

Net washing for Phase II LLIN Trials

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WHOPES: Guidelines for Laboratory and Field-Testing of Long Lasting Insecticidal Nets

Enough nets should be prepared for each treatment arm. The nets should be from three different production batches. One net from each batch should be retained for laboratory bioassays and chemical analysis. After netting pieces (30 x 30 cm) have been removed for baseline bioassays and chemical assays, the net is washed 20 times (or more, depending on the manufacturer's claim) as described below; then bioassays and chemical assays are performed on pieces cut from adjacent positions. The other five nets are then washed 20 times (or more as appropriate) and used in the hut trial. At the end of the trial, one of the five nets from each treatment arm is selected at random for bioassays and chemical assays. The pieces that are used for bioassays should be stored individually in aluminium foil at 4 °C until chemical assays are performed.

For washing, the nets should be placed in non-plastic bowls (e.g. aluminium) with 10 l of water and 2 g/l of soap such as savon de Marseille. The water should have a maximum hardness of 5 dh. The nets should be washed for a total of 10 min. It is suggested that the nets be agitated for 3 min, allowed to soak for 4 min and agitated again for 3 min. The nets should be agitated by stirring them with a pole at 20 rotations per minute. They should then be rinsed with clean water by a similar procedure, dried horizontally in the shade and stored at ambient temperature between washes. The time between two consecutive washes should correspond to the regeneration time determined in phase I studies.

Preparation of nets may take several weeks or even months for LN nets that have a long regeneration time and must be washed 20 times. To maintain equivalence between the various treatments in the trial, washing in each treatment arm should be completed at the same time, which means starting the washing of long-regeneration nets weeks or months earlier than those with a shorter regeneration time or those that are washed fewer times.

Once washing has been completed, bioassays should be conducted again (as described above) on all nets. One net is removed from each treatment arm and retained for chemical analysis (Fig. 1), and the remaining five nets are used in the experimental hut study. Six holes (4 cm x 4 cm) should be cut in each net, with two holes on each of the long side panels and one on each of the short side panels. The holes in the short side panels are located in the centre of the net, while those in the long side panels should be spaced evenly along the length of each panel; i.e. the first hole is made at one third of the distance and the second at two thirds of the distance from the edge of the side panel. The holes should be centred vertically on all sides of the net.

Net washing should be performed in the shade or indoors

Ensure that the washing is performed at a designated place where water can be safely disposed of with no access to passing people / animals

1. Net Washing Preparation

- a. Use a permanent marker and label aluminium washing bowls and paddles with the net type. Use only that bowl for washing that type of net to avoid contamination.
- b. Calibrate the buckets by pouring ten litres of water into each bowl with a calibrated measuring cylinder. Mark the 10-litre level with a permanent marker.
- c. Check the water hardness. Pour 100mL of water into a clean glass beaker and use the calibrated water hardness meter. Record this on the form. Water hardness must be ≤ 89 ppm. Water greater than 89ppm cannot be used for net washing.
- d. Check the water pH. Tear a strip from the pH test papers booklet and dip into the same beaker for 1-2 seconds. Estimate the value to the nearest whole number and record.
- e. Spray 70% ethanol into the aluminium washing bowl and wipe around the container with a paper towel to remove any possible contaminants. Leave for 5 minutes and then rinse the bowls twice with 10L of clean water.
- f. Weigh 20g of Savon de Marseille or a suitable locally available soap using a calibrated balance. Grind soap with a hand blender.
- g. Fill a jug with 1 litre warm water and pour the 20g of soap in. Stir until the soap dissolves. This is the “soap solution.”
- h. Pour the 1 litre of soap solution into an aluminium washing bowl.
- i. Repeat steps f-h for each aluminium washing bowl for each net type.
- j. Pour 9L of tap water into each aluminium container that contains 1L of “soap solution.”

2. Net washing

- a. Put on gloves.
- b. Prepare stop watch/ timer.
- c. Put the net in the bowl. Start the timer.
- d. Agitate the net for 3 minutes using the wooden paddle at 20 rotations per minute, taking care not to damage the net.
- e. Let the net soak for 4 minutes. Ensure all the net is submerged in the water.
- f. Agitate the net for a further 3 minutes using the paddle at 20 rotations per minute, taking care not to damage the net.
- g. Remove the net from the bowl and hang over a steel or nylon line.
- h. Empty the water into the insecticide-rinsate tank designated for waste disposal.
- i. Pour 10 litres of fresh water into the aluminium washing container.

- j. Put the net back in the bowl.
- k. Repeat steps d-e for the rinsing of the net, taking care not to damage the net.
- l. Remove the net from the bowl and hang over a steel or nylon line.
- m. Empty the water from the bowl into the designated insecticide-rinsate tank for disposal.
- n. Put 10 litres of fresh water into the bowl.
- o. Repeat step d-e to further rinse the net, taking care not to damage the net.
- p. Remove the net from the bowl and hang over a steel or nylon line.
- q. Empty the water from the bowl into the designated insecticide-rinsate tank for disposal.
- r. Remove the net from the bowl and hang over a steel or nylon line. Make sure the nets are left to dry in a place where they cannot be disturbed by passing cattle or children, or in an open area where they can be stolen.
- s. When net is completely dry, pack in the original packaging, then in a sealable plastic bag.
- t. Label the net bag using a permanent marker- write the date of washing, net code, number of this wash, and technician initials.
- u. Fill in the wash log
- v. Store net at ambient temperature between washes.

Fig. 1. Washing, bioassay and chemical assay of each of six net replicate nets for candidate and reference long-lasting insecticidal nets in an experimental hut trial

