Standard operating procedure for testing the susceptibility of adult sand flies to insecticides in WHO bottle bioassays

World Health Organization

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Contents

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Main contributors (organized alphabetically by country): Mr Debashis Ghosh, Dr Dinesh Mondal (International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh); Mr Rajib Chowdhury (Department of Public Health, Independent University, Bangladesh, Dhaka, Bangladesh); Dr Ademir J Martins, Dra Margarete Martins dos Santos Afonso (Fundação Oswaldo Cruz, Rio de Janeiro, Brazil); Dr Martha Liliana Ahumada (Grupo de Entomología, Instituto Nacional de Salud, Bogotá, Colombia); Dr Sisay Dugassa (Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia); Dr Vincent Corbel, Mr Stephane Duchon, Dr Dominique Cerqueira (Institut de Recherche pour le Développement, Montpellier, France); Dr Kamaraju Raghavendra, Dr Vaishali Verma (Indian Council of Medical Research – National Institute of Malaria Research, New Delhi, India); Dr Kesari Shreekant, Dr Diwakar Singh Dinesh, Dr Krishna Pandey (Indian Council of Medical Research – Rajendra Memorial Research Institute of Medical Sciences, Patna, India); Dr Luna Kamau, Dr Damaris Matoke-Muhia, Dr Josyline C. Kaburi, Ms J. Milkah Mwangi (Centre for Biotechnology Research and Development, Kenya Medical Research Institute, Nairobi, Kenya); Dr Elyes Zhioua, Dr Ifhem Chelbi (Vector Ecology Unit, Institut Pasteur de Tunis, Tunis, Tunisia).

External contributors (experts who participated in a WHO consultation and peer review): Prof Arti Prasad (Public Health Entomology Laboratory, Mohanlal Sukhadia University, Udaipur, India); Prof Ahmad Ali Enayati (School of Public Health, Mazandaran University of Medical Sciences, Sari, Islamic Republic of Iran); Dr Adanan Che Rus (Vector Control Research Unit, Universiti Sains Malaysia, Penang, Malaysia); Dr João Pinto (Chair) (Instituto de Higiene e Medicina Tropical, Lisbon, Portugal); Dr Thomas Churcher (Imperial College, London, United Kingdom of Great Britain and Northern Ireland); Dr Rosemary Lees (Liverpool School of Tropical Medicine, Liverpool, United Kingdom); Dr Audrey Lenhart (Entomology Branch, Centers for Disease Control and Prevention, Atlanta (GA), United States of America).

Other stakeholders:[1] Dr Susanne Stutz (BASF SE, Limburgerhof, Germany); Dr Sebastian Horstmann (Envu, Mosquito Management, Monheim, Germany); Dr Mark Hoppe (Syngenta, Basel, Switzerland); Dr John Lucas (Consultant, Sumitomo Chemical Co. Ltd, London, United Kingdom); Dr Barnabas Zogo (Sumitomo Chemical Co. Ltd, London, United Kingdom); Dr James W. Austin (BASF Corporation, Durham (NC), United States of America).

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1 The other stakeholders were representatives of the commercial entities who provided technical comments on the SOP with observer status. The stakeholders did not participate in finalization of the SOP.

Declarations of interest and confidentiality undertaking

WHO reported that it had received and reviewed declarations of interest and confidentiality undertakings from all the external contributors (i.e., experts who participated in the WHO consultation and peer review process) and had concluded that none could give rise to a potential or reasonably perceived conflict of interest related to the subjects discussed at the meetings.

1. Introduction, scope and purpose

This SOP describes the process for evaluating the susceptibility of adult sand fly vectors (*Phlebotomus* spp. and *Lutzomyia longipalpis*) to insecticides in the WHO bottle bioassay. The WHO bottle bioassay is a direct response-to-exposure test, in which sand fly mortality is measured after 1 h of exposure to a known standard concentration (e.g., the discriminating concentration, DC) of an insecticide. The mortality of test sand flies is recorded at 24 h (or more for slow-acting compounds) after the 1-h exposure period. Instructions are provided on preparing and coating bottles with insecticide, exposing sand flies, and recording and interpreting test results.

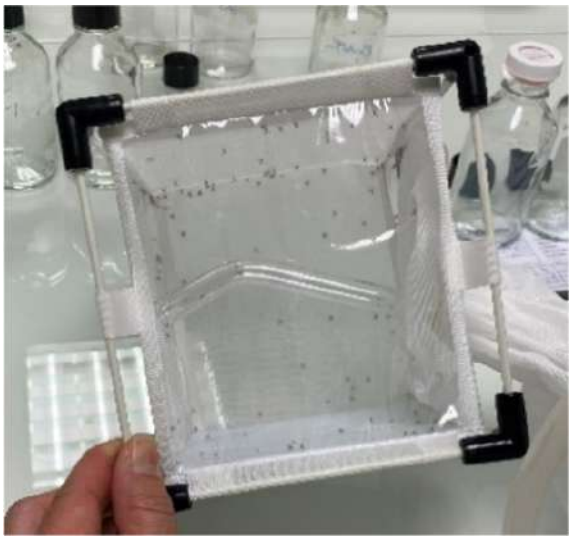
The WHO bottle bioassay was first developed for testing mosquitoes by modifying the bottle bioassay of the United States Centers for Disease Control and Prevention to harmonize the test end-points with those of the WHO tube tests, so that vector mortality can be evaluated and recorded at the same time after exposure *(1,2)*. The bottle bioassay SOP for mosquitoes has now been further modified to test the susceptibility of sand flies to insecticides that are unstable or cannot be impregnated on usual Whatman No. 1 filter papers.

The initial draft of the SOP was prepared by Dr Vincent Corbel (IRD) and Dr Rajpal S. Yadav (WHO) and discussed at a WHO consultation on 29 June 2022 that was convened to review results of a WHO multi-centre study on determining discriminating concentrations of insecticides for monitoring resistance in sand flies *(3)*. Thereafter, a revised draft of the SOP incorporating the technical comments of study investigators, experts to WHO and other stakeholders listed above was peer reviewed. The peer reviewed SOP was finalized in consultation with the main contributors and the experts who had previously participated in the WHO consultation.

Although the WHO bottle bioassays for mosquitoes are also used to test vector susceptibility to insect growth regulators (e.g., juvenile hormone mimic compound pyriproxyfen), certain additional steps are required to measure the sterilizing properties of juvenile hormone mimics (i.e., oviposition inhibition and reduced fecundity) post-exposure. An SOP is available for testing insect growth regulators with mosquitoes *(4)*, but it has not yet been validated for sand flies. Therefore, this SOP should not be used for testing the susceptibility of sand flies to insecticide growth regulators that reduce insect fecundity and fertility. For insecticides that can be impregnated on filter papers, the SOP for WHO tube tests with sand flies should be followed *(5)*.

2. Equipment, reagents and consumables

☐	250 mL Wheaton® bottles with screw caps – 6 bottles per insecticide compound to be tested. If the bottles are washed and reused, ensure that they have been cleaned properly by following the "Bottle washing" procedure described below. **Note:** The total volume of a Wheaton bottle graduated up to 250 mL is 310 mL.
☐	technical-grade insecticide (active ingredient [AI]) or its stock solution
☐	butter (greaseproof) paper for weighing insecticide (or aluminium micro cups)

☐	2 weighing spatula
☐	a microbalance (electronic balance)
☐	appropriate surfactant, if necessary (e.g., MERO®, 81% rapeseed oil methyl ester, manufactured by Envu)
☐	a vortex mixer
☐	a set of calibrated micropipettes (e.g., 100 µL, 200 µL and 1000 µL)
☐	2 amber glass bottles (volumes 50–100 mL each). Alternatively, any clean glass bottle wrapped in aluminium foil can be used.
☐	6 labels (4 for insecticide coated bottles; 2 for control bottles)
☐	2 permanent marker pens for labelling bottles, caps and pipettes
☐	paper cups or bowls for holding adult sand flies (250 mL; 9 cm height x 7 cm width) (i.e., a storage chamber for a batch of 25 test sand flies)
☐	narrow mesh netting (< 500 µm hole size) in sufficient quantities to cover the mouth of the bottles and cups or bowls to prevent escape of sand flies and rubber bands to secure the netting.
☐	sand fly cages (minimum dimensions 20 cm length x 20 cm width x 20 cm height)  *Source*: Photo courtesy of Stéphane Duchon, Institut de Recherche pour le Développement, Montpellier, France

☐	2 aspirators with bent end for collecting sand flies (Note: battery-powered glass aspirators should be used to avoid any exposure to insecticides or inhalation of allergens (Note: use glass aspirators and mouthpieces to prevent generation of static electricity).
☐	glucose to prepare 10–50% (w/v) sugar solution in water (Note: different laboratories use different concentrations of sugar solution. Select a concentration that causes the lowest mortality rate in sand flies in control replicates within the range of acceptable mortality as per WHO guidelines).
☐	2 pieces of rubber tubing, each 60 cm long
☐	testing chamber or a climate-controlled insectary at 27 °C ± 2 °C and 75% ± 10% relative humidity
☐	calibrated, traceable humidity and temperature monitors and data loggers
☐	a timer (stopwatch) a magnifying lens or binocular loupe
☐	data recording sheet, pens and pencils
☐	medical-grade cotton wool
☐	aluminium foil
☐	1 roll of adhesive tape
☐	appropriate personal protective equipment (e.g., laboratory coat, disposable latex gloves, safety glasses, face masks)
☐	acetone for preparing insecticide stock solution, rinsing glass bottles after use
☐	antibacterial cleaner, such as 70% isopropyl alcohol or ethanol for cleaning work table and fume hood
☐	a 20-L plastic container or a 20-L sink
☐	1 rack for holding bottles for drying

☐	disposable plastic tips for micropipettes (e.g., 100–1000 µL)
☐	disposable glass pipettes of various volumes (e.g., 2 mL, 5 mL, 10 mL)
☐	TFD4 or Decon 90 (for cleaning equipment in contact with chemical compounds including AIs)

3. Health, safety and environmental protection

✓	Before using any chemical compound, laboratory staff should read and understand the risk assessment, material safety data sheets and the control of substances hazardous to human health.
✓	Appropriate personal protective equipment must be worn at all times when handling insecticides, including laboratory coat, gloves, safety glasses and a face mask when weighing out chemicals.
✓	Laboratory staff/technicians should ensure that ensure that all working areas are clear of other materials and cleaned before performing the test.
✓	All staff working in the laboratory must have received laboratory induction training, and the training must be documented in the individual's training file.
✓	All staff who use this procedure must be trained in safe operation of chemical fume hoods.
✓	Laboratory staff/technicians should ensure that dispose of all waste materials appropriately according to national and institutional safety guidelines.
✓	When working with sand flies, minimize their escape by keeping all doors and windows shut. If any sand flies escape, immediately use an electric bat to electrocute them.

4. Sand flies

For monitoring the resistance of a wild population of sand flies, F1 adults (progeny of wild-caught females) of the same age should be used. The bottle bioassays require 150 non-blood-fed adult female sand flies aged 2–7 days[2]. The sand flies should be starved (no sugar meal) for 2 h before testing.

During rearing, sand flies must be well nurtured and maintained in uncrowded pots or trays during the larval stages and in uncrowded cages during the adult stage. This is important to minimize mortality due to causes other than exposure to the insecticide.

During the 1-h exposure period, the number of sand flies per bottle should be 25 or as close to 25 as possible, but it should not exceed 25 to avoid crowding. Ideally, 150 active sand flies are aspirated (in batches) from a sand fly cage into six bottles (4 coated and two control bottles), to give six replicate samples of 25 sand flies per bottle.

5. Preparation of stock solutions of discriminating concentrations

Some insecticides can be dissolved in acetone alone, but others (e.g., clothianidin) tend to crystallize, and uptake of a crystallized insecticide by the insect's body is very low. Therefore, a surfactant such as MERO® should be added to acetone before preparing the stock solution in order to prevent crystallization of the insecticide. Preliminary studies in a WHO multi-centre study with sand flies *(5)* indicated that a concentration of 800 ppm was adequate for WHO bottle bioassays with sand flies (*Phlebotomus* spp. and *Lutzomyia* spp.). This concentration may, however, be readjusted as new evidence appears.

Preparing and storing the stock solution

☐ 5.1 **Check the insecticide DC and quantity of surfactant:**

For each insecticide compound to be tested, check its DC, and add a suitable quantity of surfactant, if recommended.

In a WHO multi-centre study with sand flies *(3)*, the DC of clothianidin was found to be 4–10 µg/bottle dissolved in acetone + 800 ppm MERO (Table 1).

Annex 1 contains a spreadsheet for calculating the amounts of insecticide, solvent (acetone) and surfactant oil (MERO®) required to prepare the stock solution.

☐ 5.2 **Prepare a glass bottle to store the stock solution:** Take a clean light-proof glass bottle (amber-coloured or wrapped in aluminium foil if transparent) of appropriate volume, and label it with the stock solution concentration and date.

2 The WHO-recommended age for insects subjected to bioassays is generally 3–5 days old *(6)*. For practical reasons and because of the difficulty of obtaining large number of sand flies of synchronized age, the test procedures were developed and validated with 2–7-day-old, non-blood-fed sand fly females in a WHO-coordinated multi-centre study involving eight participating laboratories worldwide *(3)*.

☐ 5.3 **Prepare the stock solution:**

Prepare an electronic balance: Place a piece of clean weighing paper (or aluminium micro cup) on the scale and set the reading to zero.

With acetone as the solvent: To prepare a test concentration of 25 µg of AI for a 250-mL glass bottle, weigh 25 mg of AI with a clean spatula, and dissolve it completely in 1 L of acetone (solvent) or any other solvent/surfactant mixture recommended by the manufacturer (e.g., MERO® + acetone, see below). To prepare a small volume of the stock solution (e.g., 5 mL), put 125 µg of AI in the bottle and add 5 mL of solvent or surfactant. This stock solution will contain 25 µg of AI per mL of stock solution.

With acetone + MERO® (800 ppm concentration) for testing all sand fly species: Pipette out 89 µL of MERO® and add it in 100 mL of acetone to a bottle or, for a smaller volume, pipette out 8.9 µL of MERO® add it in 10 mL of acetone.

☐ 5.4 **Store and use the stock solution**

Keep the stock solution bottle tightly closed to avoid evaporation of the acetone, and store the bottle in a refrigerator at 4–8 °C for a maximum of 2 months.

To use the stock solution, take the bottle out of the refrigerator, bring it to room temperature unopened (usually for 30 min to 1 h), pipette out the required volume, immediately close the bottle and put it back in the refrigerator for future use.

Note: If the bottle containing the stock solution is opened without being brought to room temperature, water vapour may condense in the bottle and hydrolyse the insecticide, or the coating solution in the bottle may not dry properly because of the presence of water molecules.

Table 1. Insecticide discriminating concentrations for WHO bottle bioassays with susceptible *Phlebotomus* spp. and *Lutzomyia* spp. sand flies (24 h bottle drying time; 1 h exposure time; 24 h holding and recording time)

Insecticide class	Insecticide	Species	Discriminating concentration (1-h exposure)[a]	Solvent and surfactant
Neonicotinoids	Clothianidin	*Phlebotomus papatasi*	4 µg/bottle	Acetone + MERO 800 ppm
		Phlebotomus argentipes	4 µg/bottle	
		Phlebotomus dubosqui	6 µg/bottle	
		Phlebotomus longipes	10 µg/bottle	
		Lutzomyia longipalpis	4 µg/bottle	

MERO: 81% rapeseed oil methyl ester.

[a] These DCs are based on a recent WHO multi-centre study on sand flies *(3)* in which clothianidin only was tested. This table will be updated in subsequent versions of the SOP when DCs for more insecticides become available.

6. Bottle bioassay procedure

Step 1. Coating of bottles

☐ 6.1 **Prepare clean, dry bottles:** Take clean, empty 250-mL glass bottles with screw caps in the required numbers for the bottle assay, and dry them in an oven for 20 min or in open air for 1–2 h, depending on the humidity (Fig. 1).

Fig. 1. Open bottles and caps being dried horizontally in a dark place

Source: Photo courtesy of Stéphane Duchon, Institut de Recherche pour le Développement, Montpellier, France

☐ 6.2 **If the stock solution and surfactants have been stored in a refrigerator:** Take the bottles out of the refrigerator and bring them to room temperature by leaving them for 30 min to 1 h without opening the container cap.

☐ 6.3 **Label both the bottles and the caps with the insecticide name, insecticide concentration (µg/bottle) or control and the date of the test.**

6.4 **Coat the interior and cap of the control and exposure bottles:** Coat the control bottles first with solvent solution and then the test bottles with insecticide. Coat the bottles one by one following the steps below and shown in Fig. 2.

6.4.1 Hold the bottle at an approximately 45° angle with its mouth pointing upwards. Add 1 mL of acetone to the control bottle and 1 mL of insecticide stock solution to the exposure bottle with a pipette. Put the cap on the bottle and rotate the bottle at least five times to coat the base of the bottle (Fig. 2, step 1).

6.4.2. Tilt the bottle at a slight angle with the cap facing downwards, and rotate the bottle at least five times to coat the top portion of the bottle and the cap (Fig. 2, step 2).

6.4.3 Tilt the bottle at an approximately 45° angle with the lid pointing downwards, and rotate at least five times to coat the lid and neck of the bottle (Fig. 2, step 3). Repeat steps 6.4.1 to 6.4.3 once.

6.4.4 Lay the bottle on its side on a flat surface, and rock it back and forth at least 10 times; then, flip it once and rock it an additional 10 times or more to ensure that all sides are coated (Fig. 2, step 4).

6.4.5 Repeat steps 6.4.1–6.4.4 three times. Place the bottle in a fume hood, and turn the power on to start the evacuation fan.

6.4.6 Remove the bottle cap to evaporate the acetone. Place the cap of the bottle face up inside the fume hood with the inner side of the cap exposed to the air.

Note: Acetone evaporation might build some pressure in the bottle. This is a normal condition, so be cautious when removing the cap from the bottle.

6.4.7 Hold the bottle at approximately a 45° angle with the neck of the bottle pointing upwards. Rotate at least 5 times to coat the base of the bottle (Fig. 2, step 5).

6.4.8 Tilt the bottle at a slight angle with the neck of the bottle facing downwards, ensuring not to drain any remaining liquid, and rotate at least 5 times to coat the top portion of the bottle (Fig. 2, step 6).

6.4.9 Repeat steps 6.4.7 and 6.4.8 twice.

6.4.10 Lay the bottle on its side in the fume hood. Rock the bottle back and forth at least 10 times before flipping and rocking it an additional 10 times or more to ensure that all sides are coated (Fig. 2, step 7).

6.4.11 Repeat steps 6.4.7–6.4.10 three times until the solvent has evaporated and is no longer visible. Check for solvent by holding the bottle at an approximately 45° angle with the neck pointing upwards, and wait to see if any solvent pools at the base of the bottle.

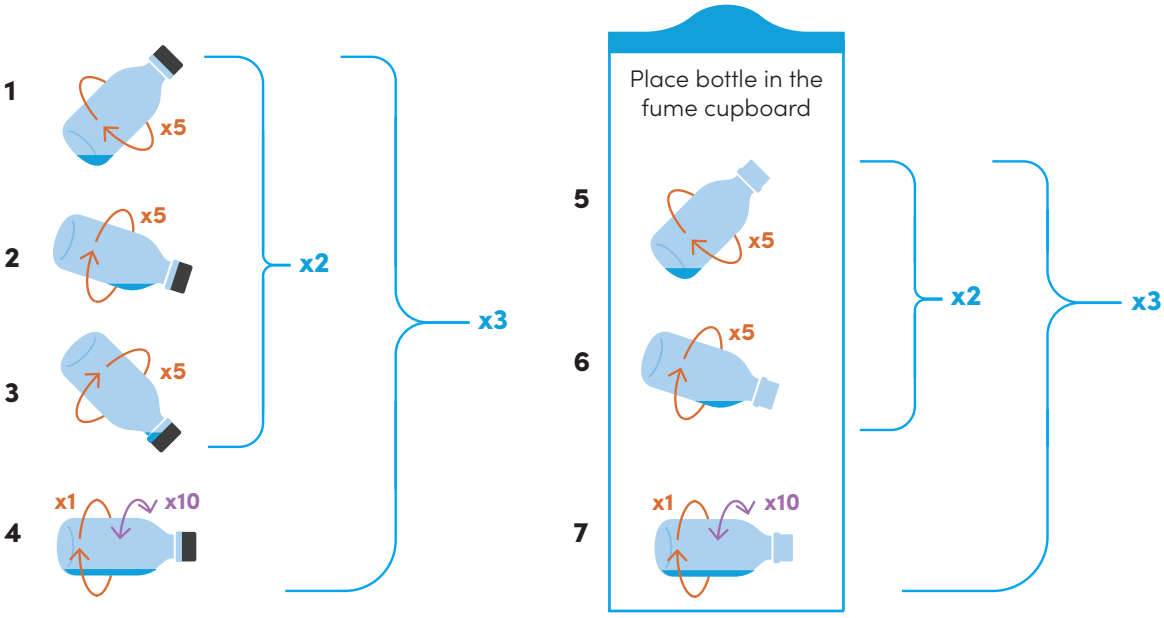
☐ 6.5 **Let the bottle dry for 24 h:** Wrap the bottle in aluminium foil to protect the contents from light. Leave the open bottle and the cap in an undisturbed fume hood (or separate room with ventilation) for 24 h.

Note: The bottles and caps should dry for 24 h; therefore, the interval between bottle impregnation (coating) and the bioassay is 24 h. While the bottles and caps are drying, the fume hood must remain switched on and should not be cleaned or used by other operators. Other equipment and glassware, e.g., pipettes, may be cleaned and removed from the fume hood, but cleaning must not interfere with the bottles drying.

☐ 6.6 **Store the bottles:** Put the caps on the bottles, and keep them in a dark, cool, dry place until use.

Note: The procedure for uniformly coating the bottles can be practised initially by coating one or more bottle with a mixture of acetone and some colouring agent (e.g., a dye or ink).

Fig. 2. Procedure for evenly coating bottles with the acetone or surfactant mixture



Source: Photo courtesy of Institut de Recherche pour le Développement, Montpellier, France

Step 2. Exposure of sand flies

6.7 **Prepare cages with female sand flies:** Female sand flies are more difficult than mosquitoes to identify and pick up directly from the rearing cages in order to introduce them into the bottles because of their small size and high agility. It is therefore recommended a cage containing only adult female sand flies be prepared just before a bioassay to avoid inadvertent selection of males.

6.8 **Prepare the bottles for introduction of sand flies:** Line up the bottles with their caps screwed on loosely so that the sand flies can be introduced easily. Place a piece of netting over the mouth of each bottle, and fasten it with a rubber band.

Note: Adult female sand flies aged 2–7 days should be starved for 2 h before testing.

6.9 **Aspirate and insert sand flies into the bottles**: Insert sand flies first into all the control bottles and then into the insecticide-coated bottles (Fig. 3). Use separate aspirators for the control and insecticide-coated bottles.

6.9.1 Preferably with a mechanical aspirator, introduce 25 non-blood-fed sand flies in batches of 5 into each bottle.

6.9.2 Insert the aspirator with sand flies into the bottle through the hole in the netting piece (Fig. 4A) or a rubber flap (Fig. 4B) that covers the mouth. Position the aspirator's opening in the central part of the bottle, and gently tap the sand flies into the bottle (Fig. 4A).

6.9.3 Quickly place the cap on the bottle to prevent the sand flies from escaping.

6.9.4 Place the bottle in a vertical position, and start the stopwatch to record an exposure time of 1 h.

Fig. 3. Exposure of sand flies in bottles

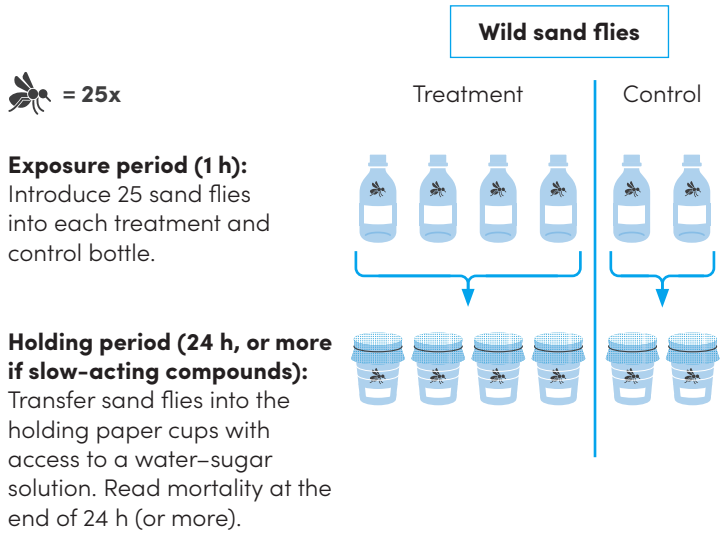
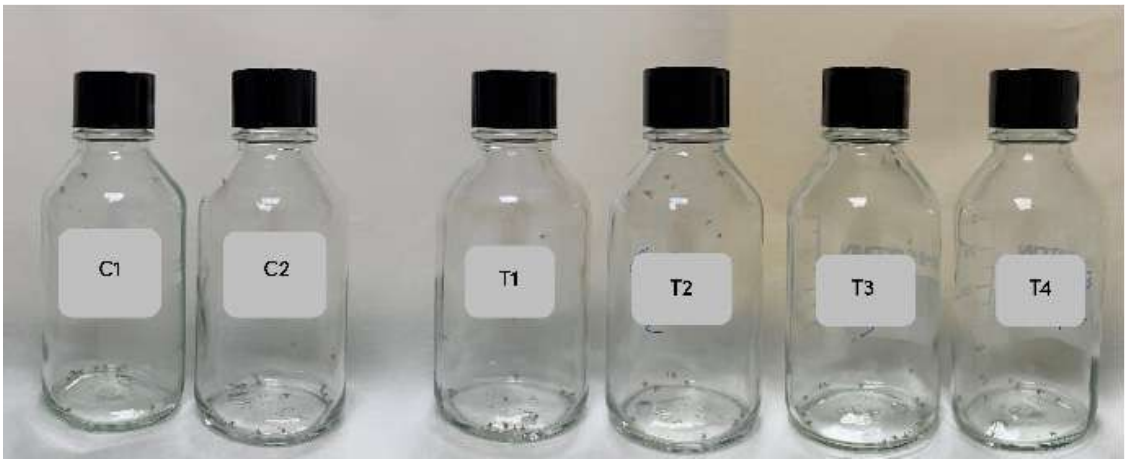


Fig. 4. Technique showing introduction of sand flies into a glass bottle[a] with a holed netting piece (A); a glass bottle with a holed cap and a rubber flap for introduction of sand flies as an alternative technique (B), and bottles with sand flies (C)

A

B

C



Source: A: © WHO/Rajpal S. Yadav; B,C: Photos courtesy of Stéphane Duchon, Institut de Recherche pour le Développement, Montpellier, France

[a] The total volume of a 250-mL graduated Wheaton glass bottle is 310 mL.

☐	6.10 **Leave the sand flies for 1 h of exposure**.
☐	6.11 **During the exposure, prepare the six holding paper cups:** Place a piece of netting (hole size < 500 µm) over the opening of each 250-mL paper cup, and fasten it with a rubber band.
☐	6.12 **Transfer sand flies to the holding paper cups and hold them for 24 h (or longer for slow-acting compounds)**: Gently suck the sand flies out of the bottle, preferably with a mechanical aspirator, and release them into the net-covered holding cups.

☐	6.13 **Record sand fly knockdown at 60 min:** Immediately after transferring the sand flies to the paper cups, record the number of insects that have been knocked down, as per the definition in Table 2.
☐	6.14 **Provide access to sugar solution:** Place a piece of cotton wool (1 cm x 1 cm) soaked in a 10–50% sugar solution on the netting cover. To ensure consistent results, hold the treatment and control cups strictly at 27 °C ± 2 °C and 75% ± 10% relative humidity throughout the test. Record temperature and relative humidity during the exposure and holding periods.
☐	6.15 **Record mortality after 24 h:** Count and record the numbers of sand flies found dead and alive 24 h after exposure as per the definitions in Table 2. Enter the data on the recording sheet or electronic data collection system.

Table 2. WHO guidelines for knockdown and mortality of sand flies in the bottle bioassay[a]

Sand flies considered to be alive after 1 h of exposure or 24 h after exposure	Sand flies considered to have been knocked down after 1 h of exposure or dead at 24 h after exposure
• Can both stand and hop in a coordinated manner	• No sign of life; immobile; cannot stand • Any sand fly that cannot hop in a coordinated manner • Any sand fly that lies on its back, moving legs and wings but unable to take off • Any sand fly that can stand and hop briefly but falls down immediately

[a] *Source*: adapted from p. 78 of reference *7*

7. Criteria for test rejection

For all insecticides, the test must be discarded and repeated if the control mortality is > 20%.

8. Data recording and calculation of test results

During the test, data should be entered on paper or digital data recording forms. A paper template is shown in Annex 2 of this SOP.

The end-point of the test is sand fly mortality at 24 h (or longer for slow-acting compounds) after 1 h of exposure to the insecticide. Therefore, the number of dead sand flies is counted 24 h after the 1-h exposure period. Sand fly mortality should be calculated separately for the treatment and control bottles.

Treatment mortality is calculated by summing the number of dead sand flies in all replicates with insecticide-coated bottles and expressing this as a percentage of the total number of sand flies in such replicates. Mortality in the control is calculated similarly.

$$\textit{Treatment mortality (\%)} = \frac{\textit{Number of treated female sand flies dead}}{\textit{Total number of treated female sand flies}} \times 100$$

$$\textit{Control mortality (\%)} = \frac{\textit{Number of control female sand flies dead}}{\textit{Total number of control female sand flies}} \times 100$$

- If the control mortality is <5%, no correction of test results is necessary.
- If the control mortality is ≥5% and ≤20%, the test mortality should be corrected with the control mortality using Abbott's formula as follows:

$$\textit{Corrected mortality} = \frac{(\%\ \textit{treatment mortality} - \%\ \textit{control mortality})}{(100 - \%\ \textit{control mortality})} \times 100$$

9. Interpretation of test results

- **Susceptibility:** A sand fly population is considered to be susceptible to an insecticide if mortality in the treatment group (corrected with Abbott's formula, if necessary) is ≥ 98%.
- **Possible resistance:** If the observed mortality rate in the treatment group (corrected with Abbott's formula, if necessary) is ≥ 90% but < 98%, resistance is possible but not confirmed. The results should be confirmed by repeating the test with a new sample from the same sand fly population. If two tests consistently show mortality in the treatment group < 98%, resistance is confirmed.
- **Confirmed resistance:** A vector population is considered to be resistant to an insecticide if the mortality rate in the treatment group (corrected with Abbott's formula, if necessary) is < 90%, provided that at least 100 sand flies were tested.

10. Bottle washing procedure

Below, we provide two options for washing bottles according to the availability of washing agents.

Generic washing method with Decon or TFD4

1. Remove any adhesive labels from the bottles before washing and dispose of them safely.
2. Under a fume hood, add approximately 10 mL of acetone to each bottle to be washed, and close with a cap.
3. Shake the bottles vigorously one by one.
4. Discard the acetone safely.
5. Prepare a 2–5% Decon solution (or equivalent product, e.g., 10% alkaline detergent TFD4) in hot water in a 20-L open container or sink.
6. Submerge the acetone-rinsed bottles and caps in the Decon solution overnight.
7. The next day, remove the bottles and caps from the solution, scrub every bottle and cap vigorously with the Decon solution and a cleaning brush, and rinse them thoroughly three times with tap water.

8. Submerge the bottles and caps in clean tap water in a container or sink for 24 h.

9. Remove the bottles, rinse them with clean tap water, and dry them for 6–8 h upside down on a rack (as shown in Fig. 4), or dry the bottles and caps in an oven at 50 °C for 20–30 min. Increase the drying time if moisture is still visible in the bottle.

Washing procedure when Decon or TFD4 is not available

1. Remove any adhesive labels from the bottles before washing.

2. Rinse the bottles with acetone, if available, as described above.

3. Prepare a 10% soap solution in hot water in a 20-L open container or sink.

4. Submerge the bottles and caps in the soapy water for 24 h.

5. Remove them from the soapy water, and either scrub each bottle and cap vigorously with soap solution and a clean brush and rinse them thoroughly three times with tap water, or wash them in a washing machine with hot water.

6. Submerge the bottles and caps in clean tap water in a container or sink for 24 h.

7. Remove the bottles, rinse them with clean tap water, and dry them for 6–8 h upside down on a rack (as shown in Fig. 4), or dry the bottles and caps in an oven at 50 °C for 20–30 min. Increase the drying time if moisture or droplets are still visible in the bottles.

To ensure that no insecticide residues are left in the washed bottles, check the quality of washing by selecting some dry bottles at random and exposing five sand flies in each, recording their knockdown at the end of 1 h of exposure and mortality at 24 h of holding after exposure.

Fig. 4. Method of drying glass bottles upside down on raised steel rods

Source: Photo courtesy of Stéphane Duchon, Institut de Recherche pour le Développement, Montpellier, France

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2. Standard operating procedure for testing insecticide susceptibility of adult mosquitoes in WHO bottle bioassays. Geneva: World Health Organization; 2022 (https://apps.who.int/iris/handle/10665/352312, accessed 3 June 2023).

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For further information, comments or suggestions, please contact: VVE@who.int

Annex 1. Calculations for preparing solutions for WHO bottle bioassays

A1.1 Calculations for the amount of active ingredient (AI), solvent and surfactant for preparing the initial stock solution and subsequent dilutions for serial concentrations for coating bottles

The Excel calculation spreadsheet is available online at: https://cdn.who.int/media/docs/default-source/ntds/vector-ecology-mangement/calculation-tables-paper-impregnation-bottles-17jan2022-locked.xlsx

In the Excel spreadsheet, you will enter values in the green cells and the values in the white cells will be automatically calculated.

											Calculation for AI weight in g adjusted for purity of AI					Calculation for AI weight in mg adjusted for purity of the AI		
Insecticide class group	Surfactant	Insecticide	Targeted concentration of AI in the bottles (µg /bottle)	No. of bottles to be coated	Amount of surfactant per bottle* (ppm or µg)	Total weight of surfactant needed (mg)	Density of surfactant	Volume of surfactant (mL)	Volume of acetone (mL)	Total volume of coating solution (mL)	Amount of AI to weigh for coating bottles (g)	Purity of insecticide AI (%)	Adjusted amount of AI to weigh (g)*	Exact weight of AI (g)**	Total adjusted volume of coating solution (mL)***	Amount of AI to weigh (mg)**	Exact weight of AI (mg)***	Total adjusted volume of coating solution (mL)***
			a	b	c	d = (b × c)/1000	e	f = (d/e) / 1000	g = (b × 1) - f	h = f + g	i = (a x b) / 106	j	k = i x (100 / j)	l	m = (l × h) / k	n = k x 1000	o	p = (o × h) / n
	e.g. MERO[a]			1	800	0.8	0.900	0.001	1.00	1	0.000000	99.2	0.000000		#DIV/0	0.00	1	#DIV/0
		Control		1	800	0.8	0.900	0.001	1.00	1								
	None[b]			1					1.00	1	0.000000	99.8	0.000000		#DIV/0	0.00	1	#DIV/0
		Control		1					1.00	1								

AI, active ingredient
MERO, 81% rapeseed oil methyl ester
* Considering purity of the insecticide AI
** Exact weight of AI shown on the electronic balance
*** TThis is the volume adjusted for exact weight of AI; use 1 mL solution to coat a 250-mL Wheaton bottle (total volume 310 mL)
[a] For example, clothianidin
[b] With acetone alone as a solvent; for example for juvenile hormone mimics

A1.2 Dilutions of stock solutions with acetone or acetone + surfactant for preparing serial concentrations of insecticides for coating bottles

The Excel calculation spreadsheet is available online at: https://cdn.who.int/media/docs/default-source/ntds/vector-ecology-mangement/calculation-tables-paper-impregnation-bottles-17jan2022-locked.xlsx

In the Excel spreadsheet, you will enter values in the green cells and the values in the white cells will be automatically calculated.

a. With acetone alone (i.e., without a surfactant)

	Final concentration of AI (mg/m^2)**	Final volume of acetone (mL)*	AI concentration of the initial stock solution (mg/m^2)**	Volume to take from the initial stock solution (mL)	Volume (mL) of acetone to add
	a	b	c	d = (a x b)/c	e = b - d
Stock solution*			**100**		
Serial dilution no. 1	**50**	5	**100**	2.50	2.50
Serial dilution no. 2	**30**	5	**100**	1.50	3.50
Serial dilution no. 3	**20**	5	**100**	1.00	4.00
Serial dilution no. 4	**10**	5	**100**	0.50	4.50
Serial dilution no. 5	**5**	5	**100**	0.25	4.75

b. With acetone and a surfactant (e.g., MERO) – example of clothianidin

	Final concentration of AI (µg/bottle)	Final volume of acetone + surfactant (mL)**	AI concentration of the initial stock solution (µg/bottle)	Volume to take from the initial stock solution (mL)	Volume of solution acetone + surfactant to add (mL)
	a	b	c	d = (a x b)/c	e = b - d
Stock solution*			**100**		
Serial dilution no. 1	**50**	5	**100**	2.50	2.50
Serial dilution no. 2	**30**	5	**100**	1.50	3.50
Serial dilution no. 3	**20**	5	**100**	1.00	4.00
Serial dilution no. 4	**10**	5	**100**	0.50	4.50
Serial dilution no. 5	**5**	5	**100**	0.25	4.75

AI, active ingredient; MERO, 81% rapeseed oil methyl ester

* Initial stock solution is prepared by weighing adequate AI amount and adjusting required volume of solvent with or without surfactant.

** Prepare final volume according to the number of bottles to coat (e.g., 5 mL volume is needed to coat 4 bottles to account for any procedural loss of some solution).

Annex 2. Data collection form for testing susceptibility to insecticides of adult sand flies in WHO bottle bioassays

World Health Organization

Data collection form – WHO bottle bioassay for testing susceptibility to insecticide of adult sand flies

To be completed in black or blue ink only. Do not use pencil or correction fluid.

<table>
<tr><td>Bioassay date (dd/mm/yy):</td><td colspan="2">Technician's name:</td></tr>
<tr><td>Country/place of sand fly collection:</td><td colspan="2">Coordinates

Latitude: Longitude:</td></tr>
<tr><td>Period of sand fly collection:

Start date (dd/mm/yyyy):

End date (dd/mm/yyyy):</td><td colspan="2">Collection method:</td></tr>
<tr><td rowspan="2">Insecticide tested and concentration:</td><td>Date of coating bottles (dd/mm/yyyy):</td><td>Number of times the coated test and control bottles have been used previously:</td></tr>
<tr><td colspan="2">Bottle storage temperature (°C)

Max: Min:</td></tr>
<tr><td>Sand fly species and strain:</td><td colspan="2">Sand fly stage:

F0 adults (wild-caught females):

F1 adults (progeny of wild-caught females):</td></tr>
<tr><td>Age of females (days):</td><td colspan="2">Feeding status:

(unfed; sugar-fed and starved; other, specify)</td></tr>
<tr><td>Start time of exposure (hh/mm):</td><td colspan="2">End time of exposure (hh/mm):</td></tr>
<tr><td>Temperature during exposure + holding period (°C):

Max: Min:</td><td colspan="2">Relative humidity during exposure + holding period (%):

Max: Min:</td></tr>
</table>

Results per bottle

Test arm	Bottle	Number of sand flies introduced	Number of knocked down sand flies after 1-h exposure	No. of dead and alive sand flies at 24 h after 1-h exposure		Mortality at 24 h after 1-h exposure (%)
				No. dead	No. alive	
No. of sand flies exposed to bottles impregnated with the DC[a] of the insecticide	Bottle 1					
	Bottle 2					
	Bottle 3					
	Bottle 4					
No. of sand flies exposed to control bottles	Control bottle 1					
	Control bottle 2					

Final results (all bottles)

	Knocked down after 1-h exposure (%) (i.e., at the end of 1-h exposure)	Mortality at 24 h[b] after 1-h exposure (%)	Abbott's corrected mortality, if required (%)
Sand flies exposed to the DC[a] of the test insecticide			

[a] DC, discriminating concentration

[b] At 72 h for slow-acting compounds.

Test result

The vector population is _____ (susceptible/resistant/possibly resistant) to the insecticide

Comments, if any:

_____

_____

_____

_____

_____

Signed by the technician: _____

Verified and signed by the supervisor: _____

Date (dd/mm/yyyy): _____


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