SOP: Net washing for laboratory LLIN trials

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Prepared by

Name	Role	Institution
Alex Wright	Author	Consultant to I2I
Graham Small	Author	IVCC
CREC	Contributor	CREC
KCMUCo	Contributor	KCMUCo
Natalie Lissenden	Contributor	LSTM
Katherine Gleave	Contributor	LSTM
Annabel Murphy-Fegan	Contributor	LSTM

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1	30/10/2020	Angus Spiers	I2I
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Version Control[1]

Version	Date	Updated by	Description of update(s)
2	April – May 2022	Alex Wright, Natalie Lissenden	Purpose, materials & equipment, regeneration time

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

			information, data collection sheet information, glossary of terms and references added.
3	August 2024	Annabel Murphy-Fegan	Materials & equipment and purpose updated.

Related documents

- I2I Best Practice SOP Library, 30 October 2020 (https://innovationtoimpact.org/)
- WHO Guidelines for laboratory and field testing of long-lasting insecticidal nets (WHO, 2013)

1. Purpose

The objectives of laboratory testing are to determine the efficacy and wash-resistance of an LLIN and to study the dynamics of the insecticide on the netting fibre, including regeneration time (the time required to restore the biological efficacy of a net when the surface insecticide has been depleted by washing). The aim of these experiments is not to simulate washing under field conditions but rather to provide a standardized protocol to allow consistent comparisons among laboratories and among different LLIN products. This SOP details the procedure for washing net pieces for laboratory LLIN testing with the Shaker Bath Method and the CIPAC Method.

2. Background

Net samples (25 cm x 25 cm or 30 x 30cm) are introduced individually into 1-L beakers containing 0.5L deionized water, with 2g/L soap (using savon de Marseille or equivalent non-biological local soap) (pH 10-11) added and fully dissolved just before washing. The beakers are introduced into a water bath at 30°C±2°C and shaken for 10 minutes at 155 movements per minute. The samples are then removed from the bottle using tweezers and excess fluid is removed by gently shaking it several times in the air.Rinsed twice for 10 minutes in clean,

deionized water under the same shaking conditions as above, dried at room temperature and stored at 30°C in the dark between washes.

CIPAC Guidelines (in Annex 3 of WHOPES Guidelines):

The CIPAC method 4827/m was provisionally accepted in 2012 for determination of wash resistance index of LNs.31 In this method, a stock solution of the CIPAC washing agent is prepared as follows. Heat one bottle of polyoxyethylene glycol (25) monostearate (CAS number 9004-99-3 or 37231-60-0) to approximately 50°C to melt and reduce its viscosity. Turn the bottle 180° a few times to ensure homogeneity. In a suitable glass flask, add 80 ml of water, 12 g of sodium oleate (CAS number 143-19-1) and 8 g of polyoxyethylene glycol (25) monostearate. Heat the mixture to approximately 50°C, turning 180° frequently or stirring with a magnetic stir bar until the mixture becomes clear and homogeneous. The CIPAC washing agent can be used for up to 4 weeks if kept sealed in the dark at 4 °C. For each wash, 2.5 ml of the stock CIPAC washing agent solution is added to 500 ml of de-ionized water at 30 °C ± 2 °C in a 1-l glass bottle. A piece of netting (25 cm x 25 cm) is inserted and the bottle capped and inverted 10 times. The bottle is turned 180° by hand and brought back to its upright position with both these steps being completed in approximately 2 seconds. The bottle is then placed in a water bath or in an oven with thermostat at 30 °C ± 2 °C in an upright position free from vibration for 10 minutes, after which the piece of netting is removed with tweezers, and excess fluid is removed by gentle shaking. After washing, the piece of netting is rinsed twice by placing it in a 1-l glass bottle containing 500 ml of de-ionized water at 30°C ± 2°C. The bottle is capped, inverted 10 times and placed in a water bath or in an oven with thermostat in an upright position free from vibration for 10 minutes. The sample is then removed with tweezers. After the second rinse, excess water is removed by gently shaking the net sample, which is then allowed to dry on a line for 30 minutes at room temperature out of direct sunlight. Once dry, the net samples are folded once or twice in each direction, placed in a bottle which is then closed and stored at 40°C ± 2 °C for 22 hours ± 2 hours before starting the next washing cycle. The washing–rinsing–heating process is repeated 3 more times. After the 4 wash cycles, the net samples are analysed using the appropriate CIPAC method for determination of total active ingredient content, and the wash resistance index is calculated using the following equation: w =

100 x 4√(t4/t0) where: w = wash resistance index, expressed as a percentage; t4 = total active ingredient content (in g/kg) after 4 washing cycles; and t0 = total active ingredient content (in g/kg) before washing (no washing).

3. Materials & Equipment

- General
    - Calibrated weigh scale
    - Thermometer for measuring water temperature (with probe)
    - Water heater
    - Two 20L buckets
    - Lab coat
    - Gloves
    - 70% ethanol
- Shaker Bath method (in addition to above material)
    - Savon de Marseille soap (or equivalent non-biological local soap, pH 10-11)
    - Grater or blender
    - Calibrated water hardness meter
    - Calibrated pH meter or pH test strips
    - Water deionizer
    - Shaker bath
    - 1L bottles with lid
    - Label
    - Marker pen
    - Funnel
    - Forceps
    - Tweezers
    - Three small glass beakers
    - 10% bleach

- Paper towel
- Jerry can for water waste
- Washing lines
- Clothes pegs
- 70% Ethanol
- Aluminum foil

- CIPAC Method (in addition to general items)
  - Low temperature oven
  - Polyethylene glycol monostearate (PEG) stock
  - Sodium oleate powder
  - Recording sheet
  - Labels
  - Marker pen
  - +4°C Refrigerator
  - Three 1L glass bottles per net piece
  - Calibrated pipette to measure 2.5mL
  - Timer
  - Forceps

4. Procedure

a. Prepare a weighing balance, one thermometer, one water heater and two graduated buckets of 20L
b. Wear lab coat and gloves
c. Prepare wash room by cleaning surfaces with 70% Ethanol and leaving to dry for 30 minutes.

d. Net washing using Shaker Bath
    i. Preparation of washing soap solution

1. Weigh 30g Savon de Marseille soap flakes (or other suitable non-biological soap) and transfer into a 20L bucket
2. Add 1L of hot deionised water, cover the bucket to allow the soap to be dissolved and then stir until the soap is fully dissolved
3. Add 14L of deionised water to the bucket for a total volume of 15L giving a soap solution of 2g/L (The mass of soap and volume of water can be adjusted according to the number of net pieces to be washed)
4. If using tap water, this should have a maximum hardness of 5 dH
5. Ensure the temperature of the soap solution is always within the range of 30 ± 2 °C. This can be controlled by water heater or ice pack to increase or decrease the temperature respectively.
6. Take the pH of the washing solution using litmus paper. Record the pH on the form for each net piece being washed

ii. Preparation of the water for rinsing

1. Add 15L of deionized water in the second bucket and ensure that the temperature is also adjusted at 30°C ±2 as described above

iii. Preparation of the water bath

1. Add tap water into the water tank of the shaker (maximum level of the tank according to the manual)
2. Set the temperature of the water bath to 30°C and the shaking frequency at 155 / min. (Check by counting)
3. Turn on the shaker and wait until the temperature reached 30°C
4. Use a calibrated thermometer and adjust the temperature if required

iv. Washing net pieces using WHO method

1. Set up a 1L glass graduated bottle with lid for each net piece to be washed.

2. Retrieve the net sample to be washed. Record the test item code on the test form.
3. Write the test item code on a label and stick it onto the 1L glass bottle. Position the label on the neck of the bottle to prevent it getting wet.
4. Put 500 ml of the soap solution into each bottle using a funnel if necessary
5. Put each piece of net to be washed into the bottle labelled accordingly
6. Close the bottle, invert it and shake by hand to wet the sample
7. Add the bottles to the shaking carriage. The bottles should be covered by the water up to the 500mL mark on the bottles.
8. If washing less than eight net pieces use bottles containing 500mL water to fill all the slots. Close the lid.
9. Once the bath is up to temperature, put a probe of a calibrated temperature logger inside one of the bottles. Once the temperature of the washing solution inside the bottles reaches the same temperature, the net pieces can be added to the bottles. Record the temperature for each net piece being washed. The acceptable range is ±2°C.
10. Prepare the control net pieces.
    a. Prepare three small glass beakers for cleaning forceps: one for distilled/deionized water, one for 10% bleach solution and one for tap water. Fill each beaker with corresponding liquid and label the beakers.
    b. Using forceps, place the control net pieces into the 1L bottle. Replace the bottle lid and invert the bottle to ensure net is completely submerged.

c. Repeat this for each of the other net pieces and ensure forceps are cleaned between each use to prevent any possible cross contamination. Wash the forceps in the bleach solution for a few seconds, then in tap water for a few seconds, and finally in deionised/distilled water. Wipe the forceps on a clean paper towel and be careful not to recontaminate them.

11. Begin the washing cycle. Set the required duration.
12. Once time is up the alarm of the shaker will ring. Turn the shaker bath off. Lift lid and remove bottles.
13. Empty the solution contents of the bottles into the jerry can. Hold the net pieces using forceps so they don't fall out of the bottles. Clean the forceps between each use.
14. Begin rinsing stage.
    a. Add 500mL of deionised water to each 1L bottle. Place 1L bottles back into the water bath and repeat steps xvi to xx.
    b. Repeat for a second rinse. The default is wash (10 minutes), rinse one (10 minutes), rinse two (10 minutes).
15. Once all the washing and rinsing steps have been completed, remove the nets from the bottles one at a time (using forceps and cleaning forceps between each use). Carefully remove any remaining water drops by gently shaking the net pieces.
16. Hang the pieces on the washing lines in the Wash Room. Make sure pieces do not touch each other and make sure control pieces are hung in a control area. Discard clothes pegs after single use or decontaminate with 70% ethanol.

17. After one hour, remove the nets from the lines, wrap in aluminium foil, and label the foil on the outside with the net piece ID number. Store pieces at 30 ±2°C between washes.
18. Regeneration time may differ between AIs. Check the regeneration time requirements from sponsor and in the protocol before planning wash schedule.

e. Net washing using CIPAC method

i. Calculations

1. Determine the amount of net pieces/ whole nets that are being washed from the protocol and record on the form.
2. Multiply the number of pieces with the number of required washes to determine the number of piece-washes.
3. Determine the amount of CIPAC solution needed by reading the line for the corresponding number of piece-washes in the table for Phase I or Phase II.
4. Record this as the amount of CIPAC solution needed e.g. for 80 pieces-washes 200mL of CIPAC solution is needed.

ii. Preparing the CIPAC washing solution (Stock solution)

1. One day before the washing, heat the low temperature oven to 50°C to melt the polyethylene glycol monostearate (PEG) overnight.
2. Once oven is 50°C, put the PEG stock bottle in the middle of the oven and leave for a minimum of 12 hours.
3. On the morning of washing, remove the bottle from the oven and let it cool.
4. Determine the amount of PEG and sodium oleate needed from the table on the form (as described above, when the table is inserted), e.g. for 200mL of CIPAC solution 20g of PEG and 30g of sodium

oleate are needed. Record on form the date solution is made, the chemical code for the ingredients, the amounts used of each ingredient and the equipment used to make the solution (e.g. oven, weigh scales).

5. Measure the deionized water for the CIPAC solution in a glass beaker or cylinder e.g. for 200mL solution you need 200mL deionized water. Record on the form. Take the reading from the meter, bucket and direct and record the protocol number. Pour the deionized water into the washing solution container.
6. Pour the deionized water in a 250mL glass bottle fitted with screw cap.
7. Weigh the sodium oleate powder and add this to the deionised water in the glass bottle.
8. Add the required PEG to the glass bottle.
9. Heat the mixture in the low-temperature oven at 50°C for 30 minutes.
10. Remove the mixture from the oven and shake until the mixture becomes clear and homogenous.
11. CIPAC solution can be used up to four weeks if the bottle is kept sealed in the dark in a refrigerator. Label the bottle with:
    a. CIPAC Solution
    b. Date of preparation
    c. Expiry date
    d. Initials of staff making the solution

iii. Washing the net pieces (CIPAC)

1. For each net piece being washed, set up three 1L glass bottles with lids.
2. Each piece is labelled with a unique net piece number.

3. Write the test item code for each net piece onto a label (masking tape) and stick onto the 1L glass bottles. Position the label on the neck of the bottle to avoid it getting wet.
4. Fill all the 1L glass bottles with 500mL deionised water. Three bottles should be prepared for each net piece (1 for washing solution and 2 for rinsing).
5. Switch on the water bath.
6. Set the temperature to 30°C. Acceptable range is ±2°C.
7. Insert the bottles in the water bath.
8. Check the temperature in one bottle using a calibrated thermometer probe. Once the temperature is in range, add 2.5mL of the stock CIPAC solution to all washing solution bottles.
9. Insert the net pieces into the bottles using forceps and cap the bottles. Ensure that the forceps are cleaned between each use.
10. Invert the bottles 10 times to ensure the net piece is completely submerged.
11. Leave the bottles in the water bath for 10 minutes. Use a timer.
12. After 10 minutes, remove the net samples from the water using forceps. Ensure that the forceps are cleaned between each use to prevent cross contamination between net pieces.
13. Insert the net samples in the first set of rinse bottles (i.e. with deionized water only), invert the bottles as before and leave for 10 more minutes.
14. Insert the net samples in the second set of rinse bottles (i.e. with deionized water only) invert the bottles as before and leave for 10 more minutes).
15. After the last rinsing, pull out the net samples from rinsing water with forceps, cleaning the forceps between each use.

16. Carefully remove any remaining water drops by gently shaking the net pieces.
17. Allow the samples to dry for one hour at room temperature on a line- follow same principles of the shaker bath method for drying.
18. Once dry, fold the samples carefully twice in each direction, place them loosely rolled in a glass bottles and cap them.
19. Store the bottles in the low temperature oven at 40°C ± 2°C for 22 ±2 hours before starting the next wash cycle. If all washes are complete, return the piece to the box to a 4°C refrigerator.

Collection and reporting of data

Ensure the following data is recorded in the data collection sheets:

- CIPAC method
    - Protocol number
    - Test item number
    - Amount of CIPAC solution made
    - Date CIPAC solution was made
    - Sodium oleate chemical code and amount used
    - PEG chemical code and amount used
    - Date of expiry
    - Solution made by (staff name)
    - Study Director signature
- Shaker Bath method
    - Protocol number
    - Data logger ID number
    - Soap brand
    - Staff name
    - Date of washing
    - Net piece IDs

- Temperature start and end of washing

5. Glossary of terms

AI	Active Ingredient
CIPAC	Collaborative International Pesticides Analytical Council
dH	Degree hardness
LLIN	Long-lasting insecticidal net
PEG	Polyethylene glycol monostearate
SOP	Standard Operating Procedure
WHO	World Health Organization

6. References

WHO. (2013). Guidelines for laboratory and field-testing of long-lasting insecticidal nets. In *WHO/HTM/NTD/WHOPES/20131*. World Health Organization.

Innovation to Impact
Pembroke Place
Liverpool L3 5QA UK

contact@innovation2impact.org
+44 151 702 9308

BILL & MELINDA GATES foundation

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