SOP: Experimental Hut Preparation and Running

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1	30/10/2020	Angus Spiers	I2I
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Version Control[1]

Version	Date	Updated by	Description of update(s)
3	October 2023	Katherine Gleave	Net holing methodology updated with more detail.
2	April – May 2022	Alex Wright, Natalie Lissenden	Related documents, purpose, materials & equipment, post-

[1] Historical versions of SOPs can be found on the I2I website (https://innovationtoimpact.org/)

			collection holding for nets containing PPF, data collection sheet information, glossary of terms and references added.

Related documents

- I2I Best Practice SOP Library, 30 October 2020 (https://innovationtoimpact.org/)
- SOP for Cleaning of Experimental Huts (https://innovationtoimpact.org/workstreams/standard-operating-procedures/)
- SOP for Mosquito Collections from Huts (https://innovationtoimpact.org/workstreams/standard-operating-procedures/)

1. Purpose

Experimental huts allow evaluation of LLINs (long-lasting insecticidal net) under controlled conditions that resemble those in which mosquitoes enter a human habitation and contact an LLIN in normal use. Candidate LLINs that meet the requirements of laboratory testing should be tested in semi-field studies in experimental huts, where the efficacy of LLINs against free-flying mosquitoes in terms of inhibiting blood-feding, deterring mosquitoes and inducing exophily and mortality are assessed. This SOP details the process of preparing and running an experimental hut trial.

2. Background

Once washing has been completed, a further five pieces are cut post washing from the net from which samples were cut pre-washing. Further five samples are cut for chemical analysis. The other nets are then used in the huts and then one is cut for 5x2 samples for post-trial bioassay and chemical testing. For the remaining five nets to be used in the experimental hut study, six holes (4 cm x 4 cm) should be cut in each net, with two holes on each of the long side panels and one on each of the short side panels. The holes in

the short side panels are located in the center of the net, while those in the long side panels should be spaced evenly along the length of each panel, i.e. the first hole is made at one third of the distance and the second at two thirds of the distance from the edge of the side panel. The holes should be centered vertically on all sides of the net.

Sleepers are recruited into a study preferably from the local area, and an information sheet is given or read to them to apprise them of the procedures involved. Depending on the requirements of the local ethics committee, written informed consent may be obtained. A bed or mattress should be placed in each hut and, at a specified time each night, sleepers should enter each hut. At least one sleeper is needed for each hut. To attract more mosquitoes, it may be desirable to have more than one sleeper inside each hut, although the number should be standardized for all huts and teams should be rotated between huts as a pair. The sleepers should ensure that the nets are tied to walls with strings and tucked under the mattress and should then remain inside the huts until a specified time in the morning. Provisions such as water, food and a chamber pot may be provided to minimize the risk of sleepers leaving the huts during the night. Smokers and pregnant women should be excluded to avoid potential bias in the results. At a specified time in the early morning, mosquitoes should be collected from inside the hut. Dead and live mosquitoes are first collected from inside the nets. The verandas are then closed to prevent movement of mosquitoes between the different compartments. Then, dead and live mosquitoes are collected from inside the hut including under the bed if a bed is used. Lastly, dead and live mosquitoes are collected from inside the exit and veranda traps. Mosquitoes should be scored by location as dead or alive and as blood-fed or unfed. Live mosquitoes should be placed in small cups, given sugar solution, and held at 25°C ± 2 °C and 75% ± 10% relative humidity for 24 hours to assess delayed mortality (or as stated in the study protocol).

3. Materials & Equipment

a. Hut preparation
    i. Petri dish
    ii. Ten dead mosquitoes
    iii. Bleach
    iv. Spray bottle
    v. Broom
    vi. Cotton wool
    vii. 50 lab reared mosquitoes
b. Net preparation
    i. Template for cutting nets (6 holes, 4cm x 4cm)
    ii. Scissors
    iii. Ethanol
    iv. Spray bottle
    v. Chemical waste bin
    vi. Package material for cut net pieces (aluminum foil)
    vii. Marker pen/label
c. Preparation before first collection
    i. Strings to hang nets
    ii. Rat glue
    iii. 10% glucose-soaked cotton wool
    iv. Two calibrated data loggers
    v. Mattress for sleeper
    vi. Broom to clean
d. Net hanging and collection
    i. White sheet for mattress
    ii. Nylon sheet for floor
    iii. Storage bag for whole nets
    iv. Data loggers for storage room

v. Folders for storing forms
vi. Rotation schedule
vii. Packing forms

e. Mosquito collection
    i. Torch
    ii. Torch batteries
    iii. Watch
    iv. Collection cups with netting and cotton wool secured with rubber band
    v. Aspirators- manual and battery-operated
    vi. Marker pen or label
    vii. Cup rack
    viii. 10% glucose solution-soaked cotton ball
    ix. Pen
    x. Raw data sheet
f. Post- collection holding for PPF
    i. Mosquito cages
    ii. Blood meal
    iii. Netted plastic cups (with fresh water)
    iv. Larval food
    v. Data forms

4. Procedure

Hut Preparation

a. Ensure that the moat around each hut is filled with water and check that there is no leakage.
b. Ensure huts are cleaned after last trial (SOP for experimental hut cleaning). If necessary, perform cone bioassays on the walls to ensure no residual insecticide is remaining from previous studies of IRS.

c. Search rooms, verandahs, and exit traps for ants. If ants are found, follow SOP for removal of ant infestations from experimental huts. Ants can be monitored by placing ten dead mosquitoes in an open petri dish in the experimental hut room. If they are not removed within 24-hours it is okay to start the trial when ready.
d. Remove spiders, spider webs and lizards from the rooms and verandahs of all huts.
e. Repair holes in screened verandahs and fill any large cracks or holes in the walls.
f. Clean the sugar bowls with 10% bleach and rinse well with water only.
g. Repair/patch any holes in exit traps and clean with a 10% bleach solution, then rinse with water. Hang up with no gaps around the window. Plug gaps with cotton wool.
h. Check the integrity of the huts by releasing 50 laboratory reared mosquitoes and recapturing them in the morning.

Net Preparation

a. Code nets by treatment and by net number so that rotation of the nets can be done systematically.
b. Put on disposable gloves and place net over cutting frame (Figure 1).
c. All nets to be used for the study will be cut according to WHO standards (WHO, 2013).
d. Use a cardboard hole cutting template to accurately cut holes to the correct size (Figure 2).
e. A total of six holes are to be cut in each net in the positions shown in Figure 3.
f. Cut one hole (4cm x 4cm) on each of the short sides of the net in the centre of the panel.

g. Cut two holes (4cm x 4cm) on each of the long sides of the net located halfway up the net (equidistant from top and bottom) and 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.

h. - For each net with different AI(s), use different or cleaned equipment (sprayed/wiped with 70% ethanol then water). Cut control nets first, then single AI nets, then mixture AI nets.

i. Store the 4x4cm pieces in the designated chemical waste.

j. Pack the nets and seal. Transport to the field station.

Figure 1. Net stretched over a frame for deliberately holing.

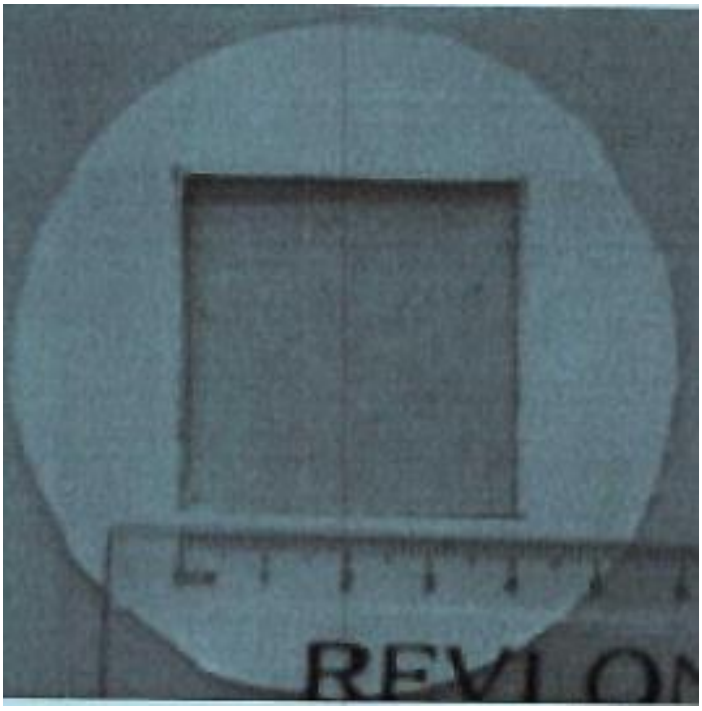

Figure 2. Hole cutting template.

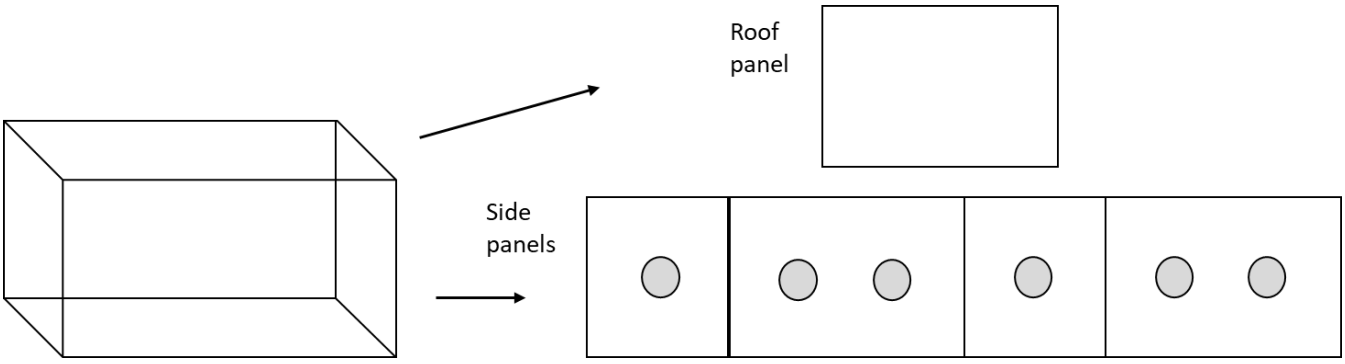

Figure 3. Location of holes in 'deliberately holed' nets uses for experimental hut studies.

Preparation 24 hours before first collection day

a. Tie strings for hanging nets into the corners of the hut rooms.
b. Add a line of rat glue to the outer edging of the verandah floor to stop ants, geckos, and spiders from invading hut rooms.
c. Ensure that moats have been kept topped up with water.

d. Add 10% glucose solution-soaked cotton wool to the sugar bowls in the verandah and exit traps to ensure that mosquitoes can sugar feed and do not die from desiccation.
e. Hang one data logger in a verandah and one inside the room of the control hut.
f. Hang nets from strings on walls. Ensure the length of the rope after tying is just enough to allow 5-10cm of the bottom of the net to be fitted under the mattress of the sleeper.
g. Ensure holding room is cleaned and ready for mosquito collection cups. Have all equipment cleaned and ready to use. Use an equipment checklist if necessary.

Collection schedules

a. Study director- Create a full collection schedule using a Latin square based on the number of arms of the hut trial.
b. Ensure that each treatment has been assigned to each hut and volunteer combination the same number of times.
c. Where it is practical avoid sequential movement of treatments or volunteers between huts to reduce the risk of "carry over."
d. Make a rotation schedule containing the date, hut, sleeper, and net code assigned for the duration of the experiment for the study manager to use in the field.
e. Use one starting grid and randomize the order of the rows, then the order of the columns, then randomly assign the treatments to the letters within the grid to create the "weekly" rotation of the treatments (note- a true weekly rotation is only possible for a hut trial with 6 arms of less, as one day each week should always be left free for hut cleaning).

f. In the weekly rotation, the nets within each arm will ideally be rotated daily or after every two days, but this depends on the availability of the nets. Because this is an unknown quantity the net rotation within each treatment arm cannot easily be randomized in advance and it is easier to rotate sequentially rather than randomly. The important thing is that each net within an arm should be used a similar number of times over the total length of the trial. As an example, in a 6x6 trial with 3 nets (A, B, C) per arm, it is best to assign AABBCC for the six nights in each of six weeks. Each individual net is therefore used on 12 nights of the 36 sampling nights.
g. The study director will take a second starting grid and repeat the steps of "b" to create the daily rotation schedule of the sleepers or cows. The two rotation schedules together constitute the full collection schedule.

Net hanging and collection (IHI)

a. Field site manager will oversee and conduct hanging the appropriate net at each hut following the treatment rotation schedule using the net code ID.
b. Ensure a white sheet is properly fitted to the mattress and a nylon sheet placed on the floor with an appropriate label is placed to ensure no contamination of chemicals from the net is transferred to the floor. Place the mattress in an appropriate position in the hut.
c. Ensure the nets are correctly hung and tucked under the mattress without damage.
d. At 09:00 each day, field site manager will make sure each coded net is collected from each hut and placed into the originally code-labelled bags. Store at ambient temperature not exceeding 35°C and away from sunlight until required for the next test. Record temperature each day. A cool dark room is best.

Sleeper and hut preparation (IHI)

a. Volunteers will report to work at 18:00 already having the evening meal and one volunteer will be allocated to an experimental hut according to the treatment rotation schedule developed by the study director following the treatment rotation schedule.
b. Assemble equipment needed for all collection periods that night (See SOP for mosquito collections).
c. Check that correct net has been placed inside each hut.
d. At 19:00 check volunteers are in the hut.
e. Field site manager checks each hut has the correct equipment, correct treatment, and correctly labelled cups.

Mosquito collections

a. Net collections

i. At 06:00 two collectors will collect mosquitoes from within the LLIN using the mouth aspirator starting in the top left corner of the net and working slowly toward the right of the hut, using the torch to show any mosquitoes in the net before moving forward. Work around the net and then the top of the net with the collection cup label including “Net”.
ii. Gently aspirate mosquitoes to avoid damaging them and gently blow them into the correctly labelled cup “Net”.
iii. Check that the netting and cotton wool is correctly placed on the cup to prevent mosquito escape.
iv. Collectors should seek not to exceed 5 minutes.
v. Use one cup for every 20 mosquitoes collected.

vi. Ensure the cup is correctly labelled with time period and date, and technician initial.

b. Floor collections

i. Using the battery powered aspirator, collect mosquitoes from the floor. Put mosquitoes into the collection cups labelled “Floor”. Collect mosquitoes by shining torch to clearly see mosquitoes.

ii. Check the netting and cotton wool is correctly placed on the cup to prevent mosquito escape.

iii. Use one cup for every 20 mosquitoes collected.

iv. Do not exceed 3 minutes for each cup used as the aspirator may kill mosquitoes if used for too long.

v. Ensure the cup is labelled with time period and date, and technician initial.

c. Resting collections

i. Use the battery powered aspirator to collect mosquitoes resting on the walls and on the roof of the hut and place them in the labelled paper cup “Wall”. Collect mosquitoes by shining the torch on the wall about 30cm away to clearly see the mosquitoes.

ii. Collect the mosquitoes starting in the top left corner then move methodically from left to right and up and down wherever possible to ensure that all mosquitoes resting are collected.

iii. Collectors should try not to exceed 3 minutes to sample each wall and/or ceiling as per protocol as the aspirator may kill mosquitoes if used for too long

iv. Use one cup for every 20 mosquitoes collected.

v. Ensure the cup is labelled with time period and date, and technician initial.

d. Trap collections

i. Collect mosquitoes by shining the torch through the netting to clearly see the mosquitoes. Insert the mouth aspirator through the trap sleeve being careful to seal the sleeve around the aspirator.

ii. Collect the mosquitoes starting in the top left corners- bottom left, top right, bottom right. Then move methodically from left to right, up and down to ensure all the mosquitoes in the traps are collected.

iii. Once finished, gently tap the sides of the exit traps to make any remaining mosquitoes fly around.

iv. Collectors should seek not to exceed 20 minutes to sample all traps. Use one cup for every 20 mosquitoes you collect.

v. Ensure the cup is correctly labelled with time period, date and technician initial.

Post-collection holding

a. When collections are completed, cups should be placed into cup racks and the mosquitoes transported in a cool box to the holding room (See SOP Transportation of mosquitoes). Ensure temperature and humidity are set to 25 ±2°C and 75% ±10% RH.

b. Put cup racks on the shelves in the holding room, making sure the treatment groups are kept on separate shelves from control cups.

c. Place 10% glucose solution-soaked cotton ball on top of each cup.

d. Record collection results onto the raw data sheet.

Post-collection holding for nets containing PPF (Ngufor et al., 2014)

a. After scoring mortality (24 h post-collection from the experimental huts), separate the live blood-fed mosquitoes of each treatment in separate cages and provide access to a second blood meal.

b. Once gravid (within 2-3 days), chamber individual mosquitoes separately in their own netted plastic cups containing approximately 50mL of fresh water.
c. Monitor daily for eggs and record the number of eggs laid by each female mosquito for up to 9 days. Add a pinch of larval food to any chamber which contains eggs.
d. Record the number of larvae (L2) which hatched for another 4-6 days.

Post-Trial cleaning

a. Remove nets and pack and seal in plastic bags with the net code labelled on the outside of the bag, then return to the net storage room.
b. Clean huts periodically when trials are not running. Keep moat filled when trials are not running.

Collection and reporting of data

Ensure the following data is recorded in the data collection sheets.

- **Mosquito packing record sheet**
    - Ensure you record the following data:
        - Trial date
        - Protocol Code
        - Recorder initials
        - Data logger ID (if applicable)
        - Trial site name
        - Treatment hut
        - Location of collection (net, room, exit trap, verandah)
        - Test system (species of mosquito)
        - Sex of mosquito
        - Abdominal status (unfed, blood fed, gravid, semi-gravid)

        - Stage when packed (dead, dead at 24h, dead at 72h; alive at 24h, alive at 72h, etc)
        - Capsule code/cup code
- **Net packing and storage**
    - Ensure you record the following data:
        - Protocol Code
        - Date net received
        - Lot or batch number
        - Active ingredient
        - Storage Shelf ID
        - Net ID number
        - Pieces cut for laboratory assays- Chemical assay, regeneration, or wash resistance
        - Disposal date
        - Initials of staff disposing

5. Glossary of terms

AI	Active Ingredient
I2I	Innovation to Impact
IHI	Ifakara Health Institute
LLIN	Long lasting insecticidal net
PPF	Pyriproxyfen
RH	Relative humidity
SOP	Standard Operating Procedure
WHOPES	World Health Organization Pesticide Evaluation Scheme

6. References

Ngufor, C., N'Guessan, R., Fagbohoun, J., Odjo, A., Malone, D., Akogbeto, M., & Rowland, M. (2014). Olyset Duo® (a pyriproxyfen and permethrin mixture net): An experimental hut trial against pyrethroid resistant Anopheles gambiae and Culex quinquefasciatus in southern Benin. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0093603

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