



# Protocol for Preparing Bed Nets for Experimental Work

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| <b>Title</b>                | <b>Protocol for Preparing Bed Nets for Experimental Work</b> |
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**Timeline**

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### Version Control<sup>1</sup>

| Version | Date | Updated by | Description of update(s) |
|---------|------|------------|--------------------------|
|         |      |            |                          |

### Related documents

- I2I Best Practice SOP Library, 30 October 2020 (<https://innovationtoimpact.org/>)

## 1. Purpose

The protocol aims to standardize the method of preparing bed nets for experimental work. It ensures consistent handling, maximizes the use of each net, improves traceability, and facilitates accurate data interpretation. This protocol is designed to standardize the method of preparing bed nets, specifically insecticide treated nets (ITNs), for experimental work including bioassays and chemical analysis. It aims to ensure consistent handling and cutting of net samples, maximizing the use of each net while maintaining the integrity required for accurate chemical analysis. The protocol helps us to design experiments to measure between- and within-net variability of insecticide concentration, through improved traceability of net pieces, facilitating accurate data interpretation for both experimental and analytical purposes.

## 2. Background

Bed nets, especially ITNs, are essential in preventing mosquito-borne diseases. Their usage in experimental settings often necessitates cutting them into pieces for tests such as bioassays and chemical analysis. To maximize consistency, material use, and maintain traceability, it is critical to adhere to a standardized method of preparation. Upon removal from their packaging, nets should immediately be processed according to this SOP for experimental work. Additionally, some areas of netting may be damaged or contain areas of shrinkage on arrival. Shrinkage can occur through use, manufacturing or storage and causes net samples to be unrepresentative of the whole net by potentially having a higher concentration (mg/m<sup>2</sup>) due to increased density of the fibres. This SOP will also detail how to identify appropriate net pieces for testing.

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

### 3. Materials and equipment

For Cutting Pieces from Whole Nets:

- Bed net.
- Lab coat
- Gloves
- Scissors
- Stencil or template cut to size e.g. 30x30cm.
- 70% ethanol
- Paper towel
- Marker pen (or Permanent markers)
- Measuring tape (if stencil/template not used)

For Preserving Net Samples:

- Labels with protocol number, position, date, etc.
- Stapler
- Aluminium foil (Tin foil)
- +4°C Refrigerator
- Bag sealer (if available)
- Resealable bags

Additional Equipment:

- Camera (for photographic documentation)
- Unique number generator (for numbering scheme), e.g., scientific calculator or smartphone

### 4. Procedure

#### 1. Initial Preparation:

- a. Wear personal protective equipment: lab coat, gloves, and respirator.
- b. Unpack a new bed net carefully to avoid any damage.
- c. Weigh the entire net and record its weight for documentation.

#### 2. Airing the Net:

- a. Hang the net in a clean, dry, and well-ventilated area for a week or according to manufacturer's instructions after unpacking. A frame can be used to hang the net for ease of cutting (see Figure 1Figure 1).



*Figure 1 - Frame (a Walk in Tunnel Greenhouse, 181 x 181 x 200cm) for hanging a full net for airing and breakdown of panels.*

### 3. **Preparation of Stencil and Supplies:**

- a. Draw and cut out cardboard to create stencils of the appropriate size for the pieces you require e.g. 30x30cm.
- b. Clean the benchtop with 70% ethanol.
- c. Clean a pair of scissors with 70% ethanol and leave to dry on the benchtop.

### 4. **Preparation of Net Sample:**

- a. Lay the aired net on a clean table.
- b. Start with the control net and then move to insecticide-treated net pieces, changing gloves when handling nets with different insecticides.
- c. Seam Cutting: Identify and cut along both sides of each seam using scissors to separate out individual panels. Discard seams.
- d. Cutting into Pieces: Using the stencil, measure and cut out sections from each panel, ensuring there is no shrinkage or seams in the pieces. Start at one

corner and work along the length of the net panel to maximize the number of pieces. (See figure)

5. **Post-Processing and Storage Streams:** Immediately after cutting, allocate the net pieces into three different storage streams based on their intended use:
  - a. **Room Temperature Storage for Bioassays:**
    1. Wrap the designated pieces in aluminium foil and label them.
    2. Store these pieces at room temperature in a clean, dry area for later use in bioassays.
  - b. **Refrigerated Storage for Chemical Analysis:**
    1. For pieces intended for chemical analysis post-experimentation:
      - a. Initially, wrap and label them in foil and store them at room temperature.
      - b. After the experiments, wrap them in aluminium foil, label them with all necessary details, and store at 4-10°C.
  - c. **Testing Temperature Storage for Ageing, Followed by Refrigerated Storage:**
    1. For pieces allocated for ageing studies:
      - a. Store them at the specific testing temperature as required by the experimental design.
      - b. After the ageing experiments, wrap these pieces in aluminium foil, label them with all necessary details, and then store at 4-10°C for subsequent chemical analysis or bioassays.
6. **Labelling and Documentation:**
  - a. Assign unique identifiers and maintain thorough records for each piece and storage stream.
7. **Storage of Remaining Net:**
  - a. Weigh any discard and record it.
  - b. Return any remaining whole net to its package and store appropriately.
8. **Usage and Data Collection:**
  - a. Accurately record which net pieces are used in each experiment.
  - b. Send samples to the laboratory for analysis as needed.
  - c. Keep detailed records in data collection sheets.

#### **Additional considerations:**

- Firstly, the entire net should be weighed before processing. This initial weight helps in quantifying the material used. Similarly, any discarded material, such as seams, should be weighed at the end of the processing. This step is crucial for calculating the total amount of net used and for maintaining an accurate record for experimental documentation.
- When cutting nets into pieces, the dimensions of the pieces are usually required to be 30 cm x 30 cm. This size is chosen to provide a sufficient area for effective testing in both WHO cone bioassays and tunnel tests while allowing for 5cm to be removed

from 2 edges for chemical testing if required while still preserving the integrity of the net piece for bioassay testing.

- Pieces should not be cut over a seam; instead, seams should be carefully cut out and discarded as they can affect the uniformity and results of the tests.
- Noticeable areas of shrinkage should also be avoided to ensure the integrity of the samples.

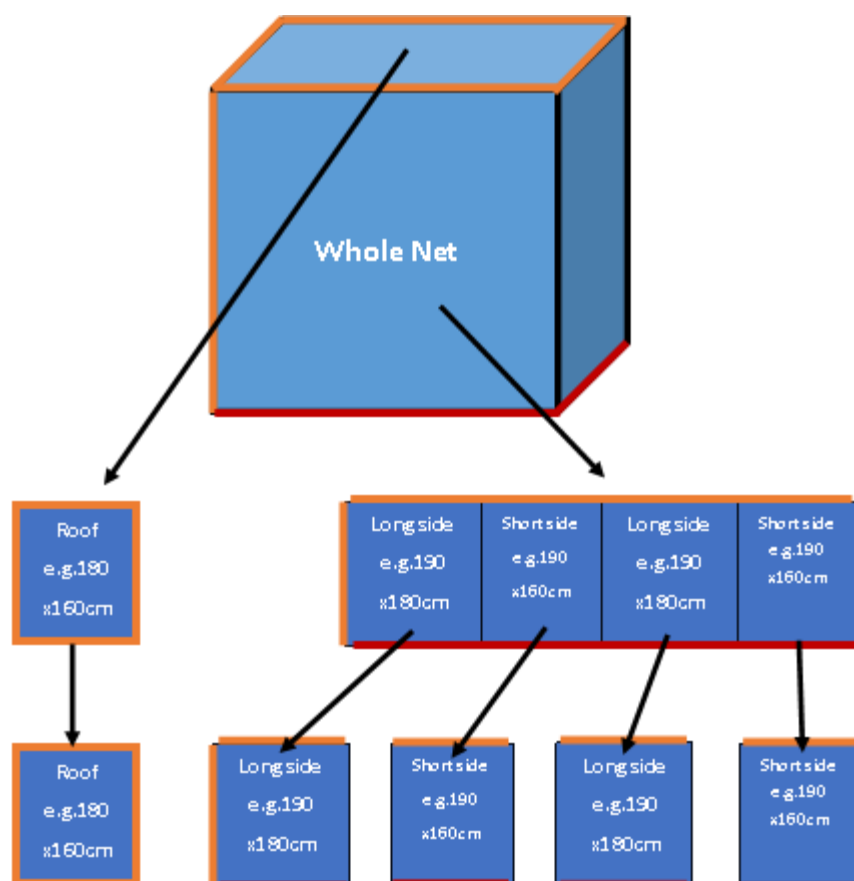


Figure 2 - Schematic showing whole net breakdown into panels for further processing into net pieces.

## 5. Additional data collection

1. Written Documentation of initial net quality: Before cutting the net, take clear, well-lit photos.
2. Record Keeping: Keep a log detailing which samples came from which nets, which panel of which net, the date of opening, the date of cutting, who performed the cutting etc. (

## 6. Worked Example for Net Cutting Protocol

### Initial Net Dimensions:

- Length: 190 cm
- Width: 160 cm
- Height: 180 cm

## Processing Steps:

### 1. Initial Panel Cutting:

- Cut one piece of 180×160 cm for the roof.
- Cut two pieces of 190×180 cm for the long sides.
- Cut two pieces of 190×160 cm for the short sides.

### 2. Modification of Side Panels:

- Remove 25 cm from the bottom edge of the two 190×180 cm panels, making them 190×155 cm.
- Remove 25 cm from the bottom edge of the two 190×160 cm panels, making them 190×135 cm.

## Resulting Panels:

1. **Roof Panel:** 1 piece of 180×160 cm.
2. **Long Side Panels:** 2 pieces of 190×155 cm.
3. **Short Side Panels:** 2 pieces of 190×135 cm.

## Cutting Panels into 30x30 cm Pieces:

### 1. Roof Panel (180×160 cm):

- Lengthwise: 6 pieces (180 cm / 30 cm).
- Width wise: 5 pieces (160 cm / 30 cm).
- Total:  $6 \times 5 = 30$  pieces.

### 2. Long Side Panels (190×155 cm):

- Lengthwise: 6 pieces (190 cm / 30 cm, with a remainder).
- Width wise: 5 pieces (155 cm / 30 cm, with a remainder).
- Total for each panel:  $6 \times 5 = 30$  pieces.
- Total for two panels:  $30 \times 2 = 60$  pieces.

### 3. Short Side Panels (190×135 cm):

- Lengthwise: 6 pieces (190 cm / 30 cm, with a remainder).
- Width wise: 4 pieces (135 cm / 30 cm).
- Total for each panel:  $6 \times 4 = 24$  pieces.
- Total for two panels:  $24 \times 2 = 48$  pieces.

## Total 30x30 cm Pieces:

- Roof Panel: 30 pieces
- Long Side Panels: 60 pieces
- Short Side Panels: 48 pieces
- **Grand Total:  $30 + 60 + 48 = 138$  pieces approx.**

This protocol ensures efficient utilization of the net material and standardizes the cutting process for creating uniform 30x30 cm pieces. It can be adapted as needed for different net sizes and requirements. Number of pieces per panel and net are approximate.

## Net Processing Record

Table 1 - Net Processing Record designed to document how each net is processed for testing, including details of subsample pieces. A blank template is located in the appendix.

| Field                          | Description                                      |
|--------------------------------|--------------------------------------------------|
| Whole net Unique ID Number     | Whole Net ID the subsample will be taken from    |
| Net subsample Unique ID number | Newly generated subsample ID                     |
| Processing Date                | Date the sample was processed                    |
| Subsample Pieces Details       | Net piece size, age etc                          |
| Processing Methods/Protocols   | SOP used to process the subsample                |
| Operator                       | Initials of the person who processed the samples |
| Study/Project Number           | Related Project Number                           |

## 7. Unique ID generation for subsamples

### Generic ID Generation Process

- A. Manufacturer Code Selection: Choose appropriate manufacturer code (e.g., **VEST** for Vestergaard).
- B. 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**).
- C. Subsample Differentiation: Extend the base ID with a unique identifier for each subsample, maintaining a clear link to the parent ITN. (e.g., PermaNet 2.0 Subsample 1: **VEST001-01**)

## 8. Requirements for specific tests

Cone tests – The test samples should be prepared as either 25×25cm or 30×30cm squares, depending on the intent of the study.

Tunnel tests – The test samples should be prepared as 25×25cm squares. In each sample nine holes of 1 cm diameter should be cut. One central hole surrounded by eight others evenly spaced around the central hole 5cm from the edge of the net piece. (Figure 2)

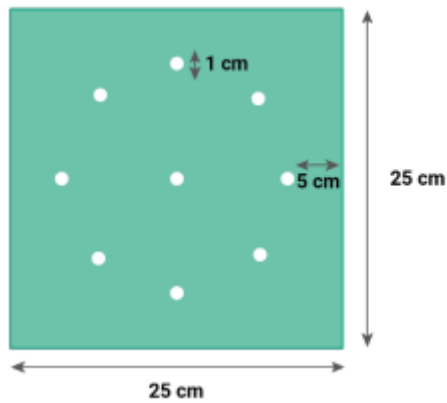


Figure 2 - Test material sample preparation for tunnel test.

Specific schematics for net sampling from a single net for regeneration and wash resistance studies are outlined in the appendix and are taken from a range of WHO documentation.

## 9. Deviations from standard protocol

While this protocol is designed to be comprehensive, modifications may be needed based on specific experimental requirements, net designs, or constraints. Any deviation from this protocol should be clearly documented and justified.

## 10. Glossary of terms

- Shrinkage: The reduction in the size of the net, typically at the edges, due to packaging or manufacturing processes.
- Seam: The line where two pieces of net are sewn together.
- Panels: The individual sections of the net, separated by seams.
- ITNs insecticide treated nets.
- SOP Standard Operating Procedure
- WHO World Health Organization

## 11. References

1. World Health Organization *Test Procedures: For Insecticide Resistance Monitoring in Malaria Vector Mosquitoes*; World Health Organization: Geneva, 2013; ISBN 978-92-4-150515-4.

## 12. Appendix

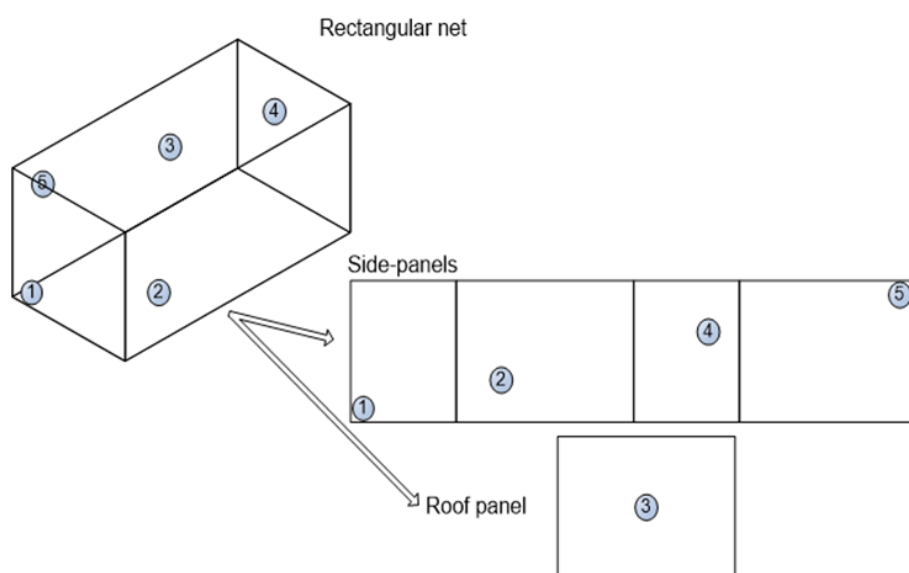


Figure 3 - Net sampling protocol used during CIPAC wash method development. Figure is from WHO 2013 "Recommended positions from which netting pieces should be taken" for phase II studies.

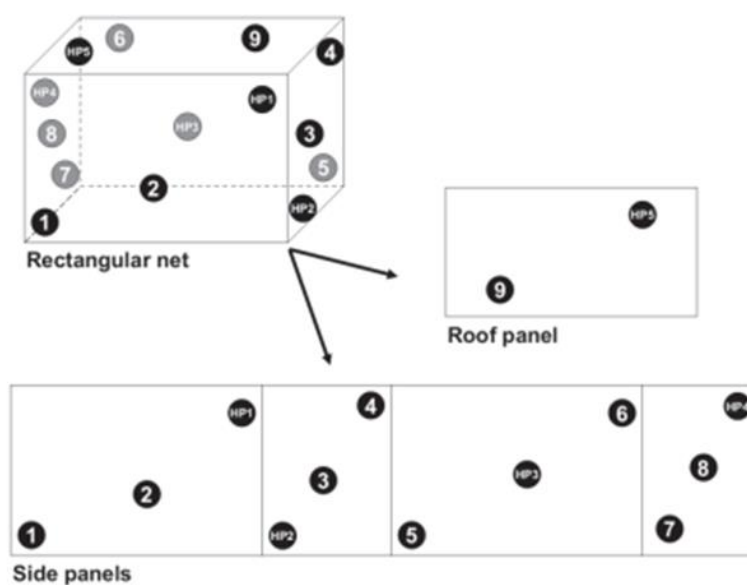
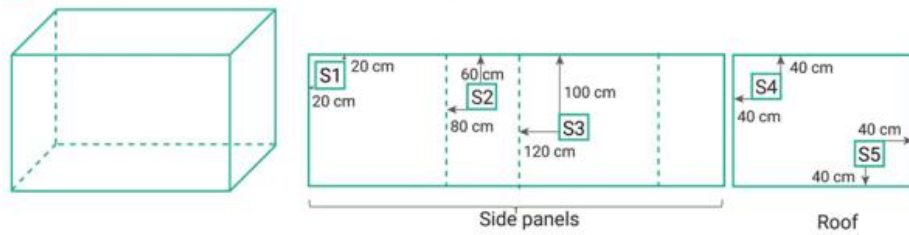


Figure 4 - Sampling scheme for fourteen pieces of netting from each net, including positions HP1–HP5 for chemical assay for Phase I studies.

### Example ITN sampling schemes for wash resistance studies

Fabric samples are cut from ITNs in defined positions to capture fabric variability. Each fabric type in the constructed ITN must be sampled and tested separately.

#### A Rectangular ITN constructed from one fabric type



#### B Mosaic ITN constructed from two fabric types

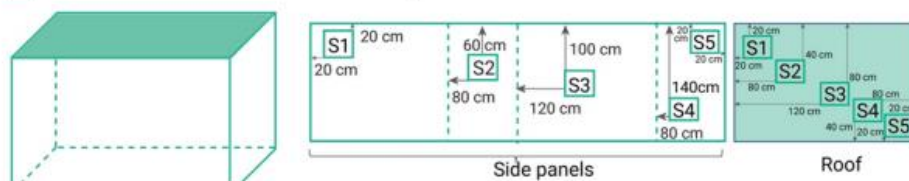
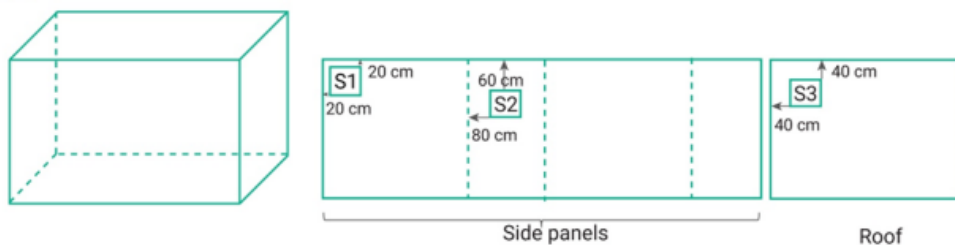


Figure 5 - Sampling scheme for wash resistance studies from 3.19 IG – Wash resistance study for ITN fabric from WHO PQ Implementation guidance documents for ITN dossier compilation.

### Example ITN sampling schemes for baseline quality checks

#### A Rectangular ITN constructed from one fabric type



#### B Mosaic ITN constructed from two fabric types

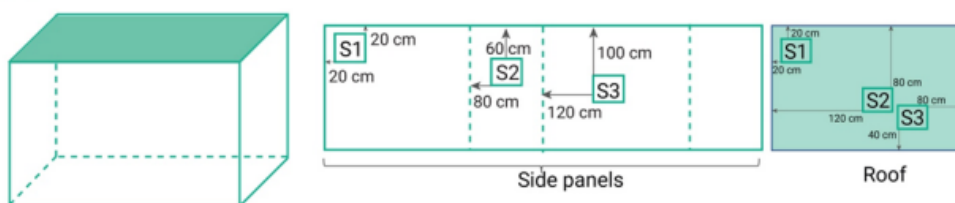
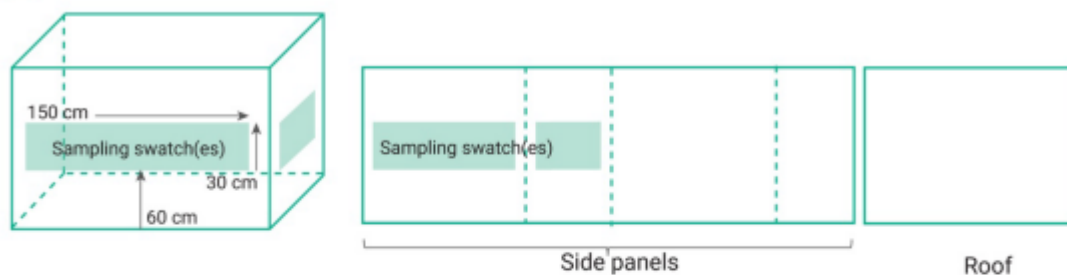


Figure 6 - Sampling scheme for baseline quality checks from 3.19 IG – Wash resistance study for ITN fabric from WHO PQ Implementation guidance documents for ITN dossier compilation.

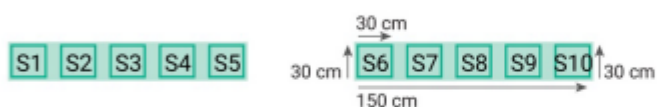
### Example ITN sampling schemes for regeneration studies

Fabric swatches are cut from ITNs in a row across the fabric to minimise fabric variability. Each fabric type in the constructed ITN must be sampled and tested separately.

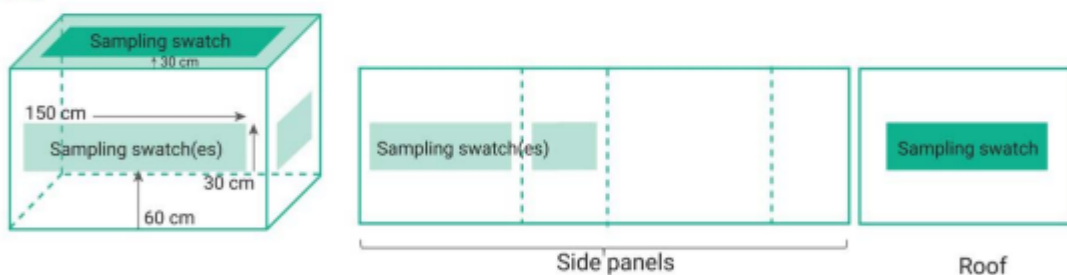
#### A Rectangular ITN constructed from one fabric type



Samples cut from fabric swatch(es)



#### B Mosaic ITN constructed from two fabric types



Samples cut from fabric swatch(es)

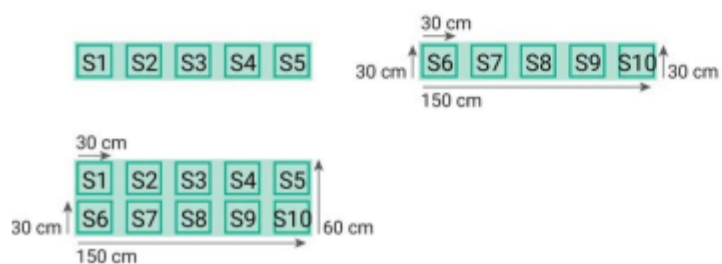


Figure 7 - Sampling scheme for wash regeneration studies from 3.12 IG – Regeneration study for ITN fabric from WHO PQ Implementation guidance documents for ITN dossier compilation.

## Net Processing Record

| Field                          | Description |
|--------------------------------|-------------|
| Whole net Unique ID Number     |             |
| Net subsample Unique ID number |             |
| Processing Date                |             |
| Subsample Pieces Details       |             |
| Processing Methods/Protocols   |             |
| Operator                       |             |
| Study/Project Number           |             |