WHO Prequalification of Vector Control Products

Semi-field studies for ITNs: experimental hut and IACT studies

Factors which may affect validity of **semi-field studies** using Experimental huts and/or IACT:

- study not conducted in compliance with GLP
- negative control mortality exceeds limits defined for the experimental hut or IACT method
- failure to conduct power calculations based on local mosquito densities
- inadequate sample sizes due to low mosquito densities (experimental hut)
- inadequate sample sizes due to low numbers of mosquitoes released or insufficient replication (IACT)
- local vector population not suitable for testing with ITN under investigation
- laboratory strain not suitable for testing with ITN under investigation
- environmental or holding conditions outside of target range
- tests not conducted in alignment with the test system's circadian rhythm
- identification of issues related to the health of test systems for IACT and supplementary bioassays

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Contents

1. Purpose of the study

For the purposes of the prequalification assessment, semi-field studies are conducted to investigate the entomological efficacy and chemical/biological consistency of an ITN by means of:

- Investigation of the biological activity of an ITN under simulated user conditions by observing the relevant effects on free-flying mosquitoes
- Supplemental bioassays to investigate the consistency of biological activity of the material's surface
- Chemical analysis to determine the retention of AI during and after artificial and operational ageing

Semi-field studies are typically conducted using unwashed ITNs and either artificially or operationally aged nets and are intended to quantify the entomological efficacy of the ITN using the selected endpoint for which all supporting laboratory bioassay data were generated.

Semi-field studies are conducted to generate semi-field efficacy data for Module 5 and as part of long-term community studies. For the purposes of the prequalification assessment and listing, the biological effect of the ITN on the target vector population at the study site after artificial ageing is considered the key efficacy criteria for demonstration of entomological efficacy.

2. Requirement for submission of experimental hut studies

Three semi-field studies are required for Module 5 submissions, of which two must be experimental hut studies conducted in diverse geographic regions. The third semi-field study can be conducted as either an experimental hut study or a study using a closed system, e.g., an IACT.

In long term community studies, IACT studies or experimental hut studies may be conducted at the end of the study to verify the entomological efficacy of the ITNs following various durations of routine use.

Semi-field studies must be GLP compliant.

3. Considerations for study site selection

Manufacturers should consider the composition of mosquito populations, including local species/strain characteristics, in the selection of sites for experimental hut studies, and the necessary characteristics of laboratory strains for use in IACT studies. The vector population (or laboratory strains) at selected sites should exhibit traits in alignment with the defined primary target(s) based on the mode of action of the AI(s) and intended effects of the product. To assist with study site selection, characterization data for

the vector population's target traits, e.g., WHO susceptibility tests, insecticide resistance intensity assays, genomic screening, etc., generated by the study site should be considered.

Additionally, manufacturers should consider requirements from other departments within WHO, and National Regulatory Authority requirements for product registration in order to prioritize generation of efficacy data which can be used to support registration and/or selection decisions across multiple countries/organizations.

4. Considerations for method selection for chemical analysis and supplemental bioassays

Chemical analysis of sampled net pieces is conducted to quantify the AI content of unwashed and artificially or operationally aged ITNs before and after use in semi-field studies. Bioassays are conducted to ensure the consistency of the bioavailability of unwashed and artificially or operationally aged ITN fabric before and after use in experimental hut studies (and optionally before and after IACT studies, refer to 4.2 for further details).

4.1. Considerations for chemistry method selection

In supplemental chemical analysis, the total AI of sampled fabric pieces should be measured using the available/validated enforcement analytical method (validation may be in-house and could require bridging to CIPAC or other methods if being validated concurrently).

4.2. Considerations for entomology method selection

Semi-field experimental hut studies can use the cone test, the tunnel test or the IACT as the bioassay method for supplemental experiments characterizing the consistency of the ITN fabric(s), based on the mode of action of the AI(s), the intent of the product, and the bioassay method used for data generation in module 3 studies. Closed system, free-flying mosquito bioassays, e.g. IACT, can be considered as a substitute for tunnel tests and therefore in experimental hut studies that use the IACT as the supplementary bioassay method, additional cone tests may not be required.

IACT semi-field studies typically use the cone test as the bioassay method for supplemental experiments, based on the mode of action of the AI (s), the intent of the product, and the bioassay method used for data generation in module 3 studies.

A single bioassay method should be selected for use in supplemental bioassays, except when there is a necessity to use multiple bioassays to demonstrate the intended effect of multiple AIs, e.g., cone tests to demonstrate the rapid toxicity of a pyrethroid insecticide coupled with IACT to demonstrate the effect of chlorfenapyr in a pyrethroid-chlorfenapyr dual-AI ITN.

Other existing or novel methods can be proposed in situations where the standard methods are not appropriate, if those methods have been used in the data generation for Module 3 studies. If another

method is being considered or augmentations to standard methods are necessary, WHO recommends that substantiating documentation be provided with a protocol review request submission.

As the prequalification assessment has evolved from a framework for decisions relying solely on bioassays results meeting preselected thresholds, prequalification will no longer accept results from a second bioassay method to verify sub-optimal bioassay results.

5. Selection of entomological endpoints

The potential **endpoint(s)** which may be selected for use in a semi-field study and the supplementary bioassay(s) must be representative of the intended effect of the product. The selection of appropriate endpoint(s) may dictate the selection of the method and/or encourage the use of multiple entomological methods.

The endpoint(s) selected for use in semi-field studies must be the same endpoint(s) as those used in the supplemental bioassays and for data generation in Module 3 entomological studies.

6. Considerations for test system species/strain selection

For the purposes of a semi-field study, the selected test systems should be relevant to the intended effect of the product, i.e., vectors of the disease(s) intended to be impacted. The selected strains should be characterized in terms of the susceptibility to the AI(s) and the specific mechanisms of resistance, if applicable. The use of multiple species/strains can provide valuable information about:

- the differences in time until effects are observed in relation to species/strain characteristics
- the differences in magnitude of effects that are observed in relation to species/strain characteristics
- identification of the potential range of response (baseline) for selected endpoints measured in the bioassay in relation to species/strains

Where multiple test system species/strains are used, the test system species/strain that will be used to determine whether the product has demonstrated the required characteristics must be clearly identified.

Further guidance on the selection of strains for use in bioassays is provided in Implementation Guidance *Considerations for the selection of mosquito strains for use in bioassays and site selection for semi-field studies*

7. Study materials

7.1. Treated fabric

Semi-field studies conducted for Module 5 data generation should include ITNs from a minimum of three production batches that have been fully characterized as part of the data generation for Module 3. For the development of a new product dossier, it is critical that the batches used in the semi-field studies are the same as those used for other data generation, e.g., the characterization of chemical and physical characteristics.

For semi-field studies conducted as part of long-term community studies, ITNs from more than three production batches may be used.

Documentation of the source, receipt and handling of ITNs prior to testing is critical.

7.2. Negative control

Negative control samples should be untreated netting made of polyethylene or polyester.

7.3. Positive control

The positive control(s) should be selected based on the intent and design of the study, including the selection of method(s), endpoint(s), and species/strains, in order to support the assessment of the validity of the study.

It is critical that the selected positive control(s) is used consistently in other studies for data generation.

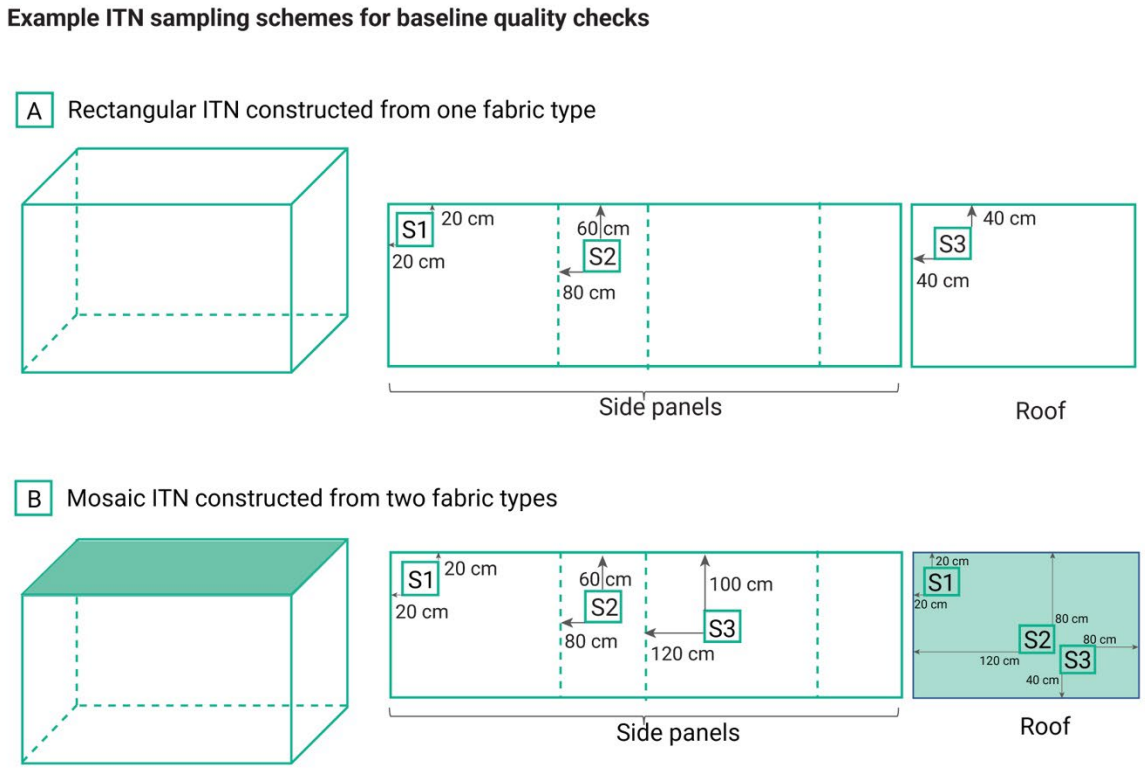
8. Baseline quality check

Prior to the commencement of the study, a baseline quality check using the selected chemical and bioassay test methods should be conducted to identify if any significant changes in the product have occurred during the transport, receipt and handling of ITNs. The baseline quality check should be conducted on 45 samples (three samples per net; five nets selected per batch of three production batches) using the sampling plan as defined below (Fig. 1)

When taking samples, the location should be measured from the left-hand seam of each panel. For a rectangular, uniform ITN comprised of one fabric (roof and sides), Sample 1 is taken 20 cm from the top of the net and 20 cm from the left-hand seam, Sample 2 is taken 60 cm from the top seam and 80 cm from the left-hand seam, and Sample 3 is taken from the roof, 40 cm from the long side and 40 cm from the left-hand seam.

For a rectangular, mosaic net comprised of two fabrics (roof and sides), for Fabric 1 (sides) Sample 1 is taken 20 cm from the top of the net and 20 cm from the left-hand seam, Sample 2 is taken 60 cm from the top seam and 80 cm from the left-hand seam, and Sample 3 is taken 100 cm from the top seam and 120 cm from the left-hand seam; for Fabric 2 (roof) Sample 1 is taken 20 cm from the left hand 'top' corner, Sample 2 is taken 80 cm from the long seam and 120 cm from the left-hand seam and Sample 3 is taken 80 cm from the right-hand seam and 40 cm from the long seam. Each fabric is evaluated separately.

Fig 1. Sampling for baseline quality checks



As the purpose of the baseline quality check is to establish a baseline for the consistency within and between the batches of ITNs that have been received at a testing facility, fewer samples are taken from a higher number of ITNs.

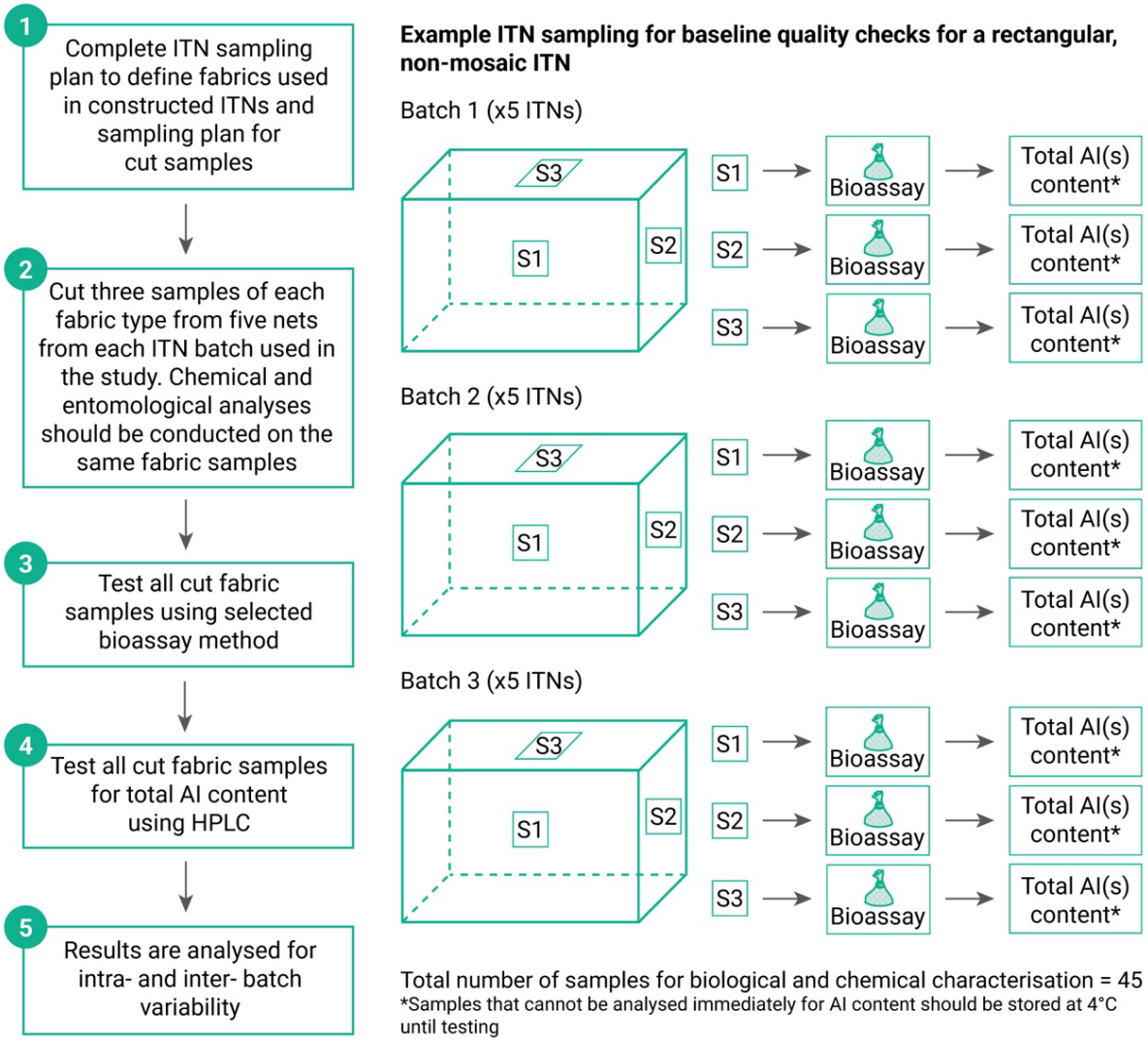
The baseline quality check should only be conducted once per testing facility, i.e., a testing facility conducting a regeneration study, a wash resistance study and a semi-field study need only conduct a single baseline quality check.

Baseline quality check results should be analysed for intra- and inter-batch variability in addition to presenting the results from each analysis with an appropriate measure of variation (Refer to Section 11).

8.1. Schematic of a baseline quality check

Fig. 2 illustrates the baseline quality check procedure for a rectangular, uniform ITN.

Fig 2. Baseline quality check



9. Sample 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.

Semi-field studies conducted for Module 5 data generation use artificially aged nets that have been washed and had in-use damage replicated by means of the cutting of holes of specified size and locations on each net. Details of washing methods and damage replication can be found in the implementation guidance method documents for each semi-field method (experimental hut, IACT).

Semi-field studies conducted as part of long-term community studies use operationally aged ITNs. Details of sample preparation for ITNs used in estimations of entomological efficacy as part of a community study can be found in the Community studies implementation guidance document.

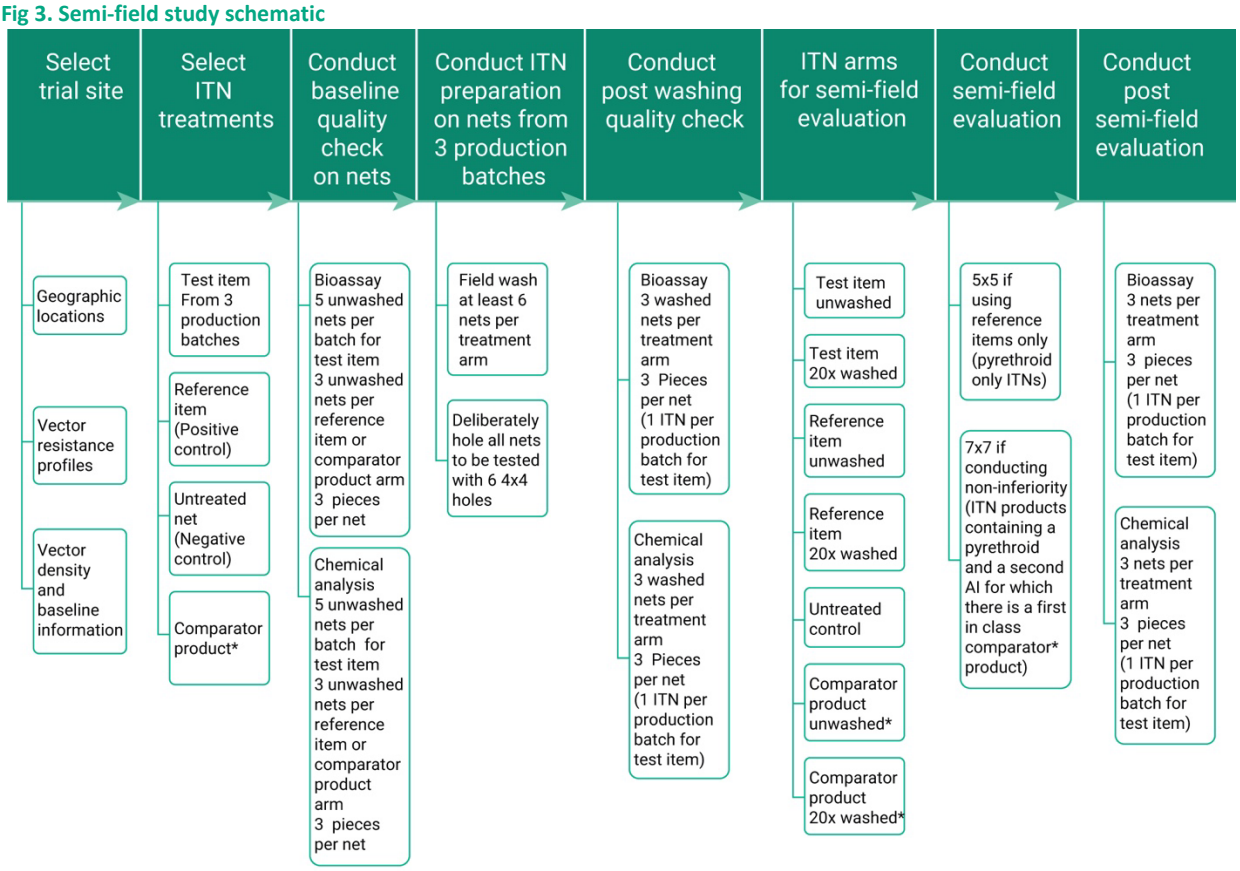
9.1. Fabric samples for chemical analysis

Fabric samples which are not immediately analysed for chemical content should individually wrapped in aluminium foil and held at 4°C.

10. Experimental procedures

10.1. Schematic of a semi-field study

Fig 3. Semi-field study schematic



10.2. Study designs for semi-field studies

Semi-field studies for ITNs are conducted using Latin Square designs (LSD). An LSD is a block study design for use with multiple treatments and subjects that allows for each subject to be measured with each treatment the same number of times, and for the same number of hours. This study design enables sources of variability, e.g. differences among hut locations and sleepers, to be appropriately handled in the eventual statistical analyses.

A 5x5 LSD is used for studies that include the test and reference items, and a 7x7 LSD is used for studies that include test, reference and comparator items, e.g., a comparative efficacy study.

10.2.1. Considerations for Latin square designs

10.2.1.1. Treatment allocation

Treatments should initially be allocated randomly to huts, and thereafter rotated sequentially to minimize potential errors in treatment allocation.

10.2.1.2. Sleeper rotation

Sleepers rotate sequentially among huts each night of the study following a prepared roster.

10.2.1.3. Treatment rotation and the number of nights for data collection

Treatments are rotated among the huts weekly. For a comparison of five treatment arms, data should be collected for five nights, to allow the rotation of each sleeper through each hut/chamber, followed by one night of rest and one night of cleaning and ventilation of huts on the seventh night before the next ITN treatment arm is introduced to the hut and the next rotation of sleepers through the huts begins. A full rotation of the 5x5 LSD therefore requires 35 nights to complete, including 25 nights of data collection.

When using a 7x7 LSD, data should be collected for seven nights, to allow the rotation of each sleeper though each hut/chamber, followed by cleaning and ventilation of the huts on the eighth night before the next ITN treatment is introduced to the hut and the next rotation of sleepers through the huts begins. A full rotation of the 7x7 LSD therefore requires 56 nights in total to complete, including 49 nights of data collection.

Note that the total number of nights required for data collection may be influenced by the power calculation conducted using local mosquito densities. In cases where mosquito densities are lower than expected, interim power analyses in order to identify the required number of additional nights of data collection are recommended.

10.3. Study arms for semi-field studies

For 5x5 LSD studies, the study arms to be used are:

1. Unwashed negative control
2. Unwashed positive control
3. 20x washed positive control
4. Unwashed test item
5. 20x washed test item

For 7x7 LSD studies, the study arms to be used are:

1. Unwashed negative control
2. Unwashed positive control
3. 20x washed positive control
4. Unwashed comparator product
5. 20x washed comparator product
6. Unwashed test item
7. 20x washed test item

Alternative LSD incorporating additional study arms can be used depending on the intent and design of the study. In these cases, WHO recommends that substantiating documentation be provided with a protocol review request submission.

10.4. Sample size calculations for semi-field studies

Sample size is estimated as the number of replicates required to measure the point estimate of the primary endpoint (usually mosquito mortality, blood-feeding inhibition occasionally considered) precisely enough that the 95% CI has a high probability of enabling the determination of non-inferiority, that is shown when the impact of a test product is no worse than that of the active comparator by a prespecified non-inferiority margin (the largest allowable difference between the test product and the active comparator in terms of the estimates of the chosen endpoints).

A power calculation should be conducted to determine the required sample size and the number of nights of collection that are required.

Semi-field studies using a 5x5 LSD should be powered to detect a precise point estimate of the selected endpoint, e.g. ± 5%, at a minimum of 80% power.

Semi-field studies using a 7x7 LSD should conduct the sample size calculation using simulations based on the primary endpoint, thus simplifying power calculations. For IACT studies variability includes the number of mosquitoes released per chamber, per replicate for each species, as well as differences between chambers, between sleepers and between nights. Data from recent hut trials or pilot studies should be used to parameterize sample size estimations.

The steps to conducting sample size calculations for comparative efficacy assessment using the example of mortality are:

i. Estimate mosquito mortality observed for the **reference product**
ii. Input the number of mosquitoes released per chamber per species
iii. Estimate the variability between chambers, sleepers and night
iv. For community studies the variability between ITNs should also be included
v. Define the number of chambers that will be used
vi. Use data i–iv together with the defined effect margin based on 7% absolute difference in mosquito mortality between **20x washed reference product and the 20x washed test product** or **20x washed ITN of the same brand as the field aged product** and field aged net to simulate theoretical results for all trial arms (assuming that the percentage mortality follows a binomial distribution). To estimate study power, the true mortality of the **test product** (i.e., the underlying actual probability that a mosquito will die) should be the same as that of the positive control
vii. Fit the logistic regression model to be used for analysis taking into consideration sources of variability to simulated data and determine whether the **20x washed test product** or **field aged product** is the same as the reference product
viii. Repeat steps v–vi 1000 times and calculate the percentage of times the test product is the same as the **reference product**. Record this as study power.

If study power is <80% then Repeat steps v–viii, adjusting the number of replicates used (increasing chambers for each trial arm, the number of rotations, the number of mosquitoes or the number of field aged nets evaluated) until the desired power of >80% has been reached, meaning that the probability of demonstrating non-inferiority to the active comparator and superiority to the standard comparator (if applicable) is at least 80%.

10.5. Quality checks

10.5.1. Pre- and post-wash and post-semi-field study bioassay and chemical quality checks for test items

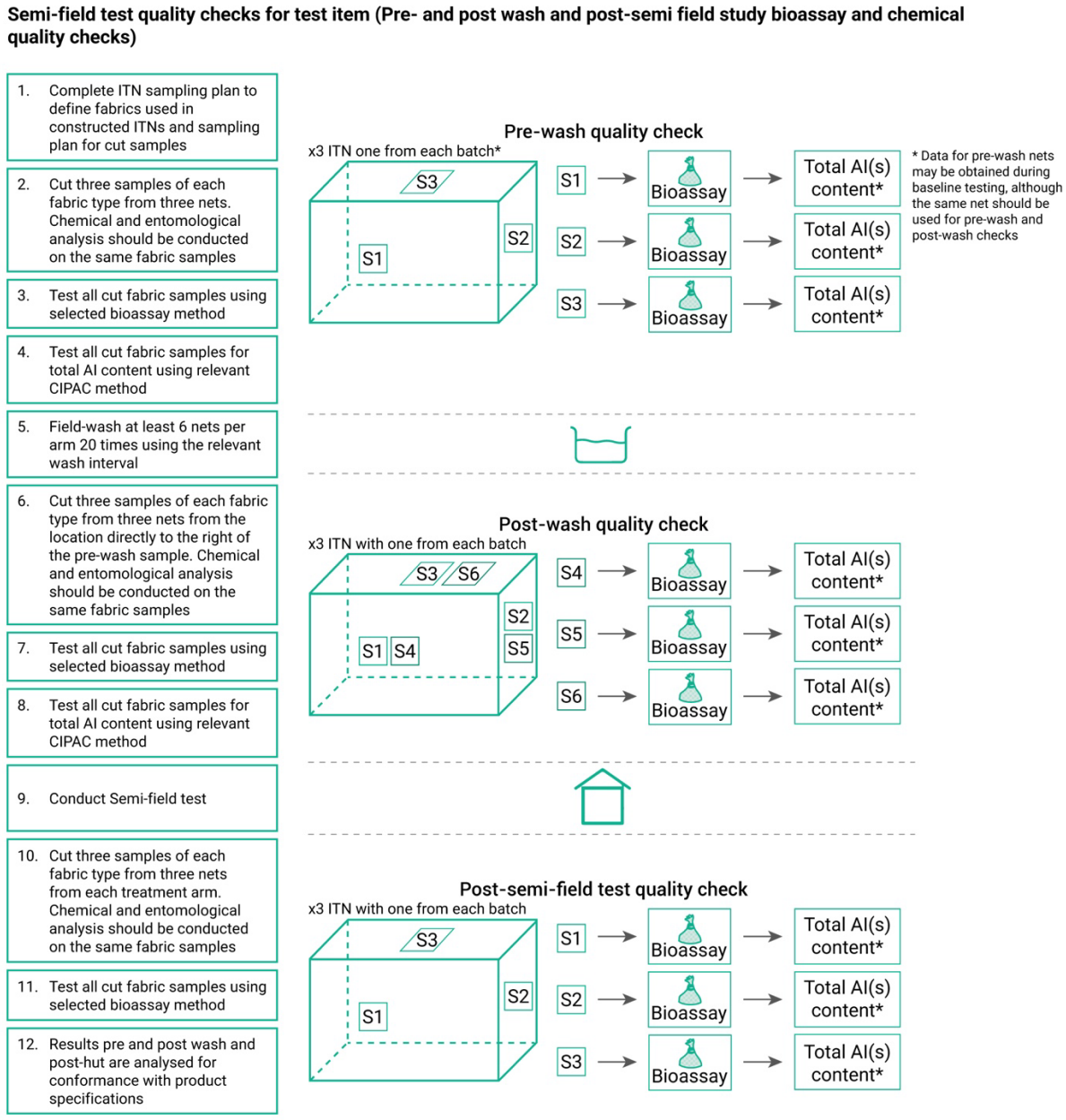
Supplementary bioassays are conducted to verify the consistency of the ITN fabric before washing, post-washing and before and after use in semi-field studies (Fig. 4).

When taking samples, the location should be measured from the left-hand seam of each panel. For a rectangular, non-mosaic ITN, Sample 1 is taken 20cm from the top of the net and 20cm from the left-hand seam, Sample 2 is taken 60cm from the top seam and 80cm from the left-hand seam, and Sample 3 is taken from the roof, 40cm from the long side and 40cm from the left-hand seam. For the post-wash quality checks, samples 4, 5, and 6 are taken from positions directly adjacent to samples 1, 2 and 3.

For a rectangular, mosaic net comprised of two fabrics (roof and sides), for Fabric 1 (sides) Sample 1 is taken 20cm from the top of the net and 20cm from the left-hand seam, Sample 2 is taken 60cm from the top seam and 80cm from the left-hand seam, and Sample 3 is taken 100cm from the top seam and

120cm from the left-hand seam; for Fabric 2 (roof) Sample 1 is taken 20cm from the left hand 'top' corner, Sample 2 is taken 80cm from the long seam and 120 cm from the left-hand seam and Sample 3 is taken 80cm from the right-hand seam and 40cm from the long seam. For the post-wash quality checks, samples 4, 5, and 6 are taken from positions directly adjacent to samples 1, 2 and 3.

Fig 4. Semi-field quality checks for test items

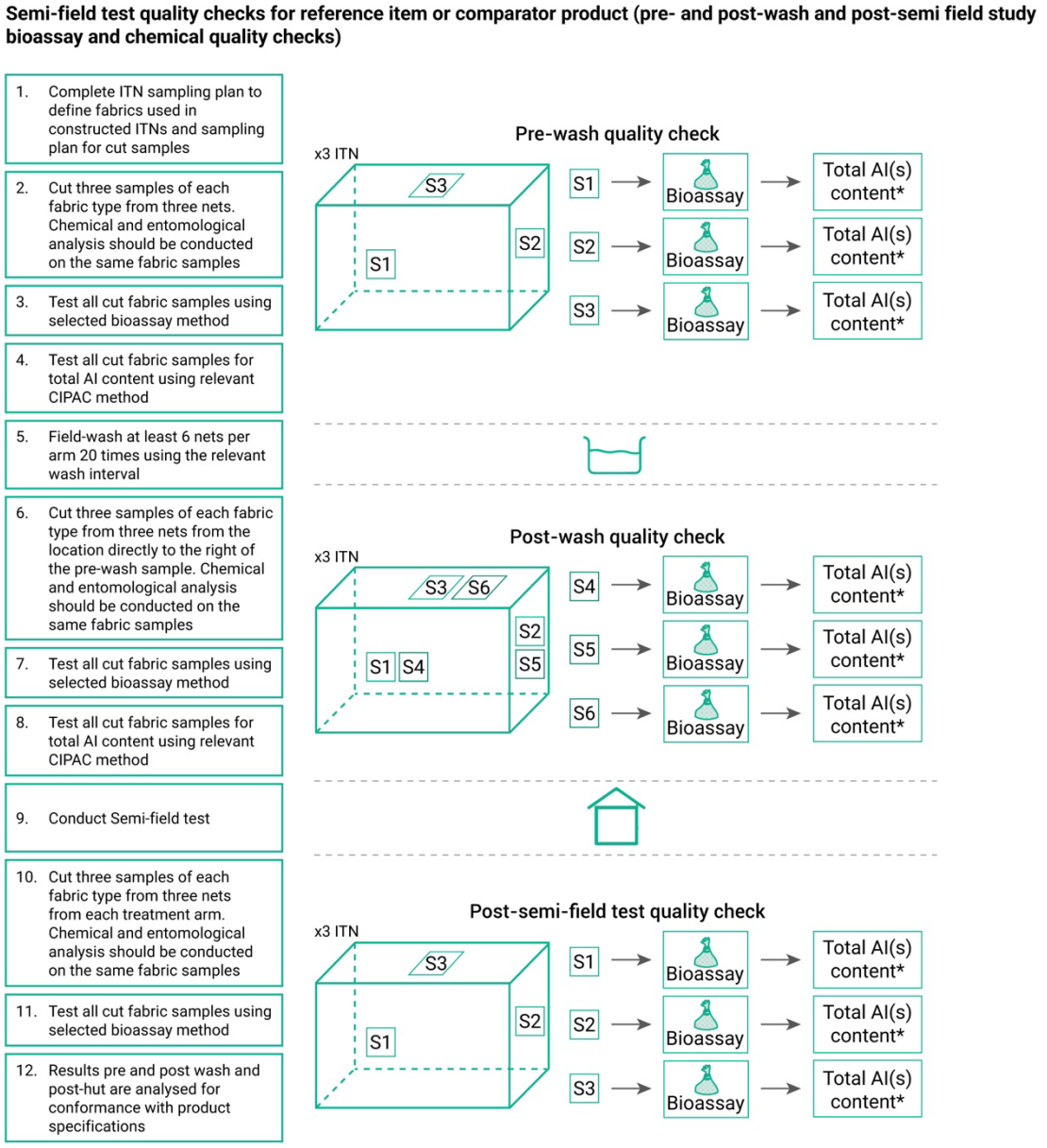


10.5.2. Quality checks for reference items or comparator products

Supplementary bioassays are conducted to verify the consistency of the ITN fabric before washing, post-washing and before and after use in semi-field studies (Fig 5).

When taking samples, the location should be measured from the left-hand seam of each panel. Sample 1 is taken 20cm from the base of the net and 20cm from the left-hand seam, Sample 2 is taken 60cm from the top seam and 80cm from the left-hand seam, and Sample 3 is taken from the roof, 40cm from the long side and 40cm from the left-hand seam. For the post-wash quality checks, samples 4, 5, and 6 are taken from positions directly adjacent to samples 1, 2 and 3.

Fig 5. Semi-field quality checks for reference item and/or comparator products



10.6. Sample sizes for supplementary bioassays

The number of replicates to be tested within the selected method must be considered as part of the protocol development and is dependent on the intent and context of the study. Sample size is estimated based on the selected endpoint for the bioassay method in use (usually mosquito mortality) and should be powered to detect a precise point estimate of the selected endpoint i.e., $\pm$ 5%. The number of ITN samples recommended here are a minimum; it is necessary to conduct supplementary bioassays using a sufficient number of replicates per samples to obtain a sufficiently precise point estimate.

10.7. LC_{50} and LC_{90} measurements of local vector populations

The LC_{50} and LC_{90} of the local vector population (for experimental hut studies) or the released laboratory strain(s) (for IACT studies) to the ITN AI(s) should be conducted prior to the beginning of the semi-field study. Results are reported in the MSMS.

11. Results and data analysis

Results for test samples and controls (negative, positive, and comparator products) should be presented in both tabular and graphical format.

If ITNs constructed from multiple fabrics have been used in the study, results for the supplementary bioassays must present the results for each fabric separately, e.g., results for the roof and sides for a mosaic net where the roof and sides have been constructed from different fabrics should be presented as [Product A roof] and [Product A sides].

Descriptive and inferential statistics with appropriate error measurements should be used to present results.

11.1. Baseline quality results

11.1.1. Baseline chemical quality check analysis

The results to be reported for baseline chemical quality checks are:

- Arithmetic mean results with respective standard deviation
- Percentage Relative standard deviation (RSD).

The inter- and intra-batch variability are analysed using RSD to measure the precision. RSD should be expressed as percentage. It is obtained by multiplying the standard deviation (SD) by 100 and dividing by product average (%RSD = SD *100/Mean).

A table showing the summary results (number of net pieces, mean concentration of AI, SD, range, %RSD) per net, production batch and overall should be included in the report.

11.1.2. Baseline bioassay quality checks

The results to be reported for baseline quality bioassays are:

- Arithmetic mean results with 95% CIs for each selected endpoint.

A table showing the summary results (number of mosquitoes exposed, number of replicates, percentage arithmetic mean and 95% confidence intervals) per net, production batch and overall should be included in the report.

11.2. Semi-field study statistical analysis

To ensure standardization of analytical approaches, a specific model must be used when performing the analysis and presenting the results for assessment. To relate the outcome variables to the intervention and covariates, generalized linear regression models (GLMs) should be used. The choice of model will depend on the endpoint(s) under investigation. For binary endpoints, such as the proportion of mosquitoes dying or the proportion fertile, a logistic model is appropriate. For outcomes that are counts, such as the number of eggs laid a Poisson or negative binomial model may be more appropriate.

It is recommended that all covariates should be categorical fixed effects and the active comparator should be used as the reference intervention (intercept). For IACT trials, covariates include **net product and preparation**, chambers, sleepers or host and night as fixed effects because these factors are sources of systematic variability that are accounted for in the experimental design. In addition, for community studies it is recommended to add net condition (good, damaged or too torn) as a covariate as net integrity influences net entomological efficacy.

Example statistical code that can be adapted for use in analysing results can be found at https://github.com/JDChallenger/WHO_NI_Tutorial.

11.2.1. Non-inferiority margin and determination of non-inferiority

The non-inferiority margin is predefined for a given comparison. The entire 95% confidence interval of the odds ratio of the endpoint for the test product must be above (for mortality endpoint) the non-inferiority margin. For non-inferiority analyses of vector control interventions assessed by WHO, this margin has been set at an absolute difference of 7%, assessed by odds ratios (Table 1).

For products for which the primary endpoint is mortality, the test product is deemed non-inferior if the lower bound of the 95% CI estimate of the OR of the test product to the active comparator is greater than the OR corresponding to a 7% non-inferiority margin (Table 1). It will be determined to be superior if the lower bound of the 95% CI estimate falls entirely above 1.

For products for which the endpoint is blood-feeding inhibition or blood feeding inhibition is being considered as a secondary outcome, the test product is deemed non-inferior if the upper bound of the 95% CI estimate of the OR of the test product to the active comparator is lower than the OR corresponding to a 7% non-inferiority margin (Table 2). It will be determined to be superior if the upper bound of the 95% CI estimate falls entirely below 1.

Table 1. The non-inferiority margin based on a fixed difference of 7% between the mortality induced by the test product and mortality induced by the active comparator product

Active comparator		Test product		Corresponding OR for a 7% non-inferiority margin
Point estimate (%)	Odds[a]	Acceptable lower bound (%) of CI with a 7% absolute difference	Odds[a]	
95	19.00	88	7.33	0.39
90	9.00	83	4.88	0.54
80	4.00	73	2.70	0.68
70	2.33	63	1.70	0.73
60	1.50	53	1.13	0.75
50	1.00	43	0.75	0.75
40	0.67	33	0.49	0.74
30	0.43	23	0.30	0.70

[a] If the percentage mortality is p, then the odds of mortality = p/(100 – p)

[b] The OR is calculated by dividing the odds in the second column by the odds in the first (e.g. 7.33/19.00 = 0.39)

Table 2. The non-inferiority margin based on a fixed difference of 7% between blood feeding for the active comparator and the test product

Active comparator		Test product		Corresponding OR for a 7% non-inferiority margin
Point estimate (%)	Odds[a]	Acceptable lower bound (%) of CI with a 7% absolute difference	Odds[a]	
5	0.05	12	0.14	2.59
10	0.11	17	0.20	1.84
20	0.25	27	0.37	1.48
30	0.43	37	0.59	1.37
40	0.67	47	0.89	1.33
50	1.00	57	1.33	1.33
60	1.50	67	2.03	1.35
70	2.33	77	3.34	1.43

[a] If the percentage of blood feeding is p, then the odds of blood feeding = p/(100 – p)

[b] The OR is calculated by dividing the odds in the second column by the odds in the first (e.g. 0.14/0.05 = 2.59)

11.3. Criteria for study validity and acceptance

Acceptance of chemical analysis results is based on the criteria for the selected available/validated enforcement analytical method.

Results for the positive and negative controls in bioassays must comply with acceptable results for the selected method. Refer to the implementation guidance documents for the selected method for details of control acceptance criteria.

Candidate ITNs must fulfil the following criteria in the semi-field test in order to meet the requirements for pre-qualification:

1. Study power:
   - The study must demonstrate sufficient power for decision-making, based on the conducted power calculation and the sample sizes of mosquitoes collected during the study (experimental hut studies), ***or***
   - The study must demonstrate sufficient power for decision-making, based on the conducted power calculation and the numbers of mosquitoes released and replications (IACT)
2. Results for free-flying mosquitoes:
   - The results for the 20x washed candidate ITN at the selected endpoint must be statistically significantly higher than the results for the negative control, ***and***
   - The results for the 20x washed candidate ITN at the selected endpoint must be ***either***:
     - For a 5x5 LSD using a pyrethroid candidate ITN and a pyrethroid ITN positive control: not significantly worse (or significantly better) than the positive control, ***or***
     - For a 7x7 LSD using a dual AI candidate ITN, a dual AI active comparator and pyrethroid positive control: demonstrated non-inferiority of the candidate ITN to the active comparator and demonstrated superiority of the candidate ITN to the positive control
3. Results from bioassays:
   - Bioassay results for the selected endpoint for the 20x washed ITN must be not less than 5% lower than the results for the unwashed net.

12. Study Report

12.1. Semi-field studies study report

The study report must be a comprehensive description of the study, procedures and include justification for specific scientific approaches and/or deviations from standardized methods.

The suggested study report sections for semi-field study reports are below. These sections are provided for guidance and do not need to be strictly followed.

- Cover page
- Table of contents
- GLP compliance statement
- Results summary
- List of abbreviations
- Background information
- Study rationale
- Study objectives

- Study endpoints
  - » If multiple strains of tests systems have been tested, identify the strain which has been used to determine the validity of the study, and provide a rationale
- Criteria for study acceptance
- Methods
  - » Test systems
    - Colony maintenance and brief summarized rearing procedures
    - Description of test system. Indicate the most recent date of insecticide resistance characterization (NB. The results of the characterization are presented in the Matrix of mosquito strains)
    - Description of any additional test systems. Indicate the most recent date of insecticide resistance characterization (NB. The results of the characterization are presented in the Matrix of mosquito strains)
    - Age and physiological status of each test system used in bioassays. Separate descriptions by method if multiple bioassay methods have been used.
  - » Test and reference items
    - Description of each test and reference item including:
      - Batch numbers
      - The number of test items received per batch
      - Source
      - Date of manufacture
      - Date of receipt
      - Storage conditions since receipt
      - Justification for choice of positive control(s)
  - » Sample preparation
    - Sampling plan
    - Net cutting procedures, including number and size of samples
    - Sample storage conditions
    - Washing method
  - » Sample shipment details for chemical analysis (if required)
  - » Chemical analysis methods (if chemical analysis was conducted on site at regeneration study testing facility)
  - » Bioassays
    - Full methodological details for selected bioassay method(s). If multiple methods are used, each should be described separately
    - Sample sizes for bioassays, including the number of replicates conducted per sample
  - » Semi-field study method
    - Study design
    - Study arms
    - LSD design
  - » Data analysis
    - Statistical analysis methods
- Results

- Baseline quality check
  - Summarised tabular results ensuring that each bioassay method is presented separately and that results for different fabric types and controls are clearly delineated
  - Graphical presentation of results
  - Narrative description of results
- Semi-field study
  - Summarised tabular results from the semi-field test
  - Graphical presentation of results
  - Narrative description of results
- Data analysis and statistical results
  - Baseline quality check
    - Summary statistics
    - Statistical analysis results
  - Semi-field study
    - Summary statistics
    - Inferential statistics
    - Non-inferiority analysis
- Results interpretation and demonstrated efficacy criteria

- Discussion and conclusions
  - The study report must include an interpretive analysis of the results. Specific discussions on any methodological deviations, anomalies in results, or other factors which may have impacted the results should be included.

13. 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 – Semi-field methods for ITNs – Experimental huts
- WHO PQT/VCP Implementation guidance – Bioassay and semi-field methods for ITNs – IACT
- WHO PQT/VCP Implementation guidance – Long-term community studies
- WHO PQT/VCP Implementation guidance – Considerations for the selection of mosquito strains
  WHO PQT/VCP Implementation guidance for use in bioassays and site selection for semi-field and community studies
- WHO PQT/VCP Implementation guidance – Supporting data considerations for novel bioassays
- WHO PQT/VCP Implementation guidance – MSMS
- WHO PQT/VCP Implementation guidance – Template MSMS

14. References

1. Challenger, J.D., Nash, R.K, Ngufor, C., Sanou, A., Hyacinthe Toe, K., Moore, S., Tungu, P.K., Rowland, M., Foster, G.M., N'Guessan, R., Sherrard-Smith, E., Churcher, T.S. Assessing the variability in experimental hut trials evaluating insecticide-treated nets against malaria vectors. Curr Res Parasitol Vector Borne Dis. 2023; 3:100115 (https://www.sciencedirect.com/science/article/pii/S2667114X23000031, accessed 15 October 2023).
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).

15. Annex. Suggested table formats for summary results

15.1. Table format for baseline quality check chemical analysis results

Table x. Baseline quality check chemical analysis results of ITNs received at [testing facility name] for [product name(s) and batch numbers [batch#1, batch#2, batch#3]]					
[Product name 1]					
Sample ID (net and batch identification)	Number of net samples	Mean [AI name] content (g/kg)	RSD (%)	Mean [synergist name, or second AI] content (g/kg)	RSD (%)
[sample IDs Batch 1 Net1]		[mean] ([SD][range lower limit] -[range upper limit])	[this value shows the intra-net variability]	[mean] ([SD][range lower limit] -[range upper limit])	[this value shows the intra-net variability]
[sample IDs Batch 1 Net2]					
[sample IDs Batch 1 Net3]					
[sample IDs Batch 1 Net4]					
[sample IDs Batch 1 Net5]					
Combined Batch [1] results		[mean] ([SD][range lower limit] -[range upper limit])	[this value shows the intra-batch variability]	[mean] ([SD][range lower limit] -[range upper limit])	[this value shows the intra-batch variability]
[sample IDs Batch 2 Net1]					
[sample IDs Batch 2 Net2]					
[sample IDs Batch 2 Net3]					
[sample IDs Batch 2 Net4]					
[sample IDs Batch 2 Net5]					
Combined Batch [2] results		[mean] ([SD][range lower limit] -[range upper limit])	[this value shows the intra-batch variability]	[mean] ([SD][range lower limit] -[range upper limit])	[value showing the effect of intra-batch variability]
[sample IDs Batch 3 Net1]					
[sample IDs Batch 3 Net2]					
[sample IDs Batch 3 Net3]					
[sample IDs Batch 3 Net4]					
[sample IDs Batch 3 Net5]					
Combined Batch [3] results		[mean] ([SD][range lower limit] -[range upper limit])	[this value shows the intra-batch variability]	[mean] ([SD][range lower limit] -[range upper limit])	[this value shows the intra-batch variability]

Table x. Baseline quality check chemical analysis results of ITNs received at [testing facility name] for [product name(s) and batch numbers [batch#1, batch#2, batch#3]]					
Combined results for all batches		[mean] ([SD][range lower limit] -[range upper limit])	[this value shows the inter-batch variability]	[mean] ([SD][range lower limit] -[range upper limit])	this value shows the inter-batch variability]
Add additional rows for additional products, if required					

15.2. Table format for baseline quality check bioassay results

Table x. Baseline quality check bioassay results for [product name(s) and batch numbers [batch#1, batch#2, batch#3]] using [bioassay method] against [species/strain(s)] mosquitoes

Sample ID (net and batch identification)	Product/Fabric [A]				Product/Fabric [B]				Product/Fabric [C]			
	N [mosquitoes]	N [replicates]	Mean M24 (%) (95% CI)	Mean endpoint 2 (%) (95%CI)	N [mosquitoes]	N [replicates]	Mean M24 (%) (95% CI)	Mean endpoint 2 (%) (95%CI)	N [mosquitoes]	N [replicates]	Mean M24 (%) (95% CI)	Mean endpoint 2 (%) (95%CI)
[sample IDs Batch 1 Net1]												
[sample IDs Batch 1 Net2]												
[sample IDs Batch 1 Net3]												
[sample IDs Batch 1 Net4]												
[sample IDs Batch 1 Net5]												
Batch [1] combined results												
[sample IDs Batch 2 Net1]												
[sample IDs Batch 2 Net2]												
[sample IDs Batch 2 Net3]												
[sample IDs Batch 2 Net4]												
[sample IDs Batch 2 Net5]												
Batch [2] combined results												
[sample IDs Batch 3 Net1]												
[sample IDs Batch 3 Net2]												
[sample IDs Batch 3 Net3]												
[sample IDs Batch 3 Net4]												
[sample IDs Batch 3 Net5]												
Batch [3] combined results												
Combined results for all batches												
Add additional rows for additional products/fabrics if required												

NB. Present results for the negative control, positive control(s) and ITN under investigation. Additional rows/columns may be added for additional products/endpoints/species/strains.

15.3. Table format for supplementary bioassay results

Table x. Supplementary Baseline quality check bioassay results for [product name(s) and batch numbers [batch#1, batch#2, batch#3]] using [bioassay method] against [species/strain(s)] mosquitoes in [location]												
	[Species/strain 1]						**[Species/strain 2]**					
	N [mosquitoes]	N [replicates]	Observed mean M24 (%)(95% CI)	Inferential Mean M24	OR(95% CI)	p	N [mosquitoes]	N [replicates]	Observed mean M24 (%)(95% CI)	Inferential mean M24	OR(95% CI)	p
Unwashed before [semi-field method]												
[Product/fabric A]												
[Product/fabric B]												
[Product/fabric C]												
[Product/fabric D]												
[Product/fabric E]												
20x washed before [semi-field method]												
[Product/fabric A]												
[Product/fabric B]												
[Product/fabric C]												
[Product/fabric D]												
[Product/fabric E]												
Unwashed after [semi-field method]												
[Product/fabric A]												
[Product/fabric B]												
[Product/fabric C]												
[Product/fabric D]												
[Product/fabric E]												
20x washed after [semi-field method]												
[Product/fabric A]												
[Product/fabric B]												
[Product/fabric C]												

Table x. Supplementary Baseline quality check bioassay results for [product name(s) and batch numbers [batch#1, batch#2, batch#3]] using [bioassay method] against [species/strain(s)] mosquitoes in [location]												
	[Species/strain 1]						**[Species/strain 2]**					
	N [mosquitoes]	N [replicates]	Observed mean M24 (%)(95% CI)	Inferential Mean M24	OR(95% CI)	p	N [mosquitoes]	N [replicates]	Observed mean M24 (%)(95% CI)	Inferential mean M24	OR(95% CI)	p
[Product/fabric D]												
[Product/fabric E]												

15.4. Suggested table formats for semi-field summary statistics

Table x. Summary statistics by arm and [endpoint(s)] for [product name(s)] in [semi-field method] against [species/strain(s)] mosquitoes in [location]					
	Negative control	[Product A]	[Product B]	[Product C]	[Product D]
Unwashed					
N females entering					
N females per hut per night					
N females dead at 24 hours					
Mean M24 (95% CI)]					
N females [indicator] at [endpoint 2]					
Mean [endpoint 2] (95% CI)					
N blood fed females					
Blood feeding rate (%)					
Blood-feeding inhibition (%)					
20x washed					
N females entering					
N females per hut per night					
N females dead at 24 hours					
Mean M24 (95% CI)]					
N females [indicator] at [endpoint 2]					
Mean [endpoint 2] (95% CI)					
N blood fed females					
Blood feeding rate (%)					
Blood-feeding inhibition (%)					

15.5. Suggested table formats for secondary outcomes in semi-field studies

Table x. Summary statistics by arm and [endpoint(s)] for [product name(s)] in [semi-field method] against [species/strain(s)] mosquitoes in [location]					
	Negative control	[Product A]	[Product B]	[Product C]	[Product D]
Unwashed					
N females entering					
N females per hut per night					
N females dead at 24 hours					
Mean M24 (95% CI)]					
N females [indicator] at [endpoint 2]					
Mean [endpoint 2] (95% CI)					
N blood fed females					
Blood feeding rate (%)					
Blood-feeding inhibition (%)					
20x washed					
N females entering					
N females per hut per night					
N females dead at 24 hours					
Mean M24 (95% CI)]					
N females [indicator] at [endpoint 2]					
Mean [endpoint 2] (95% CI)					
N blood fed females					
Blood feeding rate (%)					
Blood-feeding inhibition (%)					

Table x. Summary statistics by arm and [endpoint(s)] for [product name(s)] in [semi-field method] against [species/strain(s)] mosquitoes in [location]					
	Negative control	[Product A]	[Product B]	[Product C]	[Product D]
Unwashed					
N females entering					
N females per hut per night					

Deterrence (%)					
Total exiting					
% exiting (95% CI)					
Killing effect (%)					
Personal protection (%)					
20x washed					
N females entering					
N females per hut per night					
Deterrence (%)					
Total exiting					
% exiting (95% CI)					
Killing effect (%)					
Personal protection (%)					

15.6. Suggested table format for non-inferiority analysis results for semi-field studies designed as comparative efficacy studies

Table x. Non-inferiority analysis for [product name(s)] in [semi-field method] against [species/strain(s)] mosquitoes in [location]												
	Unwashed				20x washed				Pooled			
Indicator and reference	Target outcome	Δ for 7% difference	OR (95% CI)	Interpretation	Target outcome	Δ for 7% difference	OR (95% CI)	Interpretation	Target outcome	Δ for 7% difference	OR (95% CI)	Interpretation
M24 [standard comparator]	Superiority				Superiority				Superiority			
M24 [active comparator]	Non-inferiority				Non-inferiority				Non-inferiority			
[endpoint 2] [standard comparator]	Superiority				Superiority				Superiority			
[endpoint 2] [active comparator]	Non-inferiority				Non-inferiority				Non-inferiority			
Blood feeding rate [standard comparator]	Superiority				Superiority				Superiority			
Blood feeding rate [active comparator]	Non-inferiority				Non-inferiority				Non-inferiority			