



Net characterisation for malaria control experiments

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Prepared by

Name	Role	Institution
Katherine Gleave	Author	LSTM, I2I
Rosemary S Lees	Author	LSTM, I2I
Giorgio Praulins	Author	LSTM, I2I

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Version Control¹

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

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1. Purpose

The purpose of this Standard Operating Procedure (SOP) is to describe the methods for characterizing insecticide-treated nets (ITNs) employed in research. ITNs play a crucial role in combating malaria, a role that is increasingly challenged by the development of resistance to pyrethroids—the most common insecticides used in ITNs—and other active ingredients. Laboratory bioassays are used to evaluate the bioefficacy of ITNs, which is their capacity to kill mosquitoes in controlled tests, during product development, quality testing, and to monitor the durability of bioefficacy over time and with use. In addition, chemical analysis is

used to measure the content of insecticide in a net, again for quality and monitoring purposes.

The characterization of nets and pieces of net used for these purposes is important for ensuring the consistency and reliability of research outcomes across various study environments and conditions. If the provenance and characteristics of the net sample used for an experiment is not known, then the results may not be correctly interpreted.

As resistance to traditional insecticides becomes more prevalent, it is imperative to ascertain the effectiveness of new ITN formulations that incorporate different active ingredients or synergists like piperonyl butoxide (PBO). Precise characterization enables researchers to robustly compare and interpret results from different tests and facilities, ensuring that the effectiveness of ITNs can be measured against diverse mosquito populations, including those with phenotypic resistance.

This SOP serves to standardize the detailed recording and reporting of ITN characteristics, such as the type and concentration of insecticides and the physical properties of the nets. By establishing a uniform framework for these characterisation procedures, research groups can consistently trace data back to specific nets, pieces of nets, and batches, thereby enhancing the integrity of the data and facilitating more accurate assessments of ITN effectiveness over time and in different environmental conditions.

Related SOPs

- Strain characterisation of resistant mosquitoes for monitoring bioefficacy in ITNs treated with two active ingredients (Dual-AI ITNS) (Lees et al., 2022, I2I-SOP-016).
- Cutting and preserving net samples for chemical analysis (I2I-SOP-005).
- Protocol for Preparing Bed Nets for Experimental Work (DOCUMENT NUMBER)

2. Background

Characterizing the parameters of insecticide-treated nets (ITNs) before use in research studies is crucial for several reasons. Firstly, with the emergence of widespread resistance to pyrethroids—the primary insecticide used on ITNs—it has become imperative to evaluate the effectiveness of new ITN formulations that incorporate additional active ingredients (AIs) or synergists like piperonyl butoxide (PBO) to combat pyrethroid-resistant vector populations. Understanding the specific characteristics of ITNs, such as the types of insecticides used, their concentration, and the physical properties of the nets, is essential to assess their bioefficacy. Entomological efficacy refers to the impact of a product on the target vector species, specifically when using free-flying mosquitoes. It measures the ability of a net sample to kill mosquitoes in a bioassay. This is distinct from general efficacy, which

assesses a product's overall performance in fulfilling its intended function and meeting the claims made on its label. Detailed characterization allows researchers to robustly interpret and compare results across various tests within and between test facilities, ensuring that the ability of ITNs to meet required standards and retain their effectiveness against different mosquito populations, including those with developed resistance, can be measured accurately. Furthermore, by establishing a standardized framework for compiling and reporting net details, research groups can consistently link collected data back to specific nets, net pieces, and batches of nets, enhancing the reliability of research outcomes and facilitating more accurate assessments of ITN effectiveness over time and across different environmental conditions. This comprehensive approach to characterizing ITNs for laboratory, or semi-field use (either received directly from manufacturers or following field studies or routine use) is vital to ensure the continued success of malaria control strategies, especially given the evolving nature of mosquito resistance to traditional insecticides.

3. Materials and equipment

- 1) Data collection sheets
 - a) Net Sample Details Record
 - b) Net Inventory Master Sheet
- 2) Laboratory coat
- 3) Gloves
- 4) Permanent marker pens

4. Procedure

Upon the arrival of nets at our laboratory, a systematic approach is used for management, characterization, and documentation of nets, to ensure the integrity, consistency, and traceability of our research. This begins upon their delivery, where each net's characteristics are recorded, using criteria set out in the “Net Sample Details Record”.

Simultaneously, the receipt of each net is accurately logged into the “Net Inventory Master Sheet,” a comprehensive record that records all nets within our inventory. During this process, each net is assigned a unique “Net Reference Number,” generated according to a specific format, ensuring a robust and traceable identification system.

In scenarios where a net is broken down into smaller pieces for bioassays or chemical analysis (**CITE NET PREP SOP**), our procedure remains the same. The original Net Reference Number is transcribed onto each subsample. Furthermore, each of these subsamples is given its own unique ID, and pertinent details such as size and relative location on the

original net are recorded. This practice is vital for preserving a detailed history of each piece of the net, essential for traceability. Details for this are recorded in the “Net Processing Record”, which can be found in the associated Net Preparation SOP.

Samples and products supplied by manufacturers require a Material Transfer Agreement (MTA). The MTA number should also be noted alongside other net details.

Following these stages, the nets are stored according to specific guidelines. These guidelines might be provided in the Material Safety Data Sheet (MSDS) or as instructed by the supplier and tailored to fit the requirements of the experimental work planned.

When nets are used in experiments, detail of their usage should be carefully recorded in the relevant metadata section of the record sheet associated with the experiment. This captures essential information such as the date of the experiment, the type of experiment conducted, and any specific experimental protocols used.

This SOP outlines the approach adopted in our laboratory for handling net samples. From their receipt to their eventual use in experimental work, each step is carefully planned and executed. This ensures not only the precise logging and standardized characterization of each net but also rigorous documentation which is essential for maintaining the integrity of our research and for ensuring compliance with safety and legal protocols, while also facilitating consistent comparisons and understanding of variability in results across different nets.

Step-by-Step

1. Receipt and Characterization of Nets

- Upon delivery, immediately begin the characterization of nets.
- Record specific characteristics of each net according to the details outlined in “Net Sample Details Record.”
- Simultaneously, record the receipt of each net in the " Net Inventory Master Sheet " for prompt and precise logging.
- Check to make sure relevant MSDS is on file and a risk assessment is in place. Any certificates of analysis should also be stored on file.

2. Entry into Net Inventory Master Sheet

- Enter each net into the “Net Inventory Master Sheet.”
- Assign a unique “Net Reference Number” to each net, generated as per the specified format.

3. Division and Tracking of Net Samples

- If a net is divided into smaller pieces for detailed study:

- Carefully transcribe the original Net Reference Number onto each subsample.
- Assign a unique ID to each subsample.
- Record details about each net piece in the “Net Processing Record,” such as size and relative location, to maintain traceability, in accordance with the Net Preparation SOP.
- If a Material Transfer Agreement (MTA) pertains to the nets, mark the MTA number on each net and any subdivided pieces.

4. Storage

- Store the nets and net pieces according to the specifications in the Material Safety Data Sheet (MSDS), as per supplier instructions or as required for experimental work.

5. Use of this documentation in Laboratory Records for an experimental study

Documentation in laboratory records for an experimental study must adhere to predefined study protocols. Before commencing the study, all relevant information regarding the nets should be documented in the required format. Including the copy of the Net Sample Details Record for the nets to be used in the study.

Additionally, once experimental work begins, records must be maintained for the usage of net samples or any processing of subsamples. This is done through use of the Net Processing Record.

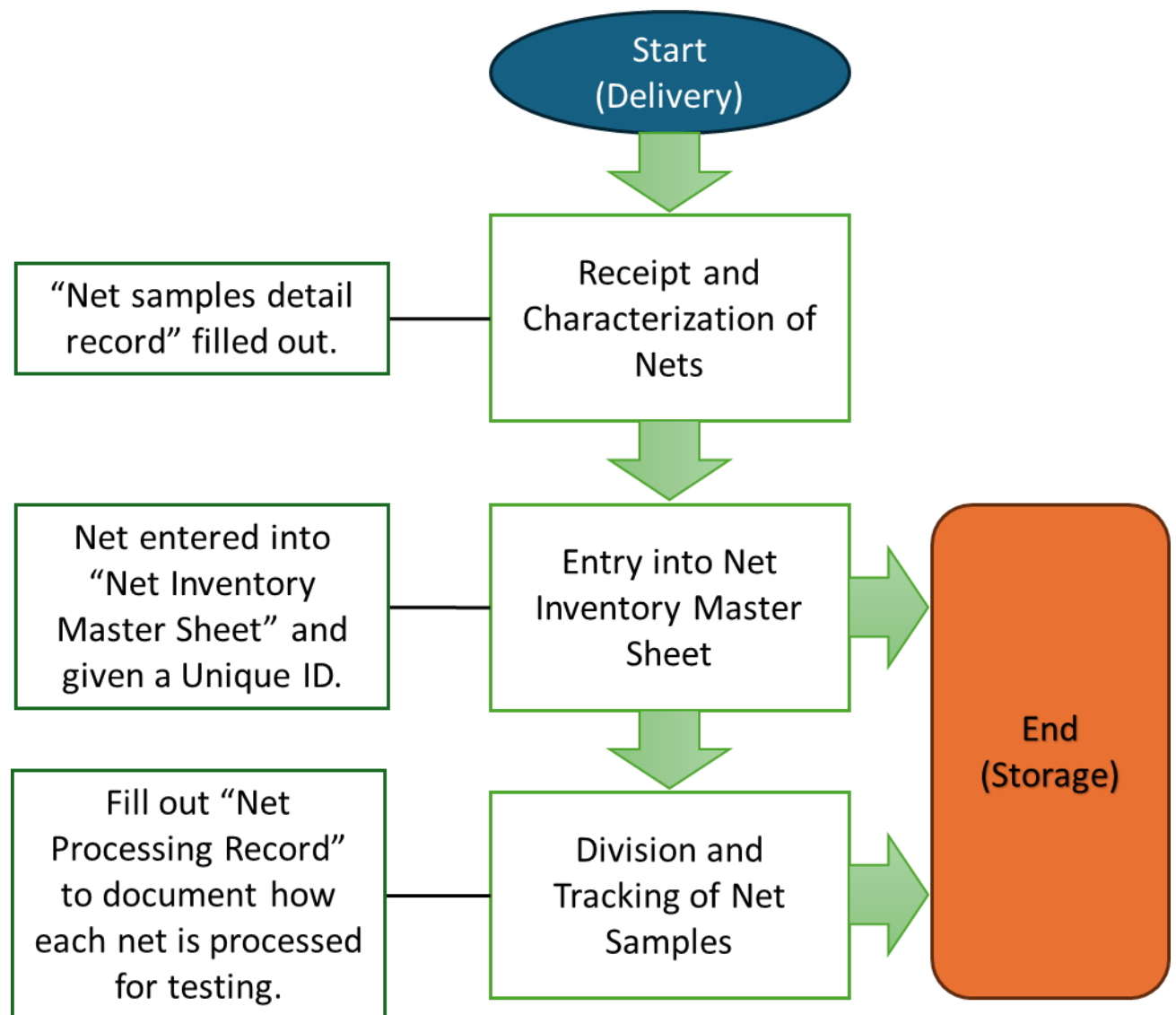
By maintaining these records, the experimental study ensures traceability, accountability, and adherence to protocols throughout the research process.

Unique ID generation

ID Generation Process

- Manufacturer Code Selection: Choose appropriate manufacturer code (e.g., **VEST** for Vestergaard).
- Sequential Number Assignment: Increment the sequential number for each new type of ITN produced by the manufacturer. (e.g., PermaNet 2.0: **VEST001** - PermaNet 3.0: **VEST002** - PermaNet Dual: **VEST003**).
- Net Differentiation: Extend the base ID with a unique identifier for each net, maintaining a clear link to the ITN. (e.g., PermaNet 2.0 Net 1: **VEST001-01**)

Figure 1 - Flowchart outlining net characterisation process



1. Net Sample Details Record

Table 1 - Net Sample Details Record designed to record detailed information about each individual net when it is received. A blank template is located in the appendix.

Field	Value
Net ID Number	To record the Net ID number created through ID Generation e.g. VEST001-01
Brand Name	The brand name of the net product e.g. PermaNet 2.0
Manufacturer Details	The name of the net manufacturer e.g. Vestergaard
Batch Number	The batch number provided by the manufacturer for the supplied nets. e.g. P0002135 KT43-23A
Supplier/Delivery Information	The name of the supplier of the net e.g. Vestergaard if it came from the manufacturer or CRID if it came from a research institute.
Material Transfer Agreement (MTA) Number	The internal LSTM MTA number.
Date Received	Date of expiry as given by the supplier in DD/MM/YYYY format or MM/YYYY format if no DD values are given.
Manufacture Date	Date of expiry as given by the supplier in DD/MM/YYYY format or MM/YYYY format if no DD values are given.
Expiry Date	Date of expiry as given by the supplier in DD/MM/YYYY format or MM/YYYY format if no DD values are given.
MTA End	Date of expiry as given by the supplier in DD/MM/YYYY format or MM/YYYY format if no DD values are given.
Active Ingredients and Target Doses (mg/m ² or g/kg)	Active ingredients and doses for the net. e.g. Deltamethrin - 1.4 g/kg $\pm$ 25%
Colour	Colour of the net e.g. White
Denier	Denier of the net e.g. 100 denier
Condition	Record of the condition of the net on arrival. And a note of any damage. Categorised by "Undamaged/Major Damage/Minor Damage" e.g. Minor damage – several small holes in net side panel OR Major damage – Large area of shrinkage on one corner of the roof panel.
Additional ITN Details (Notes)	Any additional relevant details of the net. e.g. Time in Field, Panel Number, Cut Sample Location, Net Airing Details (Y/N, airing temperature [°C] and humidity [RH%], Dates Aired [from/to]) Net Washing Details (Y/N, Washing Protocol, Date washed), Certificate of analysis supplied.
Broken down on	Date of net breakdown into subsample pieces for experimental use in DD/MM/YYYY format.

2. Net Inventory Master Sheet

Table 2 - Net Inventory Master Sheet designed to maintain a full inventory of all nets received. A blank template is located in the appendix.

[illegible]

5. Glossary of terms

AI	Active ingredient
HPLC	High performance liquid chromatography
I2I	Innovation to Impact
ID	Identification
ITN	Insecticide treated net.
LSTM	Liverpool School of Tropical Medicine
MTA	Material transfer agreement
SOP	Standard operating procedure.

References

Lees, R. S., Armistead, J. S., Azizi, S., Constant, E., Fornadel, C., Gimnig, J. E., Hemingway, J., Impoinvil, D., Irish, S. R., Kisinza, W., Lissenden, N., Mawejje, H. D., Messenger, L. A., Moore, S., Ngufor, C., Oxborough, R., Protopopoff, N., Ranson, H., Small, G., ... Spiers, A. (2022). Strain Characterisation for Measuring Bioefficacy of ITNs Treated with Two Active Ingredients (Dual-AI ITNs): Developing a Robust Protocol by Building Consensus. *Insects*, 13(5).
<https://doi.org/10.3390/insects13050434>

Appendix

Net Sample Details Record

Field	Value
Net ID Number	
Brand Name	
Manufacturer Details	
Batch Number	
Supplier/Delivery Information	
Material Transfer Agreement (MTA) Number	
Date Received	
Manufacture Date	
Expiry Date	
MTA End	
Active Ingredients and Target Doses (mg/m ² or g/kg)	
Colour	
Denier	
Condition	
Additional ITN Details (Notes)	
Broken down on	

Net Inventory Master Sheet

[illegible]