WHO Prequalification of Vector Control Products

Wash resistance study for ITN fabric

Factors which may affect the validity of a wash resistance study:

- study not conducted in compliance with GLP;
- negative control mortality exceeds limits identified for the applicable method(s);
- identification of issues related to the health of test organisms;
- environmental conditions, e.g., temperature and humidity at which the test is conducted or the mosquitoes are held for delayed mortality monitoring;
- storage conditions of ITNs and net samples during sample preparation, storage and testing.

1. Purpose of the study

For the purpose of the prequalification assessment, **wash resistance** studies are conducted to investigate how an ITN fabric performs through a series of washes by means of:

- chemical analysis to determine the rate of loss for total AI(s), and
- bioassay(s) to investigate the consistency of biological activity of the material's surface

The series of washes is intended to simulate the intended useful life of the ITN during which there is loss of AI(s) from each wash and re-establishment of surface concentrations post-wash. This is referred to as artificial ageing.

The wash resistance study is intended to measure the rate at which the AI(s) in the reservoir (bound in coating or within the yarn) is depleted through the series of washes by measuring the chemical content.

Additionally, a bioassay(s) is used to measure the biological activity of the material's surface at particular points in the series of washes in order to investigate the continuity of the induced effect on the test systems used for the bioassay(s).

Note: The loss of AI(s) per wash is influenced by the wash interval which is the number of days between washes (determined using results from the regeneration study). Additionally, the number of days between washing and conducting the bioassay can impact results, and therefore bioassays should be conducted just before the next wash in the series or for selection for storage at 4°C for a complete series method.

2. Requirement for submission of wash resistance studies

It is required that a wash resistance study, or studies, are conducted for each fabric used within the construction of an ITN. The specific formulation and manufacturing process for the fabric can significantly influence the behaviours of the treated fabric, especially the rate at which active ingredient(s) move from the reservoir to the surface and are thereby lost by means of washing.

Wash resistance studies must be GLP compliant.

3. Considerations for chemistry method selection

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. Considerations for entomology method selection

Typically, wash resistance studies use the **cone test** or **tunnel test** as the bioassay method(s) for experimentation. The IACT method can also be used. In designing a wash resistance study, the formulation of the fabric, mode of action of the AI(s) and intent of the product should be carefully considered to determine which method or methods to use.

Other existing or novel methods can be proposed in situations where the standard methods are not appropriate. 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.

A single bioassay method should be selected for use in the wash resistance study, 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 tunnel tests to demonstrate the effect of chlorfenapyr in a pyrethroid-chlorfenapyr dual-AI ITN.

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 the wash resistance study must be representative of the intended effect of the product. The selection of appropriate endpoint(s) may dictate the selection of method and/or encourage the use of multiple entomological methods.

The endpoint(s) selected for use in wash resistance studies must be the same endpoint(s) as those used for data generation in other Module 3 entomological studies

6. Considerations for test system species/strain selection

For the purposes of the wash resistance study, the selected test system species/strain should be relevant to the intended use 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 in a wash resistance study can provide valuable information about:

- the differences in time until effects 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

The study should include samples from a minimum of three batches of each fabric used in the construction of the ITN. Depending on the design of the individual fabrics, bespoke ITN sampling plans may be necessary to address different formulants and/or target effects of the various fabrics (refer to Section 7.2). For the development of a new product dossier, it is critical that the batches used in the wash resistance study are the same as those used for other data generation, e.g., the characterisation of chemical and physical characteristics and semi-field trials.

Documentation of the source, receipt, handling and storage of whole ITNs and cut netting samples prior to testing is critical.

7.2.ITN sampling for wash resistance studies

As the purpose of the wash resistance study is to measure the retention and presentation of AI(s) during a series of washes designed to simulate artificial ageing *(1)*, the sampling plan should be designed to capture any differences in the retention of AI over the wash series at different positions on the net.

Depending on the context and use of the study, samples may be cut from:

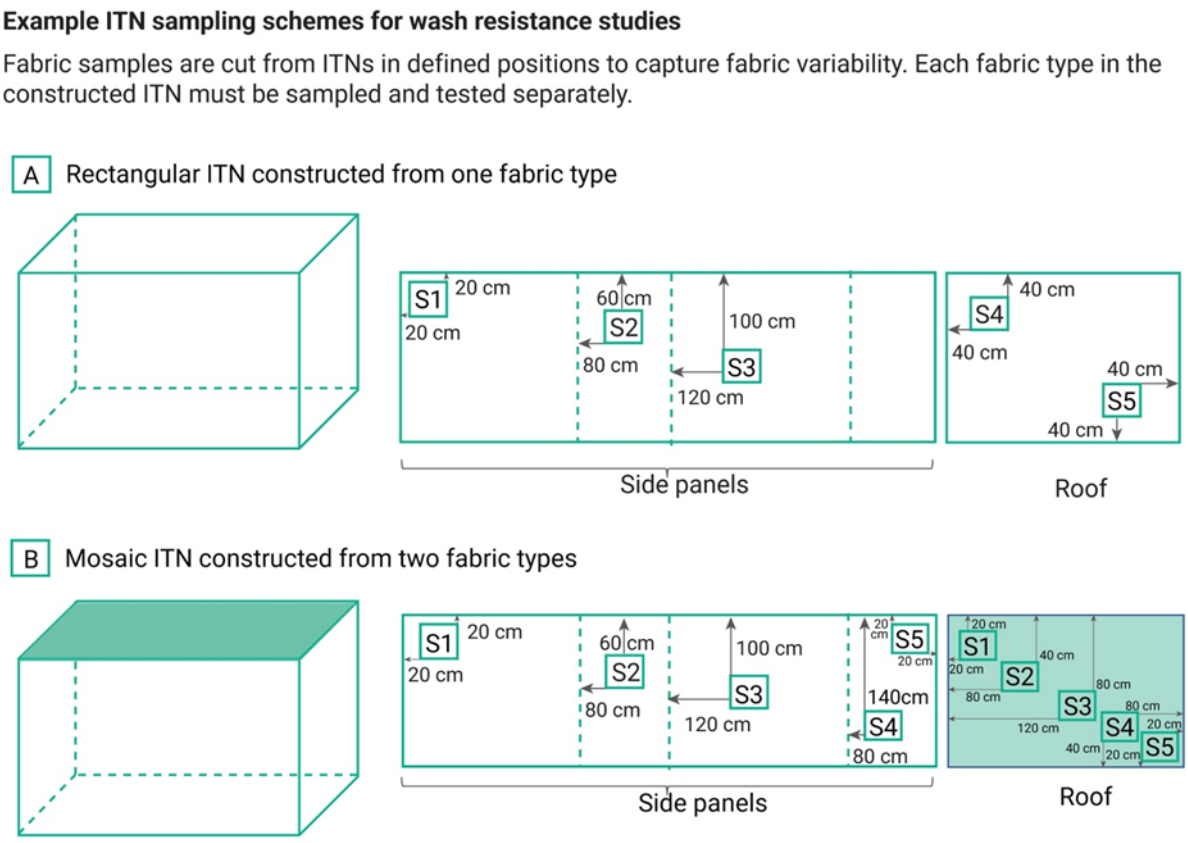
- pre-constructed treated fabrics in a described manner to ensure good representation across the batch production, or
- constructed ITNs.

Samples are cut from defined positions (Fig. 1). Measurements should be taken from the left hand and top seams when locating the positions from which to cut samples from side panels, excepting sample S5 on a mosaic net, which should be measured from the right hand and top seam. For roof samples, measurements should be taken from the long and short seams. Samples should be 25x25cm in size.

Six ITNs should be selected for use in the wash resistance study, two from each of three production batches. Five samples of each fabric type are cut from each net. A total of twenty-eight samples for each fabric are washed in the study.

Alternative sampling schemes may be proposed. In these cases, WHO recommends that substantiating documentation be provided with a PQ200 protocol review request submission prior to the commencement of prequalification data generation.

Fig. 1. Sampling schemes for wash resistance studies



7.3. Negative control

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

7.4. 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.

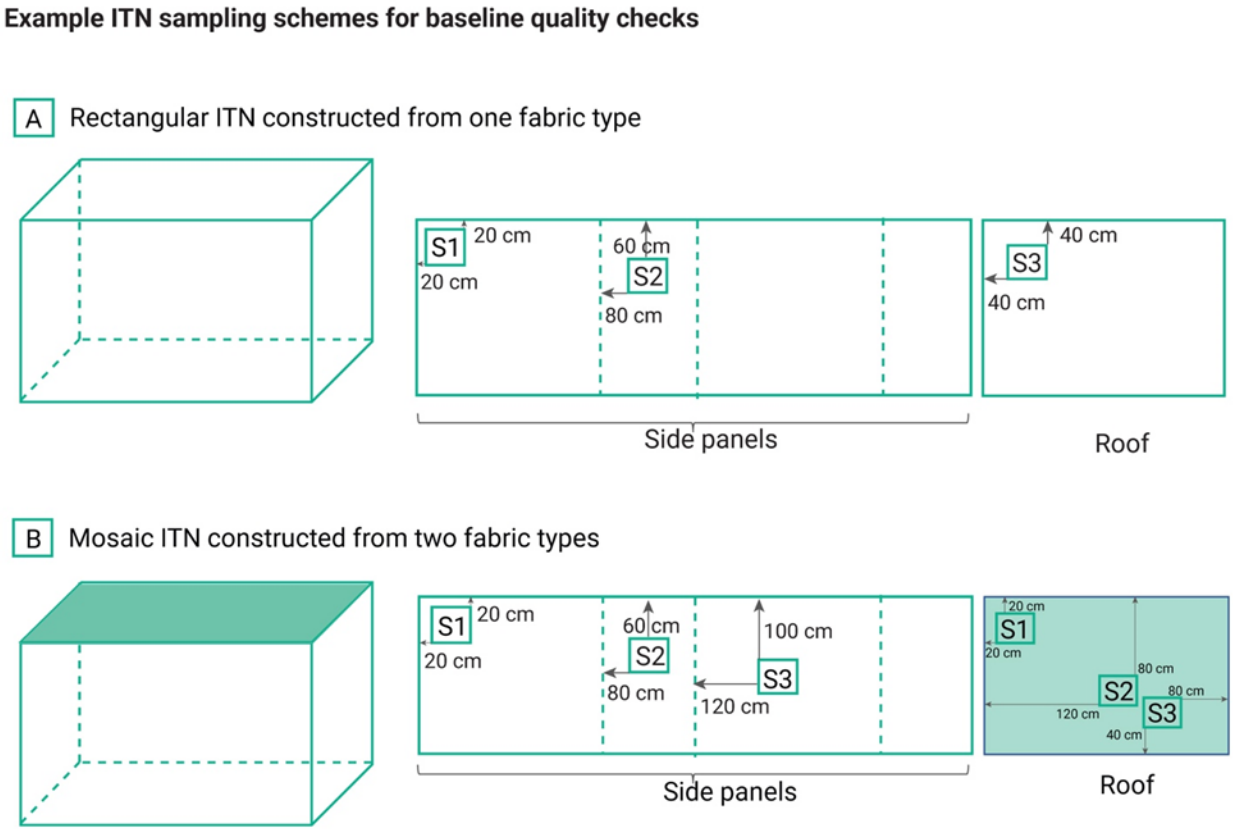
8. Baseline quality check

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 fabric samples. 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. 2).

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 2. 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 than in other Module 3 studies.

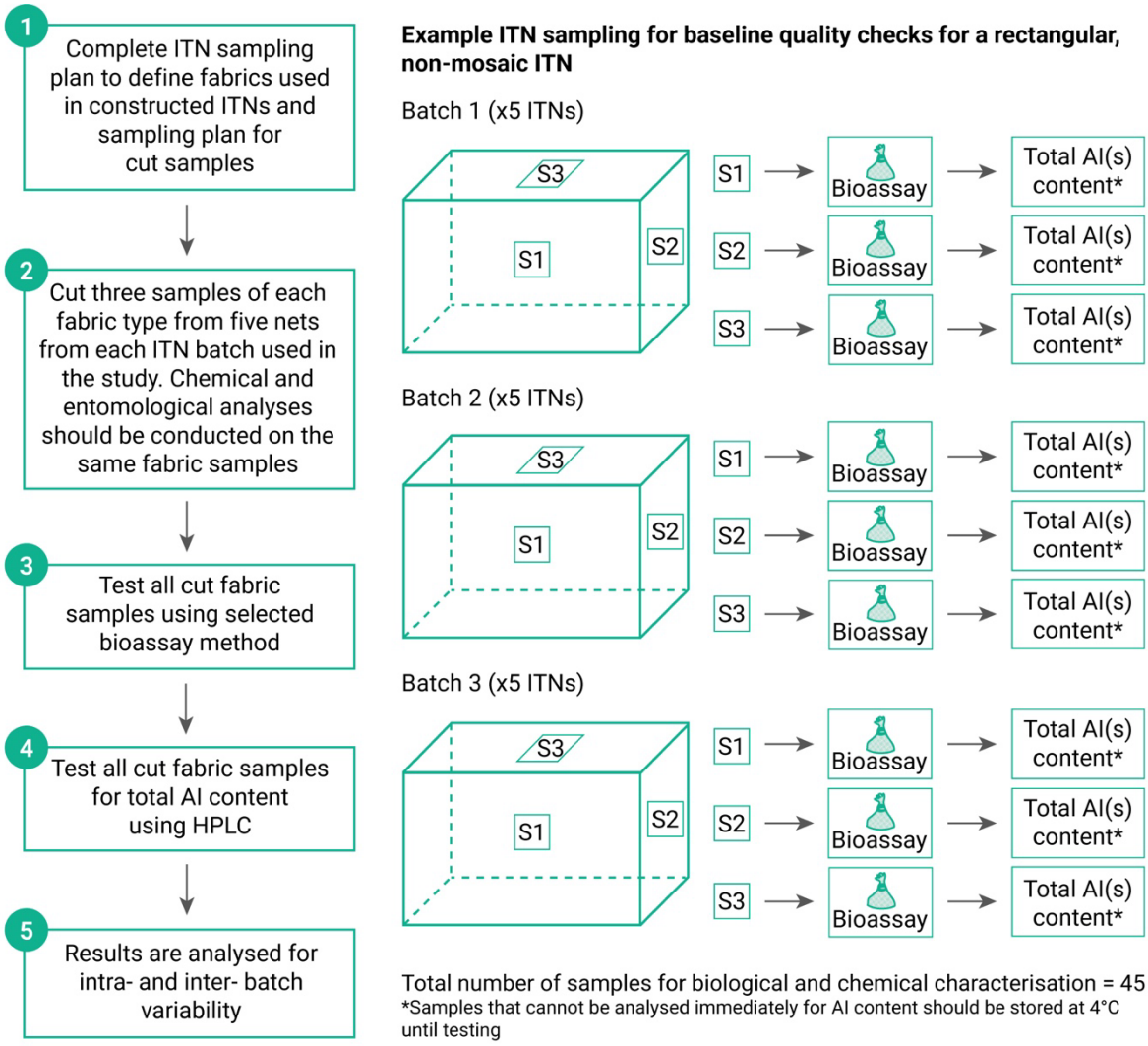
The baseline quality check should only be conducted once per testing facility, i.e., a testing facility conducting a regeneration study, a wash resistance study and a semi-field study need only conduct a single baseline quality check.

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 12).

8.1.Schematic of a baseline quality check

Fig. 3 illustrates the baseline quality check procedure for a rectangular, uniform ITN.

Fig 3. Baseline quality check



Note:Test all cut fabric samples for total AI content using HPLC or the appropriate method of analysis accoridng to the AI.

9. Sample preparation

9.1.Cutting

Fabric samples should be cut in accordance with the procedures for the selected test(s) to be performed. When cutting the test material ensure that the material is not being stretched nor compressed.

9.2. Washing of fabric samples

Fabric samples should be washed using the established washing procedure:

- Cut ITN samples (25 cm x 25 cm) are introduced individually into 1l beakers or glass bottles containing 0.5 l deionized water, with 2 g/l of soap (pH 10–11) added and fully dissolved just before washing. Place the beakers/bottles upright in a water bath heated to 30 ±2°C and shake horizontally for 10 minutes at 155 movements per minute.
- Remove the sample from the beaker/bottle using tweezers and remove excess fluid by gently shaking it several times in the air.
- Rinse the sample twice. For each rinse the sample is placed in a 1l beaker or glass bottle in 500ml deionized water at 30 ±2°C, shaken horizontally for 10 minutes at 155 movements per minute, and fresh water used for each rinse.
- After the second rinse, remove the sample from the beaker/bottle using tweezers and remove excess fluid by gently shaking it several times in the air. Dry the sample on a washing line for 30 minutes at 27 ±2°C away from direct sunlight. Ensure that the sample is completely dry before further washing, testing, or storage.

Between washes (number of days between washes results from the regeneration study), samples should be individually wrapped in aluminium foil and stored in the dark at 30 ±2°C.

9.3. Storage of fabric samples prior to testing and between washing/testing days

Fabric samples should be stored at 30 ±2°C out of the light.

9.4. 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. Environmental Conditions in Testing Room

The testing laboratory where bioassays are conducted should be maintained at a temperature of 27 ±2° C and 75% ± 10% relative humidity.

11. Experimental Procedures

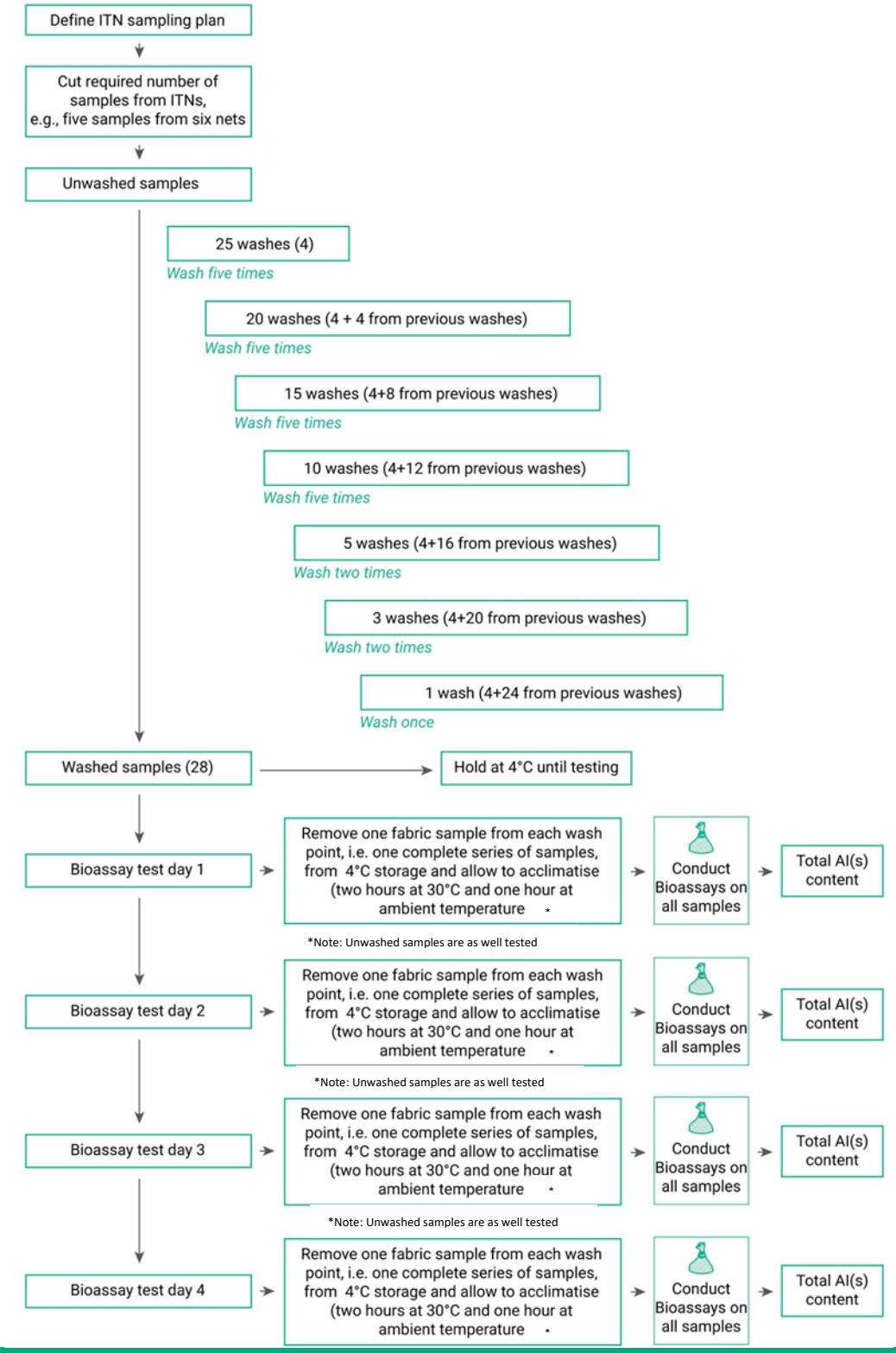
The duration of a wash resistance study is dependent on the selected wash interval for the product under investigation.

If needed and appropriate for the fabric(s) under investigation, the number of washes can be extended.

11.1. Schematic of a wash resistance study

Fig. 4 illustrates the wash resistance study procedure for a rectangular, uniform ITN.

Fig 4. Wash resistance study



11.2. Random selection of samples

The random selection of samples for testing at each stage of the study should be managed such that at least one sample from each ITN batch that is used in the study is represented at each wash point.

11.3. Complete series method for sample preparation

Fabric samples for a wash resistance study should be prepared in advance of testing using the complete series method, which is a method of sample preparation that allows for all bioassays in a series or study (wash 0, 1, 3, 5, 10, 15, 20 and 25) to be conducted on the same day. This reduces the source of variability in bioassay results that is due to bioassay test day and reduces variability due to the use of different batches of mosquitoes.

To prepare samples for a wash resistance study using the complete series method, the washing of samples is managed such that all of the study samples complete washing at the same time. This necessitates beginning the wash series for samples allocated to higher numbers of washes before the samples allocated to lower numbers of washes. The wash points used in the wash resistance series for bioassay and chemical analysis are unwashed samples and samples that have been washed 1, 3, 5, 10, 15, 20 and 25 times *(1)*. If there is a delay between the end of the washing series and the bioassay test day, samples should be wrapped in aluminium foil and stored at 4°C in the dark.

To conduct the bioassays, remove all fabric samples from cold storage and allow to acclimatise for two hours at 30°C followed by one hour at ambient temperature. Bioassays should be conducted on ITNs that have regenerated, i.e., completed an additional regeneration time post-washing. Following bioassays, the sample is wrapped in aluminium foil and stored at 4°C until chemical analysis. Care must be taken to ensure that each sample is appropriately labelled to avoid mismatches in chemical analysis results. The chemical analysis consists of measurements of total AI.

11.4. Sample sizes for 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%. While 4 ITN samples are recommended as a minimum, it is necessary to conduct wash resistance studies using a sufficient number of replicates per samples to obtain a sufficiently precise point estimate.

12. Results and data analysis

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

If multiple fabrics are investigated in the study, the results for each fabric must be presented 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.

The results of the bioassays (observed and model-derived) should be plotted on a graph (y axis - % mean response including 95% confidence intervals; x axis – number of washes) in order to visualize the response over time.

12.1. Baseline quality results

12.1.1. 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.

12.1.2. 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.

12.2. Wash resistance study results

12.2.1. Chemical analysis

12.2.1.1. Chemical analysis results

The results to be reported for chemical analyses are:

- Total AI for each piece in g/kg.

A table of summary results of the observed data (number of samples tested, arithmetic mean difference and Standard Deviation) should be included in the report.

The model-estimated mean total AI (12.2.1.2) of each fabric should be plotted on a graph together with results derived from the observed data (y axis – estimated surface concentration in g/kg; x axis – days) in

order to visualize the observed response over time. The model and observed data should include the 95% CIs and estimates of the unwashed samples in the plot.

12.2.1.2. Statistical data analysis

The mean difference between the total AI of each fabric when unwashed and at each wash point should be analysed using a linear regression model, with wash, day, and production batch as fixed effects. An additional fixed effect should be included if more than one day is used to analyse the samples.

The fitted model should be used to generate a predicted means and respective 95% CIs for the total AI (g/kg) for each day that experiments were carried out. The predicted estimates are averaged across the batches and day of analysis (if included). The means with respective 95% CIs should be plotted on a graph with the (y axis - difference in total AI in g/kg; x axis – days) together with the observed total AI in order to visualize the total AI over the wash series.

12.2.1.3. Determination of AI retention per wash

The AI retention per wash is calculated using the following equation:

$$w = 100 \times \sqrt[n]{(t_n/t_0)}$$

where: w = AI retention per wash, expressed as a percentage; t_n = total active ingredient content (in g/kg) after n washing cycles; and t_0 = total active ingredient content (in g/kg) before washing (no washing); n = number of washes.

12.2.2. Bioassay

12.2.2.1. Bioassay results

The results to be reported for bioassay are:

- Mean results with an appropriate measure of dispersion for each selected endpoint at each wash point

If multiple bioassays methods are used, the results must be presented separately. Data generated from various bioassays should not be combined.

If multiple test systems (species/strains) are used, results must be presented in relation to the bioassay method used. Summary graphs including means and 95% CIs for the arithmetic mean and model-derived mean mortality for 0, 1, 3, 10, 15, 20 and 25 washes (12.2.2.2) per test system should be presented.

A table showing the summary results of the observed data (number of net samples, number of mosquitoes exposed, number of replicates, percentage arithmetic mean and 95% confidence intervals) per product and wash point should be included in the report for all test systems exposed to a specific fabric should be presented.

12.2.2.2. Statistical data analysis

The data for the selected endpoint (usually mosquito mortality) is analysed using a logistic regression model, with wash, batch and day as fixed effects (factor variables). An additional fixed effect is included if more than one technician carried out the experiments.

The fitted model is used to generate a predicted mortality and the respective 95% CI for each wash point. These model-derived means and observed arithmetic mean % mortality (including 95% CI) should be plotted on a graph (y axis – percentage arithmetic mean endpoint (usually mosquito mortality); x axis – number of washes) to visualise the response over time. If longer holding times are used the analysis should be conducted for each holding time and the choice of holding time should be justified.

12.2.2.3. Interpretation of results

Wash resistance is defined as the number of washes for which the point estimate for the model-derived endpoint (usually mosquito mortality) is maintained within 5% of the endpoint (usually mosquito mortality) result for the unwashed ITN. This is achieved by using a variable odds ratio that translates to a fixed percentage of a mortality of not less than 5% relative to the results for the unwashed net (Annex 2).

It is also helpful to additionally report the wash resistance relative to the one-time washed ITN.

12.3. 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.

13. Study report

13.1. Wash resistance 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 wash resistance 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:
      - Name of the items, type of net, active ingredient(s)/synergist name and their concentration
      - 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
  - » Data analysis
    - Statistical analysis methods

- Results
  - » Baseline quality check
    - Summarized 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
  - » Wash resistance study
    - Summarized 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
  - » Data analysis and statistical results
    - Baseline quality check
      - Summary statistics
      - Statistical analysis results
    - Wash resistance study
      - Summary statistics
      - Inferential statistics
      - Statistical analysis results
  - » Results interpretation and the demonstrated wash resistance

- 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.

14. Related documents

- WHO PQT/VCP Implementation guidance – Regeneration Study for ITN fabric
- WHO PQT/VCP Implementation guidance – Considerations for the 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 – Bioassay and semi-field methods for ITNs: IACT
- 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 – Matrix of selected mosquito strains
- WHO PQT/VCP Matrix of selected mosquito strains - Template

15. References

1. 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).

16. Annexes.

16.1. Suggested table formats for summary results

16.1.1. 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-upper limit)])	[this value shows the intra-net variability]	[mean] ([SD] [range (lower limit-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-upper limit)])	[this value shows the intra-batch variability]	[mean] ([SD] [range (lower limit-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-upper limit)])	[this value shows the intra-batch variability]	[mean] ([SD] [range (lower limit-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-upper limit)])	[this value shows the intra-batch variability]	[mean] ([SD] [range (lower limit-upper limit)])	[this value shows the intra-batch variability]

Combined results for all batches		[mean] ([SD] [range (lower limit-upper limit)])	[this value shows the inter-batch variability]	[mean] ([SD] [range (lower limit-upper limit)])	this value shows the inter-batch variability]
Add additional rows for additional products/fabrics, if required					

16.1.2. Suggested 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.

16.1.3. Suggested table formats for wash resistance study chemistry results

Table x. AI content and retention of sampled pieces of [product name] used in the entomological wash resistance study (batch numbers [batch#1, batch#2, batch#3])

Wash No.	Mean [AI name] content (g/kg)	RSD (%)	[AI name] retention	[AI name] retention per wash	Mean [synergist name, or second AI] content (g/kg)	RSD (%)	[synergist name] retention	[synergist name] retention per wash
0	[value] ([range (lower limit-upper limit)])	[value]	-	-	[value] ([range (lower limit-upper limit)])	[value]	-	-
1	[value] ([range (lower limit-upper limit)])	[value]	[value] %	[value]%	[value] ([range (lower limit-upper limit)])	[value]	[value]%	[value]%
3	[value] ([range (lower limit-upper limit)])	[value]	[value] %	[value]%	[value] ([range (lower limit-upper limit)])	[value]	[value]%	[value]%
5	[value] ([range (lower limit-upper limit)])	[value]	[value] %	[value]%	[value] ([range (lower limit-upper limit)])	[value]	[value]%	[value]%
10	[value] ([range (lower limit-upper limit)])	[value]	[value] %	[value]%	[value] ([range (lower limit-upper limit)])	[value]	[value]%	[value]%
15	[value] ([range (lower limit-upper limit)])	[value]	[value] %	[value]%	[value] ([range (lower limit-upper limit)])	[value]	[value]%	[value]%
20	[value] ([range (lower limit-upper limit)])	[value]	[value] %	[value]%	[value] ([range (lower limit-upper limit)])	[value]	[value]%	[value]%
25	[value] ([range (lower limit-upper limit)])	[value]	[value] %	[value] %	[value] ([range (lower limit-upper limit)])	[value]	[value] %	[value] %

16.1.4. Suggested table format for wash resistance study bioassay results (observed)

Table x. Wash resistance study bioassay results for [product name(s) and batch numbers [batch#1, batch#2, batch#3]] using [bioassay method] against [species/strain] mosquitoes															
N [washes]	Product/Fabric [A]					Product/Fabric [B]					Product/Fabric [C]				
	N [net samples]	N [mosquitoes]	N [replicates]	Mean M24 (%) (95% CI)	Mean [endpoint 2] (95% CI)	N [net samples]	N [mosquitoes]	N [replicates]	Mean M24 (%) (95% CI)	Mean [endpoint 2] (95% CI)	N [net samples]	N [mosquitoes]	N [replicates]	Mean M24 (%) (95% CI)	Mean [endpoint 2] (95% CI)
UW															
1															
3															
5															
10															
15															
20															
25															
Add additional rows for additional products/fabrics/washes if required															

16.1.5. Suggested table format for wash resistance bioassay inferential statistics results

Table x. Wash resistance inferential statistics results for [product name(s) and batch numbers [batch#1, batch#2, batch#3]] using [bioassay method] against [species/strain] mosquitoes																		
N [washes]	Product/Fabric [A]						Product/Fabric [B]						Product/Fabric [C]					
	Mean M24	OR (95%CI)	p	Mean [endpoint 2]	OR (95%CI)	p	Mean M24	OR (95%CI)	p	Mean [endpoint 2]	OR (95%CI)	p	Mean M24	OR (95%CI)	p	Mean [endpoint 2]	OR (95%CI)	p
UW																		
1																		
3																		
5																		
10																		
15																		
20																		
25																		
Add additional rows for additional products/fabrics if required																		

16.2. Annex 2. Table of odds ratios to detect a 5% difference in mortality

World Health Organization

Odds ratios applicable for 5% mortality differences											
Unwashed % mortality	Odds for unwashed -5%	Odds for unwashed	Odds Ratio	Unwashed % mortality	Odds for unwashed -5%	Odds for unwashed	Odds Ratio	Unwashed % mortality	Odds for unwashed -5%	Odds for unwashed	Odds Ratio
99.9	18.6	999.0	0.02	77	2.6	3.3	0.77	54	1.0	1.2	0.82
99	15.7	99.0	0.16	76	2.4	3.2	0.77	53	0.9	1.1	0.82
98	13.3	49.0	0.27	75	2.3	3.0	0.78	52	0.9	1.1	0.82
97	11.5	32.3	0.36	74	2.2	2.8	0.78	51	0.9	1.0	0.82
96	10.1	24.0	0.42	73	2.1	2.7	0.79	50	0.8	1.0	0.82
95	9.0	19.0	0.47	72	2.0	2.6	0.79	49	0.8	1.0	0.82
94	8.1	15.7	0.52	71	1.9	2.4	0.79	48	0.8	0.9	0.82
93	7.3	13.3	0.55	70	1.9	2.3	0.80	47	0.7	0.9	0.82
92	6.7	11.5	0.58	69	1.8	2.2	0.80	46	0.7	0.9	0.82
91	6.1	10.1	0.61	68	1.7	2.1	0.80	45	0.7	0.8	0.81
90	5.7	9.0	0.63	67	1.6	2.0	0.80	44	0.6	0.8	0.81
89	5.3	8.1	0.65	66	1.6	1.9	0.81	43	0.6	0.8	0.81
88	4.9	7.3	0.67	65	1.5	1.9	0.81	42	0.6	0.7	0.81
87	4.6	6.7	0.68	64	1.4	1.8	0.81	41	0.6	0.7	0.81
86	4.3	6.1	0.69	63	1.4	1.7	0.81	40	0.5	0.7	0.81
85	4.0	5.7	0.71	62	1.3	1.6	0.81	39	0.5	0.6	0.81
84	3.8	5.3	0.72	61	1.3	1.6	0.81	38	0.5	0.6	0.80
83	3.5	4.9	0.73	60	1.2	1.5	0.81	37	0.5	0.6	0.80

82	3.3	4.6	0.73	59	1.2	1.4	0.82	36	0.4	0.6	0.80
81	3.2	4.3	0.74	58	1.1	1.4	0.82	35	0.4	0.5	0.80
80	3.0	4.0	0.75	57	1.1	1.3	0.82	34	0.4	0.5	0.79
79	2.8	3.8	0.76	56	1.0	1.3	0.82	33	0.4	0.5	0.79
78	2.7	3.5	0.76	55	1.0	1.2	0.82	32	0.4	0.5	0.79
Odds ratios applicable for 5% mortality differences (continued)											
Unwashed % mortality	Odds for unwashed -5%	Odds for unwashed	Odds Ratio	Unwashed % mortality	Odds for unwashed -5%	Odds for unwashed	Odds Ratio	Unwashed % mortality	Odds for unwashed -5%	Odds for unwashed	Odds Ratio
31	0.4	0.4	0.78	22	0.2	0.3	0.73	13	0.1	0.1	0.58
30	0.3	0.4	0.78	21	0.2	0.3	0.72	12	0.1	0.1	0.55
29	0.3	0.4	0.77	20	0.2	0.3	0.71	11	0.1	0.1	0.52
28	0.3	0.4	0.77	19	0.2	0.2	0.69	10	0.1	0.1	0.47
27	0.3	0.4	0.76	18	0.1	0.2	0.68	9	0.0	0.1	0.42
26	0.3	0.4	0.76	17	0.1	0.2	0.67	8	0.0	0.1	0.36
25	0.3	0.3	0.75	16	0.1	0.2	0.65	7	0.0	0.1	0.27
24	0.2	0.3	0.74	15	0.1	0.2	0.63	6	0.0	0.1	0.16
23	0.2	0.3	0.73	14	0.1	0.2	0.61				