

# GUIDELINES FOR EFFICACY TESTING OF SPATIAL REPELLENTS



World Health  
Organization



# **GUIDELINES FOR EFFICACY TESTING OF SPATIAL REPELLENTS**



**World Health  
Organization**

**CONTROL OF NEGLECTED TROPICAL DISEASES  
WHO PESTICIDE EVALUATION SCHEME**

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The Department welcomes feedback on the guidelines and suggestions for improvement from national programmes, research institutions and industry.



## 1. INTRODUCTION

The term 'spatial repellency' is used here to refer to a range of insect behaviours induced by airborne chemicals that result in a reduction in human–vector contact and therefore personal protection. The behaviours can include movement away from a chemical stimulus, interference with host detection (attraction inhibition) and feeding response.

While many household insecticide products, such as mosquito coils, have been used for personal protection, most are based on the insecticidal activity (knock-down and mortality) of the active ingredient.

These guidelines are designed to extend those already recommended by WHO for testing the efficacy of household insecticide products.<sup>1</sup> Expansion of those guidelines was required in order to promote and facilitate the discovery and development of novel active ingredients (AIs) with inherent spatial repellent properties for public health purposes and for further standardization of evaluation procedures for spatial repellency.

The document provides guidance and describes steps for laboratory testing and for semi-field and field evaluations of spatial repellent products (technical materials and formulated products) designed to provide protection in a specific space (indoor and/or outdoor) against mosquitoes. With some modifications, the guidelines can be used to determine the efficacy and personal protectiveness of candidate products against other flying nuisance pests. These guidelines may have to be modified when proof of principle is established (i.e. the public health value of spatial repellents for vector-borne disease control) and as new methods for assessing the spatial repellency of such products become available.

These guidelines are designed for using AIs that have already undergone safety assessment, including toxicity by inhalation. Nevertheless, any adverse-effects or undesirable

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<sup>1</sup> *Guidelines for efficacy testing of household insecticide products.* Geneva, World Health Organization, 2009 (WHO/HTM/NTD/WHOPES/2009.3; [http://whqlibdoc.who.int/hq/2009/WHO\\_HTM\\_NTD\\_WHOPES\\_2009.3\\_eng.pdf](http://whqlibdoc.who.int/hq/2009/WHO_HTM_NTD_WHOPES_2009.3_eng.pdf)).

characteristics observed during laboratory studies and field trials should be recorded and reported.

Technical material or formulated products submitted for laboratory testing and field trials should be sent with the material safety data sheet, the labelling recommendations and the manufacturer's certification that the product is within the company's manufacturing specifications. Independent physical and chemical assessment for compliance with the specifications may be required before efficacy testing.

Biological tests are subject to the variations inherent to living organisms. Test insects must be reared carefully for standardized size and good biological fitness in order to ensure representative responses to test compounds. Testing should be conducted under the close supervision of personnel familiar with biological testing of insecticides and by sound scientific and experimental procedures; the principles of good laboratory practice or other suitable quality assurance schemes should be applied.

All laboratory and field personnel should be given adequate training in safety and the standard operating procedures associated with an assay before beginning testing, and such training should be documented. Use of a standard operating procedure for data processing, management and validation is advisable, and copies of the procedures should be made available and accessible in the relevant languages for all study staff.

The quality of data reporting should be sufficient to allow comparisons of efficacy at multiple evaluation sites. The minimum data to be reported include a measure of centrality (e.g. mean), sample size and a measure of variability (e.g. standard error).

Evaluations of spatial repellents should be conducted in accordance with applicable national ethical regulations.

## 2. LABORATORY STUDIES

The primary objective of laboratory studies is to determine the inherent properties of the AI under well-controlled, standardized conditions and its activity against well-characterized mosquito strains. Such studies include measurement of movement away from a chemical stimulus, interference with host detection (attraction–inhibition) and feeding response. Laboratory studies also include determinations of the efficacy, including the duration of protection, of a formulated product in various delivery formats under well-controlled, standard conditions.

The specific objectives of laboratory studies are to:

- establish dose–response relations and determine the effective dosage (ED) of the AI for 50% and 95% (ED<sub>50</sub> and ED<sub>95</sub>) movement away from a chemical stimulus;
- establish dose–response relations and determine the ED<sub>50</sub> and ED<sub>95</sub> of the AI for host attraction–inhibition; and
- determine the efficacy and duration of protection (landing and feeding inhibition) of formulated spatial repellent products.

If the spatial repellent product is intended for application to a large surface area (e.g. fabric), determination of contact irritancy may also be required, following established WHO guidelines.<sup>1</sup>

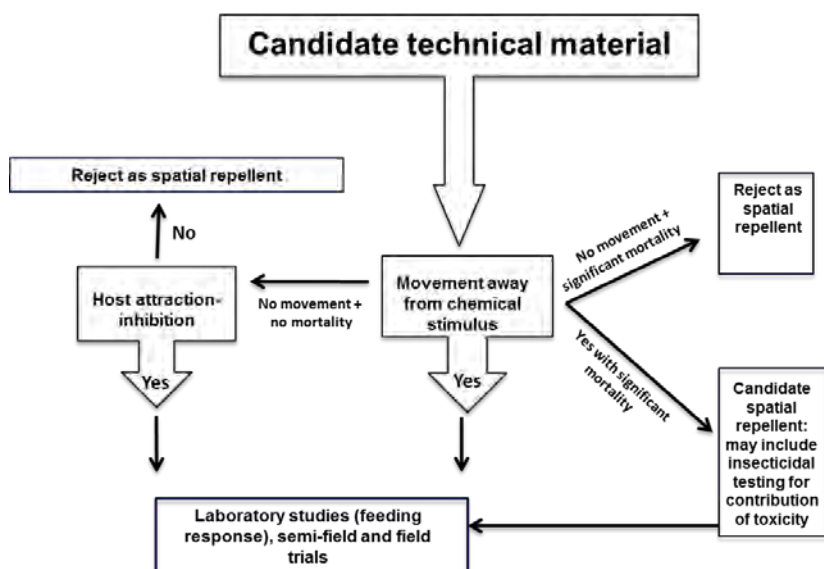
If there is any significant mortality in the spatial repellency assay (see below), the insecticidal activity (e.g. vapour toxicity) of the product should be determined by established WHO guidelines,<sup>2</sup> to fully understand its overall performance.

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<sup>1</sup> *Guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets*. Geneva, World Health Organization, 2006 (WHO/CDS/NTD/WHOPES/GCDPP/2006.3; [http://whqlibdoc.who.int/hq/2006/WHO\\_CDS\\_NTD\\_WHOPES\\_GCDPP\\_2006.3\\_eng.pdf](http://whqlibdoc.who.int/hq/2006/WHO_CDS_NTD_WHOPES_GCDPP_2006.3_eng.pdf)).

<sup>2</sup> *Guidelines for efficacy testing of household insecticide products*. Geneva, World Health Organization, 2009 (WHO/HTM/NTD/WHOPES/2009.3; [http://whqlibdoc.who.int/hq/2009/WHO\\_HTM\\_NTD\\_WHOPES\\_2009.3\\_eng.pdf](http://whqlibdoc.who.int/hq/2009/WHO_HTM_NTD_WHOPES_2009.3_eng.pdf)).

A flow-chart of the decision-making process for laboratory studies of a candidate spatial repellent product is shown in Figure 1.



**Figure 1. Flow chart for making decisions on the basis of laboratory studies of candidate spatial repellent technical material**

Ideally, tests should be conducted with three or more local anthropophilic, fully susceptible species of *Aedes* (preferably *Ae. aegypti*), *Culex* (preferably *Cx. quinquefasciatus*) and *Anopheles* (preferably *An. stephensi*, *An. gambiae* or *An. albimanus*) characterized according to WHO standard guidelines for monitoring susceptibility.<sup>1</sup>

As the physiological status of mosquitoes can significantly

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<sup>1</sup> *Test procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticide on treated surfaces*. Geneva, World Health Organization, 1988 (WHO/CDS/CPC/MAL98.12; [http://whqlibdoc.who.int/hq/1998/WHO\\_CDS\\_CPC\\_MAL\\_98.12.pdf](http://whqlibdoc.who.int/hq/1998/WHO_CDS_CPC_MAL_98.12.pdf)).

affect their behavioural response, use of the assays described below for measuring various physiological states (e.g. parity) should be considered.

### ***Mosquito rearing and physiological status***

Use of standardized mosquito rearing and testing conditions for laboratory assays is essential to ensure the reliability and reproducibility of data. The conditions are generally a temperature of  $27 \pm 2$  °C, a relative humidity of  $80 \pm 10\%$ , and a photoperiod of 12 h light : 12 h dark. Temperate species may have different requirements, and the assay conditions should be matched as closely as possible to the environmental conditions of the target locale. Adults are maintained on sugar solution (typically 10% glucose on cotton wool or filter paper).

Assays should be carried out on female mosquitoes that are nulliparous and of uniform age, preferably 5–8 days post-emergence. Actively host-seeking females should be selected from general colony groups to ensure a maximum behavioural response. This can be done with an aspirator or an appropriate airflow apparatus while holding a hand close to (but not touching) the cage and collecting those mosquitoes that actively probe. Spatial repellency should be observed in female mosquitoes starved for the preceding 12 h, preferably during times in the diel period that correspond to the biting activity of that species. Mosquitoes must be transferred to holding containers or assay devices with care to avoid physically damaging them.

### ***Laboratory test procedures***

All test chambers and other assay instruments should be properly cleaned and decontaminated after the completion of each test according to assay-specific instructions. Test chambers must be checked for contamination by performing assays under blank conditions before the start of each subsequent test. Under chemical-free conditions, knock-down (see Annex 1 for a full definition of knock-down response) must not exceed 5% among mosquito populations held for 10 min.

Inclusion of an appropriate, well-characterized AI as a reference or positive control is highly recommended.

The temperature and humidity at the time of testing as well as

time of each replicate should be reported for each laboratory test.

## **2.1 Spatial repellency of active ingredients (technical material)**

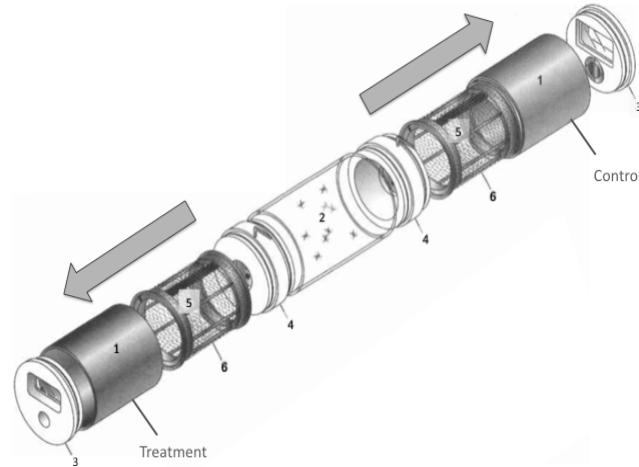
### **2.1.1 Movement away from a chemical stimulus**

The objective of this test is to measure the movement away from an AI in a spatial repellency assay, which is a modular test system (Figure 2). Specifications for the system components and full standard operating protocols are provided in Annex 2.

A central clear cylinder is connected to two metal test cylinders (one control (C) and one treatment (T)) with gated funnels to build one test unit (C–T). The funnel bevels (interior slope) are positioned towards the clear cylinder to facilitate mosquito movement into the metal cylinders of the test unit. A blank is placed within a metal spool at one end of the system and a treated substrate within another metal spool at the other end, creating a concentration gradient between the two ends. Pieces of opaque felt can be wrapped around the clear cylinder and over the rectangular ports in the end caps (with Velcro attachments) to simulate darkness, depending on the diel pattern of the target species.

As a negative control, substrate treated only with diluent is placed at both ends of the test unit (C–C) simultaneously to ensure that no greater proportion of mosquitoes moves into a particular end of the test system for a given test population, time of day and assay conditions (i.e. temperature and humidity). If multiple assays are being run at the same time, the metal cylinders should be labelled  $C_1, C_2 \dots C_n$  and  $T_1, T_2 \dots T_n$  to facilitate data recording.

Serial dilutions of AI are made with acetone (or another suitable diluent recommended by the manufacturer) and tested to identify the effective dose range. Minimum of five concentrations covering a range of responses should be chosen, i.e. two to three concentrations resulting in <50% spatial repellent response and two to three concentrations that give >50% response (excluding 0% and 100% response).



**Figure 2. Schematic drawing of the spatial repellency assay** The components include: 1, metal cylinder; 2, clear (Plexiglas) cylinder (with mosquito introduction portal); 3, end cap; 4, gated funnels (with butterfly valve); 5, metal spool; and 6, treatment substrate. Grey arrows indicate direction of potential mosquito movement during repellency evaluation.

Adapted from Grieco JP et al. A novel high-throughput screening system to evaluate the behavioral response of adult mosquitoes to chemicals. *Journal of the American Mosquito Control Association*, 2005, 21(4):404–411.

Aliquots of 1.5 ml of the repellent AI and of the diluent are applied evenly to 11 x 25 cm (275 cm<sup>2</sup>) pieces of Whatman No. 1 paper with a pipette. All filter papers, both control (diluent only) and treatment (AI and diluent), are allowed to sit for 30 min (for acetone) or less (depending on the diluent) before the first test replicate is initiated, to ensure that the diluent has completely evaporated, leaving only the chemical of interest on the filter paper. Other substrates that can be used in the spatial repellency assay include polyester and cotton, depending on the textile or product format into which the AI is expected to be incorporated.

The spatial repellency assay is typically performed under static airflow under a chemical hood. Groups of 20 female mosquitoes are introduced from holding tubes into the clear cylinder (with an aspirator) and are allowed to acclimatize to the test environment for 30 s. The number of mosquitoes that are physically damaged and are incapable of flying or walking is recorded to correct for the total mosquito sample size available to respond to the AI in that replicate. The butterfly valves are simultaneously opened for 10 min to allow chemical vapours to flow through the test unit and also to allow free movement of the mosquitoes throughout the unit, as indicated by the grey arrows in Figure 2. The gates are closed after 10 min, and the number of mosquitoes in each cylinder is recorded. The number of knock-down mosquitoes (see Annex 1) in each cylinder is also recorded. All mosquitoes are kept overnight to check for 24-h mortality. If the mortality is significantly higher than among controls, insecticidal activity must be evaluated by established WHO efficacy guidelines<sup>1</sup> (Figure 1).

Between replicates, the metal cylinders are disconnected from the clear cylinder and the end cap is removed from the control metal cylinder for 3 min to allow passive ventilation of the AI from the clear and control metal cylinders before the next replicate. During the ventilation period, all treated substrates remain in place within the metal cylinders under the chemical hood. Successive replicates are carried out without delay.

The cylinders are washed at the end of each evaluation. The metal spools are washed with acetone solution. The clear cylinders, end caps, gated funnels and metal cylinders are

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<sup>1</sup> Available at <http://www.who.int/whopes/guidelines/en/>.



washed with non-fragrant laboratory detergent solution. Component sections are allowed to dry overnight before reuse for testing other AIs and/or dosages.

Nine replicates are performed for each AI dosage. At the conclusion of testing, the proportion of mosquitoes repelled by the treatment is determined. Spatial repellency is expressed as the proportion of mosquitoes prevented from entering the treatment space in relation to all mosquitoes moving within the system and is calculated from a 'spatial activity index' (Equation 1):

$$SAI = \left[ \frac{(Nc - Nt)}{(Nc + Nt)} \right] \times \left( \frac{Nm}{N} \right),$$

*(Equation 1)*

where SAI is the spatial activity index,  $Nc$  is the number of mosquitoes in the control metal chamber,  $Nt$  is the number of mosquitoes in the treatment metal chamber,  $Nm$  is the total number of mosquitoes in the two metal chambers, and  $N$  is the total number of mosquitoes in the test unit.

The spatial activity index varies from  $-1$  to  $1$ : zero indicates no response;  $-1$  indicates that all mosquitoes moved into the treatment chamber, resulting in an attractant response; and  $1$  indicates that all the mosquitoes moved into the control chamber (away from the treatment source), resulting in a spatial repellent response. If no movement is recorded within the system (i.e.  $Nt = 0$ ,  $Nc = 0$ ), the test is valid but the spatial activity index is  $0$  (see example in Annex 2).

The spatial activity index is calculated for each replicate, and the mean index for each AI dosage is analysed by probit-plane regression analysis, from which the  $ED_{50}$  and  $ED_{95}$  and corresponding 95% confidence limits can be estimated. The number of replicates, the total number of mosquitoes and the mean spatial activity index ( $\pm$  standard error) for each AI dosage and negative control should be reported.

### 2.1.2 Host attraction–inhibition

The objective of this test is to measure the ability of an AI to inhibit mosquito attraction to a host. This is achieved by use of a Y-tube olfactometer to measure attraction to host odours in the absence and presence of the AI. A dual port design, such as a Y-tube, is recommended, and a variety of suitable olfactometers can be used.<sup>1</sup>

A scheme of a Y-tube olfactometer is presented in Figure 3. Specifications of a Y-tube olfactometer with photographs and sources of components are provided in Annex 3.

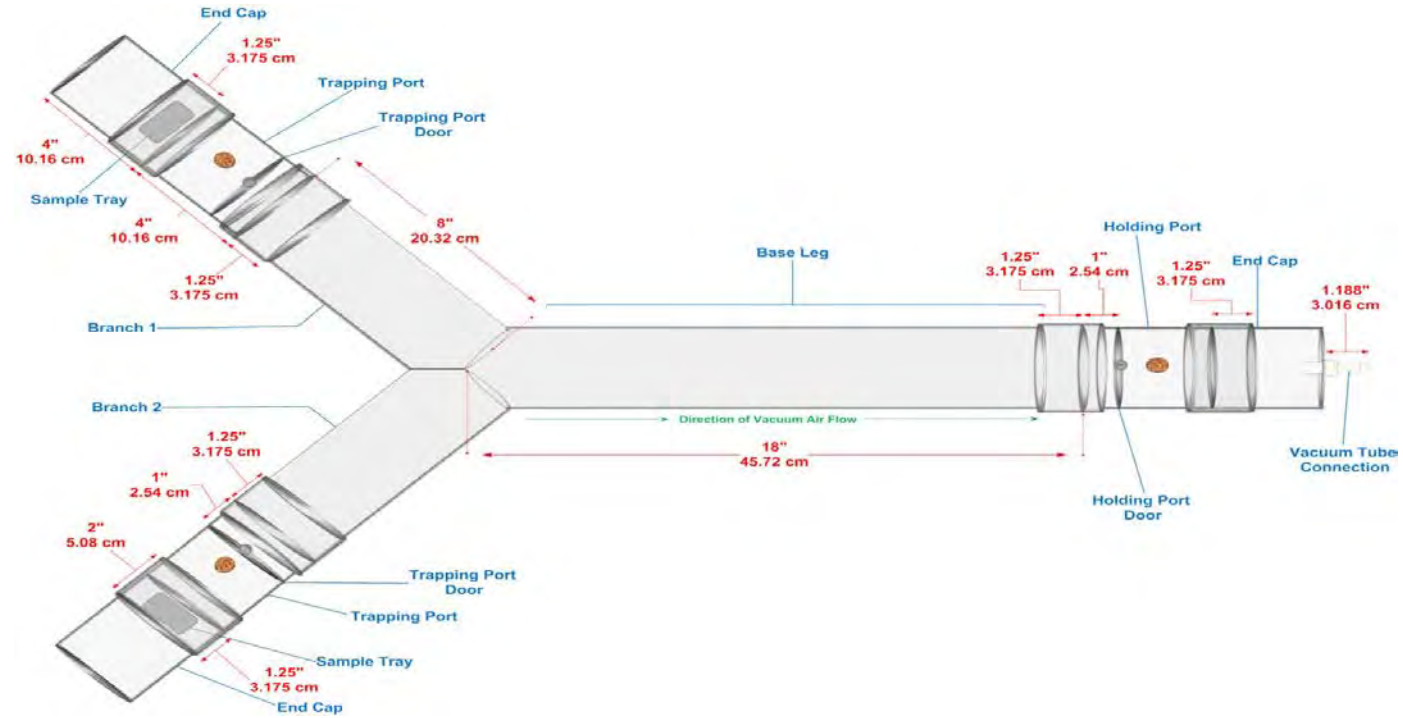
A central base leg made of acrylic plastic constitutes the main body of the olfactometer. Two branches meet the base leg at a decision point. Each branch has a trapping port with a mesh screen over the end, and each trapping port has a rotating circular door that also has a mesh screen. The mesh on the control and treatment trapping ports allows odours from the air input to pass through these areas and also prevents direct contact between the mosquitoes and the odour sources. During a test, odours in the air migrate down the branches, through the base leg and then to the holding port, from which test mosquitoes are released at the start of the test.

The time of day and assay conditions (i.e. temperature and humidity) should be recorded, and an attempt should be made to maintain these conditions in all subsequent tests in a single evaluation.

A preliminary test with diluent only at one port of the test unit should be performed, with a sufficient number of replications to ensure no response to the solvent (indicated by an attraction response  $\leq 10\%$ ).

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<sup>1</sup> Bernier UR et al. Human emanations and related natural compounds that inhibit mosquito host-finding abilities. In: Debboun M et al., eds. *Insect repellents: principles, methods and uses*, Boca Raton, Florida, CRC Press, Taylor & Francis Group, 2007:31–46.



**Figure 3. Schematic drawing of a Y-tube olfactometer used to measure attraction–inhibition from the ability of a chemical to inhibit attraction to a port containing odours from a host**

Two negative control assays are performed: one with no diluent (blank control) before AI testing to ensure no contamination (indicated by  $\leq 10\%$  attraction to either port) and a second in which host odour is released from both ports (positive control) to ensure no contamination by attraction–inhibitor from a previous test (indicated by equal responses to both ports and  $> 50\%$  response overall). The study design for these assays is shown in Table A3.1 in Annex 3.

Serial dilutions of AI are made with methanol (or another suitable diluent based on AI chemistry and the manufacturer's recommendation) and tested to identify the effective dose range. Dosages that give responses between 10% and 95% are used for this analysis, preferably with two or three dosages that induce an attraction response of  $> 50\%$  and the remaining two or three dosages that induce an attraction of  $< 50\%$ .

A pipette is used to deliver 400  $\mu\text{l}$  of the repellent AI solution or diluent to a 9-mm vial cap. All vial caps, both control (diluent only) and treatment (AI and diluent), are placed in position in the end caps and attached to the trapping ports before a test. The placement of spatial repellent and control treatments should be fully randomized in replicates, following the example in Table A3.1 of Annex 3.

The olfactometer is operated with airflow in a chemical hood. Hoods are equipped with vacuum and air lines that can be used to provide this airflow. The vacuum line in a hood can be connected to the base leg trap by an end cap fitted with a gas nozzle. An anemometer is used to adjust the vacuum so that the airflow through the control and treatment ports is  $0.20 \pm 0.05$  m/s. The air velocity through the base leg should be  $0.40 \pm 0.10$  m/s.

Lots of 10 female mosquitoes are introduced from holding tubes into the holding port (with aspirators) and are allowed to acclimatize to the test environment with clean air for 15 min with no treatment. The numbers of mosquitoes that are physically damaged and are incapable of flying or walking are discounted; only those mosquitoes that are able to respond are observed and recorded for each replicate. After the acclimatization period, the treatment and control are positioned in corresponding ports, and the doors are opened for the exposure period, usually 30 s. The exposure period is species-specific and is determined by the measurement in the negative control assay of the initial attraction response (i.e. 50% total

movement). The total numbers of mosquitoes in the treatment trapping port, the control trapping port and outside these trapping ports are recorded.

Between replicates, the end caps and trapping ports are disconnected from the branches of the Y-tube, and the end cap and holding port are disconnected from the base leg. Clean holding and trapping ports are then placed on the unit, with the end caps reinstalled, and the unit is passively cleaned (with airflow) for a minimum of 3 min under the chemical hood to allow odours from the AI and/or host to clear from the olfactometer before the next replicate.

During the ventilation period, all traps remain in place. Successive replicates are done by repeating the process of loading a new trap with 10 mosquitoes onto the base leg and allowing the mosquitoes to acclimatize.

The Y-tube body and individual trapping ports are washed at the end of each chemical evaluation. As the unit is made of acrylic, all parts should be washed with non-fragrant laboratory detergent solution. Component sections are allowed to dry overnight before reuse for testing other AI and/or dosages.

A minimum of six replicates is performed for each AI dosage. At the end of testing, the proportion of mosquitoes attracted to the treatment is determined. The percentage attracted to the treatment port is calculated by dividing the number of mosquitoes trapped in the treatment port by the total number of mosquitoes in the test (minus damaged mosquitoes). Spatial repellency is indicated by a lower percentage attraction of mosquitoes to host odours plus AI than to host odours with diluent only (i.e. no AI). The mean percentage attraction is calculated from the responses of the six replicates to each treatment.

The percentage attraction for each AI dosage is determined by probit-plane regression analysis, from which the  $ED_{50}$  and  $ED_{95}$  and corresponding 95% confidence limits can be estimated. The number of replicates, the total number of mosquitoes and the mean percentage attraction ( $\pm$  standard error) for each AI dosage and negative control should be reported.

## **2.2 Spatial repellency of active ingredients against insecticide-resistant strains**

The objective of this study is to determine any difference in spatial repellency between susceptible and insecticide-resistant strains to provide information for disease protection and control strategies. The difference is determined by comparing the dose–response curves generated in the spatial repellency assay and the olfactometer assay, as outlined above (sections 2.1.1 and 2.1.2) against mosquito populations that are known to be resistant.

## **2.3 Protective efficacy of formulated products**

The objective of this study is to determine the optimum dosage(s) to be applied<sup>1</sup> and the duration of protective efficacy in a specified treated space under well-controlled and standardized conditions. Protective efficacy (personal protection) is measured by the difference in the inhibition of landing or feeding between treated groups and controls over time. The reduction in vector entry into or resting inside test spaces can be compared<sup>2</sup> when appropriate.

Products should be evaluated and placed in the free-flight room (Figure 4) according to the label instructions. The efficacy of a product is assessed from a minimum of four replicates with 50 mosquitoes for both treatment and control. Comparison with a standard product or suitable positive control is necessary. The duration of protective efficacy over time can be measured by making collections at various times after product activation from laboratory findings and/or label claims. For example, if the product performance in laboratory testing indicated 18-week efficacy, tests should be performed at systematic sampling

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<sup>1</sup> The necessity for testing various dosages of a formulated product depends on whether a final product or a product that is formulated but still under development is being evaluated.

<sup>2</sup> A decision to include measures of reduced entry and resting in addition to or in place of landing or feeding inhibition for estimating protection time should be based on product claims. If a claim states that the product prevents insects from entering a space, entry evaluation is necessary.

times (e.g. weekly) throughout the 18-week expected efficacy period. Products should be stored according to the label claims between evaluations under environmental conditions similar to those during evaluation. Longer-lasting products can be stressed or aged experimentally in environmental chambers before testing.



**Figure 4. Free-flight testing area, showing a configuration of two rooms that can be used to evaluate whether mosquitoes enter a treated space** (Photo courtesy of Arthropod Control Test Centre, London School of Hygiene and Tropical Medicine)

Free-flight rooms constructed within testing facilities should be mosquito-proof and well ventilated, with an extraction fan for safety to clear vapour remaining from spatial repellent formulations between tests. Rooms should measure 30 m<sup>3</sup>,<sup>1</sup> with smooth, light coloured surfaces such as tiles (walls, ceilings and floors) that make it easy to see mosquitoes and easy to clean with detergent or solvent. Before evaluation of repellents, the chambers should be cleaned with suitable detergent or

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<sup>1</sup> This minimum volume space was set on the basis of the WHO *Guidelines for efficacy testing of household insecticide products*. Geneva, World Health Organization, 2009 (WHO/HTM/NTD/WHOPES/2009.3; [http://whqlibdoc.who.int/hq/2009/WHO\\_HTM\\_NTD\\_WHOPES\\_2009.3\\_eng.pdf](http://whqlibdoc.who.int/hq/2009/WHO_HTM_NTD_WHOPES_2009.3_eng.pdf)).

solvent and ventilated to clear residual traces of cleaning product. Rooms should be maintained at  $27 \pm 2$  °C and  $80\% \pm 10\%$  relative humidity during the test period and evaluations conducted at appropriate times in the diel period for the target mosquito species. Instrumentation should be mounted in the room to ensure consistent comparison of measurements over time.

A domestic extractor fan or passive ventilator is switched on (air exchange of about  $110 \text{ m}^3/\text{h}$ ), and a single person remains in the room for the duration of the tests, either to conduct a human landing catch for measuring landing inhibition or to measure feeding inhibition. The placement of the product in the free-flight room should be in accordance with the label instructions. The duration of the evaluation and sampling periods depends on the product label specifications. Biological efficacy should be assessed at several times, until it has fallen to  $< 50\%$ .

Mosquitoes should be allowed to acclimatize for 1 h under conditions similar to those of the test space before they are released into the test area. Mosquitoes are released into the room containing a human volunteer or an adjacent room if vector entry into a space is the objective of the evaluation. If human landing catch is being measured, mosquitoes are collected for 1 h continuously. If feeding inhibition is being measured, the volunteer remains in the room for the period of interest, and blood-fed mosquitoes are collected by aspiration from the interior space at the end of the test.

$$\% \text{ landing inhibition} = 100 \times \left[ \frac{(Cl - Tl)}{Cl} \right]$$

(Equation 2)

where  $Cl$  is the number of mosquitoes landing in the control space and  $Tl$  is the number of mosquitoes landing in the treatment space.

$$\% \text{ feeding inhibition} = 100 \times \left[ \frac{(Cf - Tf)}{Cf} \right]$$

(Equation 3)



where  $Cf$  is the number of blood fed mosquitoes in the control space and  $Tf$  is the number of blood fed mosquitoes in the treatment space.

An appropriate statistical analysis (e.g. probit-plane regression or linear mixed-effects regression with an appropriate error structure and link function) at a significance level of  $p = 0.05$  should be used. Data should include the duration of the test, the duration of protective efficacy, the number of replicates of the control and treatment and the mean percentage landing inhibition (*Equation 2*) or feeding inhibition (*Equation 3*) with the 95% confidence interval. The size of the room(s) used in the evaluation should be reported in  $m^3$ . If more than one interconnected room is used, the distance between the source of the spatial repellent and the human should be stated.

Usually, the effective dose that provided 99.9% protection in a defined time in laboratory testing is used to establish the dosages for semi-field trials.

### 3. SEMI-FIELD TRIALS OF FORMULATED PRODUCTS

The objective of semi-field trials is to extend the results of laboratory efficacy studies and to test formulated products against free-flying populations of one or more target species under simulated indoor or outdoor conditions. The specific objectives of these trials are to determine the optimum application dosage(s)<sup>1</sup> and duration of protective efficacy in a specific treated indoor space or outdoor area. Efficacy (personal protection) is measured by comparing landing inhibition or feeding inhibition in treatment and controls over time. Alternatively, a reduction in vector entry into or resting in the test space may be compared.<sup>2</sup>

Semi-field trials are conducted in screened enclosures (with or without experimental huts) using the release of well-characterized mosquitoes, ideally in the natural ecosystem of a target disease vector.<sup>3</sup> The advantages of using screened enclosures for semi-field evaluation ensures that the mosquitoes are pathogen-free, that a known number of mosquitoes of fixed physiological status (e.g. parity) is used and there is a known distance between the point at which the mosquito populations originate and the source of the chemical stimulus, allowing estimation of the protective area (especially important in outdoor evaluation).

Appropriate arthropod containment guidelines should be followed.<sup>4</sup> The use of netting around the enclosure allows tests to be conducted in local conditions at ambient temperature,

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<sup>1</sup> The necessity for testing various dosages of a formulated product depends on whether a final product or a product that is formulated but still under development is being evaluated.

<sup>2</sup> A decision to include measures of reduced entry and resting in addition to or in place of landing or feeding inhibition for estimating protection time should be based on product claims. If a claim states that the product prevents insects from entering a space, entry evaluation is necessary.

<sup>3</sup> Ferguson HM et al. Establishment of a large semi-field system for experimental study of African malaria vector ecology and control in Tanzania. *Malaria Journal*, 2008, 7(1):158.

<sup>4</sup> American Society of Tropical Medicine and Hygiene. Arthropod containment levels. *Vector-borne and Zoonotic Diseases*, 2003, 3:75–90.

light, humidity and air movement. The enclosure should be sufficiently large to reflect the area over which the product is intended for use.

The dimensions of screened enclosures should be reported in m<sup>3</sup>, with a minimum size of 10 x 10 x 2 m<sup>2</sup> per compartment and, ideally, three identical compartments to evaluate simultaneously: the spatial repellent, a negative control and a positive control. It is important to evaluate each treatment independently of the others and to avoid interaction between treatments, especially as spatial repellents may exert an effect over several metres. Testing a spatial repellent and a control in the same space can result in a push–pull effect,<sup>1</sup> resulting in overestimation of the repellent's efficacy.

To facilitate sampling, it is preferable to conduct evaluations in enclosures with cement floors surrounded by a water-filled moat to prevent entry of ants (Figure 5), so that resting or blood-fed mosquitoes are not scavenged by them. Evaluations should be conducted at appropriate times in the diel period for the target mosquito species. Temperature, humidity and airflow<sup>2</sup> should be recorded throughout the test. Instrumentation should be mounted in each compartment in the same location to allow consistent comparisons of measurements.

### 3.1 Study design

A minimum of three semi-field trials in three geographical setting is recommended. Human landing catches are performed during the natural diel pattern of the target species. For target species that have a short period of main biting activity, tests with spatial repellent material should be conducted such that the expected end-points occur within the biting period.

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<sup>1</sup> Kitau J et al. The effect of Mosquito Magnet Liberty Plus trap on the human mosquito biting rate under semi-field conditions. *Journal of the American Mosquito Control Association*, 2010, 26(3):287–294.

<sup>2</sup> Hand-held vane anemometers are available for indoor use, which also measure temperature and humidity.



**Figure 5. (Above) Measuring landing inhibition by human-landing catch methods in a semi-field enclosure. A netted cage is used to hold mosquitoes before release. (Lower left) Semi-field systems can be made from local materials or (lower right) large enough to contain experimental huts, depending on the use.**

The duration of protective efficacy (inhibition of landing and feeding) can be measured by collecting mosquitoes at various times after product activation as shown in laboratory findings and/or label claims. For example, if a product is claimed from laboratory testing to be effective for 18 weeks, field trials should be performed at systematic sampling times (e.g. weekly) throughout the 18-week expected efficacy period.

Products should be stored between evaluations according to the label claims under environmental conditions similar to those during evaluation. Longer-lasting products can be stressed or aged experimentally in environmental chambers to facilitate logistics.

The dosage(s) for evaluation of spatial repellent products should be based on laboratory studies (see section 2.1.1).

The number of replicates per product being evaluated should be based on sample size estimates, which are required to ensure that a statistical evaluation has sufficient power and depend on the expected efficacy of the repellent. It is highly desirable that all field operatives be 'blinded' regarding the allocation of treatments to avoid bias during the evaluation. If that is not possible owing to the characteristics of the product (e.g. odour), the data should be coded by an independent person before analysis.

For each evaluation, a randomized Latin square design is used (see Annex 4 for example). The number of volunteers is equal to the number of products to be tested plus both positive (standard product if available) and negative (no product) control(s). Negative controls are used to monitor mosquito landing or feeding response, depending on the outcome of interest. If overall landing or biting in the controls is < 50% or < 25%, respectively, the data should be discarded and another replicate performed.

Collections are performed by volunteers skilled in the use of aspirators, so that they catch all mosquitoes landing on an exposed limb (for landing inhibition) or all those that are blood-engorged and resting (for biting inhibition). Records should be made throughout each trial of wind speed and direction, temperature, relative humidity and precipitation for consideration and analysis if appropriate.

### **3.2 Indoor effective dosage and duration of protective efficacy**

Indoor trials can be performed in experimental huts within a screened enclosure (Figure 5, lower right). Ideally, several huts should be available to allow comparison of several treatments simultaneously. In order to estimate efficacy in local houses, the experimental huts at the test site should be similar in design (e.g. number, orientation and size of windows and doors), volume (a minimum of 30 m<sup>3</sup>, unless local homes are much smaller) and materials and be constructed in the fashion of indigenous homes at the site with locally acquired materials when possible. Although hut designs may vary by test site, depending on local culture, it is critical that the design used at a specific evaluation site is standardized to allow direct comparison of AIs or formulated products.

An appropriate description of the design, size, furnishings, wall and ceiling characteristics and layout should be reported. Huts should be checked for contamination by an appropriate control test before evaluation of each new product. The attractiveness of the experimental huts to the target species should be tested before the trial using landing rates under control conditions.

The study design for indoor evaluation of point-source treatments (e.g. coils and emanators) differs from that for non-point source treatments (e.g. treated textiles or wall surfaces). Point-source treatments can be rotated between huts, whereas rotation of non-point source treatments may be limited by format. For point-source trials, rotation between huts should be in accordance with a Latin square design, in which every treatment is tested in every hut an equal number of times (Annex 4).

Rotation schedules should be based on the expected protection time from laboratory findings or product claims, with 1 or 2 days between rotations to clean and ventilate the hut and to remove contamination from previous treatments. When non-point source products cannot be rotated, it is essential to demonstrate before the evaluation that the attractiveness of experimental huts has little or no variation. (This also shows the importance of optimum positioning during selection or construction.)

Human volunteers are positioned at the centre of each hut throughout a single test to either conduct a human landing

catch for measuring landing inhibition or to rest or sleep for measurement of feeding inhibition. Product placement in the hut should be in accordance with the label instructions. The duration of the evaluation and sampling periods will depend on the product label specifications.

Each replicate consists of releasing 100 mosquitoes outside the hut, for both the treatment and negative control. A positive control can be used when appropriate. The mosquitoes collected are placed in separate holding cups for each sampling period. If insecticidal activity is indicated in laboratory studies, knock-down must also be monitored inside huts. All mosquitoes are held 24 h at optimum temperature and humidity conditions for observation of mortality. Data should be reported for knock-down, mortality, blood-fed status and location of collection (to estimate movement into or out of the test structure).

Marking each release population with a different coloured fluorescent powder can facilitate recapture; however, the effect of marking on mosquito behaviour and mortality should be evaluated before use in testing. Mechanical aspiration can be used to recapture all mosquitoes that were not collected during testing and remain inside the screened enclosure.

At the end of a trial, the number of mosquitoes collected during each observation period (i.e. at hour 1, hour 2 and hour 3) in the treatment and control groups can be averaged for each replicate (i.e. full treatment rotation). Landing inhibition (*Equation 2*) or feeding inhibition (*Equation 3*) is reported as a percentage with a 95% confidence interval (see section 2.3). An appropriate statistical analysis (e.g. probit-plane regression or linear mixed-effects regression with an appropriate error structure and link function) at a significance level of  $p = 0.05$  should be used for comparison.

The data reported should include the duration of the test, the age of the product (duration of protective efficacy), the number of replicates of the control and treatment, mean percentage landing inhibition (*Equation 2*) or mean percentage feeding inhibition (*Equation 3*) with 95% confidence interval.

### 3.3 Outdoor effective dosage and duration of protective efficacy

In a semi-field system, volunteers are positioned singly at a collection station at a specified distance from the spatial repellent product, according to the label recommendations, or act as a control (Figure 6) for the duration of the test to either conduct human landing catch for measurement of landing inhibition or to rest or sleep for measurement of feeding inhibition. The duration of the evaluation and sampling periods depends on the product label specifications.

Two semi-field compartments are used simultaneously to monitor landing inhibition at a set distance from a spatial repellent or control.

Each replicate consists of releasing 100 mosquitoes, for both the treatment and the negative control. Mosquitoes are released from netted boxes at a set distance from the volunteer by a pulley system (Figure 5). Plastic sheeting is used to separate semi-field compartments to ensure the independence of samples. Control and treatment can be rotated by day to prevent interference among collection stations. Temperature, humidity, wind speed and direction should be recorded for the duration of each replicate. For each defined distance, evaluations should be repeated.

At the end of a trial, the number of mosquitoes collected within each observation period (e.g. at hour 1, hour 2 and hour 3), for treatment and control, can be averaged for each replicate (i.e. full treatment rotation). The data reported should include the duration of the test, the age of the product (duration of protective efficacy), the number of replicates of the control and treatment, the mean percentage landing inhibition (*Equation 2*) or mean percentage feeding inhibition (*Equation 3*) with 95% confidence interval (see section 2.3). The size of the semi-field system used during the evaluation should be reported in m<sup>3</sup>. Efficacy at each distance between the source of the spatial repellent and the volunteer should be stated. An appropriate statistical analysis (e.g. probit-plane regression or linear mixed-effects regression with an appropriate error structure and link function) at a significance level of  $p = 0.05$  should be used for comparison.



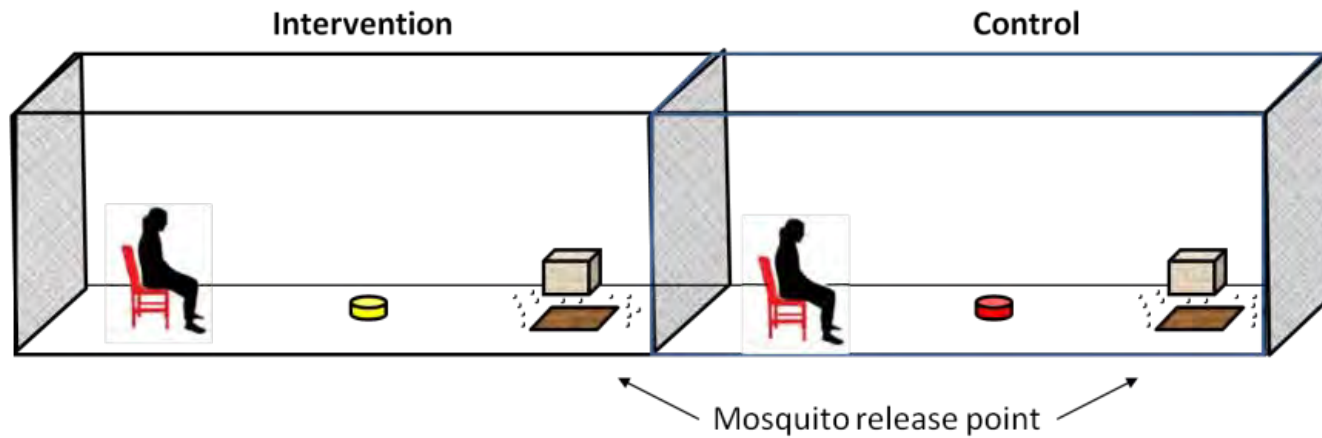


Figure 6. Scheme of an outdoor (semi-field) spatial repellent evaluation in two separate compartments.

#### **4. FIELD TRIALS OF FORMULATED PRODUCTS**

The objectives of field trials are to measure the personal protection offered by a spatial repellent product in operational settings and against free-flying natural indoor and/or outdoor populations of a target species. These are measured by comparing landing inhibition with treatment and with control.<sup>16</sup>

The specific objectives of such tests are to:

- confirm the effective dosage under operational conditions;
- observe and record the ease of application, handling and perceived adverse-effects during product application and use; and
- observe and record community acceptance.

Operational trials may have to be conducted in different ecological settings (e.g. urban or rural and indoor or outdoor), depending on the target species. The area and location of trial sites should be representative of the target species' habitat and the expected conditions of human exposure. Trials should be sufficiently replicated to allow robust statistical analysis with relevant sample size estimations based on predicted product efficacy. The outcomes are locale-specific, and the results may not be applicable to other settings. The environmental conditions of temperature, humidity and wind speed should be reported during both indoor and outdoor evaluations. Information on the insecticide resistance profile of the target species is desirable.

For indoor evaluations, several houses should be used, when feasible. Houses should be well described, especially with regard to the conditions relevant to product efficacy, including estimates of indoor volume and air ventilation (e.g. sealed or gapped walls, number of windows, doors or eave area). Houses are randomized to receive either active (formulated spatial

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<sup>16</sup> Because of ethical considerations, alternative collection methods and measures might be required to assess personal protection in locations with known active circulation of arboviruses (i.e. dengue), such as monitoring indoor resting density, use of outdoor traps or monitoring mosquito entry with window traps.

repellent) or blank (placebo; inert ingredients alone) treatment during the trial. Collectors should be 'blinded' to treatment allocation. In outdoor evaluations, spatial repellent product or blank should be allocated randomly to comparable outdoor spaces with human exposure.

A minimum of three replicates is required on different occasions at the optimal dose required for a 90% reduction in landing inhibition or a statistically significant difference between treatment and control at the dosage recommended on the label. The initial dosage for operational trials should be based on the dosage(s) recommended on the label or that which inhibited feeding by at least 90% in semi-field trials. The number and placement of spatial repellent products should be based on label claims.

Human landing catch methods should be used to measure protective efficacy. The method, statistics and minimum data reporting for evaluation of landing inhibition are outlined in sections 3.2 and 3.3 for indoor and outdoor trials, respectively.

It is advisable that the health status of volunteers be examined before, during and after product use. Brief records of exposure should be kept for each volunteer, including the spatial repellent product used, the dosage, total exposure in hours and any perceived adverse-effects. A list of mild, moderate and severe signs and symptoms of poisoning can be kept for reference. Problems encountered in use and application should be reported. The material safety data sheet issued by the manufacturer should be consulted if necessary.

Perceived adverse-effects (and other adverse events) due to use of the spatial repellent product indoors or outdoors and the general acceptability of treatment by local inhabitants in the trial area should also be observed and recorded.

## **5. ETHICAL CONSIDERATIONS**

Participants' well-being must be assured and their autonomy respected. The inclusion and exclusion criteria for participation in a test, informed consent for risk of pathogen infection, pathogen detection and monitoring as well as chemoprophylaxis and treatment should follow national guidelines, and the study protocol should be approved by the relevant research ethics committee in the country or institution in which the study is taking place.

Like all studies in which infectious agents are involved, efficacy testing often entails an occupational risk of acquiring infection in both laboratory and field settings. Measures for reducing such risks have been developed and widely implemented and include insect containment and manipulation, worker training and using known uninfected target species, when possible.

When human participants are hired or recruited, they must be informed about any responsibilities that may expose them to vectors, such as colony maintenance or conducting human landing catches in natural or field conditions. Participants are then expected to follow standard protocols, as outlined by the project leader or national guidelines; they will therefore be protected under occupational health standards to control exposure to biohazards.

### **5.1 Informed consent**

Volunteers should be given the full details of the project, including the purpose of the study, the procedures, product to be evaluated, the risks and benefits, reporting adverse effects and the voluntary right to refuse or withdraw from the study. Informed consent is usually documented on a signed and dated consent form. An example of an informed consent document is provided in Annex 5.

### **5.2 Human landing catch in semi-field and field testing**

Evaluations of landing and feeding inhibition should be based on the pathogen-specific risks of volunteers. By using laboratory-reared mosquito populations in semi-field trials, however, it may be acceptable to allow mosquitoes to feed with

minimal or no greater risk of disease for the volunteers. In all instances, ethical clearance from relevant ethics committees and informed consent from participating individuals is mandatory. In malaria-endemic countries, all participants must be screened for malaria before participation, and only parasite-free individuals should be allowed to participate.

For field trials with human landing catch in areas endemic for vector-borne disease, it is recommended, when feasible, to use healthy male recruits aged between 18 and 45 years. Males from outside the trial area (with potential exposure to unmatched vectors) and pregnant women should be excluded. Volunteers should be given protective clothing (i.e. a screen mesh jacket to protect the upper body, head and face, and closed-toe shoes to prevent bites to the feet). In malaria-endemic countries, it is recommended that participants in field trials be given appropriate prophylaxis, when possible under supervision to ensure correct compliance. When applicable, participants should be screened for parasitaemia by WHO standard microscopy or a parasite-appropriate rapid diagnostic test;<sup>17</sup> malaria treatment should be provided subsequently for infected volunteers, according to national ethical guidelines. Alternative methods for assessing personal protection may be required in field trials conducted in locales with known active circulation of arboviruses (i.e. dengue), such as monitoring indoor resting density, use of outdoor traps or monitoring mosquito entry with window traps.

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<sup>17</sup> *New perspectives: malaria diagnosis. Report of a joint WHO/USAID informal consultation 25–27 October 1999.* Geneva, World Health Organization, 2001 (WHO/CDS/RBM/2000.14 and WHO/MAL/2000.1091; [http://whqlibdoc.who.int/hq/2000/WHO\\_CDS\\_RBM\\_2000.14.pdf](http://whqlibdoc.who.int/hq/2000/WHO_CDS_RBM_2000.14.pdf)).

## **ANNEX 1: DEFINITION OF KNOCKDOWN AND MORTALITY FOR ADULT MOSQUITOES**

For the purpose of insecticide bioassays, the definition of knock-down and mortality involves not only the state of the insect but also the time at which the observation is made.

A mosquito is classified as dead or knocked down if it is immobile or unable to stand or take off (Table 1). The distinction between knocked down and dead is defined only by the time of observation. The assessment of knock-down is made within 60 min post-exposure. Mortality is determined at least 24 h post-exposure. The holding container may be tapped a few times before a final determination is made.

In the case of slow-acting insecticides, the recovery period may be extended beyond 24 h. Control mortality should be measured over the same recovery period. Mortality after 24 h should be recorded and, in some cases, repeated observations may be appropriate.

Table 1. **Classification of adult mosquitoes as alive, knocked down or dead in bioassays**

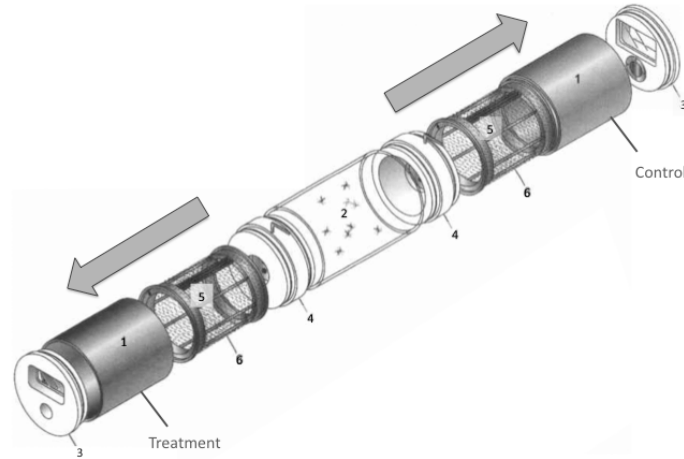
Alive	Knocked down after 60 minutes or dead after 24 hours of exposure	
	Moribund	Dead
Can both stand on and fly in a coordinated manner	<ul style="list-style-type: none"> <li>Any mosquito that cannot stand (e.g. has 1 or 2 legs)</li> <li>Any mosquito that cannot fly in a coordinated manner</li> <li>A mosquito that lies on its back, moving legs and wings but unable to take off</li> <li>A mosquito that can stand and take off briefly but falls down immediately</li> </ul>	No sign of life; immobile; cannot stand

## **ANNEX 2. SPATIAL REPELLENCY ASSAY SPECIFICATIONS**

The spatial repellency assay is modular, thus allowing examination of both spatial repellent and contact irritant responses. The main components of the modular system are illustrated and numbered in Figure A2.1. The required number and assembly of the components vary depending on the type of assay to be used. Each treatment cylinder (no. 1) is constructed of aluminium tubing (10.2 cm outside diameter, 0.6 cm thick, 14.0 cm long). Each clear cylinder (no. 2) is constructed of Plexiglas tubing with the same outside diameter and thickness as the treatment cylinders but with a length of 15.9 cm. Midway along the length of the clear cylinders, a hole covered with dental dam is provided for transferring mosquitoes. The end caps (no. 3) and gated funnels (no. 4) are constructed of Delrin® (Dupont, Wilmington, Delaware). The end caps (1.9 cm thick x 10.2 cm diameter) have been milled to slide part of the way inside either the treatment or clear cylinder and modified to provide a circular port for transferring mosquitoes and a rectangular port for viewing. The gated funnels (4.4 cm thick x 10.2 cm diameter) have also been milled, similar to the end caps, to slide partly into the treatment or clear cylinders. These sections are modified to form a funnel that leads to a 3.7-cm opening, in which an aluminium butterfly valve is installed.

Treatment filter paper (Whatman No. 1 or similar) is cut to 11 x 25 cm to fit inside the metal spool and is held in place by a 1.3 x 11.4-cm flexible magnetic strip (5699K15, McMaster-Carr, Dayton, New Jersey) (no. 7). The cradle (no. 8), constructed of 1.3-cm thick Plexiglas, holds the assembled test system steady and parallel to the bench top during assays. Pieces of opaque felt (not shown) are wrapped around the clear cylinder and the rectangular port in the end caps with Velcro attachments.





**Figure A2.1. Scheme of the spatial repellency assay**

The components are: 1, treatment (metal) cylinder; 2, clear (Plexiglas) cylinder (with mosquito introduction portal); 3, end cap (with Velcro to allow attachment of felt cover); 4, gated funnels (with butterfly valve); 5, metal spool; 6, treatment filter paper; 7, magnetic strip; and 8, cradle. Adapted from Grieco JP et al. A novel high-throughput screening system to evaluate the behavioral response of adult mosquitoes to chemicals. *Journal of the American Mosquito Control Association*, 2005, 21(4):404–411. Grey arrows indicate directional movement expected during corresponding evaluations.

### Example Spatial repellency assay datasheet

Dose	Chamber (control #/treatment #)	REP	N	CCNT	TCNT	KD CLEAR	KD CONT	KD TRT	TEMP	HUM	TIME	GEN/AGE	KD PRIOR	COMMENTS
X	C1:T1	1	20	0	0	0	0	0	27	60	0800	F2/5DO	0	
	C1:T1	2	20	5	5	0	0	0	27	60	0815	F2/5DO	0	
	C1:T1	3	20	1	0	0	0	0	27	60	0830	F2/5DO	0	
	C1:T1	4	20	13	4	0	0	0	27	60	0845	F2/5DO	0	
	C1:T1	5	20	11	4	0	0	0	27	60	0900	F2/5DO	0	
	C1:T1	6	20	11	3	0	0	0	27	60	0915	F2/5DO	0	
	C1:T1	7	20	14	1	0	0	0	26	60	0930	F2/5DO	0	
	C1:T1	8	20	14	3	0	0	0	27	60	0945	F2/5DO	0	
	C1:T1	9	20	17	3	0	0	0	26	60	1000	F2/5DO	0	

Dose: Concentration of technical material; Chamber: Labels on metal test chambers (C1, C2...Cn and T1, T2....Tn when conducting multiple tests); Rep: Number of replicates; N: Number of mosquitoes introduced into clear cylinder; CCNT: Number of mosquitoes in the control metal cylinder at the end of the assay sampling period; TCNT: Number of mosquitoes in the treatment metal cylinder at the end of the assay sampling period; KD Clear: Number of knock-down mosquitoes in clear cylinder at end of assay sampling period; KD Cont: Number of knock-down mosquitoes in control metal cylinder at end of assay sampling period; KD Trt: Number of knock-down mosquitoes in treatment metal cylinder at end of assay sampling period; Temp: Temperature at beginning of assay; Hum: Humidity at beginning of assay, Time: Time of assay; Gen/Age: Generation (F1-FN) and age of mosquito test population in days; KD prior: Number of knock-down mosquitoes in central cylinder immediately after introduction.

Sample calculations for spatial activity index

$$SAI = \frac{(N_c - N_t)}{(N_c + N_t)} \times \% \text{ Responding}$$

Replicate	Nc	Nt	% Responding	Calculation	Spatial activity index
1	0	0	0 (0 of 20)	$\frac{(0 - 0)}{(0 + 0)} \times 0$	0
2	5	5	0.5 (10 of 20)	$\frac{(5 - 5)}{(5 + 5)} \times 0.5$	0
3	1	0	0.05 (1 of 20)	$\frac{(1 - 0)}{(1 + 0)} \times 0.05$	0.50
4	13	4	0.85 (17 of 20)	$\frac{(13 - 4)}{(13 + 4)} \times 0.85$	0.45
9	17	3	1.0 (20 of 20)	$\frac{(17 - 3)}{(17 + 3)} \times 1.0$	0.70

### ANNEX 3. Y-TUBE OLFACTOMETER SPECIFICATIONS

The olfactometer is made of acrylic. The main Y-tube with the decision point is constructed from clear cast acrylic tubing: 3.25" (8.26 cm) outer diameter x 1/8" (0.32 cm) wall (US Plastics part #44590). An 18" (45.72 cm) segment of tubing (base leg) is joined to two 8" (20.32 cm) segments (branches) with IPS Weldon acrylic cement (#10315, Amazon.com) to produce a 90° angle at the decision point (Figure A3.1). At the terminal point of each branch, a 2" (5.08 cm) collar of 3.5" (8.89 cm) outer diameter x 1/8" (0.32 cm) wall (US Plastics part #44593) is cemented onto the Y-tube base leg. At the terminus of base leg and branches, collars are attached to allow attachment of trapping ports (Figure A3.2).

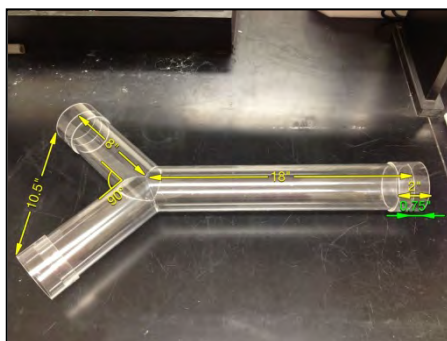


Figure A3.1. Main body of Y-tube olfactometer with decision point

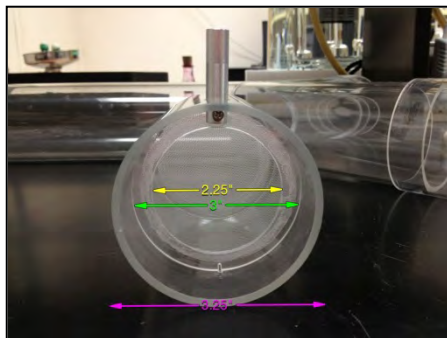
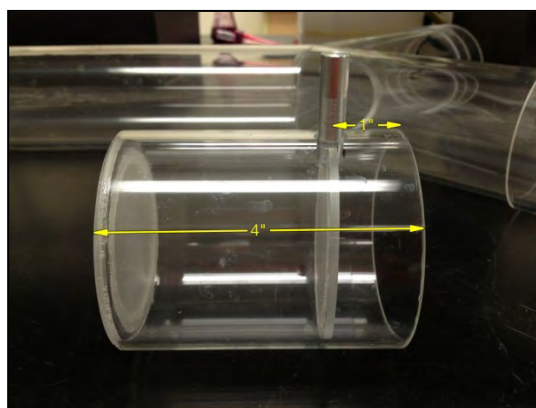


Figure A3.2. Edge view of a trapping port

Trapping ports are constructed from a 4" (10.16 cm) segment of 3.25" (8.26 cm) outer diameter acrylic. On one end of the trapping port, a screen mesh is glued to a clear 3" (7.62 cm) diameter acrylic circle (US Plastics part #44185), and the screened circle is glued into place at the edge of the housing (Figure A3.3). On the opposite side of the trapping port, a hole is drilled 1" (2.54 cm) from the edge to allow insertion of a 3/8" (0.95 cm) diameter aluminium rod (Grainger part #6ALN0). The rod is attached to another screened circle to function as a door that opens and closes to allow passage of mosquitoes during tests. A 1/4" (0.64 cm) diameter hole is drilled in the centre of the top wall of the trap (1" [2.54 cm] internal from the aluminium rod that opens the door) to allow mosquitoes to be loaded into the trapping port (from a mouth aspirator). A cork is used to seal the hole.



**Figure A3.3. Side view of trapping port**

The trapping ports are attached to the Y-tube body with the doors on the sides closest to the Y-tube. External to each trap are end caps, which contain a 2" (5.08 cm) collar constructed of 3.5" (8.89 cm) acrylic tubing and are attached to a 4" (10.16 cm) segment of 3.25" (8.26 cm) acrylic tubing (similar to the traps). The end caps for the treatment and control trapping ports of the Y-tube, however, contain a screened circle to seal the end (to allow for airflow) and are open on the side with the 2" (5.08 cm) collar. This allows placement of a small metal stand (1.5" x 1" [3.81 x 2.54 cm]) at the base of the end cap to allow treatments

and controls (usually delivered with a vial cap) to be placed on the stands inside each end cap, followed by attachment of the end caps to the traps.

The end cap for the trapping port on the base leg is made of a 4" (10.16-cm) piece of 3.25" (8.26-cm) acrylic tubing with a 2" (5.08-cm) collar. This end cap has no screen on the side closest to the trap where mosquitoes are held before an experiment. The other end is sealed with a solid acrylic circle with a hole drilled into the centre to allow a 3/8" (9.65-cm) male adapter nylon fitting 1/4" x 3/8" (0.63 x 9.65 cm) gas nozzle (US Plastics part#64198) to be screwed into the centre of the end cap. Tygon® tubing (1/4" [0.63 cm] ID x 1/2" [1.27] OD x 1/8" [0.32 cm] (US Plastics part #57111) is attached from the vacuum line to the nozzle.

When the unit is fully assembled, the airflow can be checked with an anemometer by inserting the test rod of the anemometer into the mosquito loading hole of the trap. The air velocity for treatment and control traps is set to  $0.20 \pm 0.05$  m/s. The air when checked at the trap of the base leg should be set to  $0.40 \pm 0.05$  m/s.

**Table A3.1. Example study design for assessing attraction–inhibition of an AI with a Y-tube olfactometer**

Test type	Port A	Port B	Outcome
Diluent effect (for each new diluent)	C <sub>0</sub>	No treatment	< 10% attraction to diluent and < 10% attraction to port B
Contamination (before each AI)	No treatment	No treatment	< 10% summed attraction to ports A and B
Host-seeking response (before tests with a new AI)	Host	Host	(1) > 50% summed attraction to ports A and B  (2) Equal numbers of mosquitoes collected in A and B

Test condition 1	Hand +C <sub>0</sub>	C <sub>0</sub>	<div> <div></div> <div></div> <div></div> <div></div> <div></div> <div></div> </div> <p>Treatment order is fully randomized before conducting these tests.</p>
Test condition 2	Hand +C <sub>0</sub>	C <sub>0</sub>	
Test condition 3	Hand +C <sub>0</sub>	C <sub>0</sub>	
Test condition 4	C <sub>0</sub>	Hand +C <sub>0</sub>	
Test condition 5	C <sub>0</sub>	Hand +C <sub>0</sub>	
Test condition 6	C <sub>0</sub>	Hand +C <sub>0</sub>	

Test condition 31	Hand +C <sub>5</sub>	C <sub>0</sub>
Test condition 32	Hand +C <sub>5</sub>	C <sub>0</sub>
Test condition 33	Hand +C <sub>5</sub>	C <sub>0</sub>
Test condition 34	C <sub>0</sub>	Hand +C <sub>5</sub>
Test condition 35	C <sub>0</sub>	Hand +C <sub>5</sub>
Test condition 36	C <sub>0</sub>	Hand +C <sub>5</sub>

C<sub>0</sub>, diluent only; C<sub>1</sub>–C<sub>5</sub>, concentrations of AI in diluent; H, hand odour





## **ANNEX 5. EXAMPLE OF INFORMED CONSENT FORM**

Informed consent form for participants in human landing catch  
for

[insert project title\_\_\_\_\_]

[Name of principal investigator]

[Name of organization]

[Name of sponsor]

[Name of proposal and version]

This informed consent form has two parts:

- Information sheet (to share information about the research with you)
- Certificate of consent (for signature if you agree to take part)

You will be given a copy of the full informed consent form

Date\_\_\_\_\_

### **PART I: Information sheet**

#### **Introduction**

Briefly state who you are, and explain that you are inviting them to participate in the research you are doing. Inform them that they may talk to anyone they like about the research and that they can take time to reflect on whether they want to participate. Assure the person that if he or she does not understand some of the words or concepts, you will take time to explain them as you go along and that they can ask questions now or later.

*(Example: I am ....., working for the ..... <name of the research institute>. We are doing research on ..... disease, which is very common in this country. I am going to give you information and invite you to be part of this research. You do not have to decide today whether you will participate in the research. Before you decide, you can talk to anyone you like about the research.*

*There may be some words that you do not understand. Please ask me to stop as we go through the information, and I will take time to explain. If you have questions later, you can ask [name of responsible staff]).*

### **Purpose of the research**

Explain in lay terms why you are doing the research. The language used should clarify rather than confuse. Use local, simplified terms for a disease, e.g. local name instead of malaria, mosquito instead of anopheles, “mosquitoes help in spreading the disease” rather than “mosquitoes are the vectors”. Avoid using terms like ‘pathogenesis’, ‘indicators’, ‘determinants’ and ‘equitable’. There are guides on the Internet to help you find substitutes for words that are overly scientific or are professional jargon.

*(Example .....disease is transmitted through the bites of .....mosquitoes. Knowledge of mosquito behaviour and how new methods to keep mosquitoes away from humans will help improve mosquito control in the area. In this study, we are testing a new repellent that will keep mosquitoes from biting humans. Studies in laboratories have shown this repellent is .....effective, and we want to check this result with mosquitoes in this area to make sure it is useful for people to use here.)*

### **Type of research intervention and procedures**

Briefly state the type of intervention that will be undertaken.

- As a participant you will be asked to collect mosquitoes landing on you before they bite you between \_\_ : \_\_ and \_\_ : \_\_ h. This involves collecting mosquitoes that land on your legs with a tube and a torch.

- You will be asked to not smoke cigarettes or drink alcohol for the days or weeks that you are participating.
- We will provide you with ..... medicine to stop you getting malaria if a mosquito with malaria does manage to bite you. You will have to take this every week and sign a form to show that you have taken the medicine; it will be paid for by the study.
- You will have to take a malaria test every week that you are working on the study and sign a form to show that you have taken the test; it will be paid for by the study. If you are sick we will give you the correct medicine: ....., and you will not be allowed to continue in the study.
- You can leave the study at any time without explanation. It is your choice to take part.

➤ ***Example of question to improve understanding:*** *Do you know why we are asking you to take part in this study? Do you know what the study is about?*

## **Voluntary participation**

Indicate clearly that the person can choose to participate or not. This can be repeated and expanded upon later in the form as well, but it is important to state clearly at the beginning of the form that participation is voluntary so that the other information can be heard in this context.

*(Example: Your participation in this research is entirely voluntary. It is your choice whether to participate or not. You may change your mind later and stop participating even if you agreed earlier.)*

➤ ***Examples of question to improve understanding:*** *If you decide not to take part in this research study, do you know what your options are? Do you know that you do not have to take part in this research study, if you do not wish to? Do you have any questions?*

## Risks

Explain and describe any possible or anticipated risks. Describe the level of care that will be available in the event that harm does occur, who will provide it, and who will pay for it. A risk can be thought of as being the possibility that harm may occur. Provide enough information about the risks to allow the participant to make an informed decision.

*(Example: The risk of this study is you may be made uncomfortable by mosquito bites, and you have a small risk of getting ..... disease, even though you are taking ..... medicine to prevent it. You will be given protective clothing to make sure mosquitoes can bite only on your legs, where you can catch them before they have time to bite. If you do become ill at any time during the study or during 1 month after the study, you will receive the correct treatment from ..... hospital. All costs will be paid by the study. There is also a risk that you might have some side-effects from taking medicines to stop getting ..... disease. If you feel unwell, you will see a doctor at ..... hospital who will give you the correct care and change the medicines you are taking.)*

- **Examples of question to improve understanding:** Do you understand that, while the research study is under way, you will receive free health care from ..... hospital? Do you understand that you may have some unwanted side-effects from the medicine to stop getting ..... disease? Do you have any other questions?

## Benefits

Mention only those activities that will be actual benefits and not those to which the prospective participants are entitled regardless of participation. Benefits can be categorized as those to the individual, those to the community in which the individual resides and those to society as a whole as a result of finding an answer to the research question.

*(Example: If you participate in this research, you will have the following benefits: you will be paid \_\_\_\_\_ for your work each night for up to \_\_\_\_\_ nights and will have a taxi home or accommodation at night after you have finished work.)*

- **Examples of question to improve understanding:**  
*Can you tell me if you have understood correctly the benefits that you will have if you take part in the study? Do you know if the study will pay for your travel costs and time lost, and do you know how much you will be reimbursed? Do you have any other questions?*

### **Right to refuse or withdraw**

This is reconfirmation that participation is voluntary and includes the right to withdraw.

*(Example: You do not have to take part in this research if you do not wish to do so. You may also stop participating in the research at any time you choose. It is your choice and all of your rights will still be respected.)*

### **Who to contact**

Provide the name and contact information of someone who is involved, informed and accessible (a local person who can actually be contacted). State that the proposal has been approved and how.

*(Example: If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact any of the following: [name, address/telephone number/e-mail]).*

**This proposal has been reviewed and approved by [name of the local ethical review board], which is a committee that makes sure that research participants are protected from harm. If you wish to find more about the ethical review board, contact [name, address, telephone number.]].**

- **Example of question to improve understanding:** *Do you know that you do not have to take part in this study if you do not wish to? Do you know that you can say 'No' if you wish to? Do you know that you can ask me questions later, if you wish to? Do you know that I have given the contact details of the person who can give you more information about the study?*

You can ask me any questions about any part of the research study, if you wish to. Do you have any questions?

## **PART II: Certificate of consent**

This section should be written in the first person and contain a statement similar to the one in bold below. If the participant is illiterate but gives oral consent, a witness must sign. A researcher or the person going over the informed consent forms must sign each form. **The certificate of consent should avoid phrases starting with "I understand....". Understanding is better tested by targeted questions during the reading of the information sheet (Some examples of questions are given above) or by questions asked at the end of the reading of the information sheet, if the potential participant is reading the information sheet him- or herself.**

*(Example I, ....., have clearly been informed of the aims of the project entitled "....." and I agree to participate in the study. During my participation in these studies, I have been told that mosquitoes can bite me and they may be carrying parasites. I am fully aware that I may revoke my consent and leave the study at any stage.*

*Participant name:*

\_\_\_\_\_

*Participant signature:*

\_\_\_\_\_ *Date:* \_\_\_\_\_ *DD/MM/YY*

Witness name:

\_\_\_\_\_

Witness signature:

\_\_\_\_\_ Date: \_\_\_\_\_ DD/MM/YY)

### **If illiterate**

A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Participants who are illiterate should appose their thumb print as well.

*(Example: I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.)*

Print name of witness: \_\_\_\_\_ AND thumb  
print of participant

Signature of witness: \_\_\_\_\_

Date: \_\_\_\_\_ DD/MM/YY)

Statement by the researcher or other person taking consent:

I have accurately read out the information sheet to the potential participant and to the best of my ability made sure that the participant understands that the following will be done:

1. Human landing catch will be conducted between \_\_ :\_\_ and \_\_ :\_\_.
2. The participant has been requested to refrain from smoking and consuming alcohol for the study duration.
3. The participant will be given free malaria prophylaxis,

screening and treatment for the duration of the study.

4. The participant will be reimbursed \_\_\_\_\_ for the working time taken up by the study.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant were answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this informed consent form has been given to the participant.

Print name of researcher or other person taking the consent:

\_\_\_\_\_

Signature of researcher or other person taking the consent:

\_\_\_\_\_

Date: \_\_\_\_\_(DD/MM/YY)







WORLD HEALTH ORGANIZATION  
CONTROL OF NEGLECTED TROPICAL DISEASES (NTD)  
WHO PESTICIDE EVALUATION SCHEME (WHOPES)

