WHO Prequalification of Vector Control Products

Semi-field methods for ITNs: Experimental hut tests

Factors which may affect validity of studies using the experimental hut method:

- negative control mortality must not exceed 10% after 24 hours holding or 20% at extended holding times;
- blood feeding in the control should be monitored;
- sample size needs to be calculated before each trial based on local mosquito densities;
- mosquito phenotypic resistance should be characterized before or during each trial *(1).*

1. Purpose of the method

The purpose of the hut test is to investigate the biological activity of an insecticide-treated net (ITN) under simulated user conditions by means of observing relevant effects on wild free flying mosquitoes representative of a natural interaction with the host and test material while host seeking.

2. Considerations for use of the method

2.1 Classification as a bioassay

The hut test is best considered as a standardized bioassay which is useful in characterizing the entomological mode of action (MoA) of material and active ingredients on the surface an ITN. The method:

- can be conducted in a consistent manner across testing facilities;
- allows for variations in test **sample preparation** in order to investigate how ITN fabrics may change through their life stages, e.g. new, in/post storage, in-use and end-of-life;
- allows for the investigation of multiple observable endpoints in the free-flying mosquito.

The hut test can be used to measure the entomological efficacy of ITNs with active ingredients that are intended to kill, reduce blood feeding, and/or sterilize mosquitoes, including those pro-insecticides that require mosquitoes to be metabolically active for conversion to an active form. The use of a human volunteer or bait animal in the hut allows the entomological efficacy measurement(s) using free-flying mosquitoes to mimic conditions found when mosquitoes are host-seeking.

The consistency of the method supports the analysis across samples, geographic locations, and vector resistance profiles, thereby providing useful information about the changes in the biological performance of an ITN throughout the intended useful life in multiple settings.

The hut test measures the entomological efficacy of the ITN on wild free flying mosquitoes under controlled semi-field conditions. The hut test is designed to evaluate ITNs under conditions that include variability and heterogeneity in wild vector population structures (species/strains), density and behavioural/resistance characteristics. While the semi-field setting through which the exposure of mosquitoes to the ITN has a high degree of heterogeneity (mosquito densities, environmental conditions) and requires careful experimental design to ensure adequate measurement of outcomes in the mosquito, results from the experimental hut test have been extrapolated to conditions typically found in operational use of ITNs.

2.2 Use of the method

The use of the experimental hut test method can be employed within a variety of studies. These may include:

- **Experimental hut study** – investigation of product biological efficacy under user conditions when new and after artificial or operational ageing.
- **Comparative efficacy studies** – investigation of product biological efficacy under user conditions as compared to the biological efficacy of a comparator product, e.g. first in class.
- **Long-term community studies** – investigation of entomological efficacy following durations of routine ITN use.

Within each type of study, several ITN treatments and/or preparations can be tested simultaneously with positive and negative controls. The method is an appropriate choice for investigating products formulated with active ingredients with all modes of action proposed to have public health benefit for malaria control.

Supplemental bioassays, e.g. cone, tunnel, or IACT tests conducted alongside experimental hut tests provide information characterizing the presence and bioavailability of active ingredients on the fabric surface of ITNs of different preparations used in the experimental hut tests.

3. Materials

3.1 Huts

Experimental huts allow evaluation of ITNs under standardized conditions that resemble those in which mosquitoes enter a human habitation and contact an ITN in normal use. Experimental huts have structural features that enable collection of mosquitoes that have entered and interacted with an ITN and measure multiple endpoints.

There are several kinds of experimental huts in use and all have design features in common.

The huts are identical and multiple huts in a single location are used for a single evaluation.
Huts have eave gaps or entry slits that allow host-seeking mosquitoes to enter and forage, but that minimize egress so that mosquitoes are retained.

Huts are positioned in proximity to mosquito breeding sites to allow a uniform rate of mosquito entry into each hut. A water-filled channel surrounds each hut to prevent entry of ants that would scavenge incapacitated or dead mosquitoes, which would result in underestimates of mosquito mortality. Each hut has traps at exits (eaves, window or veranda) to capture exiting mosquitoes, allowing accurate estimates of exiting rates of mosquitoes. The size of experimental huts should approximate existing designs to minimize variability in results.

There are several different designs of huts used in experimental hut studies. Each of the designs described below has been tested in a suitable geographical region and shown to be effective for sampling local mosquito populations (Table 1). The hut design(s) may be adapted, or novel designs may be used in different settings. In such cases, appropriate baseline information should be generated to characterize the suitability of the design to support interpretation of data generated in studies using the new/adapted huts.

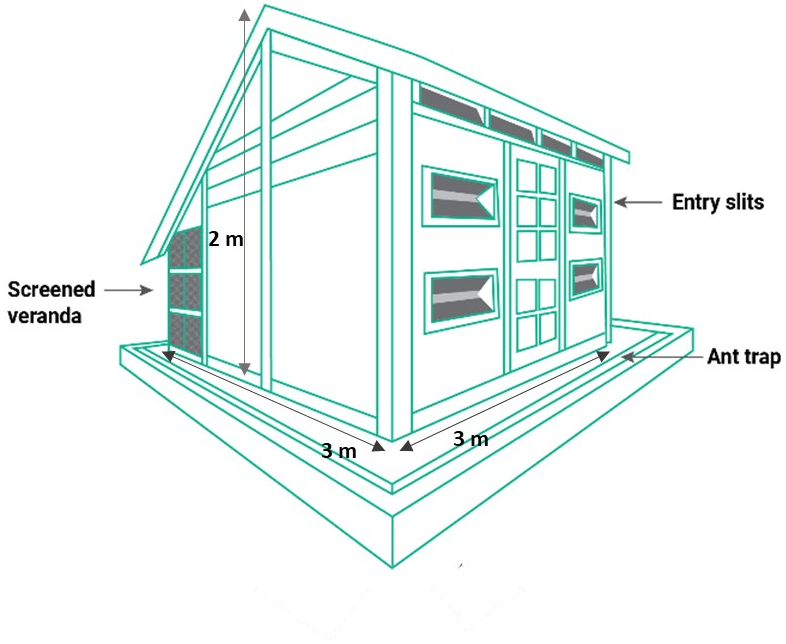
Table 1. Characteristics of experimental huts

	Asian hut	East African hut	Ifakara hut	Rapley hut	West African hut
Size (W x L x H)	3 m x 3 m x 2 m	3 m x 3 m x 2 m	3.25 m x 3.5 m x 2 m	2.5 m x 2.5 m x 2 m	2. 5m x 1.75 m x 2 m
Roof material	Cement tiles	Corrugated iron	Corrugated iron overlaid with thatch	Corrugated iron overlaid with thatch	Corrugated iron
Wall material	Wood	Bricks lined with mud	Bricks lined with mud	Concrete blocks	Concrete blocks
Ceiling material	Wood	Wood lined with hessian or plastic sheeting	Corrugated iron lined with foil	Corrugated iron lined with plastic sheeting	Corrugated iron lined with plastic sheeting
Entry points	5 entry slits funnelling to 1cm entry on the front 1 x 3 m entry at the eave 4 x 0.75 m either side of the door	Eave entry points on each wall of 140 cm wide and 20 cm height that funnels to 20 cm x 5 cm entry on two sides	Eave entry points 300 cm x 10 cm that funnels to 300 cm x 5 cm entry on three sides	Eave entry points 200 cm x 15 cm that funnels to 200 cm x 3 cm entry on four sides	4 entry slits (1 cm x 60 cm) 2 slits on the front and one on each of two sides
Area of Entry	600 cm^2	400 cm^2	4,500 cm^2	2,400 cm^2	240 cm^2
Exit point	Veranda	Veranda & window exit traps	Window exit traps	Window exit traps	Veranda
Area of exit	150 cm^2	880 cm^2	240 cm^2	320 cm^2	225 cm^2 (150 x 150 cm)
Re-entry possible?	no	no	no	no	yes
Number of sleepers	1	1	1	1	1

3.1.1 Asian-style huts

Each hut measures 3 m x 3 m x 2 m (Fig. 1) and is built of wood on a concrete floor. The roof is covered with cement tiles with wooden ceiling. The front of the hut has four entry slits (0.75 m), two on each side of the door, and one long slit over the entire width of the front above the door (3 m). The back also has three exit slits (0.75 cm each), with two on the wall and one in the eaves between the wall and roof. A screened veranda is connected to the hut and can be closed by a door.

Fig. 1. Design of experimental huts commonly used in Asia



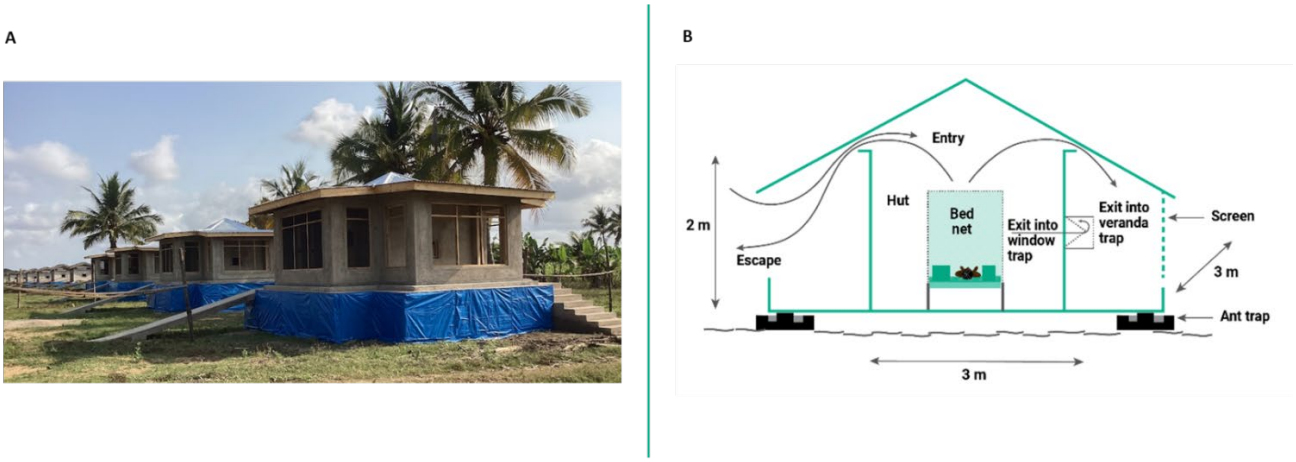
Credit: Courtesy of Dr Marc Coosemans, Institute of Tropical Medicine, Antwerp, Belgium, as presented in the Guidelines for laboratory and field-testing of long-lasting insecticidal nets *(2)*.

3.1.2 East African-style huts

East African style huts have veranda traps on all four sides, but two sides remain open each night to allow entry of mosquitoes (Fig. 2). The huts have brick walls plastered with mud on the inside, a wooden ceiling lined with hessian sackcloth or plastic sheeting, an iron roof, open eaves and window and veranda traps on each side.

Two opposite sides of the huts have closed verandas, screened to capture mosquitoes that leave via the eaves; the other two verandas are left open so that mosquitoes can enter through the eaves. Baffles are attached to the eaves on the open sides to prevent exit of mosquitoes through these points. The screening of the verandas may be rotated to compensate for possible selective entry/exit in one direction.

Fig 2. Design of experimental huts commonly used in East Africa (United Republic of Tanzania).
A. **Row of East African huts. B. Hut schematic.**

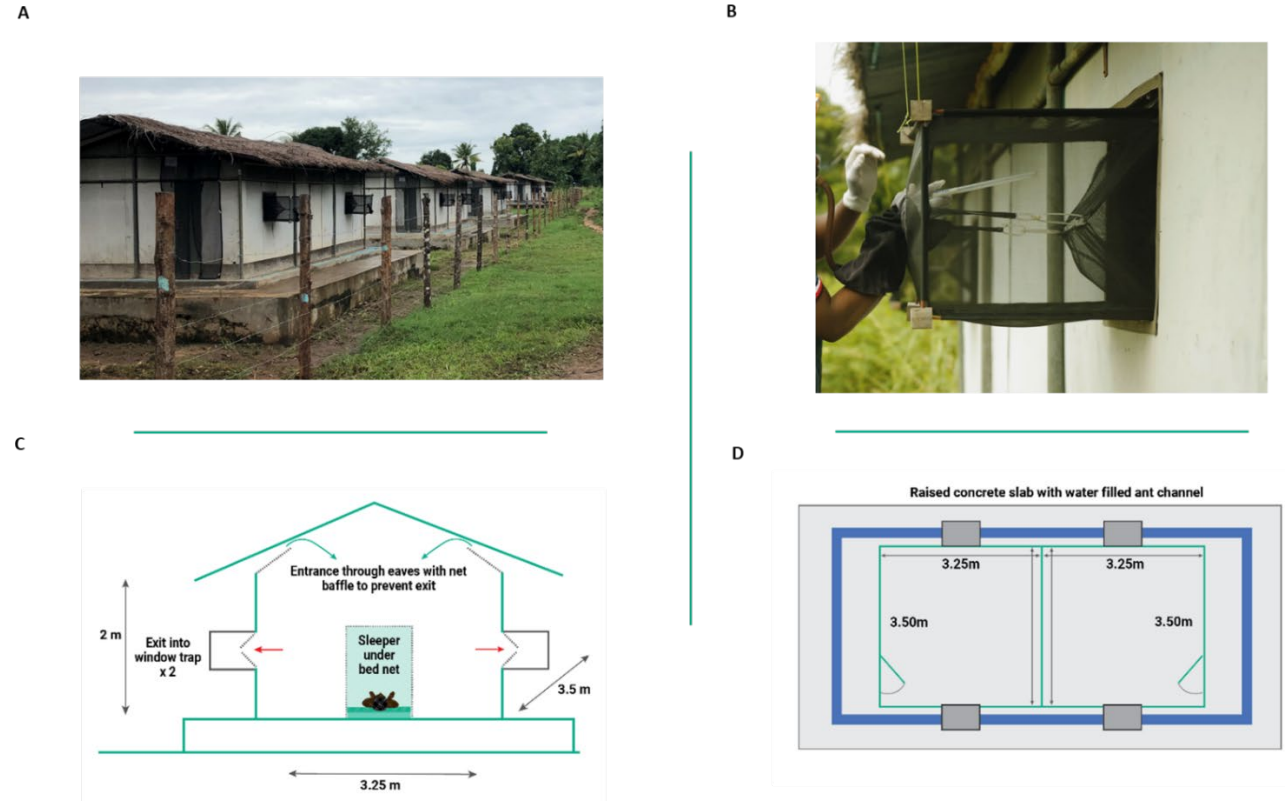


Credit: A: Ifakara Health Institute, Bagamoyo, United Republic of Tanzania, 2023. B: Courtesy of Professor C.F. Curtis, London School of Hygiene and Tropical Medicine, London, England, as presented in the Guidelines for laboratory and field-testing of long-lasting insecticidal nets (2).

3.1.3 Ifakara huts

Ifakara huts measure 6.5m by 3.5m wide, 2.0m on the sides and 2.5m to the apex of the roof (Fig. 3). The huts are divided into two 3.5 x 3.25m rooms, designated as individual huts each with its own entrance and two exit traps. The floors are covered with white linoleum to facilitate the recovery of knocked down or dead mosquitoes. The interior walls are coated with mud or plaster to simulate local hut conditions or may be lined with white cloth to facilitate recovery of resting mosquitoes while the roof is covered with grass thatch to reduce daytime temperatures inside the hut. The huts have one door, two windows and open eaves. The windows are fitted with exit traps.

Fig. 3. Design of Ifakara experimental huts
A. **Row of Ifakara huts. B. Mosquito collection from window trap. C. Hut schematic. D. Concrete base with ant trap schematic.**



Credit: A-D: Ifakara Health Institute, Bagamoyo, United Republic of Tanzania, 2023.

3.1.4 Rapley huts

Rapley huts are made from concrete bricks with a corrugated iron roof, a ceiling of polythene sheeting (Fig. 4). Mosquitoes can enter the huts through 10 cm eaves on all four sides that narrow over 20 cm depth to a 3 cm letterbox opening inside the huts that prevents mosquito egress. They measure 2.5 m x 1.75 m x 2 m. The huts have three windows fitted with window traps to collect exiting mosquitoes.

Fig. 4. Design of Rapley experimental huts
A. **Row of Rapley huts. B. Mosquito collection from window trap.**

A

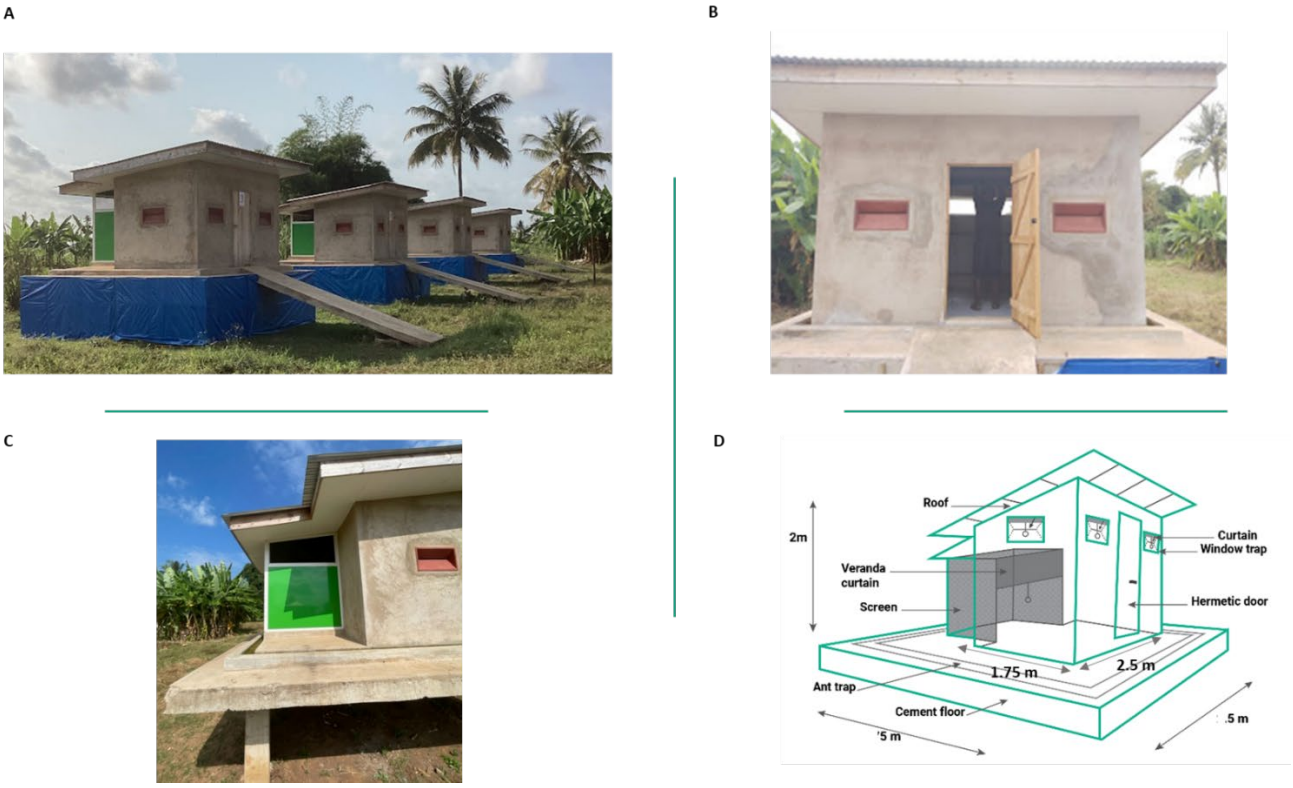
B

Credit: A-B: Ifakara Health Institute, Bagamoyo, United Republic of Tanzania, 2023.

3.1.5 West African-style huts

West African huts are made from concrete bricks with a corrugated iron roof, a ceiling of polythene sheeting (Fig. 5). They measure 2.5 m x 1.75 m x 2 m. Mosquitoes can enter the huts through four window slits constructed from pieces of metal fixed at an angle to create a funnel with a 1cm gap. The design of the window slits allows easy entry but limits the egress of mosquitoes once they have entered the hut. A veranda trap made of polythene sheeting and screening mesh (2 m long, 1.5 m wide and 1.5 m high) is fitted at the back of each hut. Mosquitoes are allowed to move unimpeded to and from the veranda trap during the night.

Fig. 5. Design of the experimental huts commonly used in West Africa.
A. **Row of West African huts. B. Front view of West African hut. C. Side view of West African hut. D. Hut schematic.**



Credit: A-C: Ifakara Health Institute, Bagamoyo, United Republic of Tanzania, 2023. D: Courtesy of Dr J.M. Hougard, Institut de Recherche pour le Développement, Benin, as presented in the Guidelines for laboratory and field-testing of long-lasting insecticidal nets *(2)*.

3.2 Hut preparation

Huts should be decontaminated and refurbished prior to an experimental hut study commencing. Baffles, verandah and window traps should be repaired and walls may be replastered, especially if the huts have previously been used for an IRS study. Following refurbishment, contact bioassays with 30-minute exposure times should be conducted on each hut wall to rule out contamination that could affect the outcome of the study.

3.3 Baseline information

Baseline information regarding the attractiveness of the huts and the recapture rates of mosquitoes released in the huts, i.e. hut retention, should be collected prior to each experimental hut study. The scavenging rate should be estimated by placing dead mosquitoes in petri dishes in huts overnight. The baseline period serves to re-train staff and increase the attractiveness of the huts to mosquitoes through indoor sleepers. The environmental conditions in experimental huts (temperature and humidity) should be continuously monitored with a data logger.

3.4 Human volunteers and bait animals

The hut test requires the presence of a human or animal as an attractant force. This is preferably a human volunteer, medically supervised who does not smoke, drink alcohol or use perfumed skin care products for the duration of their involvement in the study as these factors can affect human attractiveness to mosquitoes. If female volunteers are involved then provision should be made to ensure that no pregnant women are involved in the study due to the complications of malaria in pregnancy. It is advised that experimental hut tests are not conducted at times when arbovirus vectors are active in areas of active arbovirus transmission.

Sometimes cows are used as baits in experimental hut studies although in these cases experiments demonstrating that the mosquito response to the ITN is not different to the response to the ITN in the presence of humans are required. The way that the cattle are cared for should be documented including the steps taken to ensure that the cattle have not been treated with endectocides for worm control. Animals should be supervised by a veterinarian and fed on a diet free from pesticides. The animals should be gently restrained in a pen and may have a means of soaking urine away from them using a sponge mattress or cloth diaper. Care should be taken so that the animal is calm and cannot injure itself during the test. The use of a restraint or sedation is advised for this means.

Institutional ethical approval for the study must be sought from the local Ethical Review Board. Written informed consent must be obtained from each volunteer sleeper prior to their participation in the study. The consent form is explained to each volunteer in her/his local language by an interpreter. Sleepers should be monitored for possible adverse effects from mosquitoes or the ITNs and excluded if they experience any discomfort. In malaria endemic areas sleepers should be regularly monitored for malaria parasitemia to ensure that no individual carrying parasites participates in the study.

3.5 Aspirators

Aspirators comprise a clear Perspex tube with an aperture of around 1cm that will fit into the WHO cone that has an opening of 1.5cm. Mosquitoes are entered into the Perspex tube through aspiration either through mouth aspiration or mechanical aspiration. If mouth aspiration is conducted then it is recommended that a HEPA filter is used to prevent technical staff inhaling mosquito scales, dust or pesticide residues.

Mechanical aspirators such as the Prokopack are often preferred for collecting mosquitoes from larger areas including floors, walls and ceilings. The use of a 6 volt battery may be considered to minimise mechanical damage to resting mosquitoes that are to be held for delayed mortality monitoring.

3.6 Holding receptacle

Paper cups or other inert holding receptacle lined with paper that allows mosquitoes to comfortably rest are used prior to exposure procedures and during post-exposure monitoring. The top must be an untreated netting which can be affixed to the receptacle. The holding receptacle has a 1.5-2 cm hole sealable with cotton wool or another means that allows removal or introduction of test insects using a mechanical aspirator. It is recommended that no more than 1 mosquito per 20 cm^3 is retained in the cups to minimize mosquito mortality though overcrowding.

3.7 ITN preparation

The test materials to be used in the method should be prepared as whole, constructed ITNs. Ensure that all prepared samples are adequately labelled and stored appropriately, as improper storage may impact the results of the test and invalidate the study. In between testing it is advisable to keep the products out of sunlight, e.g. wrapped in foil and in a temperature-controlled room. The environmental parameters of the storage room should be recorded.

3.7.1 Damage Replication

For standard evaluations of ITNs, the nets are deliberately holed to simulate operational ageing, using 4 cm x 4 cm holes.

Cut six holes in each net. Each hole should measure 4 x 4 cm in length and width. One hole is cut on each of the short sides of the ITN and two holes are cut on the longer sides of the ITN. These should be equidistant from the top of the net, i.e. for a 160 cm net with 10 cm of the net tucked under the mattress the holes should be at 75 cm from the top of the ITN. On the long sides, the holes are equally spaced, i.e. the first hole is made at one third of the distance and the second at two-thirds of the distance from the edge of the side panel.

When cutting the test material ensure that the material is not being stretched nor compressed. Holes are cut using scissors or a razor, preferably using a template, taking care that the holes are standardized. Larger or smaller holes than standard may invalidate the results.

3.7.2 Washing

Preparation of nets may take several weeks or even months as the ITNs must be washed 20 times following the selected and justified wash interval. To maintain equivalence between the various treatments in the trial, washing in each treatment arm should be completed at the same time, which means starting the washing of ITNs requiring a longer wash interval earlier than those with a shorter wash interval. A minimum of six test nets are used for each preparation to measure between net heterogeneity.

Washed ITNs should be allowed to rest for two weeks between the completion of washing and the hut test commencement.

3.8 Sampling procedure for supplementary bioassays

Sampling procedure for ITNs is dependent on the design and construction of the ITN, including the presence of multiple fabrics in the ITN design. Sampling schemes are described in the relevant study guidance documents and are designed to ensure that any differing fabrics in the ITN are adequately represented. The total number of samples required is dependent upon the study and the product.

3.9 Storage of test and reference materials

Since the hut test is a method for understanding efficacy of an ITN, the history of and conditions to which test materials and samples have been exposed are critical pieces of information for the interpretation of the resulting data. The means by which samples (including reference items) were obtained and the storage conditions prior to testing should be documented, with certificate of conformity and batch numbers reported.

3.10 Reference items (controls and comparator products)

The purpose of the **reference items** (positive and negative controls, and comparator products in the case of comparative efficacy studies) is to validate the experimental procedures. In comparative efficacy studies, the reference items serve a further purpose, as the results from reference items are used in statistical analyses to determine the non-inferiority of the ITN under investigation to the reference item.

The means by which **reference items** were obtained and the storage conditions prior to testing should be documented, with certificate of conformity and batch numbers reported.

Negative control samples should be untreated netting made of polyethylene or polyester. Positive controls should be selected in accordance with the study that is being conducted. All positive controls should be prequalified products that have an entomological mode of action consistent with the entomological mode of action and the intended effect of the product that is under investigation.

4. Environmental conditions

4.1 Environmental monitoring

The environmental conditions of the ITN storage area, the testing room, and insectaries should be continuously monitored and reported.

4.2 Test room environmental conditions

The temperature and humidity of the post-exposure holding room should be maintained at 27 ± 2° C and 80 ± 20%, respectively.

All environmental conditions at which the tests are conducted should be documented and reported.

5. Test systems for supplementary bioassays

The selection and preparation of test systems for use in supplemental bioassays to characterise the presence and bioavailability of active ingredients on the surface of ITNs used in hut tests should align with the:

- entomological mode of action of the product under investigation;
- characteristics, e.g. insecticide resistance status, of the test systems used in laboratory studies;
- characteristics, e.g. insecticide resistance status, of the vector population at the experimental hut site;
- age and nutritional status of the selected bioassay method.

It is recommended that alongside laboratory strains, adult mosquitoes reared from larvae collected at the experimental hut site are used in supplemental bioassays to characterise ITN properties.

6. Selection of endpoints and considerations

Table 2 provides information pertaining to the relevant endpoints which may be observed and measured when using experimental huts. The endpoint to be used for decision-making purposes must be selected based on the intended entomological mode of action of the product under investigation and be used consistently across all laboratory studies, semi-field studies and semi-field supplemental bioassays. Justification for the selection of the decision-making endpoint must be presented in the study report.

Regardless of the intended entomological effect of the product, M24 should be observed and documented for the purpose of monitoring the experimental controls and thereby experimental acceptability.

Table 2. Experimental hut endpoints

Endpoint	Time it is measured	Purpose/definition	Considerations

Mortality at 24 hours (M24)	24 hours after the 9-hour experimental hut test exposure has ended	The measurement of mortality in a cone test is an indicator of the lethal effects of the net.
		
		Mortality is observed by the following indicators:
		
		• No sign of life; immobile; cannot stand.
		• Moribund mosquitoes are also classed as dead after 24 hours of holding as it is unlikely that they would survive in nature, i.e.
		  • Any mosquito that cannot stand (e.g. has 1 or 2 legs).
		  • Any mosquito that cannot fly in a coordinated manner.
		  • A mosquito that lies on its back, moving legs and wings but unable to take off.
		  • A mosquito that can stand and take off briefly but falls down immediately.
			The standard exposure time in the experimental hut test for measuring mortality is 9-12 hours. Extension of the exposure time, or inclusion of multiple exposure times must be declared and scientifically justified in the context of the product being tested and study being conducted.
			
			The standard holding time post-exposure in the experimental hut test is 24 hours. Control mortality should not exceed 10% after 24 hours. Otherwise, the test is invalidated.
			
			Extension of the post-exposure holding time must be declared and scientifically justified in the context of the product being tested and study being conducted.
			
			e.g. Mortality at x hours after exposure - Mx
			
			Control mortality should not exceed 20% after extended holding times. Otherwise, the test is invalidated.

Blood feeding inhibition	At the end of the exposure period	The proportion of unfed females. Blood fed includes partially or fully blood engorged mosquitoes. Blood feeding inhibition is the proportion of mosquitoes that are not fed. Blood feeding inhibition induced by the intervention may also be calculated as follows: 1) calculate average blood feeding in the control (C) arm, 2) calculate the blood feeding for each observation for each intervention (T) relative to the average blood feeding rate in the control using the formula 100x(C-T/C), 3) calculate the mean blood feeding inhibition (% and 95% CI) from all the observations in each arm.	
Fertility – eggs per female	Blood fed mosquitoes are held for 72 hours	The measurement of eggs per female is an indicator of fertility. Fertility is observed by the following indicators:	Blood fed female mosquitoes are provided with an egg laying substrate for oviposition.

Endpoint	Time it is measured	Purpose/definition	Considerations
	after overnight exposure in a hut test.	Number of eggs laid / females live at each period of observation.	
Fecundity – Proportion of fertile females	Number of females with Christopher's stage 5 eggs when dissected. Blood fed mosquitoes are held for 72 hours after overnight exposure in a hut test.	The measurement of proportion of fertile females in a hut test is an indicator of reduction of fecundity. Fecundity is observed by the following indicators: Number of females that have fully developed (viable eggs) at each period of observation measured using the Christopher's classification. Stage 4 and Stage 5 eggs are classified as viable.	
Other	Applicants may propose other endpoints to be measured by means of the experimental hut test with adequate justification		

7. Experimental method

Before each night of mosquito collection:

- Experimental huts and exit traps should be cleaned to remove predators such as spiders and lizards.
- ant channels should be checked to ensure that scavenging ants are unable to enter the hut.
- In huts with exit traps and verandas, these should be checked to ensure integrity and the veranda curtain rolled up before the hut is used if the West African Hut design is used.
- The equipment needed for each sleeper should be assembled. This includes mattress, sheet and pillow as well water, food and a chamber pot for comfort and to minimize the risk of sleepers leaving the huts during the night.
- The aspirators and any other equipment must be cleaned in 10% bleach or appropriate cleaning agent and rinsed well with clean water. Separate aspirators must be prepared and labelled for the negative control, positive control, and each treatment. Each individual must have a separate aspirator to avoid contamination of samples and to safeguard participants from respiratory diseases.
- Mosquito holding receptacles are prepared and adequately labelled.

7.1 ITN allocation and hanging

Ensure all samples are adequately labelled.

The study supervisor will allocate the ITN to each hut. Nets should be tied in place with string ensuring that the hanging method standardises the surface area that is available to mosquitoes. Nets should be used tucked in with the holes approximately 75 cm from the floor.

After each collection night, each ITN should be taken down and stored in its original packaging or wrapped in foil in a suitable storage area out of direct sunlight. The environmental conditions of the storage area should be continuously monitored with a data logger and should not exceed 32^0C. Incorrect handling and storage of test items will invalidate the method.

7.2 Test systems

The selection of mosquito species/strains, i.e. study location, should be informed by the intended effect of the test material as well as the purpose of the study in which the hut method is applied. The mosquito resistance profile should be well characterized and regularly checked using WHO tube test or bottle bioassay. Species composition of alive and dead mosquitoes should be determined if there are multiple sympatric vectors (including sibling species), to evaluate whether the net is equally effective against all.

7.3 Mosquito collections

The timing of the hut method tests within a study should be aligned with the circadian rhythm of the test system and be consistent when tests are conducted across multiple days/sample periods. The WHO hut test is normally conducted overnight between 21:00 and 06:00 hours.

At a specified time early in the morning, mosquitoes should be collected from inside the hut. The verandas should be closed to prevent movement of mosquitoes between the different compartments. Dead and live mosquitoes are first collected from inside the nets and then from the floor. Then, dead and live mosquitoes are collected from inside the hut. Lastly, dead and live mosquitoes are collected from inside the exit and veranda traps. Collections should be performed in a systematic way, e.g. starting consistently in the left corner of the net, hut or veranda and working slowly clockwise searching up and down with a torch.

The collection period should be for a consistent number of minutes each day and carefully supervised to ensure that mosquitoes are not missed. Correct training and high motivation of staff is key to conducting good quality experimental hut evaluations.

7.3.1 Transit between semi-field sites and laboratories

Holding receptacles containing live mosquitoes should be labelled with the hut number, test item identifier and the date of collection and placed into a cooler box lined with damp towels and with a data logger to monitor the temperature and humidity to which mosquitoes are exposed during transit from the field site.

Transfer of collected mosquitoes to the laboratory should be done as soon as practically possible to minimize the effects of temperature and humidity during transit on mortality. If mosquitoes need to be transferred from the huts to a laboratory or insectary it is important to minimize stress caused by sudden changes of temperature, humidity, sunlight, or wind through using closed receptacles to transport holding cups or holding cages.

7.4 Mosquito scoring

Mosquitoes are scored by species and collection location as dead unfed, dead blood fed, alive unfed and as alive blood fed. After recording initial mortality and blood feeding status, place sugar source on top of the holding receptacle. Sugar source is 10% glucose or sucrose solution made with sterile water.

7.5 Post-exposure

The post-exposure holding time should begin, and be documented, at the time that mosquitoes are removed from the hut and transferred to the holding receptacle.

Live mosquitoes should be placed in holding receptacles, given sugar solution and held at 27 ± 2 °C and 75 ± 10% relative humidity. Holding conditions should be continuously monitored with a data logger. Control mortality should not exceed 10% for up to 24 hours and 20% for up to 168-hour holding.

High control mortality is generally linked to poor mosquito handling or suboptimal holding conditions (too hot, not sufficiently humid or poor cleanliness) and will invalidate the test.

Delayed mortality of mosquitoes should be assessed every 24 hours for up to the specified holding time dependent on the chemical mode of action of the active ingredient(s) on the ITN. If reduction in fecundity is an endpoint under investigation, blood fed mosquitoes should be held in individual glass or plastic tubes with oviposition pads. The proportion of mosquitoes that lay eggs and the total number of eggs per female should be monitored after 4 days or mosquitoes are dissected at 72 hours and the proportion with mature eggs (Christopher's stage 5) is measured.

8. Results

All endpoints should be presented with an appropriate measure of centrality and dispersion e.g. arithmetic mean % and 95% confidence intervals for percentages; median and interquartile range for count data (e.g. number of eggs). Data for the control arm provides data needed to appraise the quality of study conduct and should always be presented.

Mosquito mortality at each holding period should be presented by arithmetic mean % mortality and 95% confidence intervals. Negative (untreated) control mortality and feeding success should also be reported at each post-exposure holding time. If mortality at 24 hours exceeds 10% the test should be discarded and repeated. If 24-hour mortality (M24) exceeds 5% then mortality should be control corrected using Abbotts Formula.

The number of replicates conducted per arm or per ITN preparation should be presented.

Presentation of test environmental conditions is useful to enable understanding of test conduct.

Tables of full results are presented in an annex if figures are used in the report.

Suggested summary data presentation for critical endpoints. Note that detailed guidance for presenting results from studies is given in the relevant study guidance document.

Outcome	Intervention and preparation	N	Mean	Odds ratio (95% CI)	p-value
Mortality at 24 hours	Control				
Blood feeding					

9. Related documents

- WHO PQT/VCP Implementation guidance – Regeneration study for ITN fabric
- WHO PQT/VCP Implementation guidance – Wash resistance study for ITN fabric
- WHO PQT/VCP Implementation guidance – Selection of controls for use in ITN studies
- WHO PQT/VCP Implementation guidance – Bioassay methods for ITNs: cone test
- WHO PQT/VCP Implementation guidance – Bioassay methods for ITNs: tunnel test
- WHO PQT/VCP Implementation guidance – Considerations for the selection of mosquito strains for use in bioassays and site selection for semi-field and community studies
- WHO PQT/VCP Implementation guidance – MSMS
- WHO PQT/VCP Implementation guidance – Template MSMS

10. References

1. Manual for monitoring insecticide resistance in mosquito vectors and selecting appropriate interventions. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO https://iris.who.int/bitstream/handle/10665/356964/9789240051089-eng.pdf?sequence=1
2. Guidelines for laboratory and field-testing of long-lasting insecticidal nets. Geneva: World Health Organization & WHO Pesticide Evaluation Scheme; 2013 (https://iris.who.int/handle/10665.80270).

11. Annexes

1. Definition of endpoints

Annex 1. Definition of endpoints

Definition of endpoints

Critical endpoints

Mortality: Mortality is currently the primary endpoint for all ITNs and IRS with the exception of those that have effects on reproduction. A mosquito is classified as dead (at 24 hours or longer, post exposure) if shows no movement, if it is immobile, or if it is unable to stand or fly in a coordinated manner. This post-exposure period may be extended up to 168 hours depending on the chemical mode of action of the active ingredient (AI) in the vector control product under evaluation provided control mortality is less than 20% at the longest holding time. However, mortality should always be recorded and reported at 24-hour intervals when a longer holding time is selected. It is also useful to consider when mortality occurs: killing before biting (pre-prandial mortality) provides personal protection and community protection, whereas killing after biting (post-prandial mortality) provides only community protection. Mortality is calculated in experimental hut studies by comparing the proportion of mosquitoes that die of all the mosquitoes that entered that hut at a set time after the morning of collection. An untreated control is also conducted to ensure that poor experimental conduct does not result in high mosquito mortality.

Blood-feeding: A reduction in blood feeding occurs when adult female mosquitoes are inhibited by a vector control product from completing the sequence of behaviours that result in a blood meal. This is a result of interference with the sequence of olfactory and gustatory processes that result in successful host location and/or blood feeding. The impact of an insecticide on mosquito feeding is calculated in experimental hut studies by comparing the proportion of mosquitoes that are fed of all the mosquitoes that entered that hut. This proportion may be evaluated relative to other trial arms, e.g. the active comparator. Data are checked against an untreated control to ensure that it is the insecticide that is inducing the change in mosquito blood feeding behaviour. The blood feeding rate is used for comparative efficacy assessment and should always be reported. Data on reduction in blood-feeding is to be included as a secondary end-point to assist in informing programmatic and procurement decisions; it is not the key driver on which decisions are to be based.

Blood feeding inhibition induced by the intervention may also be calculated as follows: 1) calculate average blood feeding in the control (C) arm, 2) calculate the blood feeding for each observation for each intervention (T) relative to the average blood feeding rate in the control using the formula 100x(C-T/C), 3) calculate the mean blood feeding inhibition (% and 95% CI) from all the observations in each arm.

Effect on fecundity: Reductions in fecundity are decreases in the proportion of females with viable eggs produced by a blood-fed adult female mosquito. Fecundity is calculated by dissection of mosquitoes to look for viable eggs at a set time (72 hours) after the morning of collecting blood-fed females from an experimental hut. It may also be measured by counting the number of eggs laid by each blood fed mosquito that remained alive long enough to complete egg development and lay eggs. Data are checked against an untreated control to ensure that it is the insecticide that is inducing the change in mosquito fecundity.

Supporting endpoints to understand intervention mode of action (MoA)

Effect on fertility: Reductions in fertility are decreases in the number of viable offspring produced by a blood-fed adult female mosquito. Fertility is calculated by the proportion of mosquitoes that produce viable eggs. It can also be calculated by counting the number of viable larvae produced by each blood fed mosquito that remained alive long enough to lay eggs. Data is checked against an untreated control to ensure that it is the insecticide that is inducing the change in mosquito fertility.

Deterrence: Deterrence is reduced likelihood of adult female mosquitoes to enter a treated house because of an active ingredient applied inside the house. Deterrence is calculated in experimental hut studies by comparing the number of mosquitoes that enter the control hut to those that enter the treated huts. As mosquito densities vary in space and time it is critical to 1) rotate ITNs between huts, 2) rotate volunteers between huts, 3) ensure adequate replication of IRS huts, 4) monitor mosquito escape from huts does not differ between huts to ensure that deterrence is adequately calculated.

Excitorepellency (irritancy, induced exophily): Excitorepellency is when mosquitoes exit a space due to excitation that results in them moving away from the repellent source. Excitorepellents induce three types of movement: (1) taxis: directional movement of mosquitoes away from the treated space, (2) unidirectional movement due to orthokinesis (change in flight speed), and (3) unidirectional movement due to klinokinesis (change in turning during flight) that results in random movement of mosquitoes away from the treated space, often toward light. Induced exophily is calculated in experimental hut studies by comparing the number of mosquitoes that enter the exit traps or verandahs in treated huts relative to control huts.

Personal protection: Personal protection is when adult female mosquitoes move away from an otherwise attractive host. This may be due to contact or noncontact irritancy, sublethal incapacitation of the mosquito olfactory system or an avoidance reaction to the active ingredient. Personal protection is calculated in experimental hut studies by comparing the number of mosquitoes that are fed in treated huts relative to control huts.