITY MONITORING OF ITNS IN COUNTRY**

**FOLLOW-UP INTERVIEW WITH SELECTED HOUSEHOLD (24-MONTHS AND 36-MONTHS)**

**CONSENT FORM**

Study Title: *Streamlined durability monitoring of insecticide-treated nets (ITNs) distributed during the [year] mass campaign in [Country]*

**ORAL CONSENT TO PARTICIPATE**

**QUESTIONS**

If you have questions or concerns after the interview, you may contact the study investigators:

|  |  |
| --- | --- |
| [Name of local study investigator][Name of implementing partner][Telephone contact][Email contact] | [Name of HQ study investigator][Name of implementing partner][Telephone contact][Email contact] |

If you have any questions about your rights as a research participant, or if you think you have not been treated fairly, you may contact the [Name of Country IRB] at [Telephone contact].

Do you agree to participate in the study at this time?

Check if the respondent accepts to participate □

“I have read the consent form completely to the study participant and the study participant voluntarily agreed to participate in the study.”

|  |
| --- |
|  |
| *Print name of person obtaining consent* |
|  |
| *Signature of person obtaining consent* |
|  |
| *Date* |

# Annex 5: I2I-SOP-001: Methods for monitoring the durability of dual AI insecticide-treated nets containing a pyrethroid plus piperonyl butoxide (PBO)



[1. Purpose 1](#_Toc89425408)

[2. Background 2](#_Toc89425409)

[3. Materials & Equipment 2](#_Toc89425410)

[4. Procedure 3](#_Toc89425411)

[4.1. Test mosquitoes 3](#_Toc89425412)

[4.2. Collection and storage of test net samples (for bioefficacy testing) 3](#_Toc89425413)

[4.3. Control net samples 4](#_Toc89425414)

[4.4. Cone bioassay setup 6](#_Toc89425415)

[4.5. Cone bioassay procedure 6](#_Toc89425416)

[4.6. Measured outcomes 7](#_Toc89425417)

[5. Data priority list 8](#_Toc89425418)

[6. Deviations from standard protocol 9](#_Toc89425419)

[7. Supplementary data 9](#_Toc89425420)

[8. Glossary of terms 9](#_Toc89425421)

[9. References 9](#_Toc89425422)

1. Purpose

This standard operating procedure (SOP) describes the methods to determine the bioefficacy of the pyrethroid and piperonyl butoxide (PBO) components of insecticide-treated nets (ITNs) used under operational conditions. The process used to determine the methodology detailed in this SOP, and justifications for key methodological parameters can be found in ‘I2I-MD-001: Durability monitoring method development: Dual AI insecticide-treated nets containing a pyrethroid plus piperonyl butoxide (PBO)’.

1. Background

Several ITNs containing pyrethroid plus PBO (pyrethroid + PBO nets) are prequalified (PQ) by the WHO (WHO, 2020). These nets products have different specifications. They contain different pyrethroid insecticides at various concentrations, and PBO is located on varying parts of the net (e.g. roof only). Monitoring the bioefficacy of the active ingredients (AI) in the nets is a vital part of establishing the durability of these nets under operational conditions.

1. Materials & Equipment

**General**

* Data collection sheets
* Lab coat
* Gloves
* Test pyrethroid + PBO nets
* Control untreated net
* Control new pyrethroid + PPO net
* Control new pyrethroid only net
* Aspirator (manual/electronic), separate for each insecticide
* Mosquito strains
* Pen/permanent markers

**Collection and storage of net samples**

* Net frame
* Scissors
* Paper labels
* Aluminium foil

**Cone bioassay**

* Tape
* Mosquito holding containers (e.g. paper cups covered with untreated netting held by elastic bands)
* Cone holding frame (x 2), with holes to hold standard WHO plastic cones
* Cone holder frame stand, which holds frame at 45°
* WHO plastic cones
* Binder clips or clamps
* Cotton wool or rubber stoppers
* Temperature and humidity data logger
* Timer
* 10% sucrose solution (e.g. sugar or honey and water)
* Cotton wool
1. Procedure
	1. Test mosquitoes
* Use 2-to-5-day-old non-blood fed female *Anopheles* mosquitoes. Mosquitoes should be well characterized lab strains with respect to insecticide susceptibility (Lees et al, In Prep). F0 adults collected from larval breeding sites should only be used when lab strains are unavailable and should following the same insecticide resistance characterisation methods as lab strains (see Section 6. Deviations from standard protocol).
* For a pyrethroid-only test net panel: Use pyrethroid-susceptible mosquito strains (Lees et al, In Prep).
* For a pyrethroid + PBO test net panel: Use pyrethroid-susceptible and pyrethroid-resistant mosquito strains (Lees et al, In Prep).
* Where resources allow it and mosquitoes are available a second resistant and susceptible strain should be tested (See Section 5. Data priority list).
* For negative and positive control net panels: Test all mosquito strains being used on that experimental day against each control panel.
	1. Collection and storage of test net samples (for bioefficacy testing[[11]](#footnote-12))
* Whole nets and net pieces may need to be stored before and after testing and may be transported between study sites. When collecting and storing whole net and net samples always ensure they are kept separately to avoid cross-contamination of AIs. Store nets in a cool dry place at <5°C out of direct sunlight.
* Gloves and a lab coat should always be worn when handling the nets and should be changed between handling nets/net panels with different AIs to avoid cross-contamination.
* Hang sample net on net frame. Net frame should be cleaned between nets as specified by the labs cleaning protocols.
* Cut 4 pieces (30 x 30 cm) from each test net (2 from the roof panel, 2 from the sides panels). Scissors should be changed or cleaned between cutting net panels with different AIs. Recommended sampling positions can be found in Figure 1.
* Label net pieces with the sample position (i.e. 1 - 4) and net ID on paper labels secured to the corner of each piece.
* Wrap each piece individually in aluminium foil and refrigerate. If a refrigerator is not available store nets a cool dry place at <5°C.



*Figure 1. Recommended sampling position of net pieces from bednet. The lower 25 cm of the net should not be sampled as it is likely to have been exposed to abrasion from being tucked under a bed. Two samples should be taken from the net roof panel and two samples should be taken from the net side panels for bioassays. Two samples should be taken adjacent to each bioassay piece for chemical content testing. Image adapted from (WHO, 2011).*

* 1. Control net samples
* Gloves and a lab coat should always be worn when handling the nets and should be changed between handling different nets/net panels with different AIs to avoid cross-contamination.
* Control nets should be aired, but unwashed. Air new nets away from direct sunlight for a minimum of 7-days before testing.
* Only one piece of control netting is needed per assay. However, control pieces should not be used >5 times, so multiple pieces will be needed.
	+ Hang net on net frame. Net frame should be cleaned between nets as specified by the labs cleaning protocols.
	+ Cut 10 pieces (30 x 30 cm) from each control net.
	+ Label net pieces with the control net ID on paper labels and secure to the corner of each piece.
	+ Wrap each piece individually in aluminium foil and refrigerate. If a refrigerator is not available store nets a cool dry place at <5°C.
* On each experimental testing day, a negative control and two positive control nets (Table 1) should be tested alongside test nets.

*Table 1. Specifications of control nets.*

|  |  |
| --- | --- |
| **Net Type** | **Description** |
| Negative control: Untreated net | Untreated netting of the same material as the test netting (e.g. polypropylene). Record the number of times the net piece has been used and do not use the same piece >5 times. If 24-hour mortality in the negative control on a particular testing day is >10% results should be discarded and testing repeated. If 24-hour control mortality for the day is <10% the test results should be corrected using Abbot’s formula[[12]](#footnote-13) (Abbott, 1925; WHO, 2013). |
| Positive control 1: New pyrethroid + PBO net panel | Brand new pyrethroid + PBO netting of the same brand as the test net. Air new nets away from direct sunlight for a minimum of 7-days before testing. Record the number of times the net has been used and do not use the same piece >5 times. |
| Positive control 2: New pyrethroid-only net panel | Brand new pyrethroid-only netting of the same material, treated using the same impregnation method, insecticide, and dose as the test netting. If such netting is not available, the closest non-PBO commercial equivalent should be used (e.g. if testing PermaNet 3.0, a new PermaNet 3.0 side panel could be used). Air new nets away from direct sunlight for a minimum of 7-days before testing. Record the number of times the net has been used and do not use the same piece >5 times.  |

* 1. Cone bioassay setup
* Gloves and a lab coat should always be worn when handling the nets and should be changed between handling different nets/net panels with different AIs to avoid cross-contamination.
* Clean testing area and equipment as specified by the labs cleaning protocols.
* Prepare test mosquitoes. The numbers of mosquitoes required for testing different types of pyrethroid + PBO net can be found in Table 3, Section 5. ‘Data priority list’). Carefully transfer required mosquitoes to holding containers, 5 mosquitoes per container using an aspirator.
* Test mosquitoes and net samples should be acclimatised to the climatic conditions of the testing room for a minimum of one hour before testing. Remove any knocked-down mosquitoes from holding containers before testing.
* Prepare cone testing board(s).
	+ Place 1st cone holder frame in stand.
	+ Secure control and test nets to 1st cone holder frame with tape. Make sure nets do not overlap to avoid cross-contamination, that they are correctly labelled, and that the labels are visible.
	+ Place the plastic cones over the nets and secure the cones in place by placing the 2nd cone holder frame over the top. The two cone holder frames can be secured together using binder clips or clamps.
	+ Make sure that the board is stable and situated at a 45˚ angle.
	+ Cover the opening of the plastic cones with a stopper (e.g. rubber plug or cotton wool).
	1. Cone bioassay procedure
* Record the temperature and humidity during testing. Preferably continuously with a data logger, or alternatively manually at the start and end of exposure, and the end of the mosquito holding period.
* Exposed batches of 5 mosquitoes to netting pieces for 3 minutes for a total of 2 replicates per net piece:
	+ Remove the stopper from the cone and transfer 5 mosquitoes from the holding container into the plastic cone using an aspirator. Take care not to touch the net with the aspirator end as this may result in contamination.
	+ Cover the cone with the stopper to prevent mosquitoes from escaping.
	+ Expose mosquitoes to the netting sample for 3 minutes.
	+ Transfer mosquitoes from the cone back to their holding container with an aspirator. Take care not to touch the net with the aspirator end as this may result in contamination. Ensure containers are correctly labelled with the net sample ID (Net ID and position), test rep, mosquito species, and testing date.
	+ Repeat until 2 replicates of 5 mosquitoes have been exposed to each net sample.
* Provide mosquitoes with a sugar meal (10% sucrose solution soaked onto a relevant substrate such as cotton wool).
* Record the number of mosquitoes in each holding container to give the total numbers exposed.
* After 1 hour post-exposure record the number of mosquitoes knockdown (Table 2).
* After 24 hours post-exposure record the number of dead mosquitoes (Table 2).
* At the end of testing, ensure mosquitoes are stored correctly (i.e. in individual tubes with silica gel) for future analysis. If mosquitoes are not required for future analysis, discard mosquitoes safely.

*Table 2. The definitions used for classifying alive, knocked down or dead mosquitoes, adapted from* (WHO, 2013)

|  |  |
| --- | --- |
| **Mosquito status** | **Definition** |
| Alive | The mosquito is mobile or able to stand or fly in a coordinated manner |
| Knocked down | The mosquito is immobile or unable to stand or take off, at 1-hour following net exposure |
| Dead | The mosquito is immobile or unable to stand or take off, at 24-hours following net exposure |

* 1. Measured outcomes
* The number of mosquitoes exposed, knocked down after 1-hour, and dead after 24-hours should be recorded for each net piece and replicate individually.
* For each panel type (i.e. pyrethroid-only panel or pyrethroid + PBO panels) sample and replicate data should be pooled and the 1-hour knockdown %[[13]](#footnote-14) and 24-hour mortality %[[14]](#footnote-15) calculated for each panel type.
* Panel types should then be pooled to calculate the 1-hour knockdown %[[15]](#footnote-16) and 24-hour mortality %[[16]](#footnote-17) calculated for each net.
1. Data priority list
* All testing should be carried with the same resistant and susceptible strains over time. Where resources allow it and mosquitoes are available a second resistant and susceptible strains should be tested. However, it is more important to have a full data set with one strain, so resources should be prioritised to ensure this before considering testing with secondary strains.
* Ad hoc testing with secondary strains when available will provide useful data.
* The ideal methodological parameters (i.e. net samples, replicates, controls) can be found in Table 3. All methodological parameters and deviations from standard testing should be recorded at the time of testing.

*Table 3. The number of mosquitoes required per test net and for daily controls for a pyrethroid + PBO net treated with PBO all over and for a pyrethroid + PBO net treated with PBO on the roof only. Numbers are based on testing 5 mosquitoes per replicate, with 2 replicates per net piece. For test nets 4 net pieces (2 from the roof, 2 from the sides) are used. For control nets 1 piece is used (3 control nets tested per day: untreated, new pyrethroid + PPO net, and new pyrethroid only net).*

|  |
| --- |
| **Net with PBO on all panels** |
| Strain | Test net | Daily control | Total |
| Resistant | 40 | 30 | 70 |
| Susceptible | 40 | 30 | 70 |
| Total | 80 | 60 | 140 |
| **Net with PBO only on roof panel** |
| Strain | Test net | Daily control | Total |
| Resistant | 20 | 30 | 50 |
| Susceptible | 40 | 30 | 70 |
| Total | 60 | 60 | 120 |

1. Deviations from standard protocol
* All deviations from the standard protocol should be noted in the data collections sheets.
* When insecticide characterised lab strains are unavailable, wild larval collected mosquitoes could be used. Details on larval collected should be recorded, such as location of sampling sites (including co-ordinates), number of sampling sites, and type of sampling site (e.g. rainwater puddle, permanent water body). The wild larval collected population should be insecticide characterised using the same methods as those used to characterise lab strains (Lees et al, In Prep).
1. Supplementary data
* Additional information that should be recorded:
	+ Time of testing
	+ The light-dark rearing cycle of test mosquitoes (including times where possible)
1. Glossary of terms

AI Active ingredient

I2I Innovation to Impact

ITN Insecticide-treated net

PBO Piperonyl butoxide

PQ Prequalification

SOP Standard operating procedure

WHO World Health Organization

1. References

Abbott, W.S. (1925) “A method of computing the effectiveness of an insecticide.,” *Journal of Economic Entomology*, 18(2), pp. 265–267. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3333059 (Accessed: January 4, 2017).

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WHO (2011) *Guidelines for monitoring the durability of long-lasting insecticidal mosquito nets under operational conditions Control of Neglected Tropical Diseases WHO Pesticide Evaluation Scheme and Global Malaria Programme Vector Control Unit*.

WHO (2013) *Guidelines for laboratory and field-testing of long-lasting insecticidal nets*, *WHO/HTM/NTD/WHOPES/20131*. Geneva: World Health Organization.

WHO (2020) *List of WHO Prequalified Vector Control Products*. Available at: https://www.who.int/pq-vector-control/prequalified-lists/VCP\_PQ-List\_26August2020.pdf?ua=1 (Accessed: February 18, 2021).

# Annex 6: I2I-SOP-002: Methods for monitoring the durability of dual AI insecticide-treated nets containing a pyrethroid plus pyriproxyfen (PPF)



[1. Purpose 1](#_Toc89433933)

[2. Background 2](#_Toc89433934)

[3. Materials & Equipment 2](#_Toc89433935)

[4. Procedure 3](#_Toc89433936)

[4.1. Test mosquitoes 3](#_Toc89433937)

[4.2. Collection and storage of test net samples (for bioefficacy testing) 4](#_Toc89433938)

[4.3. Control nets samples 5](#_Toc89433939)

[4.4. Mosquito blood-feeding 6](#_Toc89433940)

[4.5. Cone bioassay setup 6](#_Toc89433941)

[4.6. Cone bioassay procedure 7](#_Toc89433942)

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1. Purpose

This standard operating procedure (SOP) describes the methods to determine the bioefficacy of the pyrethroid and pyriproxyfen (PPF) components of insecticide-treated nets (ITNs) used under operational conditions. The process used to determine the methodology detailed in this SOP, and justifications for key methodological parameters can be found in ‘I2I-MD-002: Durability monitoring method development: Dual AI insecticide-treated nets containing a pyrethroid plus pyriproxyfen (PPF)I2I-MD-002: Durability monitoring method development: Dual AI ITNs containing pyriproxyfen’. This SOP details two methods to evaluate durability of pyrethroid + PPF nets, measuring mortality, and either (i) oviposition or (ii) dissection of ovaries following net exposure in a standard WHO cone bioassay. The same method should be used for all test nets throughout the durability trial.

1. Background

Pyrethroid + pyriproxyfen (PPF) nets are PQ listed (i.e. Royal Guard, **Error! Reference source not found.**) and being deployed in RCTs and pilot deployment schemes. The WHO cone test is a suitable method for exposing mosquitoes to pyrethroid + pyriproxyfen (PPF) nets for measuring the nets durability, but different endpoints are needed for each active ingredient. Knockdown and mortality can be used to assess the bio-efficacy of the pyrethroid but the most suitable endpoints for PPF, a juvenile hormone analogue that affects fertility and fecundity in mosquitoes, need to be defined. We are proposing two methods to evaluate durability of pyrethroid + PPF nets, measuring either (i) oviposition or (ii) dissection of ovaries following net exposure in a standard WHO cone bioassay. Monitoring the bioefficacy of the active ingredients (AI) in the nets is a vital part of establishing the durability of these nets under operational conditions.

1. Materials & Equipment

**General**

* Data collection sheets
* Lab coat
* Gloves
* Test pyrethroid + PPF nets
* Control untreated net
* Control new pyrethroid + PPF net
* Aspirator (manual/electronic), separate for each insecticide
* Mosquito strains
* Pen/permanent markers

**Collection and storage of net samples**

* Net frame
* Scissors
* Paper labels
* Aluminium foil

**Cone bioassay**

* Tape
* Mosquito holding containers (e.g. paper cups covered with untreated netting held by elastic bands)
* Cone holding frame (x 2), with holes to hold standard WHO plastic cones
* Cone holder frame stand, which holds frame at 45°
* WHO Plastic cones
* Binder clips or clamps
* Cotton wool or rubber stoppers
* Temperature and humidity data logger
* Timer
* 10% sucrose solution (e.g. sugar or honey and water)
* Cotton wool

**Measuring sterility - Oviposition using chambering.**

* Artificial egg laying chambers (e.g. falcon tubes containing damp water-soaked cotton wool covered with filter paper. Tube is covered with untreated netting secured in place with an elastic band)

**Measuring sterility - Ovary development using ovary dissection**

* Dissecting microscope
* Glass slides
* Dissection kit (e.g. dissection pins, forceps)
* Distilled water
* Plastic pipette/water dropper
1. Procedure
	1. Test mosquitoes
* Use 3-to-5-day-old blood-fed female *Anopheles* mosquitoes. Mosquitoes should be blood-fed 3-9 hours prior to exposure. Mosquitoes should be well characterized lab strains with respect to insecticide susceptibility (Lees *et al*, In Prep). F0 adults collected from larval breeding sites should only be used when lab strains are unavailable and should following the same insecticide resistance characterisation methods (see Section 6. Deviations from standard protocol).
* A pyrethroid-susceptible strain should be tested to monitor the durability of the pyrethroid insecticide, and a pyrethroid-resistant strain should be used to monitor the durability of the PPF (Lees, *et al*, In Prep). Where resources allow it and mosquitoes are available a second resistant and susceptible strain should be tested (See Section 5. Data priority list).
* For negative and positive control net panels: Test all mosquito strains being used on that experimental day against each control panel.
	1. Collection and storage of test net samples (for bioefficacy testing[[17]](#footnote-18))
* Whole nets and net pieces may need to be stored before and after testing and may be transported between study sites. When collecting and storing whole net and net samples always ensure they are kept separately to avoid cross-contamination of AIs. Store nets in a cool dry place at <5°c out of direct sunlight.
* Gloves and a lab coat should always be worn when handling the nets and should be changed between handling different nets/net panels with different AIs to avoid cross-contamination.
* Hang sample net on net frame. Net frame should be cleaned between nets as specified by the labs cleaning protocols.
* Cut 4 pieces (30 x 30 cm) from each test net (2 from the roof panel, 2 from the sides panels). Scissors should be changed or cleaned between cutting net panels with different AIs. Recommended sampling positions can be found in Figure 1.
* Label net pieces with the sample position (i.e. 1 - 4) and net ID on paper labels secured to the corner of each piece.
* Wrap each piece individually in aluminium foil and refrigerate. If a refrigerator is not available store nets a cool dry place at <5°c.



*Figure 1. Recommended sampling position of net pieces from bednet. The lower 25 cm of the net should not be sampled as it is likely to have been exposed to abrasion from being tucked under a bed. Three samples should be taken from the net roof panel and three samples should be taken from the net side panels for bioassays. Two samples should be taken adjacent to each bioassay piece for chemical content testing. Image adapted from (WHO, 2011).*

* 1. Control nets samples
* Gloves and a lab coat should always be worn when handling the nets and should be changed between handling different nets/net panels with different AIs to avoid cross-contamination.
* Control nets should be aired, but unwashed. Air new nets away from direct sunlight for a minimum of 7-days before testing.
* Only one piece of control netting is needed per assay. However, control pieces should not be used >5 times, so multiple pieces will be needed.
	+ Hang net on net frame. Net frame should be cleaned between nets as specified by the labs cleaning protocols.
	+ Cut 10 pieces (30 x 30 cm) from each control net.
	+ Label net pieces with the control net ID on paper labels and secure to the corner of each piece.
	+ Wrap each piece individually in aluminium foil and refrigerate. If a refrigerator is not available store nets a cool dry place at <5°c.
* Only one piece of control netting is needed per assay. However, the methods for preparing control nets should follow the same procedure as test nets:
* On each experimental testing day, a negative control and a positive control net (Table 1) should be tested alongside test nets.

*Table 1. Specifications of control nets.*

|  |  |
| --- | --- |
| **Net Type** | **Description** |
| Negative control: Untreated net | Untreated netting of the same material as the test netting (e.g. polypropylene). Record the number of times the net piece has been used and do not use the same piece >5 times. If 24-hour mortality in the negative control on a particular testing day is >10% results should be discarded and testing repeated. If 24-hour control mortality for the day is <10% the test results should be corrected using Abbot’s formula[[18]](#footnote-19) (Abbott, 1925; WHO, 2013).  |
| Positive control: New pyrethroid + PPF net panel | Brand new pyrethroid + PFF netting of the same brand as the test net. Air new nets away from direct sunlight for a minimum of 7-days before testing. Record the number of times the net has been used and do not use the same piece >5 times. |

* 1. Mosquito blood-feeding
* Blood-feed mosquitoes 3-9 hours before exposure. Mosquitoes should be blood fed using method of feeding standard for the test population (e.g. Hemotek membrane feeding system, arm feed, animal fed to repletion)
* Only visibly blood-fed[[19]](#footnote-20) mosquitoes should be used for the assay.
	1. Cone bioassay setup
* Gloves and a lab coat should always be worn when handling the nets and should be changed between handling different nets/net panels with different AIs to avoid cross-contamination.
* Clean testing area and equipment as specified by the labs cleaning protocols.
* Prepare test mosquitoes. The numbers of mosquitoes required can be found in Table 6, Section 5. Data priority list). Carefully transfer required mosquitoes to holding containers, 5 mosquitoes per container using an aspirator.
* Test mosquitoes and net samples should be acclimatised to the climatic conditions of the testing room for a minimum of one hour before testing. Remove any knockdown mosquitoes from holding containers before testing.
* Prepare cone testing board(s).
	+ Place 1st cone holder frame in stand.
	+ Secure control and test nets to 1st cone holder frame with tape. Make sure nets do not overlap to avoid cross-contamination, that they are correctly labelled, and that the labels are visible.
	+ Place the plastic cones over the nets and secure the cones in place by placing the 2nd cone holder frame over the top. The two cone holder frames can be secured together using binder clips or clamps.
	+ Make sure that the board is stable and situated at a 45˚ angle.
	+ Cover the opening of the plastic cones with a stopper (e.g. rubber plug or cotton wool).
	1. Cone bioassay procedure
* Record the temperature and humidity during testing. Preferably continuously with a data logger, or alternatively manually at the start and end of exposure, and the end of the mosquito holding period.
* Exposed batches of 5 mosquitoes to netting pieces for 3 minutes for a total of 2 replicates per net piece:
	+ Remove the stopper from the cone and transfer 5 mosquitoes from the holding container into the plastic cone using an aspirator. Take care not to touch the net with the aspirator end as this may result in contamination.
	+ Cover the cone with the stopper to prevent mosquitoes from escaping.
	+ Expose mosquitoes to the netting sample for 3 minutes.
	+ Transfer mosquitoes from the cone back to their holding container with an aspirator. Take care not to touch the net with the aspirator end as this may result in contamination. Ensure containers are correctly labelled with the net sample ID (Net ID and position), test rep, mosquito species, and testing date.
	+ Repeat until 2 replicates of 5 mosquitoes have been exposed to each net sample.
* Provide mosquitoes with a sugar meal (10% sucrose solution soaked onto a relevant substrate such as cotton wool). Ensure sugar meal is changed daily throughout assay, as this can impact mosquito health.
* Record the number of mosquitoes in each holding container to give the total numbers exposed.
* After 1 hour post-exposure record the number of mosquitoes knockdown (**Error! Reference source not found.**Table 2).
* After 24 hours post-exposure record the number of dead mosquitoes (Table 2).

*Table 2. The definitions used for classifying alive, knocked down or dead mosquitoes, adapted from (WHO, 2013)*

|  |  |
| --- | --- |
| **Mosquito status** | **Definition** |
| Alive | The mosquito is mobile or able to stand or fly in a coordinated manner |
| Knocked down | The mosquito is immobile or unable to stand or take off, at 1-hour following net exposure |
| Dead | The mosquito is immobile or unable to stand or take off, at 24-hours following net exposure |

* 1. Measuring sterility - Method for scoring oviposition using chambering.
* The sterilising effect of PPF can be measured in several ways. This SOP details two methods; (i) chambering to observe oviposition or (ii) ovary dissection to observe ovary development. Either method can be used, however the same method should be used for all nets throughout the durability trial.
* On Day 3 (three days post-exposure), record the number of dead mosquitoes in holding containers (Table 2).
* Set up artificial egg laying chambers using method used in your lab.
* Transfer alive mosquitoes individually into their own egg laying chamber using an aspirator.
* Ensure egg laying chambers are correctly labelled with a mosquito ID number, the net sample ID (Net ID and position), test rep, mosquito species, and testing date.
* Provide mosquitoes with a sugar meal (10% sucrose solution soaked onto a relevant substrate such as cotton wool).
* Place egg laying chambers in a dark area. Ensure sugar meal is changed daily throughout assay, as this can impact mosquito health.
* On Day 7 (four days post-chambering), record if each individual mosquito is alive or dead (Table 2). Record if each individual mosquito has laid eggs or not.
* At the end of testing, ensure mosquitoes are stored correctly (i.e. in individual tubes with silica gel) for future analysis. If mosquitoes are not required for future analysis, discard mosquitoes safely.
	+ 1. *Measured outcomes*
* The number of mosquitoes exposed, knocked down after 1-hour, and dead after 24-hours should be recorded for each net piece and replicate individually.
* Replicate data should then be pooled and the 1-hour knockdown %[[20]](#footnote-21) and 24-hour mortality %[[21]](#footnote-22) calculated for each individual net.
* On Day 3, number of mosquitoes dead, and number of alive mosquitoes chambered should be recorded for each test net.
* On Day 7, for each individual chambered mosquito status (alive or dead), and egg laying (laid eggs or did not lay eggs) should be recorded.
* Individual mosquito data should then be pooled to calculate oviposition and oviposition inhibition using the definition in Table 3.

*Table 3. The definitions used for classifying oviposition and calculation of oviposition inhibition.*

|  |  |
| --- | --- |
| **Mosquito status** | **Definition** |
| Oviposition  | $$\frac{O}{T} ×100$$Where *O* is the number of mosquitoes (living or dead) which laid eggs and *T* is the total number of surviving blood-fed mosquitoes placed into oviposition chambers.  |
| Oviposition inhibition | $$\frac{(Oc-Ot)}{Oc} × 100$$Where Oc is the proportion of surviving blood-fed females from the control which laid eggs while Ot is the proportion of surviving blood-fed females from a given treatment which laid eggs. |

* 1. Measuring sterility - Method for scoring ovary development using ovary dissection
* On Day 3 (three days post-exposure), record the number of dead mosquitoes in holding containers (Table 2).
* Dissect mosquito ovaries:
	+ Mount mosquito onto glass slide on its back
	+ Use a dissecting pin or forceps to hold the mosquito stationary by the thorax
	+ Add a drop of distilled water on to the last two segments of the mosquitoes abdomen.
	+ Use a dissection pin to gently pulling off the last two segments of the mosquito abdomen
	+ For better visualisation of the ovaries, use the needle to separate the ovaries from other internal material and wash off fat and other debris by rinsing the ovaries with distilled water.
	+ Leave the slide with dissected ovaries to air dry
* Two individuals should classify ovary development and they should be blinded to the net exposure treatment. Where disagreement on ovary classification occurs, a third individual should be consulted. If ovaries cannot be classified on the same day as dissection, photographs should be taken of the ovaries on the day and these should examined to classify ovaries.
* Record the developmental status of the eggs in each mosquitos’ ovaries according to Christopher’s stage of egg development (Figure 2).
* Record if the mosquito is fertile, Infertile, or inconclusive (Table 4)



*Figure 2. Christopher’s stages of egg development in female Anopheles mosquitoes, adapted from Christophers (1911).*

*Table 4. The definitions used for classifying egg development*

|  |  |
| --- | --- |
| **Ovary status** | **Definition** |
| Fertile | Female *Anopheles* eggs have fully developed to Christophers’ stage V = normal elongated, boat/sausage-shaped eggs with lateral floats (Figure 2). |
| Infertile | Female *Anopheles* eggs have not fully developed and remain in Christophers’ stages I –IV = less elongated, round shape, lacking floats (Figure 2). |
| Inconclusive | If both stage IV and stage V eggs are observed, record this as “inconclusive”. |

* + 1. *Measured outcomes*
* The number of mosquitoes exposed, knocked down after 1-hour, and dead after 24-hours should be recorded for each net piece and replicate individually
* Replicate data should then be pooled and the 1-hour knockdown %[[22]](#footnote-23) and 24-hour mortality %[[23]](#footnote-24) calculated for each individual net.
* On Day 3, number of mosquitoes dead, and number of alive mosquitoes dissected should be recorded for each test net.
* For each dissected mosquito the developmental status of the eggs in each mosquitos’ ovaries should be recorded, and this should be used to classify the mosquito as fertile, infertile or inconclusive.
1. Data priority list
* All testing should be carried with the same resistant and susceptible strains over time. Where resources allow it and mosquitoes are available a second resistant and susceptible strains should be tested. However, it is more important to have a full data set with one strain, so resources should be prioritised to ensure this before considering testing with secondary strains.
* Ad hoc testing with secondary strains when available will provide useful data.
* The ideal methodological parameters (i.e. net samples, replicates, controls) can be found in Table 5. When resources are reduced the number of mosquitoes required can be altered by changing the net samples required (Option B). All methodological parameters and deviations from standard testing should be recorded at the time of testing.

*Table 5. The number of mosquitoes required per test net and for daily controls. Examples are provided for a pyrethroid + PPF net treated with PPF all over. Green highlight is current ideal testing. Grey highlight shows suggested changes to reduce sample size.*

|  |  |  |
| --- | --- | --- |
|  | **A** | **B** |
| Roof panels samples | 2 | 2 |
| Side panel samples | 2 | 1 |
| Control samples (2 net types) | 2 | 2 |
| Replicate per panel | 2 | 2 |
| Mosquitoes per rep | 5 | 5 |
| Per test net | Resistant Strain | 40 | 30 |
|  | Susceptible strain | 40 | 30 |
| Daily controls | Resistant Strain | 20 | 20 |
|  | Susceptible strain | 20 | 20 |
| Total | 120 | 100 |

1. Deviations from standard protocol
* All deviations from the standard protocol should be noted in the data collections sheets.
* When insecticide characterised lab strains are unavailable, wild larval collected mosquitoes could be used. Details on larval collected should be recorded, such as location of sampling sites (including co-ordinates), number of sampling sites, and type of sampling site (e.g. rainwater puddle, permanent water body). The wild larval collected population should be insecticide characterised using the same methods as those used to characterise lab strains (Lees, *et al*, In Prep).
1. Supplementary data
* Additional information that should be recorded:
	+ Time of testing
	+ The light-dark rearing cycle of test mosquitoes (including times where possible)
1. Glossary of terms

AI Active ingredient

I2I Innovation to Impact

ITN Insecticide-treated net

PPF Pyriproxyfen

PQ Prequalification

SOP Standard operating procedure

WHO World Health Organization

1. References

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# Annex 7: I2I-SOP-003: Methods for monitoring the durability of dual AI insecticide-treated nets containing a pyrethroid plus chlorfenapyr



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1. Purpose

This standard operating procedure (SOP) describes the methods to determine the bioefficacy of the pyrethroid and chlorfenapyr (CFP) components of insecticide-treated nets (ITNs) used under operational conditions. The process used to determine the methodology detailed in this SOP, and justifications for key methodological parameters can be found in ‘I2I-MD-003: Durability monitoring method development: Dual AI insecticide-treated nets containing a pyrethroid plus Chlorfenapyr2I-MD-003: Durability monitoring method development: Dual AI ITNs containing Chlorfenapyr’.

1. Background

Pyrethroid + chlorfenapyr nets are PQ listed (i.e. Interceptor G2) and being deployed in randomised control trials (RCTs) and pilot deployment schemes. There is therefore an urgent need for a method to measure the bioefficacy of these nets, to collect baseline data and subsequently measure the durability of biological efficacy in nets collected from the field after fixed periods of use. Monitoring the bioefficacy of the active ingredients (AI) in the nets is a vital part of establishing the durability of these nets under operational conditions.

1. Materials & Equipment

**General**

* Data collection sheets
* Lab coat
* Gloves
* Test pyrethroid + CFP nets
* Control untreated net
* Aspirator (manual/electronic), separate for each insecticide
* Mosquito strains
* Pen/permanent markers

**Collection and storage of net samples**

* Net frame
* Scissors
* Paper labels
* Aluminium foil

**Tunnel Test**

* Mosquito holding/collection containers (e.g. paper cups covered with untreated netting held by elastic bands)
* 60 cm glass/plastic tunnels (25 cm x 25 cm square section), separate for each net type (i.e. untreated or dual-AI CFP net)
* Netted capture cages, 25 cm2 (2 per tunnel)
* Net frame holder (1 per tunnel)
* Animal bait (specify what animal bait is being used)
* Temperature and humidity data logger
* Timer
* 10% sucrose solution (e.g. sugar or honey and water)
1. Procedure
	1. Test mosquitoes
* Use 5-to-8-day-old nulliparous non-blood fed female *Anopheles* mosquitoes. Mosquitoes should be sugar starved for a minimum of 6 hours before exposure (the exact starvation period should be recorded). Mosquitoes should be well characterized lab strains with respect to insecticide susceptibility (Lees et al, In Prep). New IG1 & IG2 should be used to characterise strain prior to testing as these will not be used for daily controls.
* F0 adults collected from larval breeding sites should only be used when lab strains are unavailable and should following the same insecticide resistance characterisation methods as lab strains (see Section 6. Deviations from standard protocol).
* A pyrethroid-susceptible strain should be tested to monitor the durability of the pyrethroid insecticide, and a pyrethroid-resistant strain should be used to monitor the durability of the CFP (Lees et al, In Prep). Where resources allow it and mosquitoes are available a second resistant and susceptible strain should be tested (See Section 5. Data priority list). As testing is conducted overnight, the different strains may need to be conducted on different days. Test all mosquito strains being used on that experimental day against each control panel.
	1. Collection and storage of test net samples (for bioefficacy testing[[24]](#footnote-25))
* Whole nets and net pieces may need to be stored before and after testing and may be transported between study sites. When collecting and storing whole net and net samples always ensure they are kept separately to avoid cross-contamination of AIs. Store nets in a cool dry place at <5°C out of direct sunlight.
* Gloves and a lab coat should always be worn when handling the nets and should be changed between handling nets/net panels with different AIs to avoid cross-contamination.
* Hang sample net on net frame. Net frame should be cleaned between nets as specified by the lab’s cleaning protocols.
* Cut 2 pieces (20 x 20 cm) from each test net (1 from the roof panel, 1 from the sides panels). Scissors should be changed or cleaned between cutting net panels with different AIs. Recommended sampling positions can be found in Figure 1.
* Label net pieces with the sample position (i.e. 1 - 2) and net ID on paper labels secured to the corner of each piece.
* Wrap each piece individually in aluminium foil and refrigerate. If a refrigerator is not available store nets a cool dry place at <5°C.

  

*Figure 1. Recommended sampling position of net pieces from dual-AI CFP bednet,* *when the net is treated with the same AIs all over. The lower 25 cm of the net should not be sampled as it is likely to have been exposed to abrasion from being tucked under a bed. One sample should be taken from the net roof panel and one sample should be taken from the net side panels for bioassays. Two samples should be taken adjacent to each bioassay piece for chemical content testing. Image adapted from* (WHO, 2011)*.*

* 1. Control net samples
* Gloves and a lab coat should always be worn when handling the nets and should be changed between handling different nets/net panels with different AIs to avoid cross-contamination.
* Control nets should be aired, but unwashed. Air new nets away from direct sunlight for a minimum of 7-days before testing.
* Two pieces of control netting are needed per assay. However, control pieces should not be used >5 times, so multiple pieces will be needed.
	+ Hang net on net frame. Net frame should be cleaned between nets as specified by the labs cleaning protocols.
	+ Cut 10 pieces (20 x 20 cm) from each control net.
	+ Label net pieces with the control net ID on paper labels and secure to the corner of each piece.
	+ Wrap each piece individually in aluminium foil and refrigerate. If a refrigerator is not available store nets a cool dry place at <5°C.
* On each experimental testing day, two negative untreated control nets (Table 2) should be tested alongside test nets.

*Table 2. Specifications of control nets.*

|  |  |
| --- | --- |
| **Net Type** | **Description** |
| Negative control: Untreated net | Untreated netting of the same material as the test netting (e.g. polypropylene). Record the number of times the net piece has been used and do not use the same piece >5 times. If mortality in the negative control on a particular testing day is >10% (24-hours) or >20% (72-hours) results should be discarded, and testing repeated. If control mortality for the day is <10% (24-hours) or <20% (72-hours) the test results should be corrected using Abbott’s formula[[25]](#footnote-26) (Abbott, 1925; WHO, 2013). If blood-feeding in the negative control on a particular testing day is <50% the results should be discarded and testing repeated.  |

* 1. Tunnel test setup
* Gloves and a lab coat should always be worn when handling the nets and should be changed between handling different nets/net panels with different AIs to avoid cross-contamination.
* Clean testing area and equipment as specified by the labs cleaning protocols.
* Prepare test mosquitoes. The numbers of mosquitoes required can be found in Table 4, Section 5. Data priority list). Carefully transfer required mosquitoes to holding containers, 50 mosquitoes per container using an aspirator.
* Prepare test and control nets. Cut 9 holes into the 20 x 20 cm net piece (holes 1 cm in diameter; one hole is located at the centre of the square net sample, and the other eight are equidistant and located 5cm from the border). Secure the holed net onto the net holder.
* Test mosquitoes and net samples should be acclimatised to the climatic conditions of the testing room for a minimum of one hour before testing. Remove any knocked-down mosquitoes from holding containers before testing.
* Assemble the tunnel netted capture cages.
* Place the net sample in its holder into the tunnel, one third along its distance.
* Prepare and label 8 mosquito collection containers per tunnel, 4 for each compartment (compartment 1 & compartment 2[[26]](#footnote-27)): labelled dead blood-fed, dead unfed, alive blood-fed, alive unfed.
	1. Tunnel test procedure
* Record the temperature and humidity during testing. Preferably, continuously with a data logger or alternatively manually at the start and end of tunnel exposure, and the end of the mosquito holding period.
* Place the animal bait into compartment 2 (the shorter section of the tunnel). Ensure that the selected animal has not been used for testing for at least 2 weeks before this test and that it is placed in a position in the compartment where it will not injure itself.
* Secure the netted capture cages to either end of the tunnel. Ensure the netted cage on compartment 1 (the longer section of the tunnel) has an opening to allow the test mosquitoes to be added.
* Transfer 50 mosquitoes from the holding container into the netted cage on compartment 1 using an aspirator.
* Expose mosquitoes to the net in the tunnel for 12-15 hours. Record the start and end time of the exposure on the data collection sheet. Testing should be conducted in darkness during the ‘night phase’ of the mosquitoes circadian rhythm.
* At the end of the exposure period, determine the mosquitoes compartment location, mortality and blood-feeding status (Table 3). Transfer mosquitoes from the tunnel into their corresponding collection container using an aspirator (i.e. compartment 1 dead blood-fed). Return the animal to its enclosure and ensure it is provided with food and water.
* Ensure mosquito collection containers are correctly labelled with the net sample ID (Net ID and position), test rep, mosquito species, and testing date.
* Provide mosquitoes with a sugar meal (10% sucrose solution soaked onto a relevant substrate such as cotton wool).
* Record the number of mosquitoes in each holding container (i.e. compartment 1 or compartment 2, dead blood-fed, dead unfed, alive blood-fed, alive unfed).
* For remaining mosquitoes, record the number dead at 24, 48, and 72 hours from the start of exposure. Remove dead mosquitoes each day to avoid duplicate counting.
* At the end of testing, ensure mosquitoes are stored correctly (i.e. in individual tubes with silica gel) for future analysis. If mosquitoes are not required for future analysis, discard mosquitoes safely.

*Table 3. The definitions used for classifying alive, dead, unfed and blood-fed mosquitoes, adapted from* (WHO, 2013)

|  |  |
| --- | --- |
| **Mosquito status** | **Definition** |
| Alive | The mosquito is mobile or able to stand or fly in a coordinated manner |
| Dead | The mosquito is immobile or unable to stand or take off in a coordinated manner following net exposure |
| Unfed | No blood-meal is visible by eye |
| Blood fed | A partial or complete blood-meal is visible by eye |

* 1. Measured outcomes
* The number of mosquitoes in each compartment (1 & 2) on collection separated by dead blood-fed, dead unfed, alive blood-fed, alive unfed for each net/tunnel replicate.
* The number of mosquitoes dead at 24, 48, and 72hr, separated by their collection compartment (1 & 2) and blood-feeding status (unfed or blood-fed).
* Individual data should then be pooled to calculate blood-feeding %[[27]](#footnote-28), blood-feeding inhibition[[28]](#footnote-29), immediate mortality%[[29]](#footnote-30), 24-hour mortality%[[30]](#footnote-31) and 72-hour mortality %[[31]](#footnote-32) for each net/tunnel replicate.
* If control mortality is >10% or blood-feeding is <50% for the day the test results should be discarded, and the test repeated.
1. Data priority list
* All testing should be carried with the same resistant and susceptible strains over time. Where resources allow it and mosquitoes are available a second resistant and susceptible strain should be tested. However, it is more important to have a full longitudinal data set with one strain, so resources should be prioritised to ensure this before considering testing with secondary strains.
* Ad hoc testing with secondary strains when available will provide useful data.
* The ideal methodological parameters (i.e. net samples, replicates, controls) can be found in Table 4. All methodological parameters and deviations from standard testing should be recorded at the time of testing.

*Table 4. The number of net samples, and mosquitoes required from each strain per test net and for daily controls.*

|  |  |
| --- | --- |
| **Parameter**  | **Amount** |
| CFP roof panel sample | 1 |
| CFP side panel sample | 1 |
| Untreated control samples  | 2 |
| Replicate per panel | 1 |
| Mosquitoes per rep | 50 |
| Mosquito per CFP test net | 100 |
| Mosquito per untreated control net (daily) | 100 |
| Total | 200 |

1. Deviations from standard protocol
* All deviations from the standard protocol should be noted in the data collections sheets.
* When insecticide characterised lab strains are unavailable, wild larval collected mosquitoes could be used. Details on larval collected should be recorded, such as the location of sampling sites (including coordinates), the number of sampling sites, and the type of sampling site (e.g. rainwater puddle, permanent water body). The wild larval collected population should be insecticide characterised using the same methods as those used to characterise lab strains (Lees, et al, In Prep).
1. Supplementary data
* Additional information that should be recorded:
	+ Time of testing
	+ The light-dark rearing cycle of test mosquitoes (including times where possible)
1. Glossary of terms

|  |  |
| --- | --- |
| AI | Active ingredient |
| CFP | Chlorfenapyr  |
| I2I | Innovation to Impact |
| ITN | Insecticide-treated net |
| PBO  | Piperonyl butoxide |
| PQ | Prequalification |
| RCT | Randomised control trial |
| SOP | Standard operating procedure |
| WHO | World Health Organization |

1. References

Abbott, W.S. (1925) “A method of computing the effectiveness of an insecticide.,” *Journal of Economic Entomology*, 18(2), pp. 265–267. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3333059 (Accessed: January 4, 2017).

Lees, R. *et al* (In Prep) “Strain Characterisation for Monitoring Durability of Bioefficacy in ITNs Treated with Two Active Ingredients (Dual AI ITNs): Developing a Robust Protocol by Building Consensus,” *Insects* [Preprint].

WHO (2011) *Guidelines for monitoring the durability of long-lasting insecticidal mosquito nets under operational conditions Control of Neglected Tropical Diseases WHO Pesticide Evaluation Scheme and Global Malaria Programme Vector Control Unit*.

WHO (2013) *Guidelines for laboratory and field-testing of long-lasting insecticidal nets*, *WHO/HTM/NTD/WHOPES/20131*. Geneva: World Health Organization.

1. Long-lasting insecticidal nets (LLINs) have played an important role in malaria prevention since 2000. The term LLIN is reserved for WHO prequalified vector control products. As several new insecticide treated nets (ITNs) are currently in trial but not yet prequalified by WHO, we use the broader term ITN rather than LLIN in this protocol. [↑](#footnote-ref-2)
2. World malaria report 2019. Geneva: World Health Organization; 2019. [↑](#footnote-ref-3)
3. WHOPES: **Guidelines for monitoring the durability of long-lasting insecticidal mosquito nets under operational conditions**, WHO/HTM/NTD/WHOPES/2011.5 <http://whqlibdoc.who.int/publications/2011/9789241501705_eng.pdf> [↑](#footnote-ref-4)
4. World Health Organization: **WHO guidance note for estimating the longevity of long-lasting insecticidal nets in malaria control.** Geneva: 2013. <http://www.who.int/entity/malaria/publications/atoz/who_guidance_longevity_llins/en/index.html>. [↑](#footnote-ref-5)
5. World Health Organization: **Estimating functional survival of long-lasting insecticidal nets from field data**. Vector Control Technical Expert Group Report to MPAC September 2013. <http://www.who.int/malaria/mpac/mpac_sep13_vcteg_llin_survival_report.pdf>. [↑](#footnote-ref-6)
6. WHO: **Guidelines for laboratory and field testing of long‐lasting insecticidal nets.** Geneva 2013, WHO/HTM/NTD/WHOPES/2013.3 <http://www.who.int/iris/bitstream/10665/80270/1/9789241505277_eng.pdf?ua=1> [↑](#footnote-ref-7)
7. Benin, Burma, DRC, Ethiopia, Guinea, Madagascar, Malawi, Mozambique, Nigeria, Tanzania (Zanzibar), Zimbabwe (Source: www.durabilitymonitoring.org) [↑](#footnote-ref-8)
8. World Health Organization: **WHO guidance note for estimating the longevity of long-lasting insecticidal nets in malaria control.** Geneva: 2013. [↑](#footnote-ref-9)
9. WHO: **Guidelines for laboratory and field testing of long‐lasting insecticidal nets.** Geneva 2013, WHO/HTM/NTD/WHOPES/2013.3 <http://www.who.int/iris/bitstream/10665/80270/1/9789241505277_eng.pdf?ua=1> [↑](#footnote-ref-10)
10. Burkina Faso, Burma, Burundi, DRC, Ghana, Kenya, Liberia, Madagascar, Mozambique, Niger, Nigeria, Tanzania (Zanzibar). [↑](#footnote-ref-11)
11. The number of sample pieces listed is for conducting the bioefficacy testing specified in this protocol. Additional samples may be required for chemical analysis. [↑](#footnote-ref-12)
12. Abbott’s formula: Adjusted mortality (%) = 100 x (X–Y) / (100–Y), where X is the percentage mortality with the test netting, and Y is the percentage mortality with the untreated control sample [↑](#footnote-ref-13)
13. 1-hour knockdown (%) = (X/Y) x 100, where X is the total number of mosquitoes knocked down at 1-hour and Y in the total number of mosquitoes exposed to the test net. [↑](#footnote-ref-14)
14. 24-hour mortality (%) = (X/Y) x 100, where X is the total number of mosquitoes dead at 24-hour and Y in the total number of mosquitoes exposed to the test net. [↑](#footnote-ref-15)
15. 1-hour knockdown (%) = (X/Y) x 100, where X is the total number of mosquitoes knocked down at 1-hour and Y in the total number of mosquitoes exposed to the test net. [↑](#footnote-ref-16)
16. 24-hour mortality (%) = (X/Y) x 100, where X is the total number of mosquitoes dead at 24-hour and Y in the total number of mosquitoes exposed to the test net. [↑](#footnote-ref-17)
17. The number of sample pieces listed is for conducting the bioefficacy testing specified in this protocol. Additional samples may be required for chemical analysis. [↑](#footnote-ref-18)
18. Abbott’s formula: Adjusted mortality (%) = 100 x (X–Y) / (100–Y), where X is the percentage mortality with the test netting, and Y is the percentage mortality with the untreated control sample. [↑](#footnote-ref-19)
19. Visibly blood-fed mosquitoes: The mosquitoes’ abdomen is engorged, and red. Filled with a bloodmeal and not a sugar meal. [↑](#footnote-ref-20)
20. 1-hour knockdown (%) = (X/Y) x 100, where X is the total number of mosquitoes knocked down at 1-hour and Y in the total number of mosquitoes exposed to the test net. [↑](#footnote-ref-21)
21. 24-hour mortality (%) = (X/Y) x 100, where X is the total number of mosquitoes dead at 24-hour and Y in the total number of mosquitoes exposed to the test net. [↑](#footnote-ref-22)
22. 1-hour knockdown (%) = (X/Y) x 100, where X is the total number of mosquitoes knocked down at 1-hour and Y in the total number of mosquitoes exposed to the test net. [↑](#footnote-ref-23)
23. 24-hour mortality (%) = (X/Y) x 100, where X is the total number of mosquitoes dead at 24-hour and Y in the total number of mosquitoes exposed to the test net. [↑](#footnote-ref-24)
24. The number of sample pieces listed is for conducting the bioefficacy testing specified in this protocol. Additional samples may be required for chemical analysis. However, it is advisable that the same samples be used for biological and chemical testing. [↑](#footnote-ref-25)
25. Abbott’s formula: Adjusted mortality (%) = 100 x (X–Y) / (100–Y), where X is the percentage mortality with the test netting, and Y is the percentage mortality with the untreated control sample. [↑](#footnote-ref-26)
26. Compartment 1 = long section of tunnel into which mosquitoes are released; compartment 2 = short section where animal bait is housed. [↑](#footnote-ref-27)
27. Blood-feeding (%) = (X/Y) x 100, where X is the total number of blood fed mosquitoes collected from the tunnel and Y in the total number of mosquitoes exposed to the test net in the tunnel. [↑](#footnote-ref-28)
28. Blood feeding inhibition (%) = $\frac{(X-Y)}{X} × 100$, where X is the blood feeding % in the untreated net tunnel and Y is the blood-feeding % in the test net tunnel. [↑](#footnote-ref-29)
29. Immediate mortality (%) = (X/Y) x 100, where X is the total number of dead mosquitoes collected from the tunnel and Y in the total number of mosquitoes exposed to the test net in the tunnel. [↑](#footnote-ref-30)
30. 24-hour mortality (%) = (X/Y) x 100, where X is the total number of mosquitoes dead within 24 hours and Y in the total number of mosquitoes exposed to the test net in the tunnel. [↑](#footnote-ref-31)
31. 72-hour mortality (%) = (X/Y) x 100, where X is the total number of mosquitoes dead within 72 hours (including mosquitoes dead on collection and at 24- and 48-hours) and Y in the total number of mosquitoes exposed to the test net in the tunnel.

 [↑](#footnote-ref-32)