

## Cutting and preserving net samples for chemical analysis

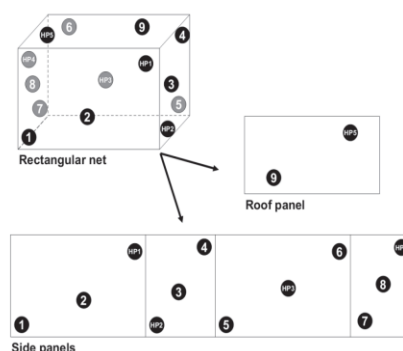
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**WHOPES Guidelines:** Guidelines for laboratory and field testing of long-lasting insecticidal nets

After cutting or testing, all netting samples should be properly labelled, wrapped individually in aluminium foil and stored at 4°C until they can be analysed for their insecticide content to determine their wash-resistance index. The insecticide content of each net sample should be analysed to estimate between- and within-net variation, and the density of netting (i.e. mass of net per unit area) should be measured. The samples should be analysed by the methods published by the Collaborative International Pesticides Analytical Council (CIPAC) or, if those are unavailable, with tests devised by the manufacturer and validated. The results should be expressed in grams of active ingredient per kilogram as well as in milligrams of active ingredient per square metre of netting material. The decrease in insecticide content after successive washes can be used to estimate the wash-resistance index of the LN.

Preparation of nets for testing four candidate LNs are required for phase I studies, from at least two different production batches. From each net, 14 pieces (25 cm x 25 cm) are sampled, as shown in Figure 1. The tests conducted on the 56 pieces are as follows: - Eight pieces (four unwashed and four washed) are used to estimate regeneration time. - 28 net pieces are used to evaluate wash-resistance. Four pieces are tested after 1, 3, 5, 10, 15, 20 and 25 washes (4 x 7 = 28 bioassays), although only 20 washes are considered standard procedure for determining wash-resistance. If the manufacturer's claim cites more than 20 washes, additional net pieces may be cut and used for further washing and bioassays. After bioassays, the net pieces are tested in chemical assays to determine the wash-resistance index. - 20 pieces (five pieces from four nets) are wrapped in aluminium foil and held at 4°C for chemical analysis in order to determine the between- and within net variability. Net pieces should be handled with care to avoid contamination or excessive abrasion. Nets should be stored wrapped in aluminium foil at 30°C between washes.

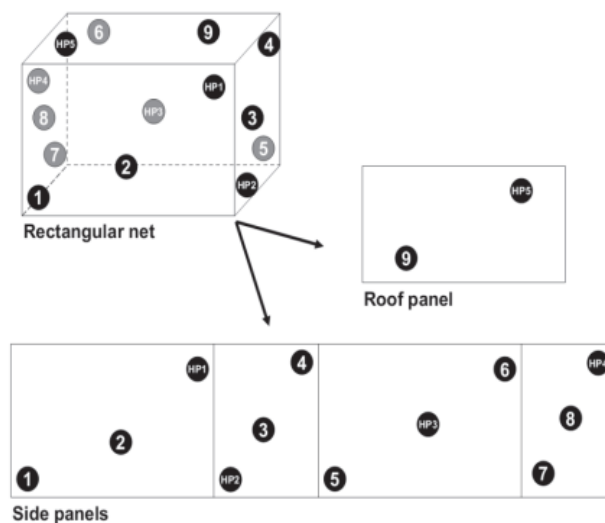
*Figure 1. Sampling scheme for 14 pieces of netting from each net, including positions HP1–HP5 for chemical assay. A different sampling scheme is required for combination nets.*



Procedure:

**1. Cutting net pieces from whole nets**

- a. **Wear personal protective equipment (lab coat, gloves, respirator)**
- b. Preparation of the cardboard stencil and supplies
  - i. Clean benchtop with 70% ethanol, then wipe with damp paper towel and dry with clean dry paper towel
  - ii. Draw squares (30cm x30cm) on two pieces of cardboard with a marker pen
  - iii. Cut out the squares to obtain two separate cardboard stencils of 30cm x 30cm
  - iv. Clean a pair of scissors with 70% ethanol and leave to dry on a clean benchtop
- c. Preparation of net sample
  - i. Remove net from package and put on clean table
    1. Start with control net and move to insecticide treated net pieces
    2. Change gloves for nets with different insecticides
  - ii. Identify areas to cut from the sides and top of the net for the chemical analysis according to the diagram below.
  - iii. Measure carefully with a ruler, mark around the cutting location with a permanent marker pen Cut out the net sample carefully following the drawn line
  - iv. Cut out five pieces of netting material (30cm x 30cm) from each net as shown below.





## **2. Preserving net samples**

- a. Prepare labels for net samples. Include the protocol number, net ID, position, date of cutting, technician initial
- b. Staple label on netting sample
- c. Wrap each sample in aluminium foil and label the foil
- d. Place all samples for each net together in one clean, labelled foil or plastic container
- e. Store at 4°C
- f. Return whole net to the original package, and seal using a bag sealer. Return to storage room.
- g. Send to laboratory for chemical analysis